12-2015

In Vivo Periodical Monitoring of Immune Cell Infiltration in Response to Feathers and Intramuscular Injection of IONPs Using the Pulp (Dermis) of Growing Feathers as Test Site Tissue in Chickens

Olfat Taleb Alaamri
University of Arkansas, Fayetteville

In Vivo Periodical Monitoring of Immune Cell Infiltration in Response to Feathers and Intramuscular Injection of IONPs Using the Pulp (Dermis) of Growing Feathers as Test Site Tissue in Chickens

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy in Cell and Molecular Biology

by

Olfat T. Alaamri
King Abdul Aziz University
Bachelor of Science in Biochemistry, 2002
University of Arkansas
Master of Science in Cell and Molecular Biology, 2011

December 2015
University of Arkansas

This dissertation is approved for recommendation to the Graduate Council.

_____
Douglas D. Rhoads
Dissertation Director

_____
Jeannine M. Durdik
Committee Member

_____
Bob Wideman Jr.
Committee Member

_____
Charles F. Rosenkrans Jr.
Committee Member

ABSTRACT

The applications of nanoparticles are growing, but little is known about their interactions with the immune system as most studies did not use suitable *in vivo* test systems. The purpose of these studies was to investigate the utility of chicken growing feathers as an *in vivo* test site for iron oxide nanoparticle (IONP) interactions with the immune system. The first objective of this study was to monitor leukocyte infiltration into the growing feather pulp upon the administration of IONP and IO-mIgG preparations. The second objective was to test the utility of IONPs as vaccine adjuvants by monitoring primary and memory immune responses in the growing feathers upon intramuscular injection of treatment preparations. For the first objective, three feather injection studies were conducted. The IONP treatment alone elicited similar responses as the IO-mIgG treatment, suggesting that IONPs can elicit immune responses without the presence of an antigen. The IO-mIgG treatment elicited a significantly higher heterophil response, $p = 0.009$, and MHCII+ macrophage response, $p = 0.0027$, compared to the Alum-mIgG group. However, Alum-mIgG elicited significantly higher adaptive immune responses compared to IO-mIgG. It is possible that IONPs were taken up by the innate immune cells before they could activate the adaptive immune cells, but the analyses of organ tissue is an ongoing study that will further clarify this finding. For the second objective, four intramuscular injection studies were conducted using IONPs, Alum-mIgG, and mIgG treatments. The mIgG concentration in Study A was 20-fold lower in the IONP group compared to the other groups. IO-mIgG elicited significantly higher MHCII+ B cell+, γδ T cell+ CD8+, and γδ T cell+ CD8- feather pulp infiltration compared to the other groups. When the mIgG concentration for all treatment groups was matched in Study B, the IO-mIgG group elicited similar or significantly lower responses compared to the other groups. These findings suggest that growing feathers in chickens can be

used to monitor immune responses to treatments periodically in one subject. The clinical utility of IONPs as vaccine adjuvants is promising, but further research is necessary to improve the knowledge of IONP biointeractions and toxicity.

Keywords: adaptive immunity, growing feather, innate immunity, IONP, primary immune response, memory immune response

ACKNOWLEDGEMENTS

I am grateful to my advisor, Dr. Rhoads, for helping me finish my dissertation under his supervision. Your help has been invaluable and much appreciated.

To the Committee Members, I wish to express my sincere gratitude for accepting to serve as my committee.

I want to express my gratitude to Dr. Erf, who taught me research techniques and design, as well as helped me in the early stages of this research.

I am also grateful to all of my colleagues at Dr. Erf's lab for their help and for creating an enjoyable working environment.

I wish to thank my mother and father for their help and their continuous unconditional support that gave me strength and helped me overcome any obstacle I came across.

I am grateful to my husband Rani Bakhsh and my daughters Monai, Maya, Mies, and Mira for their support and their help with some of the experiment preparation, sampling, and farm maintenance duties.

TABLE OF CONTENTS

PAGE

LIST OF TABLES

PAGE

LIST OF FIGURES

PAGE

INTRODUCTION

The development of *in vivo* test systems for testing bioactivities and toxicity of nanoparticles (NPs) is required to ensure that they are safe and effective in healthcare, but current animal models are invasive and do not allow monitoring bioactivities periodically in one individual. The aim of this study is to develop an avian test-system that would allow repeat access to tissue from the test-site and would enable researchers to utilize minimally invasive tissue collection methods.

Biological interactions and toxic effects of NPs are still being investigated, but NPs are often used in consumer products. Consequently, the safety of nanostructures to the health of individuals, as well as the environmental risks associated with NP manufacturing byproducts and NP waste disposal, are a major concern (Roco, 2005). In the biomedical field, previous studies found that the toxicity of NPs mostly depends on the type of NP core material (Chen, Zhen, Todd, Chu, & Xie, 2013). Although no long term adverse events have been reported, the degradation of the NPs metal cores can leave traces of toxic metals in the body.

Quantum dots show the highest toxicity because cadmium residue can be found in the organs (i.e., liver, spleen, and kidneys) up to 90 days after administration (Chen et al., 2013). Metal NPs are also a potential hazard due to toxic effects, but their toxicity is mostly regulated because it is affected by size, solubility, and level of targeted cellular uptake (Johnston et al., 2010). The lowest levels of toxicity are reported for iron oxide NPs (IONPs), which increase the risk of oxidative tissue damage by increasing cytoplasmic ferric iron levels (Zhao et al., 2011). Although some degree of oxidative stress has been reported due to IONP administration in mice and non-human primates, no long-term effects were observed upon histological analyses of organ tissues (Jain et al., 2008; Pusic et al., 2013).

The numerous potential medical applications of NPs include improved diagnosis via contrast imaging, targeted drug delivery, tissue engineering, and the use of NPs as adjuvants in vaccine preparation (Salata, 2004). Depending on the purpose of the NP in the organism, different surface modifications can be applied. For example, manufacturing NPs to deliver drugs to cancer cells needs to improve the longevity of the NPs in the blood, which is achieved by avoiding complement activation and modifying the size and surface of the particles to avoid premature elimination (Kievit & Zhang, 2011; Sim & Wallis, 2011). However, when NPs are used as vaccine adjuvants, they must be able to elicit a high immune response.

At the moment, the most common adjuvant found in the vaccines produced in the United States is aluminum, which is used in form of gels or salts in small doses to elicit an immune response so that the body can build better immunity when exposed to the antigen in the vaccine. The U.S. Food and Drug Administration (FDA) recommend an upper limit of 0.85-1.25 mg for aluminum used in vaccines, but the exact dose depends on age and bodyweight, so aluminum contents in vaccines can be as low as 0.25 mg for infants (Keith, Jones, & Chou, 2002).

Compared to aluminum, monophosphoryl lipid A (MPL-A) is a less common adjuvant in vaccines, but its effectiveness and safety have been established (Casella & Mitchell, 2008; Drachenberg, Wheeler, Stuebner, & Horak, 2001). MPL-A is derived from the *Salmonella minnesota* lipopolysaccharide (LPS). LPS induces a higher proinflammatory cytokine response compared to MPL-A, but MPL-A is safer for use in humans because it attenuates the toxic effects of LPS (Ulrich & Myers, 1995).

The key difference among adjuvants is their mode of action because they can work as antigen delivery systems or immunopotentiators, but the balance between efficacy and safety is also a major concern for healthcare practitioners and clinical researchers (Batista-Duharte,

Lindblad, & Oviedo-Orta, 2011). Depending on the patient's medical history and current situation, the administration of adjuvants in vaccines can trade off efficacy for safety when patients are at high risk for adverse events. Trading off safety for efficacy is also an option for patients with severe disease (e.g., human immunodeficiency virus, cancer patients), who need stronger adjuvants to elicit an adequate immune response compared to the general healthy population. The necessity of redistributing the efficacy and safety of adjuvants is the key issue with contemporary adjuvants, so novel adjuvants are continuously developed to address the needs of specific vulnerable populations (Grzegorzewska, 2014).

Although NPs proved to be efficient adjuvants with little or no toxic effects (Alaamri, Byrne, Falcon, & Erf, 2014; Pusic et al., 2011), the understanding of NP-immune interactions *in vivo* is still limited. The efficacy and safety of NPs as vaccination adjuvants needs to be further investigated in animal models before their utility is investigated in human clinical trials. However, the choice of animal models affects the outcomes of studies that monitor NP *in vivo* interactions as each model has certain strengths and limitations regarding procedural advantages and reliability of results.

Based on previous studies (Erf, Trejo-Skalli, & Smyth, 1995; Erf, 2010; Wang & Erf, 2004), the chicken model was identified as the least invasive model that has several immune system features similar to mammalian organisms because growing feather (GF) pulp can be used as a test-site for biological interactions. It is important to note that the use of GF as a test site is not limited to specific agents or poultry species. All young feathered birds have GF, and the only difference between the species is the age at which they develop and lose the ability to regenerate feathers (Erf, U.S. Patent No. 8,216,551, 2012). The use of GFs as a test site in chickens is common because they are immunologically well-defined and easy to obtain for research.

Specifically, the Long Brown Leghorn (LBL) chicken line (see Figure 1) is immunologically well-defined and is not susceptible to autoimmune vitiligo (Erf, 2010; Sreekumar, Smyth, Ambady, & de Leon, 2000). These advantages of the LBL line make it a suitable *in vivo* test system for monitoring the effects of NPs on cellular, molecular, and physiological events.

GFs are present in chickens between 2 and 22 weeks of age. The GF consists of the barbs and the living portion, which is ensheathed (see Figure 1). The living portion size depends on the chicken line and age, so its size varies between 8 and 10 mm in length and between 2 and 3 mm in diameter. The inner pulp arises from the dermal papilla and makes up the majority of the living portion. It consists mostly of mesenchymal reticulum, which is a loose and pliable tissue, but the pulp also has a central axial artery and a network of vascular structures, including arterioles, capillaries, lymphatic capillaries and vessels, small sinuses, venules, and veins (Lucas & Stettenheim, 1971). The nervous system in the pulp is comprised of three sets of nerves, which include the autonomic ganglion cells, short fibers that follow a sinusoidal course, and long fibers positioned along the peripheral part of the pulp. The pulp is immunologically active with a low mononuclear cell presence similar to the dermis and the ability to recruit leukocytes from the circulation (Abdul-Careem et al., 2008; Erf et al., 1995). The pulp of the feather is surrounded by an epidermal layer and an outer sheath comprised of connective tissue (see Figure 1).

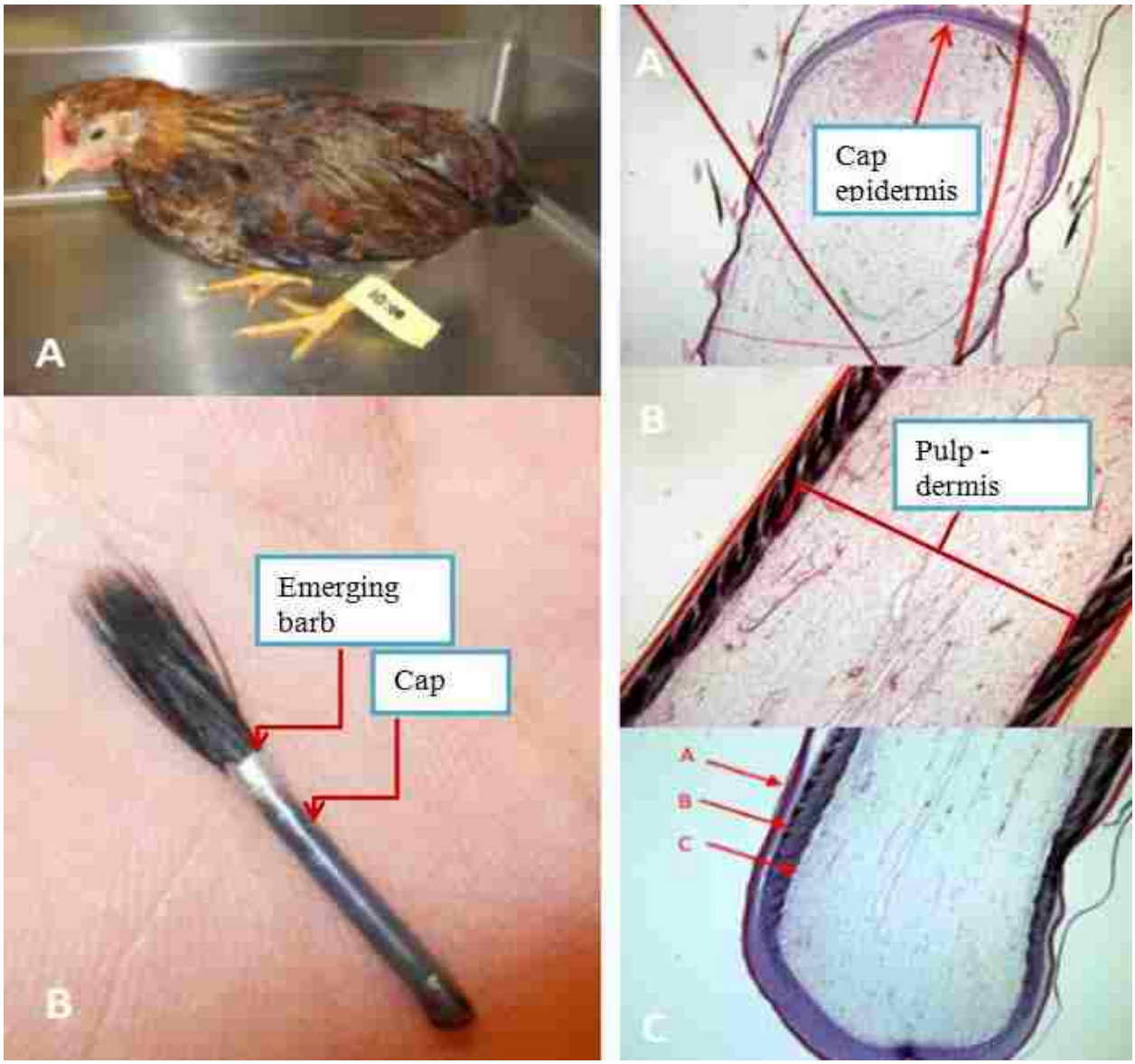

Figure 1. The chicken model and growing feather anatomy.

Left panel: The chicken model used in the current studies, showing a) one male LBL at 7 weeks of age and b) a plucked 2-3 week old GF, approximately 8-10 mm long and 2-3 mm in diameter of living tissue from bottom (newest growth) to epidermal cap.

Right panel: H/E stained sections showing the feather's a) top, b) part of the middle, and c) bottom .The red arrow in a) point to the cap portion of the epidermis. The red arrows in c) point to the sheath (A), pulp epithelium epidermis (B), and the pulp (dermis)(C).

Source: Erf (U.S. Patent No. 8,216,551, 2012)

The presence of GFs can be confirmed by plucking a feather, which should subsequently regenerate. The regenerating feathers allow researchers to periodically monitor the biological activities associated with NPs in one subject, and the procedures used are minimally invasive (Erf, U.S. Patent No. 8,216,551, 2012). The results obtained from tissue analyses of GF are more reliable than those obtained from the skin because GF injections restrict the testing area to the inner pulp, whereas the distribution of particles after skin injections cannot be controlled. As these features are specific to the chicken model, it has several advantages over other animal models that can be used to study NPs *in vivo*.

The purpose of the current studies is to demonstrate the feasibility of the chicken model and the GF as a test site for *in vivo* monitoring of immune responses to injections of antigen-conjugated IONPs. Although Furthermore, the current studies aim to determine how the effects of NPs on immune system responses compare to traditional aluminum adjuvants because it was suggested that NPs could improve targeted vaccine delivery and elicit high immune responses (Aguilar et al., 2010; Singh, Chakrapani, & O'Hagan, 2007). Two research objectives were established:

Objective 1: Monitor and assess local leukocytes infiltration into the pulp that occurs after the administration of antigen-conjugated NPs and NPs via GF injections. Upon the administration of treatments in the GF pulp, the NPs are exposed to the soluble and cellular components of the innate immune system. Using GFs as a test site allows for periodical collection of tissue samples from the same bird, and the evaluation of innate immune responses can be conducted by analyzing the pulp of the injected feathers and the peripheral blood. The immunomodulatory activity of NPs will be compared to the activity of other treatments, including antigen, antigen-conjugated NPs, antigen-conjugated Alum, and Alum. It was

hypothesized that the nature and extent of leukocyte infiltration would depend on the treatment used. Specifically, it was expected that NPs alone would achieve no inflammatory activity or moderate activity compared to other treatment preparations.

Objective 2: Monitor and assess the adaptive immune system's primary and memory responses to the intramuscular injections of antigen-conjugated NPs. This objective addresses the necessity of improving vaccine adjuvants because an efficient adjuvant should be able to achieve both humoral and cell-mediated immune responses. Just like the first objective, the goal of this of this objective was to demonstrate the feasibility of using the chicken model as a minimally invasive alternative to other *in vivo* test systems. Whereas the first objective focused on the innate immune response to NPs, this objective focuses on monitoring the humoral and cell-mediated immune activities of the adaptive system. Therefore, the goal of this objective was to demonstrate that the chicken model is suitable for simultaneously monitoring both humoral and cell-mediated immune responses periodically in each chicken. That feature makes it more versatile and cost-effective compared to conventional animal models used for *in vivo* research.

The second goal was to determine whether NPs are an effective and safe adjuvant. It was hypothesized that the primary and memory response would be higher in chickens that had received antigen-conjugated NPs compared to chickens that had received antigen-conjugated Alum or antigen injections alone.

By addressing these two objectives, it is expected that this research will make various contributions to future studies investigating the *in vivo* effects of NPs. Specifically, this study contributes to the development of an avian *in vivo* test system using GF as a test site in the LBL line of chickens. This will address the need for the development of less invasive *in vivo* test systems compared to conventional models, such as non-human primates or mice (National

Research Council, 2012). The injection of NPs and antigen-conjugated NPs into the GF pulp tissue allows for periodical monitoring of local inflammatory responses, thus creating an opportunity to develop indexes for determining *in vivo* NP toxicity and bioactivity.

In addition to enhancing the avian test system for monitoring NPs *in vivo*, these studies also assess the utility of NPs as a vaccine adjuvant. As new adjuvants need to be developed to address the problem of balancing their safety and efficacy depending on each patient's situation, NPs could prove to be a more effective vaccine platform compared to conventional adjuvants. Specifically, IONPs are used as they have not been associated with adverse events because of toxicity risks. If antigen-conjugated NPs can improve the efficiency of immune responses compared to aluminum adjuvants without displaying toxic effects, future studies can refine the use of NPs as vaccine platforms to enhance the quality of healthcare delivery.

CHAPTER 1—LITERATURE REVIEW

Nanoparticles (NPs) are a promising diagnosis and treatment tool in healthcare because of their ability to transport materials, such as pharmacological agents, and target specified structures on an atomic or molecular level. However, safety is still a major barrier to the use of NPs because the materials used to develop them are inorganic and diverse. Although some NPs are approved for commercial use, some materials can increase the risk for toxicity-related adverse events that are difficult to establish for each type of NP. At the moment, there is no consensus for lower and upper NP toxicity levels despite the numerous *in vitro* and *in vivo* studies. Even though no short-term adverse events have been reported in studies using mice and non-human primate animal models, long-term follow-ups revealed residual doses of toxic materials in various tissues (Chen, Zhen, Todd, Chu, & Xie, 2013). The interactions between NPs and biological systems, as well as their potential toxicity threats and immunological responses, require further investigation before NPs are implemented in biomedical practice.

NP Overview

NP Types and Properties

NPs are classified based on their size, shape, and construction materials. Fine particles (diameter: 100-2,500 nm) and coarse particles (diameter: 2,500-10,000 nm) are classified as nanoparticles, but ultrafine particles (diameter: <100 nm) show the most effective results for *in vivo* application (Dobrovolskaia & McNeil, 2007; Müller et al., 2007; Xiang et al., 2006). However, it is important to note that shape also determines how size affects rate of uptake in cells. For example, when fine particles are used to bind to the target, rod-shaped NPs show higher cellular uptake compared to other shapes (Gratton et al., 2008), but spheres outperform rods when ultrafine particles are used (Albanese, Tang, & Chan, 2012). The optimal size for

maximizing rate of uptake in mammalian cells and intracellular concentrations was established at 50 nm (Albanese et al., 2012). Therefore, spherical ultrafine particles appear to be the most promising NPs for biomedical applications.

Several materials can be used to build NPs, and some examples include iron oxides (IONP), gold (AuNP), dendrimers, quantum dots, polymers, and nanotubes (Llevot & Astruc, 2012). Each type of material has specific properties that determine its biomedical applications and efficiency, but also risk for toxicity.

Quantum dots (QDs) produce different emissions depending on their size and composition, so one group of QDs can produce a wide emission spectrum even when they are excited with a single light source. Because of that property, QDs were considered ideal tags for multiplex optical fluorescence imaging (Chen et al., 2013). However, the main issue with QDs is the high level of toxicity because they release cadmium during deterioration. Cadmium residue was detected in various organs, such as the liver and kidneys, of rhesus macaques monkeys 90 days after the administration (Chen et al., 2013).

IONPs improve the visibility of anatomical structures in magnetic resonance imaging (MRI) results because of their property to work as contrast agents. For example, conventional MRI can reveal tumor size and location, but IONPs in conjunction with MRI can also reveal metastases, which are too small for conventional MRI to detect (Harisinghani et al., 2003). The ability to enhance MRI images is only one feature of IONPs. Like other NPs, they can also be used for targeted drug delivery, and they are efficient vaccine adjuvants (Alaamri, Byrne, Falcon, & Erf, 2014; Kievit & Zhang, 2011).

AuNPs have multiple potential applications in healthcare because of their scattering and absorption properties. These properties vary based on their shape and size, so large AuNPs can

be used for diagnostic tests (e.g., optical coherence tomography) because they scatter light better than small AuNPs. However, small AuNPs have a higher absorption efficiency compared to large AuNPs, which makes them efficient for hyperthermal therapy (Chen et al., 2013). When both large and small AuNPs are combined, their absorption and scattering properties make them a superior contrast agent compared to fluorescein molecules and other nanoparticles based on the strength of their emissions and photostability (Gobin et al., 2007; Huang & El-Sayed, 2010).

Various other materials have also been tested, including carbon black NPs and metal oxide NPs (e.g., zinc, nickel, copper). Carbon black NPs were associated with inflammatory and genotoxic events in the primary exposed tissue, as well as secondary tissues (Bourdon et al., 2012). Zinc, nickel, and copper metallic oxide NPs were all associated with cytotoxic effects (Cho et al., 2011). However, IONPs are considered safe because no long-term or significant toxic events have been reported in previous studies (Jain, Reddy, Morales, Leslie-Pelecky, & Labhasetwar, 2008; Pusic et al., 2013).

NP Modifications

NP surface modifications are essential for overcoming barriers to the effective biomedical implementation of NPs, most notably the challenges of preventing the destruction of NPs by the immune system. The purpose of modifications is to ensure that the NPs do not activate the complement immune system while remaining hydrophilic so that they are compatible with biological fluids. Current research shows that polymers with repetitive recognition patterns can avoid complement activation by altering their conformation, thus resulting in improved NP longevity and allowing them to reach targeted cells (Sim & Wallis, 2011). Another strategy is to use evasion strategies observed in bacteria and parasites adapted to human tissue, such as

borrowing complement-regulating proteins (e.g., Factor H) to limit complement activity (Sim, & Wallis, 2011).

Surface modifications are also used for targeted drug delivery and diagnosis. For targeted drug delivery, NPs need to reach their target without interference from macrophages, so macrophage evasion techniques are currently being developed to ensure that the NPs stay in the blood long enough to reach the targeted cells. Surface modifications are currently the most effective method for improving targeted drug delivery, but methods like red blood cell hitchhiking and alternative shapes are also possible solutions for avoiding macrophages (Yoo, Chambers, & Mitragotri, 2010).

The biocompatible coating that surrounds the metal core of the particle contains therapeutic agents. For example, NPs that deliver drugs to tumor cells can contain gene therapy agents, therapeutic proteins, and chemotherapy drugs (Kievit & Zhang, 2011). In order to deliver those drugs to the target cells, the NPs need to be functionalized by attaching targeting agents to their coating. NP-antibody conjugates and membrane-permeating molecules are often used in experiments to diagnose or treat specific types of cancer based on their expression of antigens (Llevot & Astruc, 2012).

NP Potential Applications

Cancer therapy is currently one of the most promising fields because NPs allow for targeted drug delivery. Other examples of the potential applications of NPs in biomedical science that can improve human quality of life and well-being also include pathogen detection, protein detection, and tissue engineering.

Cancer therapy. Because of their properties and the ability to perform passive and active targeting via various targeting moieties (e.g., antibodies and aptamers), NPs could improve the

diagnosis and treatment of cancer. The use of NPs for cancer treatment using *in vivo* animal models found that drug delivery efficiency and survival rates improve significantly when NPs are used (Yu, Park, & Jon, 2012). In addition to targeting moieties, other effective methods for targeted cancer drug delivery include drug-encapsulated IONPs (Yigit, Moore, & Medarova, 2012) and adjusting responsiveness to pH differences between malignant and non-malignant cells (Lim et al., 2011).

AuNPs are currently the most promising type of NPs for cancer treatment in human subjects because of their ability to attach to cancer cells and induce intracellular hyperthermia once stimulated with radio frequency radiation or other stimuli (Glazer et al., 2010). Some clinical trials in palliative care patients with AuNPs have already been conducted. Biopsies were used to evaluate the targeting efficiency of AuNPs, and it was found that AuNPs were present only in tumors, but not in the healthy tissues (Libutti et al., 2009 as cited in Jain, Hirst, & O'Sullivan, 2012). However, the toxicity of AuNPs is still contradictory. While Pan et al. (2007) reported that small AuNPs (1-2 nm) are toxic, larger AuNPs (>15 nm) proved to be safe even in larger doses. However, those findings are contradictory to the findings by Vecchio et al. (2012), which indicate that AuNP-related toxicity is exclusively dose-dependent, regardless of the particles' sizes.

Pathogen detection. Biosensors using NPs that bind to DNA samples can measure concentrations and identities of pathogens. The Bead Array Counter (BARC) is an example of a biosensor that injects magnetic fine NPs (1,000 nm) into the sample placed in the instrument. Once the magnetic field removes excessive particles, the giant magnetoresistive sensor detects the intensity and location of the signal received from the remaining beads (Edelstein et al., 2000). Depending on the signal's intensity and location, the concentration and the identity of the

pathogens analyzed are determined. Although the primary purpose of developing this technology was to improve the identification process of biological warfare agents, it is possible that this technology will prove useful in healthcare diagnostics.

Protein detection. As a key variable in cellular functions and inter-cellular communication, understanding protein-protein interactions can improve the understanding of physiological mechanisms on cellular levels. Using 10 nm AuNPs, Cognet et al. (2003) demonstrated the application of photothermal interference contrast for 3D imaging of low levels of intracellular proteins. Therefore, this method can be used to detect low-expression proteins as an alternative to manipulating induction conditions or examining codon usage.

Protein detection techniques can also have therapeutic applications if they are used to detect small concentrations of protein targets that are biomarkers for disease. For example, the bio-bar-code method can detect prostate-specific antigen (PSA), which is often found in small concentrations during early stages of relapse in former prostate cancer patients, and it is also present in even smaller amounts among female breast cancer patients. The high sensitivity of the method allows it to detect PSA concentrations at 30 attomolar concentration using a 10 μL sample (Nam, Thaxton, & Mirkin, 2003). The potential application of this method is facilitating the therapeutic response to cancer relapse so that early adjuvant therapies could increase treatment success rates.

Tissue engineering. While polymer NPs are not suitable for therapeutic interventions, they have the potential to improve tissue engineering by forming natural structures corresponding to the body's native structures. For example, natural bone surfaces do not have a smooth surface, which is characterized by pores of 100 nm in diameter (Salata, 2004). Using polymer nanoparticles makes it possible to replicate these structures for bone and dental repairs.

Those nanostructures are uniform and strong so that the body can transport nutrients through the porous surface without compromising adhesion, mechanical strength, and rejecting the foreign substance (Salata, 2004).

Even if biomaterial tissue is used for implants, the use of NPs could prevent the formation of biofilm, which is recognized as a cause of bacterial adhesion and infections. Antibiotics and immune cells cannot penetrate the biofilm to cure biomaterial-associated infections, but IONPs with a polymer brush coating can significantly reduce biofilm formation to prevent bacterial adhesion or expose the pathogens to antibiotics and immune cells (Thukkaram, Sitaram, Kannaiyan, & Subbiahdoss, 2014).

Vaccine platform. The effectiveness of peptide vaccines depends on whether the adjuvant can induce an immune response, but adjuvants that prove effective in animal models are not necessarily effective in human clinical trials. For example, malaria vaccine with anti-Merozoite Surface Protein 1-42 (anti-MSP1-42) with oil-water emulsion adjuvants proved to be safe and immunogenic in animal models. However, human trials using adjuvants with anti-MSP1-42 did not demonstrate the same immunogenic efficacy (Ogutu et al., 2009). Therefore, alternative strategies need to be investigated to improve the immunological efficacy of agents in vaccine.

The use of NPs as a delivery platform to increase the immunogenic effect of vaccines is one of the promising strategies. The proof-of-concept study by Pusic et al. (2011) found that small (< 15 nm), water-soluble, inorganic QDs can be used to stimulate an immune response that could enhance the activation of T-cells or antigen presentation. The IONP-mouse IgG conjugate also proved to increase lymphocyte infiltration in Light Brown Leghorn (LBL) significantly

higher compared to other adjuvants (Alaamri et al., 2014). Aluminum gels or salts are typically used as vaccine adjuvants, but they are outperformed by NPs.

IONP Overview

IONPs are well-known for their intrinsic superparamagnetism that allows minimally-invasive, highly reliable tumor detection with MRI. These particles also have several potential applications as cancer treatment delivery agents and adjuvants for vaccine. The base of each IONP is the iron oxide core that serves as a contrast agent for diagnostic purposes and an agent carrier for therapeutic purposes. IONPs are biodegradable, which is suitable for *in vivo* applications, but the particles need to be designed to evade physiological barriers (i.e., blood, liver, kidneys, spleen, and blood-organ barriers) and cellular barriers so that their longevity inside the organism allows them to perform the intended function. This is achieved using suitable coating materials to protect the core from early interactions with native systems and increase their biocompatibility (see Figure 2). For example, lauric acid coating can increase cellular uptake and cellular interactions of IONPs *in vivo* compared to dextran coating (Pradhan, Giri, Banerjee, Bellare, & Bahadur, 2007).

The level of cellular uptake and interactions does not necessarily improve the longevity of the particles, which cannot be used for diagnostic or therapeutic purposes if they are eliminated before they reach their targets. The IONP coating usually consists of polyethylene glycol (PEG) because it protects the core from quick degradation in the blood as well as the adsorption of blood components (Kievit & Zhang, 2011). Another common threat in the blood includes the complement immune system proteins, which can trigger an immune response to eliminate the IONP if they are recognized as threats. These threats are usually evaded using

various strategies, such as modifying the shape of the particles to minimize complement activation (Sim & Wallis, 2011).

Besides blood components, elimination by the kidney, liver, and spleen is another significant concern for the longevity of IONPs. However, consistent with the size of materials taken by the reticuloendothelial system (> 100 nm) and the pore size on the kidney's basal lamina (approx. 10 nm), IONPs sized between 10 and 100 nm show reduced uptake by clearance organs (Longmire, Choyke, & Kobayashi, 2008). Therefore, coating and size are key determinants for IONP longevity and reaching the targeted cells (see Figure 2).

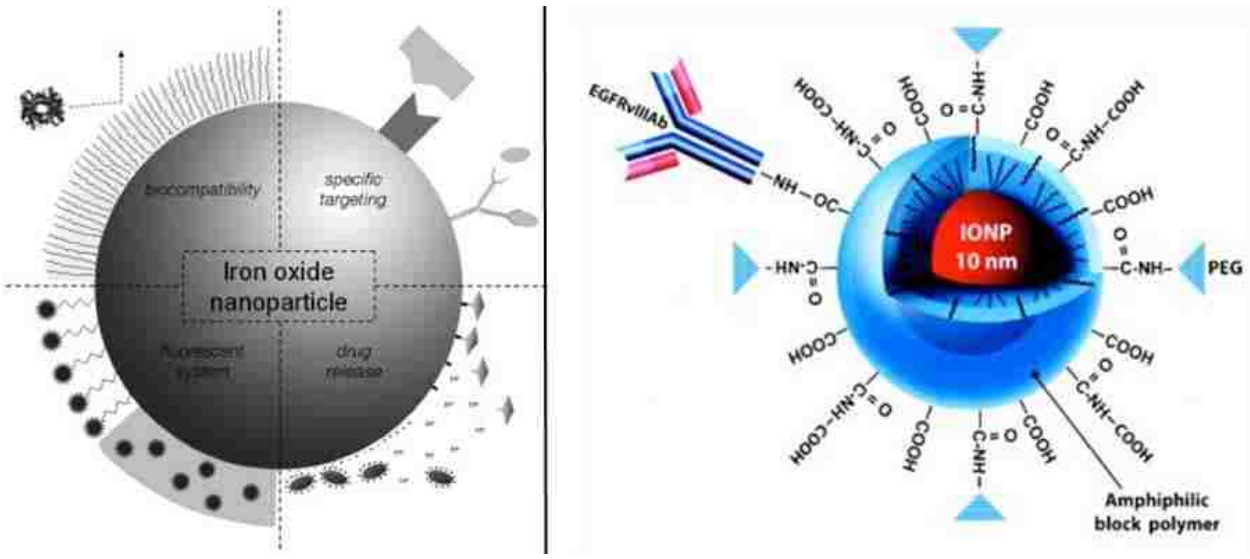

Figure 2. Overview of possible IONP modifications that could be used in clinical practice.

Left panel: Biocompatibility is achieved by coating the particles with polymers to increase their blood circulation longevity; specific targeting is achieved by attaching ligands or antibodies to the surface; organic fluorophores are conjugated to the surface for imaging purposes; drug release of attached therapeutic agents is achieved by setting up a stimuli-responsive release, so that the drugs are delivered due to enzymatic action or changes in pH value. Reproduced from Figuerola, Di Corato, Manna, and Pellegrino (2010).

Right panel: IONP design used by Hadjipanayis et al. (2010) to demonstrate how the particles can enhance glioblastoma multiforme MRI visibility and induce apoptosis in tumor cells without causing toxic effects to human tissue. The core is 10 nm in diameter and is coated using PEG, which improves the IONPs blood circulation longevity and enables them to reach the target. The surface is modified using epidermal growth factor receptor (EGFRvIII) so that the particles can bind to the tumor cells without binding to healthy brain cells. Reproduced from Hadjipanayis et al. (2010).

If the particles can reach the targeted cells after bypassing barriers, they will also need to enter the cells and identify the targeted intracellular components to perform their functions. This is achieved by using targeting agents that are placed on the coating of the particle, but various other factors determine the efficacy of cell penetration. Those factors include size, charge, and coating materials. Using tumor cells as an example, the best cellular uptake occurs with particles above 60 nm in diameter (Kievit & Zhang, 2011). Cationic particles have high cell penetration, but their distribution in the tumor is poor, so charge-reversal can be used to change anionic

particles to cationic particles at low pH levels once they distribute throughout the tumor (Mok et al., 2010). This approach enhances both particle distribution and cell penetration. Finally, coating is critical for penetrating the endosome membrane before the cell's enzymes target it for degradation.

The effects of IONPs on immune responses and their toxicity have been tested extensively. The immune response depends on various factors, such as NP charge, particle purity, particle size, and surface properties. Most studies agree that cationic IONPs induce higher inflammatory response compared to anionic and neutral IONPs (Dobrovolskaia & McNeil, 2007). IONPs should also be purified to reduce inflammatory responses associated with impurity because there are better ways to stimulate the immune system if the particles are used as vaccination adjuvants (Müller et al., 2007).

In order to activate the cell-mediated immune system, small particles (< 100 nm) should be used because they were associated with higher levels of antibodies compared to large particles (Xiang et al., 2006). However, it is important to note that both small and large IONPs are capable of activating T helper cells, which consequently activate and regulate the B cells, T cells, and macrophages (van Zijverden & Granum, 2000). Furthermore, surface modifications are essential for stimulating the immune system because the particles can be engineered to mimic pathogens. For example, Toll-like receptor (TLR) modifications with antigens are used to deliver antigens to the dendritic cells (DCs), which activate T cell proliferation (Diwan, Elamanchili, Lane, Gainer, & Samuel, 2003).

The toxicity of IONPs is still a significant concern and a barrier to their practical implications in biomedicine. Upon the degradation of the iron core, the divalent metal transport channel moves the ferric iron into the cytoplasm, thus causing an increase in cytoplasmic iron

levels (Zhao et al., 2011). This release of ferric iron into the cytoplasm results in the development of reactive oxygen species, which damage cell functionality. The level of oxidative stress is tissue-dependent, and it peaks at approximately 3 days after the injection in rats, but the effects of oxidative damage does not cause long-term abnormal changes in liver, spleen, and kidney tissues of small animals (Jain et al., 2008). The short-term increase of oxidative stress upon IONP administration could be attributed to immune responses, but the lack of long-term oxidative damage suggests that IONPs are safe for *in vivo* use. Furthermore, the size of the particles can affect clearance speed and reduce their toxic effects. Mice and non-human primates did not display signs of kidney or liver damage after high doses of IONP administration, so the use of IONPs *in vivo* is considered safe (Pusic et al., 2013).

Based on the current body of research concerning IONPs, those particles are safe and effective when used as contrasting agents for diagnosis, targeted drug delivery, or vaccine adjuvants. Teeguarden et al. (2014) measured the inflammogenic cellular doses of IONPs in mice, and the calculated adjustments for humans suggest that the acceptable exposure limit is 10 mg/m^3 of iron oxide. However, the lungs were the targeted tissue, so it is important to investigate the toxic effects of iron oxide on other tissues as well.

The applications and toxicity of IONPs are still observed using a variety of animal models to completely understand their effects on the immune system and their biological interactions with various tissues. In order to ensure the results obtained from *in vivo* studies, it is important to consider the advantages and disadvantages of each model that can be used for measuring and evaluating NPs *in vivo*.

Animal Models

Various animal models have been utilized to test NPs *in vivo*, and some of the most common ones include the mouse and non-human primate models. Other models (e.g., porcine) are also sometimes used, but they are less common. Compared to the human immune system, the mouse model appears to be one of the most reliable models for *in vivo* studies, but the avian model is responsible for various insights in immunology, especially in vaccination studies (Schat, Kaspers, & Kaiser, 2014). Using the avian model, specifically chicken, to study the interactions between NPs and the immune system is a promising alternative to other models in terms of financial advantages, procedural advantages, and reliability of results.

Mouse Models

Although the mouse model is well-known for studying NP-associated biological interactions and immune responses *in vivo*, it is an invasive model. According to the mapping of lymphatic drainage basins in mice, the hindfoot and the tail are the best injection sites for stimulating and monitoring local immune responses (Harrell, Iritani, & Ruddell, 2008). Therefore, a typical research procedure involves tail vein injections, followed by periodical blood collection from the periorbital vein for plasma analysis. The mice have to be sacrificed in order to obtain tissues from organs (e.g., liver and spleen) for histological analysis (Karmali et al., 2012).

Complex procedures are also available to study cell-mediated immunity in mice. Studying T cells is difficult because their functionality depends on the type of T cell, and it is difficult to monitor their migration and cell-to-cell communication. Technologies such as intravital microscopy (IVM) are required to visualize T cell functions and interactions *in vivo*. IVM techniques are invasive because the microscope has to be positioned above an incision

made on the animal's tail. Although IVM does not require sacrificing the animal once the experiment is completed, those techniques have other limitations. Observing antigen-experienced lymphocytes using IVM techniques does not require large numbers of purified cells, but the visibility of cells is determined by blood flow rate. The attempt to produce a 3D image and visualize the T cell migration and cell-to-cell communication can be disrupted by tissue movements of a few micrometers (Mempel, Scimone, Mora, & von Andrian, 2004). Finally, observing local immune responses in mice is not reliable because particles injected in the skin can disperse and expand the area of inflammation (Gopee et al., 2009).

There is no alternative to using invasive procedures when mice are used in NP research. Even in studies that investigated skin permeability of NPs, skin penetration in mice was not possible unless the skin was dermabraded (Smijs & Bouwstra, 2010). In order to study cell-mediated immunity in mice, the animal usually needs to be sacrificed in order to obtain tissue for histological analysis. Unless IVM is used to monitor T cell recruitment and trafficking in real time, there is no feasible method for observing cell-mediated responses multiple times in a single animal.

For NP applications in immunization, the mouse model is not always a suitable choice because of the differences in immune responses between mice and humans. For example, antitumor T-cell responses in mice are different from those that occur in humans (Lévy, & Colombetti, 2006). Furthermore, activation of antigen-specific T-cells was achieved with peptide-pulsed DCs (Degl'Innocenti et al., 2005), but not with peptide/adjuvant immunization (Grossmann, Davila, & Celis, 2001). Pusic et al. (2013) reported that IONP as an adjuvant resulted in 100% immune responsiveness for non-human primates whereas mice exhibited a high immune response only when their DCs were significantly activated.

These characteristics of the mouse immune system could also explain why studies using the mouse model often report contradictory findings. Shen, Liang, Wang, Liao, and Jan (2012) reported that IONPs suppress the infiltration and functions of T helper 1 cells and macrophages in response to the antigen. The treatments were injected in the tail veins. However, Mulens-Arias, Rojas, Pérez-Yagüe, Morales, and Barber (2015) reported that IONPs modulated macrophage formation through TLR-4 and reactive oxygen species signaling after treatment administration in the peritoneal region. The differences in findings could be attributed to the differences in IONP coatings used, but different injection sites could also account for the differences in findings.

The mouse model requires invasive procedures and the differences between mice and humans could affect the transferability of findings, so the mouse model is not the best model to study *in vivo* NP interactions and their effects on the immune system. The use of other animal models for investigating NP biointeractions could produce more reliable and transferable results compared to the mouse model.

Non-human Primate Model

The non-human primate model in NP research is often used to determine whether the *in vitro* results and small animal models can be generalized to humans, especially concerning the toxicity of NPs. Using the non-human primate model, researchers concluded that small water-soluble IONPs can deteriorate quickly, but their dispersion and exact deterioration need to be monitored using dyes (Pusic et al., 2013). In contrast, the breakdown of QDs is much slower and cadmium doses can remain stored in the liver for up to 90 days (Ye et al., 2012).

For determining the toxicity and acceptable doses, the non-human primate model is often used for measuring immune system responses because of the physiological similarities between

the human and non-human primate adaptive immunity (Messaoudi, Estep, Robinson, & Wong, 2011). Another significant advantage of the primate model is that small animals are not susceptible to infections from pathogens that affect primates only, such as the hepatitis C virus (HCV) or the human immunodeficiency virus. Although the accumulation of NPs in the hepatocytes and Kupffer cells of small animals indicates that targeted NP therapy has potential for HCV treatment, reliable results can be obtained only through primate models (Ryoo et al., 2012).

However, this model also has several disadvantages. Physical or chemical restraints are often required when handling research subjects, even though the animals can be trained to cooperate during various procedures, including vaccinations and examinations. The long life-span of non-human primates is beneficial for conducting longitudinal studies, but not for researching intervention effects throughout multiple stages of development. Finally, the restrictions and regulations for using non-human primates are constantly increasing, and the cost of those studies often exceeds the potential benefits. Therefore, the biological interactions of NPs are often studied in small animal models whereas the non-human primate model is often used to verify *in vivo* findings prior to the transition of research to human clinical trials (Ye et al., 2012).

Other Models

The *Drosophilia melanogaster* (i.e., common fruit fly) model was reported for testing the toxicity of AuNPs (Vecchio et al., 2012). Using the fruit fly model is common in biomedical research because the experiment requires little equipment and their short life span makes it easy to study inheritance. However, for the purpose of studying acute toxicity, insect models may not be the best option. It is estimated that 77% of human disease genes match to the fruit fly genome, so the fruit fly model can be useful for studying the genetics of progressive disease (Reiter,

Potocki, Chien, Gribskov, & Bier, 2001). However, that does not allow researchers to identify relevant biological processes specific to mammals, such as inflammation response pathway activation caused by NP interactions.

The porcine model is rarely used for NP research, but it is feasible because the human and pig skin show similar lipid organization. The only significant difference between the two is that the lipid organization in the pig skin *stratum corneum* has less crystalline packing compared to the *stratum corneum* in humans. That difference does not affect the permeability characteristics of the skin when exposed to NPs, so the porcine model can be used to test penetration and localization of NPs (Smijs & Bouwstra, 2010). Although this model can investigate how skin characteristics and NP characteristics affect penetration and localization, it cannot provide valuable insight into the immunological responses caused by NPs that would be relevant to humans.

These models can meet a variety of research objectives, but they are not suitable for investigating immune reactions to NPs. Most researchers prefer mice because their immune system has a similar structure to the human immune system. Even though several discrepancies in innate and adaptive immune responses between these two species have been identified (e.g., T cell signaling pathway components, Th1/Th2 differentiation), the two systems are comparable if those discrepancies are considered when interpreting results (Mestas & Hughes, 2004). Non-human primates can offer important insights into the toxicity of NPs given their weight similarity with humans, as well as the genetic and immune system similarities between the two species. However, both of those models lack a suitable test site where immune reactions can be localized and observed repeatedly. That is why using the chicken model can provide more valuable insight into the effects of NPs on immune responses compared to other models.

Chicken Model

The avian model can be used to study immunity because of the similarities birds share with mammals. Both birds and mammals produce their antibody repertoire using somatic gene conversion, and the avian major histocompatibility complex (MHC) performs cell-mediated immune functions similar to the mammalian MHC (Schat et al., 2014). Improvements in the chicken model made it possible to quantify immune system responses via various pathways. For example, Mannan-binding lectin (MBL) is produced in hepatocytes during acute infections as the first-line of immune response, and chicken share a similar MBL production and regulation pathways as mammals. Norup and Juul-Madsen (2007) established an assay for quantitatively measuring the complement activation capacity of the MBL pathway in chicken by depositing the human complement factor 4 (C4), and the high correlation between chicken MBL (cMBL) concentration and C4 deposition ($r = 0.85$, $p < 0.0001$) indicates that the assay is reliable.

The chicken model is also suitable for investigating diseases that occur in both humans and chicken, such as autoimmune disease, cancer, infectious disease, dermatitis, and various others. The avian immune system features and common disease with humans make chicken good subjects for investigating immune system responses. Besides comparable immune systems with mammals, the chicken genome was published in 2004, allowing researchers to explore the genetic factors associated with immunity in depth (Schat et al., 2014).

However, the biggest advantage of chicken compared to other models is the ability to monitor *in vivo* immune responses using the growing feather (GF) as a test site. Feather injection is a minimally-invasive procedure and feathers are easy to access as samples for histological analyses. Unlike the mouse model, which usually requires sacrificing the animals to obtain tissue samples, the test material in the chicken model can be monitored periodically for one test

subject. The use of the avian model for administering agents and measuring immune responses to determine vaccine efficiency was patented by Erf (U.S. Patent No. 8,216,551, 2012).

The procedure introduces an agent locally or systemically, followed by plucking the feather and analyzing the tissue responses in the tip of the feather. Approximately 10 µl of the agent studied can be injected directly into the feather tip as they are 1 cm long on average, depending on the age of the chickens. As a general rule, chickens between 2 and 22 weeks of age have GF, but the exact age depends on the line studied. Directly injecting an agent into the pulp of the feather does not affect the regeneration of the GF. The administration of agents does not cause bleeding or tissue damage, which usually occurs when more sensitive test sites are used, such as the wing web or wattle.

Multiple agents can be administered into the GF to monitor their effects in vivo. According to Erf (U.S. Patent No. 8,216,551, 2012), most agents can be tested using this procedure, including NPs, microbes, polypeptides, lipids, pharmaceuticals, polynucleotides, carbohydrates, and various others. In addition to direct injection, responses associated with exposure to environmental factors (e.g., pollutants) can also be monitored using the feather tip. Multiple tests on one subject can be conducted by administering tested agents into multiple GF test sites in one subject, so that each feather tip can be plucked and analyzed at different time points during the experiment.

In contrast with previous methods that required injecting agents into the birds' sensitive tissues, plucking the feather does not require euthanization and does not limit the selection of appropriate assays that can be used to analyze the tissue. The feather tips can be analyzed using a variety of tests, including histological analyses, flow cytometry, tissue culture, enzyme activity assessment, various analyses (i.e., transcriptome, protein, and lipid), and immunohistochemistry

(Erf, Trejo-Skalli, & Smyth, 1995; Lockhart & Erf, 2004; Shrestaa, Smyth, & Erf, 1997; Stepicheva, Liyanage, Lay, Dienglewicz, & Erf, 2010; Wang & Erf, 2004). The scope of agents and analyses that can be used does not depend on the chicken line because all birds have GF before reaching adulthood. However, the selection of different lines needs to be consistent with the purpose of the study.

The Smyth-Line (SL) chicken has been used extensively in autoimmune vitiligo research. Normally, the SL chickens are the intervention group whereas the Light Brown Leghorn (LBL) chickens are used as controls (Erf, 2004; Sreekumar et al., 2000; Wang & Erf, 2004). SL chicken are naturally susceptible to autoimmune vitiligo, but the LBL line appears to be vitiligo resistant, even after the attempts to induce it with 5-azacytidine (Erf, 2004).

Therefore, for the purpose of investigating immune responses, the LBL line could be a better choice compared to the SL chickens. Because SL chickens are susceptible to autoimmune disease, a spontaneous onset of vitiligo could interfere with immune responses and alter the results of analyses aimed at investigating the effects of injected agents. The resistance of the LBL line to autoimmune disease means that less random interference is expected during experiments, so the LBL could be a better option for studying immune responses to external agents.

The diversity of chicken lines that can be used in research is another advantage of this model because each line has specific genetic characteristics. The LBL line, SL line, and Brown line (BL) are examples of lines that are well-defined in terms of disease susceptibility whereas random bred lines can also be used. This enables researchers to use multiple different lines of chicken and analyze the differences in immune reactions or disease susceptibility between them.

The chicken model is a minimally invasive procedure that provides reliable results when assessing immunological responses based on various immune system activities, such as lymphocyte infiltration or antibody production (Alaamri et al., 2014). The ability to monitor immune responses over time is a specific feature of the chicken model because GF can be used as a test site whereas skin has to be used when working with other animals. Therefore, the avian model requires less complicated and less invasive procedures compared to other models for monitoring the activities of NPs *in vivo* and the outcomes of those activities.

Immune System Overview

The immune system protects the body from infections by identifying and evaluating threats, coordinating the elimination of pathogens, and preventing or limiting damage to the native tissue. The immune system can be divided into the innate and the adaptive immune systems, which are distinguished by two characteristics. First, each system has a different method it uses to recognize antigens. The innate immune system recognizes pathogens using germ-line encoded pattern recognition receptors (PRRs), which induce signals for activating adaptive immunity. The receptors used by adaptive immunity are diverse because they consistently mutate and recombine so that they can identify and eliminate specific pathogens. Second, only the adaptive immune system can develop immunological memory, which allows it to become more efficient after each exposure to pathogens with the same antigen.

The innate immune system is comprised of several immune cell subsets, which are responsible for detecting pathogen-associated molecular patterns (PAMPs). They include macrophages, natural killer (NK) cells, neutrophils (heterophils in chickens), DCs, inflammasomes, complement proteins, acute-phase proteins and various other subsets (Medzhitov, 2007). The recognition of pathogens occurs via three general mechanisms, which

include (1) microbial non-self recognition, (2) missing self recognition, and (3) altered self recognition (Medzhitov & Janeway, 2002). Non-self molecules that are specific to microbes are the easiest to recognize via the immune cells' PRRs, but most pathogens adapted to infecting humans can reduce immune system activation by binding regulating proteins (Sim, & Wallis, 2011). In those cases, the presence of pathogens is detected based on the cell surface alterations in infected cells. Infected cells usually display MHC on their surface, which triggers an immune response. However, the NK cells are able to recognize infected cells even when no obvious infection markers are present, which is referred to as the "missing self" (Vivier et al., 2011). The surface structure of the apoptotic cells can change by developing apoptotic cell-associated molecular patterns (ACAMPs) so that phagocytes recognize the pattern as "altered self" and trigger an immune response (Nakanishi, Henson, & Shiratsuchi, 2009).

Innate immunity is often referred to as the first line of defense because it is responsible for identifying threats and activating the production of antimicrobial cells that inhibit pathogen replication (Messaoudi et al., 2011). The innate immune system is also responsible for activating the adaptive immune system by associating PAMPs with the lymphocytes abilities to recognize specific antigens. The T cells of the adaptive immune systems are activated by DCs, which process the pathogens' protein constituents into antigenic peptides. The DCs also produce cytokines as a response to PRR-induced signaling. Once the DCs reach the lymph nodes, their antigenic peptides and PRR-induced signals serve as triggers for T cell activation (Medzhitov, 2007). B cell activation uses a different pathway, which involves physically linking an antigen and a specific PAMP through the coordinated effort of PRRs and B-cell receptors (Medzhitov, 2007).

Two aspects of the adaptive immune system can be distinguished, and they include humoral immunity and cell-mediated immunity. Humoral immunity is also referred to as antibody-mediated immunity because a humoral immune response leads to the production of soluble antibodies that bind to antigens. When triggered, the humoral immune system's B cells produce immunoglobulin M (IgM), a molecule that has a total of ten antigen binding sites. Because of its ability to bind many antigens and activate the complement system to assist in the elimination of the bound antigens, IgM is the first antibody produced in response to an infection (Racine et al., 2011). IgM is also used as a first response and is generally of low affinity to an infection because the B cells need to mature by mutations that increase affinity to the pathogen's antigen while they simultaneously switch to producing IgG antibodies, and those mutations are induced by T cells. The production of IgG antibodies increases the efficiency of the immune system because those antibodies have better antigen affinity compared to IgM antibodies and can also bind on leukocytes to enhance their efficiency. Because of the adaptive immune system's memory, the B cells can produce IgG antibodies immediately on secondary infection without producing IgM antibodies if the immune system recognizes the antigen.

Cell-mediated immunity protects the body by activating cells that eliminate pathogens or by activating cytokines that regulate the functions of other systems involved in the immune response. The pathogens can be eliminated directly by activating phagocytes and NK cells as long as the antigens are extracellular. Once a cell is infected, a different mechanism has to be used. In that case, antigen-specific cytotoxic T cells can be activated to induce apoptosis in infected cells with altered surfaces that display non-classical MHC molecules (Medzhitov, 2007).

Both humoral and cell-mediated immunity are monitored to examine the effects of vaccination adjuvants on the immune responses. The effects of adjuvants on the humoral immune response are easier to measure and monitor compared to the cell-mediated response. The humoral response can be replicated *in vitro* and the antibodies are easy to obtain when using *in vivo* studies. The humoral immune response usually occurs 48 hours after primary exposure to antigen or sooner for repeat exposures (Kelsoe, 2000). Depending on the purpose of the study, non-invasive procedures are available to researchers observing humoral immune responses *in vivo*. For example, MRI with positive contrasting can be used to observe the migration of DCs to activate the T cells in the lymph nodes (Tavaré et al., 2011). Because the humoral immune system's antibodies are found in extracellular fluids, blood samples and tear collection are minimally invasive procedures that can be used to obtain samples for testing humoral response levels. The assessment of serum antibodies can be performed using a variety of immunoassays, such as the enzyme-linked immunosorbent assay (ELISA), complement fixation, immunodiffusion, and serum neutralization (Koller, 1982).

Cell-mediated immunity is more difficult to study because live antigen-specific T cells need to be measured. Those T cells can identify only antigen-peptides associated with self-MHC on antigen-presenting cells (Ignatowicz, Kappler, & Marrack, 1996). Therefore, observing and measuring immune responses requires having MHC-matched target cells. Satisfying that criterion using random-bred population for *in vivo* studies is difficult.

Although both *in vitro* and *in vivo* methods can be used, the assays used to measure cell-mediated immunity have several disadvantages compared to humoral immunoassays, including susceptibility to biological variability and technical complexity (Limaye, 2010). This is especially true for studies investigating the effects of NPs on immune responses because the

presence of NPs can interfere with the assays' readout parameters (Oostingh et al., 2011). Different cell populations and population subsets can be identified using flow cytometry, but even with a precise quantification of T cells, it is difficult to determine their functional capacity and distinguish between naïve, effector, or memory T cells.

Furthermore, cell-mediated immunity is tissue-specific. Effector T cells produce cytokines or kill affected host cells at the infection site, so invasive methods of obtaining tissue for histological analyses are required, such as skin biopsy. Skin is often used as a test site for *in vivo* studies because it produces a visible response to the test material (Abbas, Lichtman, & Pillai, 2015). Although the reaction is characterized by the presence of immune response indicators (e.g., edema, redness, induration), repeat assessments in one test subject are not possible because molecular interactions can be observed only after biopsy.

Skin is a suitable and reliable test site for investigating cell-mediated immunity triggered by NPs because the small size of the NPs allows them to penetrate the *stratum corneum*, which is the upper layer of the skin. However, there are two key issues with using skin as a test site for NP bioactivity and immunological responses. First, damaged skin has reduced permeability due to structural changes or lipid organizations, so the effectiveness of permeability is dependent on the size of NPs and skin condition (Mortensen, Oberdörster, Pentland, & DeLouise, 2008). Second, the accumulation of NPs in hair follicles depends on NP size (< 50 nm) and the hair growth cycle (Smijs & Bouwstra, 2010).

Given the invasive nature of skin biopsy, the use of feathers as test sites for the effects of NPs on the immune response is preferred as a less invasive and simpler procedure. Both sites are similar in terms of mononuclear cell presence during an inactive immune response, and both sites recruit leukocytes from circulation (Erf et al., 1995). However, upon skin penetration *in*

vivo, NPs can also interact with other organs, such as the liver, so it is difficult to control the behavior of NPs after they penetrate the skin (Gopee et al., 2009). The antigen distribution and immune response locations cannot be reliably predefined when skin is used as a test site whereas using the inner pulp of the growing feather as a test site limits the immune response to a predefined area. That means multiple agents or antigens can be tested using one subject by injecting several feathers simultaneously because there is no interference between the test materials.

In terms of procedural advantages, feather collection does not require euthanization or sacrificing the animal. Even though skin biopsy is an option for tissue collection, animals can often be sacrificed for tissue harvesting (Mortensen et al., 2008). The skin also needs to be sliced to assess and analyze each layer whereas plucked feather tips are easy to remove and can be analyzed for lymphocyte infiltration using a variety of procedures, including histological analysis, immunohistochemistry, flow cytometry, and various other methods. Most importantly, there are currently no procedures that would allow researchers to analyze tissue samples from the same subject more than once when skin is used as a test site. That makes growing feathers the only test site that is both minimally invasive and allows researchers to repeatedly monitor the immune response in one animal.

Findings and Gaps in the Current Literature

Cho et al. (2011) demonstrated that *in vitro* data cannot be used to reliably predict the effect of NPs on the immune system or their toxicity because NPs tend to behave differently when *in vivo* test systems are used. However, the selection of the animal model used is critical so that *in vivo* results are reliable. The advantages of the chicken model over other models (e.g., mouse, porcine) have been discussed. The majority of studies on chicken have been conducted

using the SL line as the primary purpose of those studies was to investigate autoimmune disease (Erf et al., 1995; Lockhart & Erf, 2004; Shrestaa et al., 1997; Stepicheva et al., 2010; Wang & Erf, 2004).

Most studies that investigated immune responses and NP interactions focused on the mouse model, so more studies are required to investigate the effects of NPs on avian immune systems and tissue. Specifically, those studies should focus on Leghorn lines because they are more resistant to disease compared to other breeds, and their specific immunity is more efficient compared to other breeds (Parmentier et al., 2004). These advantages of Leghorn breeds make LBL chicken an excellent model for investigating biological interactions and pro-inflammatory effects of NPs. The cell-mediated immune response in chickens can be monitored with various instruments. Flow cytometry proved to be a reliable and repeatable procedure for monitoring, regardless of their environmental background and line (Fair, Taylor-McCabe, Shou, & Marrone, 2008).

The core and coating materials appear to be critical determinants of immune responses when NPs are used as adjuvants. Various types of NPs proved to be suitable vaccine adjuvants. Lipid-enveloped NPs with a polylactide-*co*-glycolide acid (PLGA) core can mimic the structural characteristics of pathogens to enhance a humoral immunity response (Moon et al., 2012). Using IONPs as an adjuvant results in higher immune response activities compared to mouse IgG and Alum adjuvant & mIgG (Alaamri et al., 2014).

It is important to note that the immune system responses can depend on various factors, including charge, size, and particle purity (Dobrovolskaia & McNeil, 2007; Müller et al., 2007). Current studies suggest that small particles (< 100 nm) are more efficient than larger particles for activating the cell-mediated immune system (Xiang et al., 2006). Surface modifications also

need to be documented because they influence T cell proliferation (Diwan et al., 2003). For example, protein antigens enhance the NPs ability to interact with DCs and activate antigen-presentation to T cells (Pusic et al., 2011; Tavaré et al., 2011).

The selection of appropriate materials is also critical for reducing the toxic effects of NPs *in vivo*. Hussain, Vanoirbeek, & Hoet (2012) reported that some nanomaterials can induce a pro-inflammatory response whereas other nanomaterials reduce the efficiency of the immune system. This is an important consideration for vaccinology because only materials that incude a pro-inflammatory response can be used to improve vaccination efficiency.

IONPs are considered safe because no adverse events or abnormal tissue alterations were reported in mice or non-human primates (Pusic et al., 2013). AuNP toxicity leads to DNA fragmentation and alterations in gene expressions that regulate apoptosis, DNA damage recognition, and stress response (Vecchio et al., 2012). QDs are generally considered safe, but cadmium retention in the tissue upon QD degradation is a potential issue that needs to be addressed (Chen et al., 2013). Based on the currently available evidence, it appears that IONPs could be the safest material as they have not been associated with deposition of toxic materials or increased levels of reactive oxygen species (Shen, Liang, Wang, Liao, & Jan, 2011). The exact level of oxidative stress depends on the type of tissue and peaks after approximately 3 days, but no long-term abnormal tissue changes were reported (Jain et al., 2008). Nevertheless, further investigations of IONP interactions *in vivo* are warranted to ensure their degradation does not cause significant oxidative damage to tissue.

In addition to safety, IONPs used as adjuvants have high stability and elicit high immune responses. Pusic et al. (2013) reported that IONPs conjugated with merozoite surface protein 1, elicit high immune responses in non-human primates (100%) as well as parasite inhibitory

antibodies. However, the reported immune response in mice was not as high as the one observed in primates, and the existing activation of DCs was associated with a higher immune response.

Based on the current body of knowledge, future research in the area of NPs should focus on testing IONPs as vaccine platforms because they do not show significant toxic effects. The use of IONP-antigen adjuvants was found to stimulate higher humoral and cell-mediated responses compared to Alum-antigen (Alaamri et al., 2014; Yue et al., 2012). Because most studies used mice to investigate immune responses to NPs, more studies using the chicken model are required to obtain results that explain how immune responses to NPs change over time in a single animal. The chicken model proved to be a suitable model for testing NP bioactivities and toxicity *in vivo*, so those studies will create a foundation for establishing clinical implications of NPs as vaccine adjuvants. Given the similarities between the avian and the mammalian immune systems, future studies can use the chicken model as a minimally-invasive *in vivo* test system to explore all other medical applications of NPs as well.

CHAPTER 2—MATERIALS AND METHODS FOR FEATHER AND INTRAMUSCULAR INJECTION STUDIES

A total of seven studies were conducted. Three studies monitored the innate immune response (i.e., feather injection studies). Four studies monitored the *in vivo* cell-mediated immune response (i.e., intramuscular injection studies). Two intramuscular injection studies monitored the primary response, and two studies monitored the memory response.

The majority of materials and methods used in all studies were similar. All studies used the same chicken breed (LBL line) and treatment preparations. The chicken, IONPs, and materials used to make the preparations were obtained from the same manufacturers, and the tissue collection and analysis procedures were similar for all studies. Therefore, the general materials and methods used in all studies are discussed before the specific characteristics of each study are presented.

Animals

The LBL line was used in this study. The University of Arkansas Experiment Station has been maintaining the LBL line since 1995. The animals are maintained as a closed population and frequently monitored for pathogens. The levels of biosecurity are high, and the chickens' fertile eggs, blood samples, and tissue samples meet the import requirements of Canada and European countries. The procedures used involving animals (e.g., feather sampling) have been approved by the University of Arkansas Institutional Animal Care and Use Committee (IACUC; protocol exp. Date 11/03/2014). The letter of approval is included in Appendix A.

The sample was randomly selected at hatch from different nests with one male per family, and the chicks were banded with an identification tag. Marek's disease vaccine (HVT) was not administered at hatch. The sample was raised in floor pens covered in wood shavings

where they had free access to food and water. The studies were conducted in a bio secured room at the Poultry Health Laboratory, University of Arkansas (Fayetteville, AR) where the birds reached 7 weeks of age. The birds were randomly divided into 4 groups with matching age-lines-$MHC^{101/101}$ (i.e., one bird from each nest per group). The number of birds in each group was determined based on previous experience in working with the LBL line model (Erf, personal communication) and the Power Analysis conducted using cell population data and quantitative real time reverse transcription polymerase chain reaction (RT-PCR) data (SYSTAT Power Analysis Program; Systat Software, Inc., Chicago, IL). Each group had to consist of minimum 4 and maximum 7 birds.

Nanoparticles

IONPs are approved for use in humans by the Federal Drug Administration (FDA). The IONPs for this study (10 nm Fe_2O_3 with carboxylic acid groups; IO catalog # SHP-10- 01) were obtained via Ocean NanoTech, LLC (Springdale, AR).

Treatment Preparation

The preparation of treatment materials was conducted under sterile conditions in the poultry science building's tissue culture room at the University of Arkansas (Fayetteville, AR). The treatments prepared for the birds included mIgG + IO, mIgG + Alu, mIgG, Sterile Dulbecco's phosphate-buffered saline (1X ST PBS), and endotoxin-free PBS (EF PBS).

ST PBS was used for injection and dilution. 1 ml of 2X ST PBS was made to dilute mIgG by adding 10X ST PBS (100 µl) to 1X ST PBS (900µl). ST PBS was used in two feather injection studies (Study 2 and 3, Chapter 3) and two intramuscular injection studies (Study A, Chapter 4). 1X EF PBS was used in two intramuscular injection studies (Study 1 and 3, Chapter 3) and two intramuscular injection studies (Study B, Chapter 4).

The final concentration of IO-mIgG used for intramuscular injection was 1mg/ml (i.e., 0.1 ml/dose). The stock concentration was 5mg/ml, and sterile Dulbecco's phosphate-buffered saline (1X PBS) was added to the final concentration used in the treatment. The calculation for the final concentration was dependent on the number of sampling units used. The diluted mIgG was mixed using the pipet, and each 1mg/ml IO contained 2 mIgG per one nanoparticle (0.26 mg/ml of mIgG).

The mIgG + Alu was prepared using 2% Alhydrogel, which was provided as a sterile aluminum hydroxide wet gel suspension (Cat. # vac-alu-50; InvivoGen, San Diego, CA 9212). Based on the manufacturer's instructions, a 1:1 dilution has to be made (i.e., 400 µl Alhydrogel 2% for 400 µl of Ag). The Alhydrogel was shaken before mixing it with mIgG (10 mg/ml). Pipetting up and down was used for 5 minutes so that the Alum can effectively absorb the mIgG. The final concentration used for intramuscular injections was 5mg/ml.

The mIgG was obtained as an antigen (Ag) from Rockland Antibody and Assays (Cat. # 010-0102; Gilbertsville, PA 19525). The stock concentration was 25 mg/ml, and the dilution was performed based on the manufacturer's instructions – 2.5 ml of sterile de-ionized water was added to 25 mg mIgG to obtain a concentration of 10mg/ml. 1X ST PBS was added to make the final concentration (5 mg/ml mIgG using 1:1 dilution). 10mg/ml of unused mIgG was divided into micro tubes (100 µl/aliquot) and placed in extended storage at -20 degrees. 80 µl of 10 mg/ml mIgG was added to 80 µl 2X PBS and 640 µl 1X ST PBS so that the feather injections can be performed using 800 µl of 1 mg/ml mIgG.

Immunization

Immunizations were conducted using sterile 3ml 25_G x 1 (0.5mm x 25mm) syringes, two syringes per treatment (BD, Franklin Lakes, NJ 0717). The syringes were filled with treatment

preparations at specific concentrations that were different for all studies. The syringes were inverted before each administration, and the immunization was performed in the health laboratory of the Poultry Science Department (Fayetteville, AR) in sterile conditions.

Regardless of the treatment, the intramuscular injection amount was 0.1 ml/chicken. The dose of mIgG was 20-fold higher for mIgG-Alum and mIgG compared to IO-mIgG. The treatments were administered using the breast muscles. Studies monitoring the primary response used the left breast muscle whereas studies monitoring the memory response used the left muscle for the first immunization and the right muscle for the second immunization.

Feather Injections

The barbs emerging from the sheath were cut off with scissor before the birds were injected so that the epidermal cap would be left intact (see Figure 3). Sterile-disposable insulin syringes that are 8mm in length with 31G needles (BD, Franklin Lakes, NJ 07417) were used, one syringe per bird.

The syringes were filled in the culture room at poultry science department (Fayetteville, AR) under the hood in sterile conditions. The syringes were filled with the treatment preparations using the same amount of 240µl for each treatment, but the treatment concentrations varied depending on the study. The concentrations used for the feather injection studies are shown in Table 5 whereas the concentrations used for the intramuscular injection studies are shown in Table 6 (Overview of Studies section). A total of 20 feathers were injected (10 on each side of the breast). The feathers were selected on both sides of the breast to be in a row next to each other (see Figure 3). The injection amount was 10 µl for each feather. The syringes were inverted before each administration. The syringe was inserted into the center of the pulp and 10

µl was injected to deliver the mIgG into the lower half of the pulp (see Figure 3). This procedure did not affect the tissue barrier because the pulp is surrounded by the epidermis.

In Study A, the mIgG injected into the GF test site was at 5-fold lower concentration compared to the intramuscular injection of mIgG alone (control group) and mIgG-Alum. In Study B, the mIgG concentrations were matched for all treatments. The feather injection studies used a concentration of mIgG with IO that was more than 20-fold lower compared to mIgG alone and mIgG with Alum.

Figure 3. Preparation of growing feathers for injection.

Images show: a) injected feathers (10 on each side of the breast) were selected to be in a row next to each other for easier identification during collection, b) cutting the emerging barbs above the epidermal cap, and c) injecting feathers with 10 µl of treatment for each feather.

Source: Erf (U.S. Patent No. 8,216,551, 2012)

Tissue Collection

Growing Feathers Preparation and Collection

Injected feathers were plucked 3 weeks before the feather injection. On the days of each study, the feathers were collected just before the injection and 4-6 hours after the injection, followed by feather collection at 24 hour intervals after feather injection. The number of days used to collect feathers depended on the immune response studied. Three injected feather tips were plucked from each bird, and each of the three tips were placed in separate tubes. The formalin tube was used for feathers designated for conventional histology processing at the University of Arkansas Histology Service Laboratory. The optimum cutting temperature (OCT) medium cups were used for immunohistochemistry and RNA extraction. Finally, the 1 ml cold 1X PBS tubes were used for feathers that were analyzed using flow cytometry. The tubes used for storage are shown in Figure 4.

Flow cytometry feathers were not trimmed, and the cold 1X PBS tubes were placed in ice. The procedure was conducted on the same day as the feather collection. The feathers for immunohistochemistry and RNA extraction were plucked and cut just above the epidermal cap. The feathers were stored in the bottom of aluminum cups containing Tissue-Tek® O.C.T freezing medium (SaKura Finetek Inc., Torrance, CA) and snap frozen with liquid nitrogen (see Figure 4) and stored at -80º C until used. The feathers stored in formalin were cut under the epidermal cap and placed in micro tubes filled with 1ml 10% buffered formalin to remain fixed. The tubes were stored at room temperature in biohazard place.

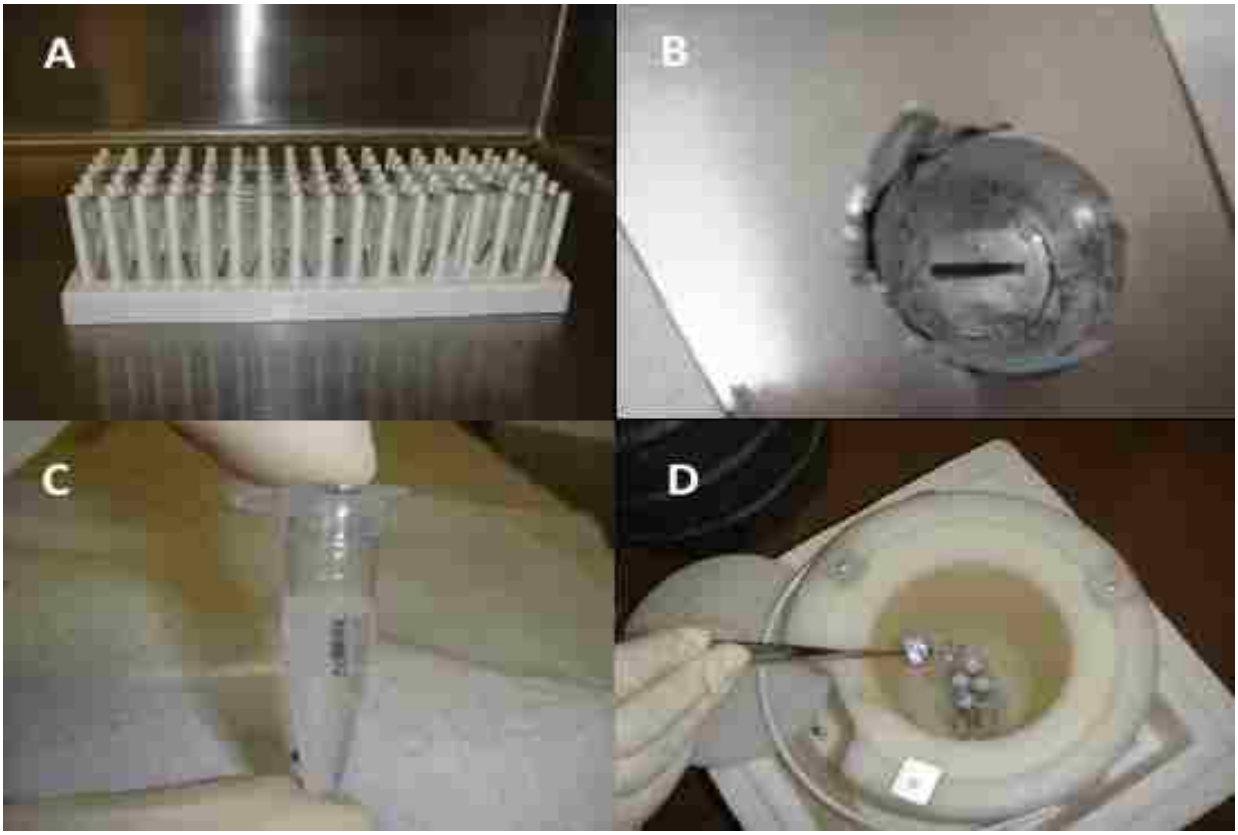

Figure 4. Feathers storage after collection at various times post-injection for *ex vivo* analysis.

Images show: a) formalin tubes, b) OCT medium cups, c) 1 ml cold 1X PBS tubes, and d) snap freezing feathers in aluminum cups for storage.

Source: Erf (U.S. Patent No. 8,216,551, 2012)

Blood

The blood (2.2 – 2.5 ml) was collected periodically from each bird in the treatment groups before and after immunization. The intervals and the duration of blood collection varied depending on the study. 1 ml of blood was stored into micro tubes, one for each chicken, and placed on ice to be used for plasma isolation. The remaining amount of blood was split into 2 microtubes with 500 µl of blood in each. Those samples were used on the same day of collection for automated hematology analysis using the CELL-DYN (Abbott Diagnostics, Abbott Park, IL) 7500 calibration for avian blood and blood smear.

The collected blood samples assigned for plasma isolation were placed in cold centrifuge at 4°C and centrifuged for 7 minutes at 750 x g so that the plasma layer could be distinctly identified above the blood cells. After the separation, the plasma was collected and placed into two separate micro tubes for each chicken. The micro tubes contained approximately 250 µL of plasma and were kept at -80°C.

Organs

Organs were collected once the birds were euthanized after blood and feather collection. The organs (liver, kidney, lung, and muscles) were collected and placed on the bottom of aluminum cups with Tissue-Tek® O.C.T freezing medium (SaKura Finetek Inc., Torrance, CA). They were snap frozen and preserved at -80ºC.

Procedures

Cell Isolation

The collected GFs (1 feather per bird) were cut longitudinally. The pulp of the feathers was pulled out for an hour enzymatic digestion with collagenase/dispase (Life Technologies, Carlsbad, CA) at 37 C° (see Figure 5). The feathers were subsequently filtered using a 60µm

pore size nylon mesh (monofilament screening fabric; Tekto Inc., Elmsford, NY) so that the single cell suspensions can be obtained (see Figure 5). The cells were washed twice with cold PBS (VWR International, Radnor, PA) and PBS+ (BSA 1% [Sigma] and 0.1% NAN3), respectively in order to prevent non-specific antibody binding and marker internalization. The samples were centrifuged at 1200 rpm for 10 minutes at 4°C in centrifuge tubes with polypropylene (Cat. # 89004-368; VWR international, Radnor, PA). The pellets obtained were re-suspended in 500µl cold PBS+.

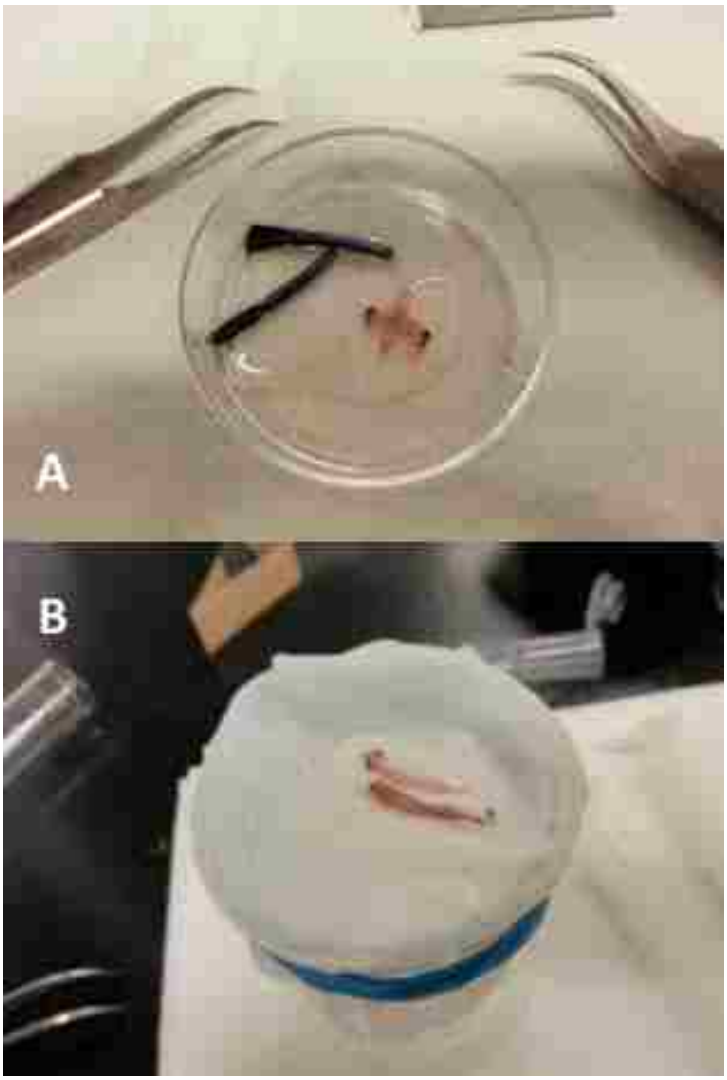

Figure 5. Cell isolation preparation.

Images show: a) removal of the pulp from the sheath, b) filtering the feather pulp for single cell suspension.

Antibodies

Antibodies (mAb) were purchased from Sothern Biotechnology (Birmingham, AL), AbD Serotec (Raleigh, NC), and Sigma (St. Louis, MO). The antibodies used are listed in Table 1. The Dual-Tag from Sigma was used as an isotype control (IC). The IC is a mix of mouse IgG_1 conjugated with fluorescein isothiocyanate (FITC) and phycoerythrin (PE), and it was used to detect non-specific binding of fluorescent labeled antibodies, as well as decide the cut-off between fluorescence positive and florescence negative populations.

Table 1. *List of antibodies used with corresponding isotype, specificity, and dilution*

Antibodies	Isotype	Specificity	Dilution
Dual-Tag	IC	no specifity to chicken molecules	1:100
Mouse anti-chicken CT-8 Ig	mouse IgG_{1k}	α chain 34kDa of chicken CD8	1:100
TCR1 Ig	mouse IgG_{1k}	γδTCR	1:100
CT-4 Ig	mouse IgG_{1k}	chicken CD4 (Mr 64 kDa)	1:200
TCR2 Ig	mouse IgG_{1k}	αβ1TCR/Vb1	1:100
TCR3 Ig	mouse IgG_1	αβ2TCR/Vb2	1:100
EP42 Ig	mouse IgG_{2a}	chicken CD8β	1:100
Cla Ig	mouse IgM_k	chicken Ia	1:100
LT40 Ig	mouse IgM_k	chicken CD45 (Mr 190-215k Da)	1:200
21-1A4 Ig	mouse IgG_{1k}	chicken Bu-1	1:100
AV6 Ig	mouse IgG_{1k}	chicken CD44	1:200
CT-3 Ig	mouse IgG_{1k}	chicken CD3	1:200
KUL01 Ig	mouse IgG_{1k}	Monocyte/Macrophage early T- cell marker	1:100
AV142 Ig	mouse IgG_{1k}	chicken CD3	1:200
Mouse anti-chicken MHC Class II*	mouse IgG_1	chicken MHC Class II	1:200
M-1 Ig**	mouse IgG_{2bk}	chicken IgM	1:100
G-1 Ig**	mouse IgG_{1k}	chicken IgG	1:100

* Antibody used for feather injection studies only
** Antibodies used for 2014 studies only

Direct Immunofluorescent Staining

Cells were stained with a panel of fluorescently labeled chicken leukocyte specific monoclonal antibodies using single, dual, and triple staining. The antibody dilutions and

conditions were provided by Sothern Biotechnology (Birmingham, AL) and AbD Serotec (Raleigh, NC). The antibodies used have been listed in Table 1.

50 µl of diluted mAb was added to 50µl of cells (approximately 2 x 10^6) in labeled 96-well round-bottom microtiter plates. The contents were mixed gently and incubated in the dark at 4°C for 30 minutes. The cells were then washed twice using cold PBS+ and centrifuged at 1200 rpm for 5 minutes at 4°C. The final pellets were re-suspended using 250µl PBS+ and transferred into 5 ml sterile Falcon round bottom tubes manufactured by VWR International, after which they were analyzed using flow cytometry.

Flow Cytometry

The FACScan with Consort 30 software package (Becton Dickinson Immunocytochemistry System, Mountain View, CA) was used for cell population analyses. The FL1 channel (530/30 nm band pass filter) and FL2 channel (585/42 nm band pass filter) were used to detect green fluorescence (from FITC) and orange fluorescence (from R-PE), respectively. Gating was used to exclude red cells, debris, and dead cells from the analyses. Cell acquisition and analysis was performed in list mode at 10,000 events per sample.

The antibody combinations used for flow cytometry were different in each study. Table 2 shows the antibody combinations used in the feather injection studies whereas Table 3 and Table 4 show the antibody combinations used in the intramuscular injection studies.

Table 2. *Antibody combinations for feather injection studies*

Study/Combination	FITC	PE	Red (SR)
Feather injection Study 1 and 2			
A	CD44 FITC	Macro PE	-
B	CD45 FITC	CD8 PE	-
C	Bu-1 FITC	Ia PE	-
D	CD8 FITC	TCR1 PE	-
E	TCR2 FITC	TCR3 PE	-
F	CD44 FITC	CD4 PE	-
Feather injection Study 3			
A	CD44 FITC	Macro PE	CD45 SR
B	CD4 FITC	CD8 PE	CD45 SR
C	Bu1 FITC	Ia PE	CD45 SR
D	TCR1 FITC	CD8a PE	CD45 SR
E	TCR2 FITC	TCR3 PE	CD45 SR
F	CD8b FITC	CD8a PE	CD3 SR
G	CD25 FITC	CD4 PE	CD3 SR

Table 3. *Antibody combinations for Study A*

Study/Combination	FITC	PE	Red (SR)	IC used for control
Primary response				
A	CD45 FITC	Macrophages PE	-	-
B	CD4 FITC	CD8 PE	-	-
C	Bu-1 FITC	Ia PE	-	-
D	CD8 FITC	TCR1 PE	-	-
E	TCR2 FITC	TCR3 PE	-	-
F	CD25 FITC	CD4 PE	-	-
G	CD44 FITC	CD4 PE	-	Yes
Memory response				
A	CD44 FITC	Macro PE	CD45 SR	-
B	CD4 FITC	CD8 PE	CD45 SR	-
C	Bu1 FITC	Ia PE	CD45 SR	-
D	TCR1 FITC	CD8a PE	CD45 SR	-
E	TCR2 FITC	TCR3 PE	CD45 SR	-
F	CD8b FITC	CD8a PE	CD3 SR	-
G	CD25 FITC	CD4 PE	CD3 SR	-

Table 4. *Antibody combinations for Study B*

Combinations	FITC	PE	Red (SR)
A	MHCII FITC	KUL01 PE	CD45 SR
B	CD4 FITC	TCR1 PE	CD8 Red
C	CD4 FITC	TCR2 PE	CD8 Red
D	CD4 FITC	TCR3 PE	CD8 Red
E	Bu-1 FITC	IgM	CD44 Red
F	Bu-1 FITC	IgG	CD3 Red

The data were expressed in percentages for the following populations: live cells, lymphocytes, CD45, macrophages, CD4+, CD8+, TCR1+, TCR2+, TCR3+, CD25, MHC class II, and B cells. The percent of CD4+ cells was divided by the percent CD8+ cells for sampling unit to obtain the CD4:CD8 ratio.

Statistical Analysis

Dependent variables. A total of 13 response factors were monitored to achieve the first research objective, which was to monitor and assess the local leukocytes infiltration into the pulp after the administration of IONPs. A total of (n) response factors were monitored to achieve the second research objective, which was to examine the immune response to IONP administration. Three gating regions were created around live pulp cells, lymphocytes, and heterophils. All types of lymphocyte cells were calculated as the percent total of the lymphocyte region. Each study monitored a different number of response factors, and the complete list of response factors by study is presented in Appendix B.

Independent variables. The analyses aimed to determine the differences in the percentage of pulp leukocyte populations depending on the type of treatment used, time of

sample collection, and their interaction. Those three variables were used as fixed effects in the analyses.

Statistical tests. The data were analyzed using SAS (SAS Inst., Inc.; Cary, NC) to conduct repeat measures procedures. The type III test of fixed effects was used to determine whether collection days, treatment, and their interaction had a significant effect on each response factor measured. Further analyses were not conducted for response factors that were not significantly affected by the fixed effects ($p > 0.05$). The differences of least square means were calculated for response factors that were significantly affected by day, treatment, or both variables. Response factors that showed a significant main effect of the interaction between treatment type and days were further analyzed using the test of simple main effects, which analyzes the mean differences in the response factor over time for each treatment and differences between treatment groups by day. It was determined that there were 4,753 possible pairs of means of treatment/day combinations, so mean separations were not performed.

Overview of Studies

The general methods and materials are the same for all studies whereas the differences included sample size, treatments, treatment concentrations, and tissue collection protocols. The treatment amounts administered to the chickens were consistent in all studies. The feather injection amount was 10 µl/GF for 10 feathers on each side of the breast, and the injection amount in the intramuscular injection studies was 0.1 ml/chicken.

All studies followed the same feather collection protocol. The feathers were collected just before the injections and 0.25 days after the injections, followed by periodical collection every 24 hours after the injection for 7 days. One feather injection study (12/02/2013) skipped feather collection on the third and fourth day.

Table 5 shows the GF injection date, total number of chickens, treatments used, and treatment concentrations in each of the three feather injection studies. Blood was collected for plasma only just before the injections, 0.25 days after the injection, and 7 days after the injection.

Table 5. *Sample size, treatment, and treatment concentrations for feather injection studies*

Study	GF Injection Date	Sample Size	Treatment	Treatment concentration
Study 1	08/09/2013	12	IO (n = 4)	0.2 mg/ml
			IOEF (n = 4)	0.2 mg/ml
			IO-mIgG (n = 4)	0.2 mg/ml
Study 2	12/02/2013	16	Alum (n = 4)	15%
			IO (n = 4)	0.2 mg/ml
			IO-mIgG (n = 4)	0.2 mg/ml
			Alum-mIgG (n = 4)	15%-0.5 mg/ml
Study 3	06/27/2014	12	Alum-mIgG (n = 4)	15%-0.26 mg/ml
			IO-mIgG (n = 4)	1 mg/ml
			mIgG (n = 4)	0.26 mg/ml

Table 6 shows the total number of chickens, treatments used, and treatment concentrations for injections in each intramuscular injection study. The GFs were injected, at the height of immune response, with mIgG (recall Ag) 10 days after the first immunization in the intramuscular injection studies and 5 days after the second immunization in studies that monitored the memory immune response.

Table 6. *Sample size, treatment, and treatment concentrations for intramuscular injection studies*

Response (Study)	Intramuscular Injection Dates	Sample Size	Treatment	Treatment Concentration
Primary (A)	06/28/2013 – treatment	18	mIgG (n = 6)	5 mg/ml
			Alum-mIgG (n = 6)	15%-5 mg/ml
			IO-mIgG (n = 6)	1 mg/ml-0.26 mg/ml
Memory (A)	10/18/2013 – first 11/15/2013 – booster	20	mIgG (n = 5)	5 mg/ml
			Alum-mIgG (n = 6)	15%-5 mg/ml
			IO-mIgG (n = 6)	1 mg/ml-0.26 mg/ml
			EF PBS (n = 3)	1X
Primary (B)	05/17/2014	12	mIgG (n = 4)	0.26 mg/ml
			Alum-mIgG (n = 4)	15%-0.26 mg/ml
			IO-mIgG (n = 4)	1 mg/ml-0.26 mg/ml
Memory (B)	05/15/2014 – first 06/18/14 - booster	16	mIgG (n = 4)	0.26 mg/ml
			Alum-mIgG (n = 4)	15%-5 mg/ml
			IO-mIgG (n = 4)	1 mg/ml-0.26 mg/ml
			EF PBS (n = 4)	1X

Blood was collected for plasma only, and the collection protocols were different for all intramuscular injection studies (see Table 7). Organs were collected only in Study A upon completing feather and blood collection for measuring the primary response (Wednesday, 8/7/2013) and the memory response (Wednesday 11/27/2013). The birds were euthanized, and their organs (liver, kidney, lung, and muscles) were collected.

Table 7. *Blood collection time points for intramuscular injection studies*

Time points (days)	Primary A	Memory A	Primary B	Memory B
0 (before immunization)	+	+	+	+
0.25	+	+	-	-
3	-	-	+	+
7	+	+	+	+
10 (before GF injection)	+	-	+	+
14	+	-	+	+
21	-	.	+	+
28	+	.	+	+
0 (before 2nd immunization)	-	+	-	+
3	-	-	-	+
5 (before GF injection)	-	+	-	+
7	-	+	-	+
10	-	-	-	+
14	-	-	-	+
21	-	-	-	+
28	-	-	-	+

CHAPTER 3—THE EFFECTS OF IONP GROWING FEATHER INJECTIONS ON LEUKOCYTE INFILTRATION

Abstract

The growing feather pulp in chickens is a minimally invasive *in vivo* test site that allows periodic monitoring of biological reactions to injected treatments in one subject. However, the majority of studies that observe iron oxide nanoparticle (IONP) interactions *in vivo* primarily use other animal models, such as the mouse and non-human primate models. The purpose of these three studies was to investigate the utility of growing feathers in light brown leghorn (LBL) chickens as a test site for the effects of IONPs on immune responses. In Study 1, a sample of 12 chickens was divided into three groups with 4 subjects in each group, and the following treatments were administered to each group: IO, IO-mIgG, and IOEF. The treatment concentration was 0.2 mg/ml for all treatment preparations. In Study 2, a sample of 16 chickens was divided into four groups with 4 subjects in each group. The following treatment preparations were administed to each group: IO (0.2 mg/ml), IO-mIgG (0.2 mg/ml), Alum (15%), and Alum-mIgG (15%-0.5 mg/ml). In Study 3, a sample of 12 chickens was divided into three groups with 4 subjects in each group. The following treatment preparations were administered to each group: IO-mIgG (1 mg/ml), Alum-mIgG (15%-0.26 mg/ml), and mIgG (0.26 mg/ml). The feather injection amount was 10 μl/feather for 10 feathers on each side of the breast in all studies. The results of Study 1 showed significantly higher immune responses to IO and IO-mIgG treatments compared to the responses observed for the IOEF treatment for the following response factors: lymphocytes, CD45+, CD4- CD8+, CD4+ CD8+, CD4+ CD8-, γδ T cell+ CD8-, αβ2 T cell+, αβ1 T cell+, and MHCII+ B cell+. The complete blood cell count analysis in Study 2 found heterophil concentration was significantly higher in the IO-mIgG group compared to the Alum-mIgG

group, $p = 0.0188$. In Study 3, the IO-mIgG treatment elicited a significantly higher heterophil response, $p = 0.009$, and MHCII+ macrophage response, $p = 0.0027$, compared to the Alum-mIgG group. However, Alum-mIgG elicited significantly higher adaptive immune responses compared to IO-mIgG, including B cell+ IgM-, γδ T cells, and αβ1 T cells. These findings suggest that the chicken growing feather is a suitable *in vivo* test site because the immune cells infiltrated the feather pulp, making longitudinal and minimally invasive observations of immune responses in each individual subject possible. The IO-mIgG conjugate elicited a stronger innate response compared to the other treatments, but it did not elicit a strong adaptive immune response. The results suggested that the nanoparticles were taken up by the innate immune cells before they could elicit an adaptive immune response. It is not clear which cells took up the IONPs, but the organ tissue analysis is an ongoing study that will further clarify the interactions between the IONPs and the immune system.

Keywords: aluminum, growing feather, IONP, leukocyte infiltration, mIgG

Introduction

The current *in vivo* test systems for monitoring the effects of NPs on cellular, molecular, and physiological processes have significant limitations. The two most popular animal models include the non-human primate and the mouse model, with the mouse model being preferred because the animals are easier to obtain and require fewer resources to maintain the animals compared to non-human primates. However, the current strategy for investigating nanomaterials calls for the development of a minimally invasive animal model that does not require terminal procedures. The mouse model does not satisfy those criteria as the assessment of NP tissue activities are usually conducted using skin tests, which require invasive procedures (e.g., skin biopsy) or post-mortem collection of tissue.

Furthermore, using skin as a test site does not ensure accurate results because it is difficult to control the behavior of NPs because they can spread unpredictably across a wide area, as well as interact with organs (Gopee et al., 2009). Therefore, the location used to monitor responses to the administration of NPs cannot be reliably predetermined. The invasive nature of the procedures used to collect tissue also does not allow for monitoring tissue responses at multiple time points in one sample subject, so it is not possible to reliably determine how responses to NPs change over time.

These limitations of current models can be addressed by introducing the avian model as a minimally invasive *in vivo* test system for monitoring events associated with NP administration. The GF in birds has similar immunological properties as the skin because it has a low mononuclear presence and can recruit leukocytes from the circulation (Erf et al., 1995). Using the pulp of GFs in chickens as a test site restricts the responses to administered agents within a predefined location, and it is possible to inject multiple agents or a single agent into multiple

feathers. This allows testing multiple agents simultaneously and periodical response monitoring within an individual subject.

The purpose of the current feather injection studies was to monitor and assess local leukocytes infiltration into the pulp upon the administration of antigen-conjugated IONPs and IONPs using GF as a test site. In addition to IONP treatments, the other treatments used included antigen, antigen-conjugated Alum, and Alum. The treatments are exposed to the soluble and cellular components of the immune system in the pulp, which makes it possible to assess and evaluate their immunomodulatory activities. It was hypothesized that the nature and extent of leukocyte infiltration would depend on the treatment used. Specifically, it was expected that IONPs alone would display little or moderate inflammatory activity compared to antigen-conjugated NPs and other treatment preparations.

Results

Blood Cell Responses

The fixed effect variables were the time of sample collection and treatment type. Time of collection was defined as a class with three levels in all studies and contained the following values: 0, 0.25, and 7 days. In Study 1, treatment type was defined as a class with three levels that contained the following values: IO, IOEF, and IO-mIgG. In Study 2, the treatment type was a class with four levels and contained the following values: Alum, Alum-mIgG, IO, and IO-mIgG. The treatment values in Study 3 were as follows: Alum-mIgG, IO-mIgG, and mIgG. The concentration of mIgG in Study 1 and Study 2 was more than 20-fold lower in the IO-mIgG group compared to the other groups in which mIgG was used, whereas the mIgG concentrations were matched in Study 3 for all treatment groups.

The results of the type III test of fixed effects are included in Appendix C. In Study 1, time of collection had a significant effect on heterophil concentration and percentage, lymphocyte concentration and percentage, and monocyte percentage, and thrombocyte percentage ($p < 0.05$).

In Study 2, significant changes by day were observed for total WBC concentration, heterophil concentration and percentage, lymphocyte percentage, monocyte concentration and percentage, basophil concentration and percentage, and RBC concentration. Treatment type had a significant effect on lymphocyte concentration, and the interaction between collection time and type of treatment was significant only for heterophil concentration ($p < 0.05$).

In Study 3, significant changes by day were observed for heterophil concentration and percentage, lymphocyte concentration and percentage, monocyte concentration and percentage, eosinophil percentage, basophil concentration, and thrombocyte concentration. Treatment type had a significant effect only on thrombocyte concentration ($p < 0.05$).

Table 8. *Blood cell responses by day, Study 1*

Response Factor	Days	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
Heterophils (K/uL)	0	5.17 ± 0.35	a
	0.25	8.84 ± 0.33	b
	7	6.40 ± 0.28	c
Heterophils (%)	0	18.66 ± 1.72	a
	0.25	35.92 ± 1.17	b
	7	29.47 ± 3.74	b
Lymphocytes (K/uL)	0	20.05 ± 1.02	a
	0.25	11.91 ± 0.54	b
	7	15.28 ± 1.50	c
Lymphocytes (%)	0	70.53 ± 1.61	a
	0.25	46.13 ± 3.15	b
	7	59.44 ± 4.17	c
Monocytes (%)	0	5.66 ± 0.40	a
	0.25	9.49 ± 0.91	b
	7	5.62 ± 0.54	a
Thrombocytes (K/uL)	0	21.51 ± 0.89	a
	0.25	29.44 ± 1.27	b
	7	23.97 ± 0.86	c

Note: different letters (a-c) represent significant differences ($p < 0.05$) between days for each response factor.

The results of the analysis of least squares means differences for response factors with significant changes in Study 1 by day are shown in Table 8. Heterophil concentration and percentage increased significantly 0.25 days after the injections, and their estimates remained significantly elevated on day 7 compared to baseline estimates. The same pattern was observed for thrombocyte concentration, which increased significantly by day 0.25 and remained significantly higher than baseline estimates by day 7.

Monocyte concentration increased significantly by day 0.25, but monocytes were the only cells that did not remain significantly elevated on day 7 compared to estimates before the injections. Lymphocyte concentration reduced significantly by day 0.25 and remained reduced by day 7 compared to baseline estimates. The same was true for lymphocyte percentage, which reduced by day 0.25 and remained lower on day 7 compared to baseline.

Table 9. *Blood cell responses by day, Study 2*

Response Factor	Days	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
WBCs (K/uL)	0	28.77 ± 1.17	a
	0.25	33.19 ± 1.38	b
	7	29.36 ± 2.16	ab
Heterophils (%)	0	19.37 ± 1.12	a
	0.25	30.98 ± 2.11	b
	7	18.18 ± 1.38	a
Lymphocytes (%)	0	67.11 ± 2.31	a
	0.25	53.88 ± 3.38	b
	7	61.88 ± 2.91	ab
Monocytes (K/uL)	0	2.30 ± 0.39	a
	0.25	3.54 ± 0.29	b
	7	3.10 ± 0.41	ab
Monocytes (%)	0	7.82 ± 1.60	a
	0.25	10.73 ± 1.46	b
	7	10.24 ± 1.65	ab
Basophils (K/uL)	0	1.72 ± 0.14	a
	0.25	1.52 ± 0.06	a
	7	2.93 ± 0.28	b
Basophils (%)	0	5.70 ± 0.55	a
	0.25	4.39 ± 0.20	b
	7	9.55 ± 1.11	c
RBC (K/uL)	0	2.31 ± 0.05	a
	0.25	2.22 ± 0.03	b
	7	2.29 ± 0.04	a

Note: different letters (a-c) represent significant differences ($p < 0.05$) between days for each response factor.

The results of the analysis of least squares means differences for response factors with significant changes by day in Study 2 are shown in Table 9. WBC concentration, heterophil percentage, monocyte concentration, and monocyte percentage increased significantly by day 0.25, but returned to baseline estimates by day 7. Lymphocyte percentage significantly decreased by day 0.25 and returned to estimates similar to those observed at baseline on day 7.

Basophil concentration did not change significantly by day 0.25 compared to baseline, but it did increase significantly on day 7 compared to previous measures. Basophil percentage showed a significant decrease by day 0.25 compared to baseline measures, but the estimates on

day 7 were significantly higher compared to both previous measurements. RBC concentration decreased significantly by day 0.25, but it returned to baseline measures by day 7.

Table 10. *Blood cell responses by day, Study 3*

Response Factor	Days	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
Heterophils (K/uL)	0	8.19 ± 0.8602	a
	0.25	12.81 ± 1.5286	b
	7	6.83 ± 0.3912	a
Heterophils (%)	0	26.34 ± 1.67	a
	0.25	47.35 ± 3.46	b
	7	26.08 ± 2.04	a
Lymphocytes (K/uL)	0	18.38 ± 0.83	a
	0.25	9.41 ± 0.97	b
	7	17.15 ± 1.54	a
Lymphocytes (%)	0	59.51 ± 1.51	a
	0.25	35.21 ± 4.01	b
	7	62.32 ± 2.39	a
Monocytes (K/uL)	0	2.48 ± 0.16	a
	0.25	3.06 ± 0.42	a
	7	1.54 ± 0.13	b
Monocytes (%)	0	8.07 ± 0.49	a
	0.25	11.81 ± 1.71	a
	7	5.87 ± 0.50	b
Eosinophils (%)	0	0.06 ± 0.02	a
	0.25	0.15 ± 0.03	b
	7	0.16 ± 0.03	b
Basophils (K/uL)	0	1.85 ± 0.10	a
	0.25	1.44 ± 0.20	ab
	7	1.47 ± 0.12	b
Thrombocytes (K/uL)	0	31.05 ± 0.90	a
	0.25	26.69 ± 1.32	b
	7	27.68 ± 1.08	ab

Note: different letters (a-b) represent significant differences ($p < 0.05$) between days for each response factor.

Table 10 shows the results of the difference of least squares means for blood cell responses in Study 3. Heterophil concentration and percentage increased significantly from baseline by day 0.25 and returned to values similar to those observed at baseline by day 7. The

same pattern of response was observed for lymphocyte concentration and lymphocyte percentage.

Monocyte concentration and percentage increased by day 0.25, but the increase was not significant compared to baseline estimates. After the slight initial increase, monocyte estimates reduced and were significantly lower on day 7 compared to baseline. Eosinophil percentage increased significantly by day 0.25, and it remained significantly elevated on day 7 compared to baseline. Basophil concentration slightly decreased by day 0.25, but the decrease was statistically significant only on day 7 compared to baseline. Thrombocyte concentration decreased significantly by day 0.25. Although the estimates on day 7 did not return to baseline estimates, the difference compared to baseline was no longer statistically significant.

Treatment type had a significant effect on lymphocyte concentration in Study 2, as well as on thrombocyte concentration in Study 3 (see Table 11). The lowest estimate of lymphocyte concentration in the 2013 study was observed in the IO treatment group, which showed a significantly lower lymphocyte concentration compared to the Alum group and the Alum-mIgG group, but not compared to the IO-mIgG group. In Study 3, thrombocyte concentration was significantly higher in the Alum-mIgG group compared to the IO-mIgG group, but not compared to the mIgG group.

Table 11. *Lymphocyte and thrombocyte concentration by treatment group, Study 2 and Study 3*

Response	Treatment	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
Lymphocytes (K/uL)	Alum	23.84 ± 1.87	a
(2013 IO vs. Alum)	Alum-mIgG	22.54 ± 1.87	a
	IO	16.20 ± 1.87	b
	IO-mIgG	18.70 ± 1.87	ab
Thrombocytes (K/uL)	Alum-mIgG	31.00 ± 1.06	a
(2014)	IO-mIgG	26.76 ± 1.06	b
	mIgG	27.66 ± 1.06	ab

Note: different letters (a-b) represent significant differences ($p < 0.05$) between treatment groups for each response factor.

Table 12. *Treatment and day interaction effect estimates and main effects p-values of heterophil concentration, Study 2*

	Treatment				
Day	**Alum (K/uL ± SE, n = 4)**	**Alum-mIgG (K/uL ± SE, n = 4)**	**IO (K/uL ± SEM, n = 4)**	**IO-mIgG (K/uL ± SEM, n = 4)**	**Day main effect P-value**
0	5.69 ± 0.53	5.75 ± 0.53	6.30 ± 0.53	5.47 ± 0.53	0.7240
0.25	10.00 ± 0.91	9.03 ± 0.91	10.37 ± 0.91	12.53 ± 0.91	0.0986
7	5.60 ± 0.76	5.86 ± 0.76	5.50 ± 0.76	5.91 ± 0.76	0.9759
Treatment main effect P-value	< 0.0001	0.0001	< 0.0001	< 0.0001	

The interaction between treatment type and time of sample collection had a significant on heterophil concentration in Study 2 (see Table 12). The simple effects comparison of heterophil concentration by treatment showed that all types of treatment increased heterophil concentration significantly 0.25 days after the injection. Seven days after the injections, the observed heterophil concentration returned to similar levels as measured before the injections in all groups. The simple effects comparison by day showed that a significant difference was observed on day 0.25 between the Alum-mIgG group and the IO-mIgG group (see Figure 6).

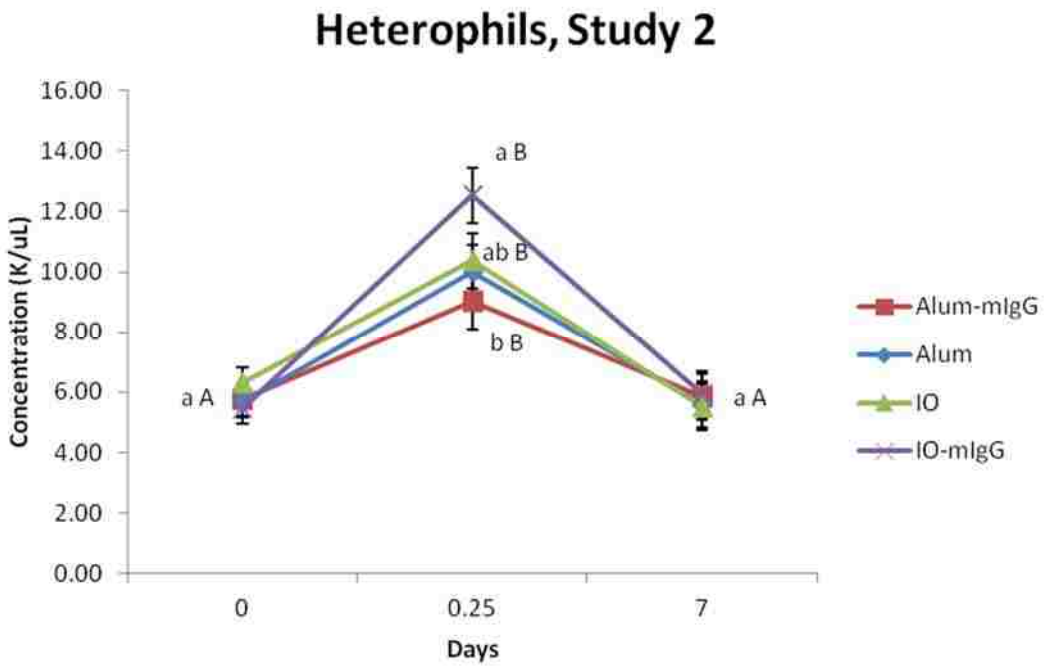

Figure 6. Heterophil concentration by day and treatment group, Study 2.

The graph shows mean differences of the treatment*day effect, not the main effect. Heterophil concentration increased by day 0.25 in all groups, but the increase was significantly higher in the IO-mIgG group compared to the Alum-mIgG group. Different lowercase letters (a-b) within one time point represent significant differences ($p < 0.05$) between treatment groups. Different uppercase letters (A-B) represent significant changes between days for each treatment group.

Most blood cell response factors measured in all three studies were affected significantly by time of collection, but no significant differences were observed in the responses between treatment groups. Significant differences by treatment group were observed only for lymphocyte concentration in Study 2 and thrombocyte concentration in Study 3. Lymphocyte mean concentration was significantly higher in the Alum-mIgG and Alum groups compared to the IO group, but not compared to the IO-mIgG group. Thrombocyte mean concentration was significantly higher in the Alum-mIgG group compared to the IO-mIgG group. The interaction between treatment and time of collection was a significant fixed effect only for heterophil

concentration in Study 2 (see Figure 6). Differences between the groups were observed on day 0.25 as the IO-mIgG group elicited a higher heterophil response compared to the Alum-mIgG group. The difference of least squares means results and simple effect comparison results for significant responses are included in Appendix D.

Pulp Cell Responses

Time of collection was defined as a class with eight levels in Study 1 and Study 3 (0, 0.25, 1, 2, 3, 4, 5, and 7 days) and with six levels in Study 2 (0, 0.25, 1, 2, 5, and 7 days). Type of treatment was a class with three levels in Study 1 (IO, IOEF, and IO-mIgG) and in Study 3 (Alum-mIgG, IO-mIgG, and mIgG). Study 2 defined type of treatment as a class with four levels (Alum, Alum-mIGg, IO, and IO-mIgG). The concentration of mIgG in Study 1 and Study 2 was more than 20-fold lower in the IO-mIgG group compared to the other groups in which mIgG was used, whereas the mIgG concentrations were matched in Study 3 for all treatment groups. The results of the type III test of fixed effects for innate immune response factors are presented in Appendix E.

Live cells. Live cells were significantly affected by the interaction between treatment and time of sample collection in Study 1, whereas no significant changes in live cells were observed in Study 3. Significant changes in live cell estimates by day were observed in the IO and IO-mIgG groups, but not in the IOEF group (see Table 13).

Table 13. *Treatment and day interaction effect estimates and main effects p-values of live cells, Study 1*

Day	Treatment			
	IO ($\bar{x} \pm SE$, n = 4)	**IO-mIgG ($\bar{x} \pm SE$, n = 4)**	**IOEF ($\bar{x} \pm SE$, n = 4)**	**Day main effect P-value**
0	37.47 ± 1.79	28.63 ± 1.79	30.34 ± 2.00	0.0124
0.25	35.90 ± 2.55	27.71 ± 2.55	28.21 ± 2.85	0.0833
1	44.89 ± 2.21	40.83 ± 2.21	45.61 ± 2.48	0.3162
2	38.94 ± 2.95	33.11 ± 2.95	38.51 ± 3.29	0.3430
3	35.73 ± 2.31	35.93 ± 2.31	35.57 ± 2.59	0.9945
4	32.90 ± 2.78	34.46 ± 2.78	29.28 ± 3.11	0.4769
5	32.65 ± 1.55	35.53 ± 1.55	32.72 ± 1.74	0.3717
7	32.82 ± 2.18	31.77 ± 2.18	28.62 ± 2.44	0.4459
Treatment main effect P-value	0.0027	0.0150	0.0730	

The only significant difference between the treatment groups was observed just before the injections. The IO treatment group had a higher baseline of live cells compared to other groups, and no further differences were observed between the treatment groups by day (see Figure 7).

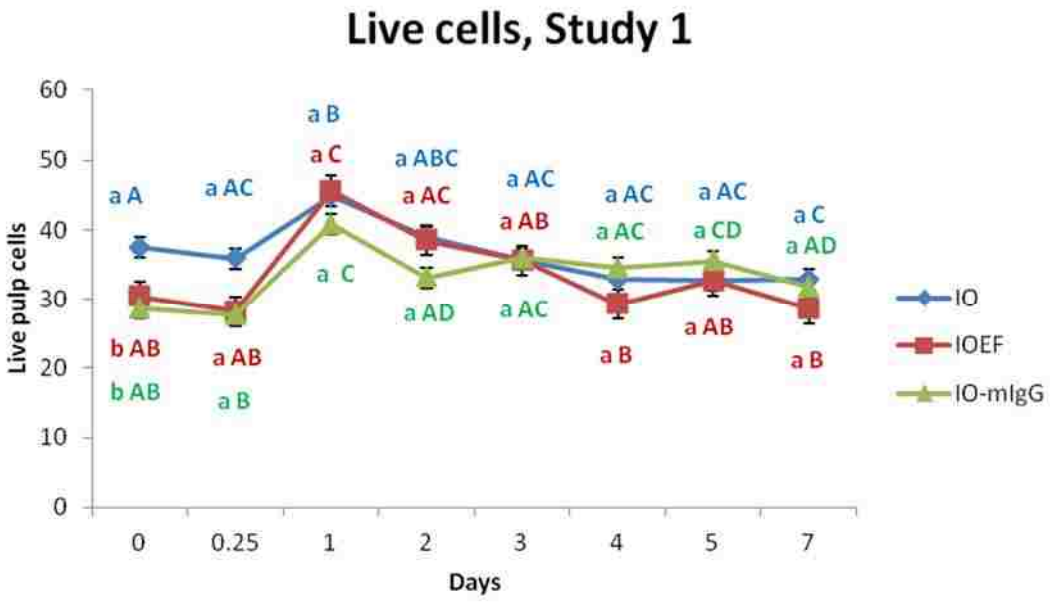

Figure 7. Live cells by day and treatment group, 2013 IO.

The graph shows mean differences of the treatment*day effect, not the main effect. Live pulp cells are measured by excluding dead and red cells. The only significant difference between treatment groups was observed prior to the injections as the IO group had an elevated live cell count compared to the other groups. Different lowercase letters (a-b) within one time point represent significant differences ($p < 0.05$) between treatment groups. A significant increase of live pulp cells occurred on day 1 and continued decreasing by day 7 in all treatment groups. Different uppercase letters (A-D) represent significant changes between days for each treatment group.

All leukocytes. All fixed effects had a significant effect on CD45+ estimates in Study 2 and Study 3, but only treatment type had a significant effect on those cells in Study 1. The CD45+ cells were significantly different by treatment group in Study 1, with the IOEF group showing the lowest response (see Table 14). The response in the IO group was significantly higher compared to the IOEF group, and the response in the IOEF group was significantly lower

compared to the IO-mIgG group. There were no significant differences in CD45+ responses between the IO and the IO-mIgG group.

Table 14. *All leukocytes by treatment group, Study 1*

Response	Treatment	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
CD45+	IO	17.58 ± 1.06	a
	IOEF	12.43 ± 1.19	b
	IO-mIgG	16.82 ± 1.06	a

Note: Different letters (a-b) represent significant differences between treatment groups.

In Study 2, the interaction between treatment type and time of collection significantly affected CD45+ estimates. The interaction effect estimates showed that CD45+ varied significantly each day for all treatment groups, and the differences between the treatment groups were significant from day 1 until day 7 (see Table 15). The highest estimates were observed in the Alum-mIgG group, which showed a significantly higher CD45+ response compared to other groups between days 1 and 5. On day 7, only the Alum group had significantly lower CD45+ estimates compared to the other groups (see Figure 8, top).

The test of interaction effect results for CD45+ estimates in Study 3 are shown in Table 16. There were no significant differences between Alum-mIgG and IO-mIgG groups by day. Compared to the mIgG treatment, the Alum-mIgG treatment and the IO-mIgG treatment elicited a significantly higher CD45+ response on day 0.25. However, only the response in the Alum-mIgG group was still significantly higher compared to the mIgG group on days 2 and 3 (see Figure 8, bottom).

Table 15. *Treatment and day interaction effect estimates and main effects p-values of CD45+, Study 2*

Day	Treatment				Day main effect P-value
	Alum ($\bar{x} \pm SE$, n = 4)	**Alum-mIgG ($\bar{x} \pm SE$, n = 4)**	**IO ($\bar{x} \pm SE$, n = 4)**	**IO-mIgG ($\bar{x} \pm SE$, n = 4)**	
0	9.41 ± 2.59	6.87 ± 2.59	8.62 ± 2.59	9.51 ± 2.59	0.8783
0.25	39.50 ± 7.41	50.36 ± 7.41	31.03 ± 7.41	39.59 ± 7.41	0.3717
1	35.26 ± 6.09	60.45 ± 6.09	25.31 ± 6.09	29.95 ± 6.09	0.0068
2	27.24 ± 5.35	55.31 ± 5.35	16.63 ± 5.35	23.64 ± 5.35	0.0013
5	34.47 ± 5.15	64.42 ± 5.15	45.41 ± 5.15	44.93 ± 5.15	0.0106
7	19.06 ± 4.15	41.29 ± 4.15	34.51 ± 4.15	32.68 ± 4.15	0.0174
Treatment main effect P-value	0.0023	< 0.0001	0.0005	0.0006	

Table 16. *Treatment and day interaction effect estimates and main effects p-values of CD45+, Study 3*

Day	Treatment			Day main effect P-value
	Alum-mIgG ($\bar{x} \pm SE$, n = 4)	**IO-mIgG ($\bar{x} \pm SE$, n = 4)**	**mIgG ($\bar{x} \pm SE$, n = 4)**	
0	10.54 ± 1.74	11.36 ± 1.74	7.76 ± 1.74	0.3531
0.25	37.76 ± 2.79	41.19 ± 2.79	16.02 ± 2.79	0.0003
1	53.15 ± 8.68	37.42 ± 8.68	25.47 ± 8.68	0.1317
2	50.24 ± 5.20	35.42 ± 5.20	21.19 ± 5.20	0.0109
3	60.83 ± 7.24	43.15 ± 7.24	26.16 ± 7.24	0.0248
4	40.46 ± 12.03	35.67 ± 12.03	31.39 ± 12.03	0.8695
5	58.91 ± 9.49	39.77 ± 9.49	30.63 ± 9.49	0.1547
7	31.85 ± 8.05	37.50 ± 8.05	16.97 ± 8.05	0.2652
Treatment main effect P-value	< 0.0001	< 0.0001	0.0218	

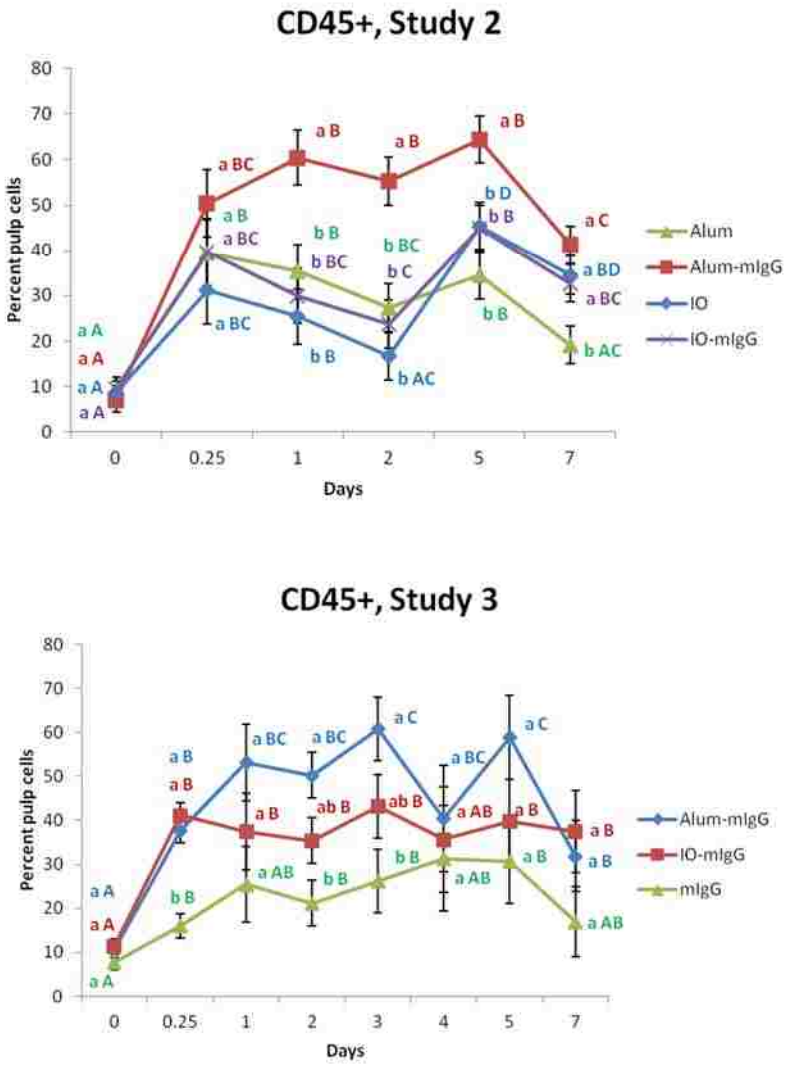

Figure 8. All leukocytes by day and treatment group.

The graph shows mean differences of the treatment*day effect, not the main effect. Different lowercase letters (a-b) within one time point represent significant differences ($p < 0.05$) between treatment groups. Different uppercase letters (A-D) represent significant changes between days for each treatment group.

Top: The highest increase of CD45+ estimates Study 2 was observed in the Alum-mIgG group. The response was significantly higher compared to other groups from day 1 until day 5.

Bottom: The highest increase of CD45+ estimates in Study 3 was observed in the Alum-mIgG group. The response was significantly higher compared to the mIgG group, but not compared to the IO-mIgG group.

Macrophages. Macrophages measured in Study 1 and Study 2 varied significantly only by day (see Table 17). In Study 1, the level of macrophages decreased significantly on day 2 compared to baseline estimates. Macrophage continued to increase until day 5. The next significant decrease in macrophages occurred on day 5, and the values remained significantly elevated until day 7. All treatment groups showed similar trends in macrophage estimates by day. The macrophage response in Study 2 showed a significant increase by day 0.25. Although the response reduced over the 7 days of taking measurements, the macrophage levels were still significantly higher on day 7 compared to baseline measures.

Table 17. *Macrophage estimates by day, Study 1 and Study 2*

Response Factor	Days	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
Macrophages (Study 1)	0	1.87 ± 0.13	a
	0.25	1.85 ± 0.18	ac
	1	1.57 ± 0.14	ab
	2	1.03 ± 0.23	bc
	3	1.36 ± 0.12	bc
	4	1.63 ± 0.45	ab
	5	1.07 ± 0.20	b
	7	1.22 ± 0.12	b
Macrophages (Study 2)	0	1.31 ± 0.09	a
	0.25	3.79 ± 0.39	b
	1	3.01 ± 0.38	bc
	2	1.94 ± 0.28	d
	5	2.55 ± 0.15	c
	7	2.02 ± 0.13	d

Note: Different letters (a-d) represent significant differences ($p < 0.05$) between days for each response factor.

Heterophils. All fixed effects were associated with changes in heterophil estimates in Study 3, but changes in heterophil estimates in Study 2 were associated only with collection time. Heterophils increased increased in Study 2 significantly by day 0.25 compared to baseline estimates. As collection time increased, heterophil estimates consistently decreased, and their levels returned to those observed at baseline on day 5 (see Table 18).

Table 18. *Heterophil estimates by day, Study 2*

Response Factor	Days	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
Heterophils	0	0.3838 ± 0.05	a
	0.25	15.5662 ± 1.94	b
	1	7.6544 ± 1.32	c
	2	1.8488 ± 0.42	d
	5	0.6787 ± 0.13	a
	7	0.4906 ± 0.12	a

Note: Different letters (a-d) represent significant differences ($p < 0.05$) between days.

In Study 3, the effect of the interaction between treatment type and collection time on heterophil estimates was significant. The treatment main effect was significant for the Alum-mIgG and IO-mIgG groups (see Table 19). No significant changes by day were observed in the mIgG group. Differences between treatment groups were observed only on day 0.25 and day 1.

Table 19. *Treatment and day interaction effect estimates and main effects p-values of heterophils, 2014*

Day	Treatment			Day main effect P-value
	Alum-mIgG ($\bar{x} \pm SE$, n = 4)	IO-mIgG ($\bar{x} \pm SE$, n = 4)	mIgG ($\bar{x} \pm SE$, n = 4)	
0	2.55 ± 0.63	2.33 ± 0.63	2.61 ± 0.63	0.9502
0.25	7.97 ± 1.66	19.25 ± 1.66	4.80 ± 1.66	0.0004
1	4.00 ± 1.83	15.34 ± 1.83	4.58 ± 1.83	0.0029
2	1.47 ± 0.25	1.87 ± 0.25	1.69 ± 0.25	0.5398
3	1.44 ± 0.38	1.98 ± 0.38	1.88 ± 0.38	0.5842
4	1.85 ± 0.39	1.46 ± 0.39	1.21 ± 0.39	0.5190
5	1.19 ± 0.30	2.12 ± 0.30	1.34 ± 0.30	0.1265
7	2.12 ± 0.54	2.19 ± 0.54	2.12 ± 0.54	0.9957
Treatment main effect P-value	0.0329	< 0.0001	0.2289	

There were no significant differences in heterophil baseline estimates among the treatment groups, and by day 0.25, the IO-mIgG group elicited a significantly stronger response than the Alum-mIgG group and the mIgG group (see Figure 9). Heterophil levels on day 1 were still significantly higher than those observed in the Alum-mIgG group and the mIgG group. No significant differences in heterophils were observed between the treatment groups on subsequent collection times.

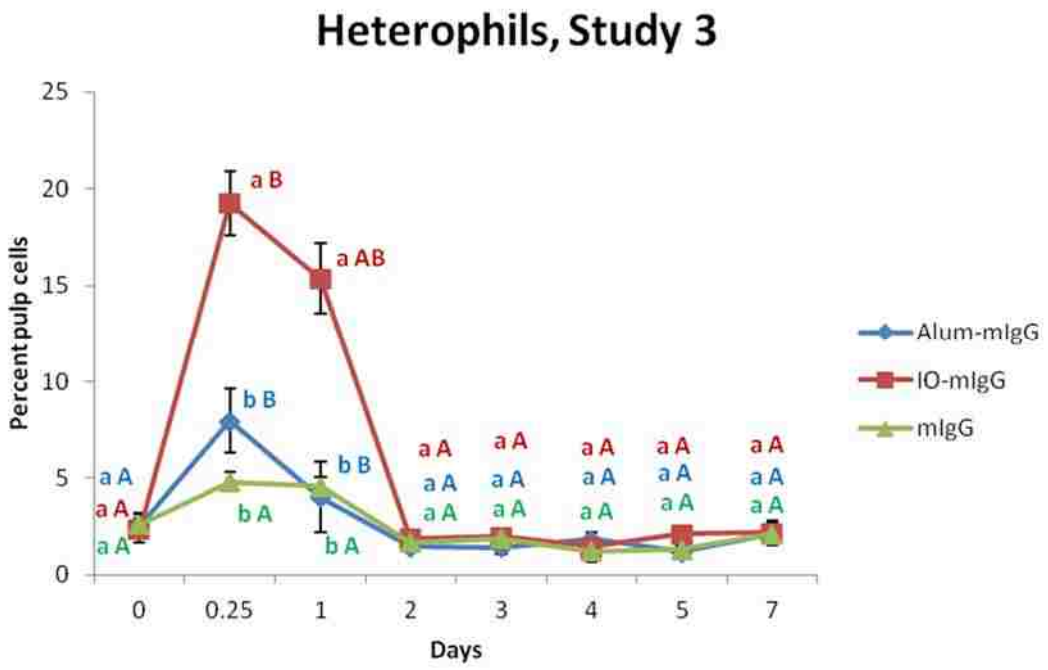

Figure 9. Heterophil estimates by day and treatment group, Study 3.

The graph shows mean differences of the treatment*day effect, not the main effect. The highest response was observed in the IO-mIgG group. After day 2, estimates in all groups returned to values similar to those observed at baseline. Different lowercase letters (a-b) within one time point represent significant differences ($p < 0.05$) between treatment groups. Different uppercase letters (A-B) represent significant changes between days for each treatment group.

Lymphocytes. Lymphocyte estimates varied significantly by day and treatment group in Study 1. Lymphocyte estimates in Study 1 increased significantly within 0.25 days after the injections (see Table 20). The estimates continued to increase until they peaked on day 2, after which they continued to decrease. On day 7, the lymphocyte estimates were no longer significantly different compared to baseline. The differences between treatment groups were significant as the IOEF group had a significantly lower lymphocyte response compared to the IO group and the IO-mIgG group.

Table 20. *Lymphocyte estimates by day and treatment group, Study 1*

Response Factor	Fixed Effect	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
Lymphocytes	*Days*		
	0	2.55 ± 0.16	a
	0.25	4.65 ± 0.68	b
	1	6.70 ± 0.84	bc
	2	9.42 ± 1.14	cd
	3	8.78 ± 1.25	cd
	4	8.64 ± 0.93	d
	5	7.90 ± 1.35	cd
	7	2.90 ± 0.77	ab
	Treatment		
	IO	8.13 ± 0.76	a
	IO-mIgG	7.45 ± 0.76	a
	IOEF	3.74 ± 0.85	b

Note: Different letters (a-d) represent significant differences ($p < 0.05$) between days and between treatment groups

Major histocompatibility complex. In Study 1, the MHCII+ macrophage estimates were significantly affected by time of collection only (see Table 21). A significant decrease was observed on day 0.25 compared to baseline, followed by a significant decrease on day 1 compared to day 0.25. No significant changes were observed after day 1, but the estimates were significantly higher on day 7 compared to baseline estimates.

For MHCII+ macrophages in Study 2, the effect of collection time was the only significant fixed effect (see Table 22). Total MHCII+ estimates increased significantly by day 1 compared to baseline, after which they continued declining each day. Compared to baseline estimates, MHCII+ macrophage estimates were still significantly higher on day 5, but not on day 7.

Table 21. *MHCII+ macrophage estimates by day, Study 1*

Response Factor	Days	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
MHCII+	0	0.54 ± 0.07	a
Macrophages+	0.25	0.32 ± 0.04	b
	1	0.72 ± 0.08	ac
	2	0.52 ± 0.13	abc
	3	0.74 ± 0.09	ac
	4	0.66 ± 0.15	ac
	5	0.71 ± 0.13	ac
	7	0.79 ± 0.06	c

Note: Different letters (a-d) represent significant differences ($p < 0.05$) between days.

Table 22. *MHCII+ macrophage estimates by day, Study 2*

Response Factor	Days	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
MHCII+	0	0.6537 ± 0.03	a
Macrophages+	0.25	0.6950 ± 0.03	ac
	1	1.4669 ± 0.18	b
	2	0.9750 ± 0.12	d
	5	0.9381 ± 0.12	cd
	7	0.6325 ± 0.11	ad

Note: Different letters (a-d) represent significant differences ($p < 0.05$) between days.

Table 23. *MHCII+ macrophage estimates by day and treatment group, Study 3*

Response Factor	Fixed Effect	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
MHCII+	*Days*		
Macrophages+	0	2.29 ± 0.29	a
	0.25	4.95 ± 0.31	b
	1	4.70 ± 0.31	b
	2	1.55 ± 0.19	ac
	3	1.72 ± 0.24	ac
	4	1.38 ± 0.20	c
	5	1.75 ± 0.12	ac
	7	2.37 ± 0.16	a
	Treatment		
	Alum-mIgG	2.77 ± 0.14	a
	IO-mIgG	3.02 ± 0.14	a
	mIgG	1.99 ± 0.14	b

Note: Different letters (a-c) represent significant differences ($p < 0.05$) between days and between treatment groups.

In Study 3, the MHCII+ macrophage estimates were significantly affected by time of collection and treatment type, but not by their interaction (see Table 23). Estimates peaked on days 0.25 and 1, and the increase was significantly higher compared to the baseline measure. After day 1, the estimates decreased and were significantly lower compared to baseline values only on day 4. On day 7, MHCII+ macrophage estimates returned to values similar to those observed at baseline.

The MHCII+ B cell+ response was significant in Study 1 and Study 2 by day only, whereas no significant fixed effects for the MHCII+ B cell+ response were observed in Study 3. In Study 1, the estimates of MHCII+ B cell+ increased by day 0.25 compared to baseline and peaked on day 2 (see Table 24). The observed response was still significant on day 7 compared to baseline estimates. In Study 2, the estimates of the MHCII+ B cell+ response showed that a decreased by day 0.25, after which the estimates continued to increase (see Table 25). The estimates on day 2 were significantly higher compared to baseline estimates, and they peaked on day 5. The estimates on day 7 were still statistically significant compared to values observed at baseline.

Table 24. *MHCII+ B cell+ estimates by day, Study 1*

Response Factor	Fixed Effect	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
MHCII+ B cell+	0	0.15 ± 0.04	a
	0.25	0.42 ± 0.05	b
	1	0.67 ± 0.08	c
	2	1.09 ± 0.11	d
	3	0.74 ± 0.06	c
	4	0.77 ± 0.07	c
	5	0.44 ± 0.17	abc
	7	0.45 ± 0.10	b

Note: Different letters (a-d) represent significant differences ($p < 0.05$) between days.

Table 25. *MHCII+ B cell+ estimates by day, Study 2*

Response Factor	Fixed Effect	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
MHCII+ B cell+	0	0.18 ± 0.03	ac
	0.25	0.14 ± 0.02	a
	1	0.31 ± 0.07	cd
	2	0.43 ± 0.04	d
	5	0.85 ± 0.14	b
	7	0.65 ± 0.07	b

Note: Different letters (a-d) represent significant differences ($p < 0.05$) between days.

T cells.CD4- CD8+ T cell estimates increased significantly by day 0.25 in Study 1, and the highest estimates were observed on days 2 and 4 (see Table 26). The estimates were still elevated on day 7 compared to baseline, but the difference was not statistically significant. The highest response was observed in the IO treatment group. The difference between the IO and the IO-mIgG group was not significant, but the elicited response in both groups was significantly higher compared to the response observed in the IOEF group.

Table 26. *CD4- CD8+ estimates by day and treatment group, Study 1*

Response Factor	Fixed Effect	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
CD4- CD8+	*Days*		
	0	0.17 ± 0.03	a
	0.25	0.57 ± 0.07	b
	1	0.70 ± 0.12	bc
	2	1.05 ± 0.16	cd
	3	0.82 ± 0.12	bcd
	4	1.06 ± 0.12	d
	5	0.99 ± 0.20	bcd
	7	0.33 ± 0.09	a
	Treatment		
	IO	0.96 ± 0.11	a
	IO-mIgG	0.81 ± 0.11	a
	IOEF	0.37 ± 0.13	b

Note: Different letters (a-d) represent significant differences ($p < 0.05$) between days and between treatment groups.

In Study 2, CD4- CD8+ T cell estimates increased significantly by day 0.25, and the highest value was observed on day 5. Despite the significant decrease from day 5 to day 7, the CD4- CD8+ T cell estimates were still significantly elevated on day 7 compared to baseline estimates (see Table 27). In Study 3, CD4- CD8+ T cell estimates increased by day 0.25, and continued increasing until they peaked on day 3 (see Table 28). Although the estimates were still elevated on day 7, they were no longer significantly higher compared to baseline. The highest response was observed in the Alum-mIgG group, and it was significantly higher compared to the other groups.

Table 27. *CD4- CD8+ estimates by day, Study 2*

Response Factor	Days	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
CD4- CD8+	0	0.65 ± 0.11	a
	0.25	1.21 ± 0.16	b
	1	2.13 ± 0.37	cd
	2	1.72 ± 0.20	c
	5	2.51 ± 0.18	d
	7	1.65 ± 0.19	bc

Note: Different letters (a-d) represent significant differences ($p < 0.05$) between days.

Table 28. *CD4- CD8+ estimates by day and treatment group, Study 3*

Response Factor	Fixed Effect	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
CD4- CD8+	*Days*		
	0	0.69 ± 0.07	a
	0.25	1.28 ± 0.18	b
	1	2.74 ± 0.42	c
	2	3.50 ± 0.57	c
	3	6.34 ± 0.87	d
	4	4.95 ± 1.19	cd
	5	4.34 ± 0.64	cd
	7	1.42 ± 0.60	ab
	Treatment		
	Alum-mIgG	4.62 ± 0.56	a
	IO-mIgG	2.55 ± 0.57	b
	mIgG	2.30 ± 0.59	b

Note: Different letters (a-d) represent significant differences ($p < 0.05$) between days and between treatment groups.

CD4+ CD8+ cells changed significantly by day and treatment group in Study 1 (see Table 29). The estimates increased significantly by day 1 compared to baseline and peaked on day 2, after which they continued decreasing. No significant differences were observed after day 5 compared to baseline. The response observed in the IO-mIgG group was significantly higher compared to the IOEF group. In Study 2, significant changes in CD4+ CD8+ cells estimates were observed by day 0.25, and the estimates continued increasing until they peaked on day 5. On day 7, the CD4+ CD8+ estimates decreased significantly compared to day 5, but they were still significantly higher compared to baseline (see Table 30).

Table 29. *CD4+ CD8+ estimates by day and treatment group, Study 1*

Response Factor	Fixed Effect	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
CD4+ CD8+	*Days*		
	0	0.13 ± 0.03	a
	0.25	0.32 ± 0.10	abc
	1	0.24 ± 0.04	b
	2	0.40 ± 0.04	c
	3	0.33 ± 0.03	bc
	4	0.26 ± 0.04	b
	5	0.16 ± 0.04	ab
	7	0.11 ± 0.02	a
	Treatment		
	IO	0.25 ± 0.02	ab
	IO-mIgG	0.30 ± 0.02	a
	IOEF	0.19 ± 0.03	b

Note: Different letters (a-c) represent significant differences ($p < 0.05$) between days and between treatment groups.

Table 30. *CD4+ CD8+ estimates by day and treatment group, Study 2*

Response Factor	Days	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
CD4+ CD8+	0	0.14 ± 0.03	a
	0.25	0.22 ± 0.04	b
	1	0.39 ± 0.11	be
	2	0.64 ± 0.16	c
	5	1.01 ± 0.17	d
	7	0.46 ± 0.07	e

Note: Different letters (a-e) represent significant differences ($p < 0.05$) between days.

CD4+ CD8- cells varied significantly by day and treatment group in Study 1 (see Table 31). A significant increase compared to baseline was observed by day 0.25. The values increased until day 2 and remained significantly elevated until day 5. A significant decrease was observed between day 5 and day 7. The estimates on day 7 were still higher compared to baseline, but the difference was not significant. The response was significantly higher in the IO and IO-mIgG groups compared to the IOEF group.

Table 31. *CD4+ CD8- estimates by day and treatment group, Study 1*

Response Factor	Fixed Effect	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
CD4+ CD8-	*Days*		
	0	0.82 ± 0.08	a
	0.25	1.60 ± 0.13	b
	1	2.23 ± 0.38	bd
	2	3.61 ± 0.49	c
	3	3.90 ± 0.60	c
	4	3.91 ± 0.41	c
	5	3.85 ± 0.82	cd
	7	1.40 ± 0.42	ab
	Treatment		
	IO	3.51 ± 0.40	a
	IO-mIgG	3.12 ± 0.45	a
	IOEF	1.36 ± 0.40	b

Note: Different letters (a-d) represent significant differences ($p < 0.05$) between days and between treatment groups.

In Study 2, CD4+ CD8- cell estimates increased significantly by day 0.25, and they continued to increase until day 5 (see Table 32). A significant decrease was observed from day 5 to day 7, but the values were still significantly higher on day 7 compared to baseline estimates. The highest response was observed in the IO group, but the response was not significantly higher compared to the Alum-mIgG and IO groups. The weakest response was observed in the Alum group, and it was significantly lower compared to the other groups. In Study 3, CD4+ CD8- cells increased significantly on day 2, and the highest estimate was observed on day 3, after which the values continued to decline. The estimates on day 7 were no longer significantly higher compared to initial estimates (see Table 33).

Table 32. *CD4+ CD8- estimates by day and treatment group, Study 2*

Response Factor	Fixed Effect	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
CD4+ CD8-	*Days*		
	0	1.71 ± 0.42	a
	0.25	3.27 ± 0.47	b
	1	5.04 ± 0.64	c
	2	5.44 ± 0.68	ce
	5	11.99 ± 0.89	d
	7	6.98 ± 0.51	e
	Treatment		
	Alum	3.72 ± 0.47	a
	Alum-mIgG	6.59 ± 0.47	b
	IO	7.04 ± 0.47	b
	IO-mIgG	5.61 ± 0.47	b

Note: Different letters (a-e) represent significant differences ($p < 0.05$) between days and between treatment groups.

Table 33. *CD4+ CD8- estimates by day, Study 3*

Response Factor	Days	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
CD4+ CD8-	0.25	3.90 ± 0.39	a
	1	5.75 ± 1.04	a
	2	8.69 ± 1.10	b
	3	15.74 ± 2.33	c
	4	10.85 ± 2.35	bc
	5	11.59 ± 1.63	bc
	7	5.58 ± 1.05	a

Note: Different letters (a-c) represent significant differences ($p < 0.05$) between days.

The CD3+ response factor was observed only in Study 3, and the effect of the interaction between treatment and time of collection. Estimates changed significantly by day in all three groups, and differences between the groups were observed from day 0.25 until day 3 (see Table 34). The highest response was observed in the Alum-mIgG group, but the treatment main effect was significant for all groups. Significant differences between the groups were observed between days 0.25 and 3 as the Alum-mIgG group elicited a stronger response compared to the other groups (see Figure 10).

Table 34. *Treatment and day interaction effect estimates and main effects p-values of CD3+, Study 3*

Day	Treatment			Day main effect P-value
	Alum-mIgG ($\bar{x} \pm SE$, n = 4)	IO-mIgG ($\bar{x} \pm SE$, n = 4)	mIgG ($\bar{x} \pm SE$, n = 4)	
0	2.83 ± 1.00	4.83 ± 1.00	2.70 ± 1.00	0.2892
0.25	16.29 ± 1.12	6.31 ± 1.12	5.68 ± 1.12	0.0001
1	27.23 ± 5.12	7.95 ± 5.12	12.53 ± 5.12	0.0613
2	30.88 ± 3.14	16.54 ± 3.14	12.67 ± 3.14	0.0063
3	37.91 ± 4.24	23.95 ± 4.24	15.89 ± 4.24	0.0153
4	22.67 ± 6.60	17.49 ± 6.60	15.24 ± 6.60	0.7247
5	24.52 ± 4.63	14.91 ± 4.63	15.95 ± 4.63	0.3358
7	8.13 ± 3.41	16.67 ± 3.94	6.76 ± 3.41	0.1934
Treatment main effect P-value	< 0.0001	0.0012	0.0128	

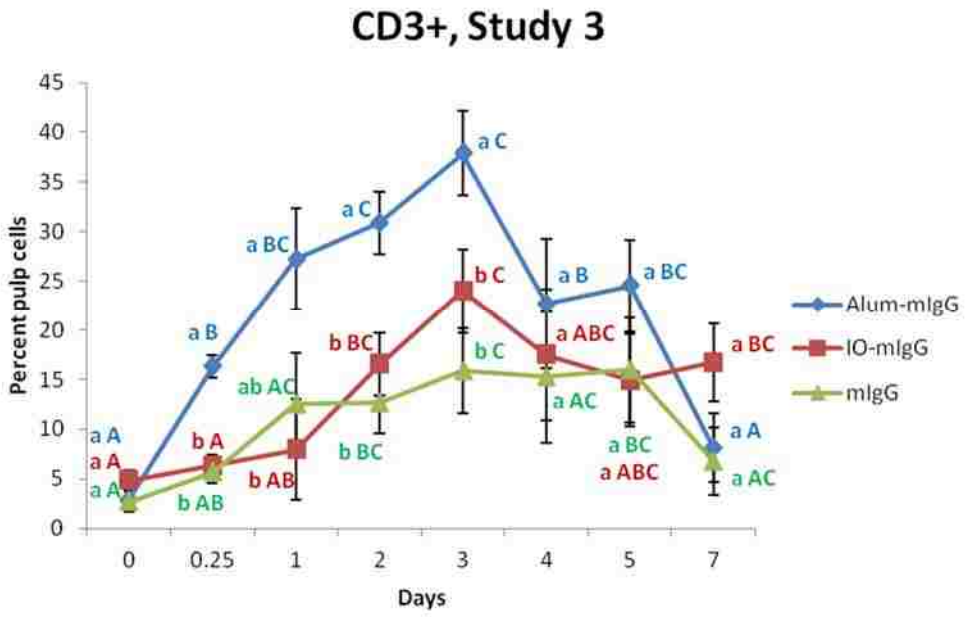

Figure 10. CD3+ estimates by day and treatment group, Study 3.

The graph shows mean differences of the treatment*day effect, not the main effect. The highest response was observed in the Alum-mIgG group, and it was significantly higher compared to other groups No significant differences were observed between the IO-mIgG and mIgG treatments. Different lowercase letters (a-b) within one time point represent significant differences ($p < 0.05$) between the treatment groups. Different uppercase letters (A-C) represent significant changes between days for each treatment group.

The CD4/CD8 ratio was measured in all studies, and the effect of sample collection time was significant in all studies, but the interaction between time of collection and treatment type was significant only in Study 3. In Study 1, the CD4/CD8 ratio decreased significantly by day 0.25, but the difference was no longer significant on day 2, after which the ratio reduced significantly compared to baseline once on day 5 (see Table 35). In Study 2, a significant increase of the CD4/CD8 ratio compared to baseline was observed on day 2, and the ratio remained significantly higher compared to baseline on day 7 (see Table 35).

Table 35. *CD4/CD8 ratio estimates by day, Study 1 and Study 2*

Response Factor	Days	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
CD4/CD8 ratio	0	6.35 ± 1.31	a
(Study 1)	0.25	3.10 ± 0.28	b
	1	3.27 ± 0.21	b
	2	3.69 ± 0.39	ab
	3	4.87 ± 0.29	a
	4	4.26 ± 0.43	ab
	5	3.69 ± 0.42	bc
	7	4.43 ± 0.31	ac
CD4/CD8 ratio	0	2.41 ± 0.24	a
(Study 2)	0.25	2.77 ± 0.26	a
	1	2.66 ± 0.26	ab
	2	3.44 ± 0.31	b
	5	5.02 ± 0.42	c
	7	4.45 ± 0.47	c

Note: Different letters (a-c) represent significant differences ($p < 0.05$) between days for each response factor.

In Study 3, significant changes in the CD4/CD8 ratio by day were observed in the Alum-mIgG group and the IO-mIgG group, but not in the mIgG group (see Table 36). The ratio increased non-significantly in the mIgG group. In the Alum-mIgG group, the ratio decreased significantly by day 1 and remained significantly lower compared to day 0.25 on day 7. The ratio also decreased significantly in the IO-mIgG group, but it increased on days 5 and 7, on which it was no longer significantly lower compared to day 0.25. The ratio was significantly higher in the IO-mIgG group compared to the Alum-mIgG group on days 0.25, 5, and 7 (see Figure 11).

Table 36. *Treatment and day interaction effect estimates and main effects p-values of CD4/CD8 ratio, Study 3*

Day	Treatment			Day main effect P-value
	Alum-mIgG ($\bar{x} \pm SE$, n = 4)	**IO-mIgG ($\bar{x} \pm SE$, n = 4)**	**mIgG ($\bar{x} \pm SE$, n = 4)**	
0.25	3.39 ± 0.54	4.49 ± 0.54	2.11 ± 0.54	0.0372
1	2.01 ± 0.43	2.15 ± 0.43	2.26 ± 0.43	0.9225
2	2.57 ± 0.52	3.11 ± 0.52	2.91 ± 0.52	0.7664
3	2.33 ± 0.47	2.84 ± 0.47	3.25 ± 0.47	0.4225
4	1.92 ± 0.33	1.94 ± 0.33	2.96 ± 0.33	0.0806
5	2.08 ± 0.29	3.59 ± 0.29	2.79 ± 0.29	0.0164
7	2.01 ± 0.60	3.72 ± 0.60	2.37 ± 0.60	0.1570
Treatment main effect P-value	0.0200	0.0044	0.5944	

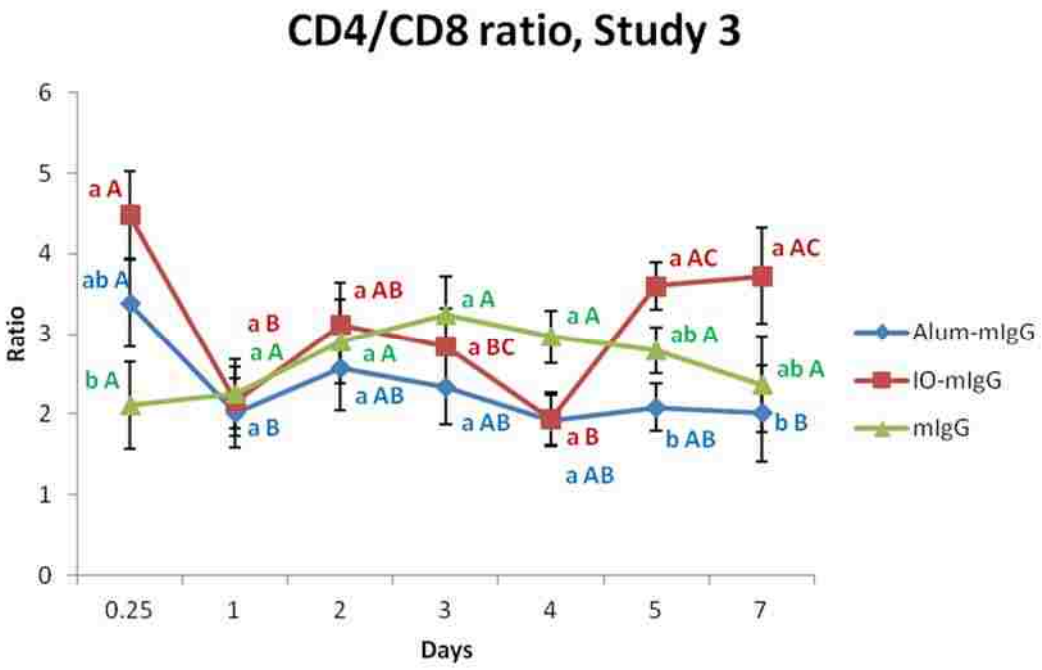

Figure 11. CD4/CD8 ratio by day and treatment group, Study 3.

The graph shows mean differences of the treatment*day effect, not the main effect. The CD4/CD8 ration was significantly higher in the IO-mIgG group compared to the Alum-mIgG group on days 0.25, 5, and 7. Different lowercase letters (a-b) within one time point represent significant differences ($p < 0.05$) between the treatment groups. Different uppercase letters (A-C) represent significant changes between days for each treatment group.

T cell receptors. Time of collection had a significant effect on the γδ T cell+ CD8+ response in Study 2, whereas the interaction between collection time and treatment type was significant in Study 1. In Study 2, the increase in γδ T cell+ CD8+ estimates was significant by day 0.25, and the estimates continued increasing until day 5 (see Table 37). On day 7, the estimates were still significantly higher compared to baseline estimates.

Table 37. *γδ cell+ CD8+ estimates by day, Study 2*

Response Factor	Days	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
γδ T cell+ CD8+	0	0.17 ± 0.03	a
	0.25	0.37 ± 0.05	bc
	1	0.56 ± 0.08	c
	2	0.58 ± 0.17	bd
	5	0.66 ± 0.05	d
	7	0.41 ± 0.05	b

Note: Different letters (a-d) represent significant differences ($p < 0.05$) between days and between treatment groups.

Table 38. *Treatment and day interaction effect estimates and main effects p-values of γδ T cell+ CD8+, Study 1*

Day	Treatment			Day main effect P-value
	IO ($\bar{x} \pm SE$, n = 4)	IO-mIgG ($\bar{x} \pm SE$, n = 4)	IOEF ($\bar{x} \pm SE$, n = 4)	
0	0.20 ± 0.09	0.26 ± 0.09	0.13 ± 0.10	0.6158
0.25	1.88 ± 0.78	1.17 ± 0.78	1.83 ± 0.87	0.7838
1	1.18 ± 0.40	1.34 ± 0.40	0.67 ± 0.44	0.5269
2	1.52 ± 0.34	1.66 ± 0.34	0.59 ± 0.38	0.1202
3	1.45 ± 0.27	0.99 ± 0.27	0.54 ± 0.30	0.1184
4	1.11 ± 0.20	1.31 ± 0.20	0.27 ± 0.22	0.0132
5	1.48 ± 0.26	0.62 ± 0.26	0.23 ± 0.29	0.0215
7	0.77 ± 0.20	0.22 ± 0.20	0.26 ± 0.22	0.1330
Treatment main effect P-value	0.0089	0.0749	0.2241	

γδ T cell+ CD8+ in Study 1 did not change significantly by day in the IO-mIgG group and the IOEF group (see Table 38). The differences between the groups were observed only on days 4 and 5. The response was significantly higher in the IO and IO-mIgG group compared to the IOEF group on day 4, but only the IO group had significantly higher estimates on day 5 compared to the other groups (see Figure 12).

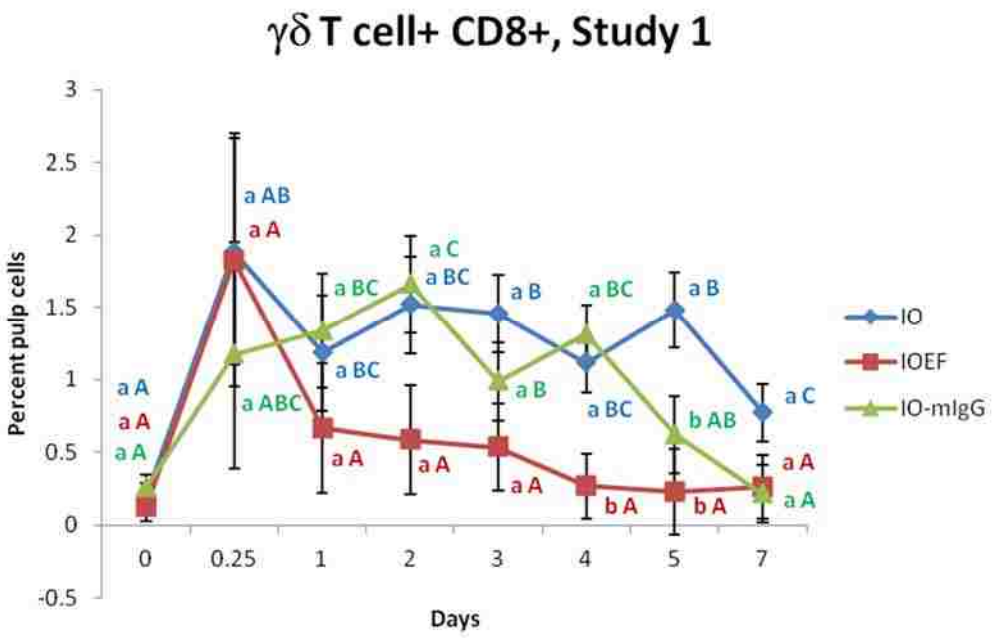

Figure 12. γδ T cell+ CD8+ estimates by day and treatment group, Study 1.

The graph shows mean differences of the treatment*day effect, not the main effect. The initial response was similar in all groups. The longest duration of the response was observed in the IO group. Different lowercase letters (a-b) within one time point represent significant differences ($p < 0.05$) between the treatment groups. Different uppercase letters (A-C) represent significant changes between days for each treatment group.

The treatment main effect for the γδ T cell+ CD8+ response factor in Study 3 was significant for all groups (see Table 39). Significant differences between the groups were observed only on day 0.25 as Alum-mIgG elicited a higher response compared to other treatments. However, no further differences between the groups were observed as the response in other groups started increasing significantly after day 1 (see Figure 13).

Table 39. *Treatment and day interaction effect estimates and main effects p-values of γδ T cell+ CD8+, Study 3*

Day	Treatment			Day main effect P-value
	Alum-mIgG ($\bar{x} \pm SE$, n = 4)	IO-mIgG ($\bar{x} \pm SE$, n = 4)	mIgG ($\bar{x} \pm SE$, n = 4)	
0	0.28 ± 0.08	0.36 ± 0.08	0.26 ± 0.08	0.6990
0.25	0.81 ± 0.10	0.29 ± 0.10	0.48 ± 0.10	0.0172
1	1.43 ± 0.25	0.67 ± 0.25	0.96 ± 0.25	0.1384
2	1.01 ± 0.21	0.82 ± 0.21	0.91 ± 0.21	0.8123
3	1.33 ± 0.20	0.81 ± 0.20	0.74 ± 0.20	0.1172
4	0.72 ± 0.28	0.96 ± 0.28	1.00 ± 0.28	0.7558
5	0.76 ± 0.19	0.82 ± 0.19	1.02 ± 0.19	0.6032
7	0.45 ± 0.22	0.92 ± 0.24	0.66 ± 0.22	0.4052
Treatment main effect P-value	< 0.0001	0.0402	0.0104	

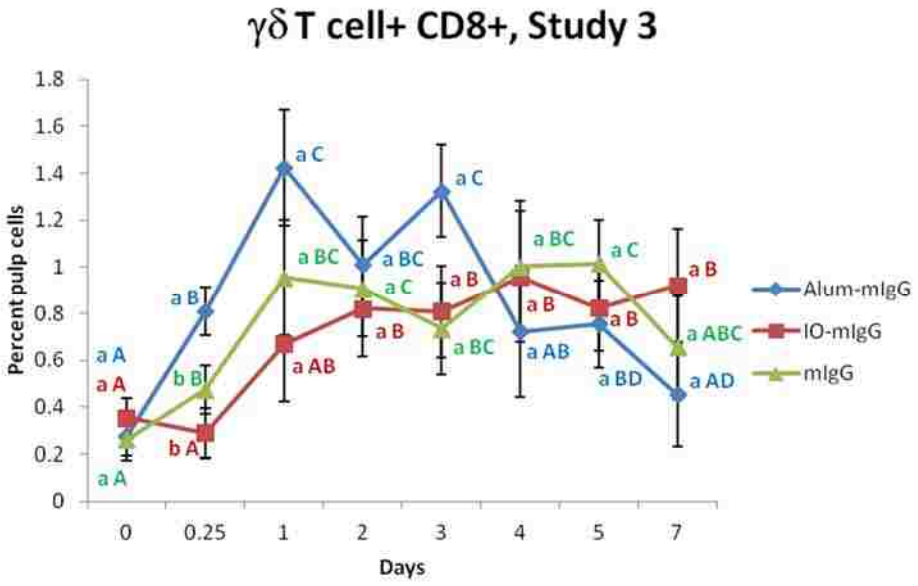

Figure 13. γδ T cell+ CD8+ estimates by day and treatment group, Study 3.

The graph shows mean differences of the treatment*day effect, not the main effect. The highest response was observed in the Alum-mIgG group, and it was significantly higher compared to other groups, but only on day 0.25. No significant differences between the groups were observed from days 1 to 7. Different lowercase letters (a-b) within one time point represent significant differences ($p < 0.05$) between the treatment groups. Different uppercase letters (A-D) represent significant changes between days for each treatment group

The γδ T cell+ CD8- response factors were significantly affected by day and treatment type in Study 1, whereas a significant effect of the interaction between time of collection and treatment was observed for those cells in Study 2. In Study 1, a significant increase in γδ T cell+ CD8- estimates was observed on day 2 compared to baseline (see Table 40). However, the estimates returned to values similar to those observed at baseline by day 3 and remained consistent until day 7. The strongest response was observed in the IO-mIgG group, and it was significantly higher compared to the IOEF group, but not compared to the IO group.

Table 40. *γδ T cell+ CD8- estimates by day and treatment group, Study 1*

Response Factor	Fixed Effect	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
γδ T cell+ CD8-	*Days*		
	0	0.20 ± 0.07	acd
	0.25	0.34 ± 0.04	a
	1	0.46 ± 0.09	ab
	2	0.52 ± 0.05	b
	3	0.33 ± 0.05	a
	4	0.39 ± 0.05	ab
	5	0.22 ± 0.04	c
	7	0.14 ± 0.02	d
	Treatment		
	IO	0.36 ± 0.05	ab
	IOEF	0.21 ± 0.05	a
	IO-mIgG	0.41 ± 0.05	b

Note: Different letters (a-d) represent significant differences ($p < 0.05$) between days and between treatment groups.

In Study 2, γδ T cell+ CD8- estimates did not change significantly by day in the IO-mIgG group, but they did change significantly in other groups (see Table 41). The differences between groups were observed from day 0.25 until day 2. Estimates in the Alum-mIgG group were significantly higher compared to other groups during that period, but the estimates in the Alum

group were also significantly higher on day 2 compared to the IO and IO-mIgG groups (see Figure 14).

The treatment main effect for γδ T cell+ CD8- in Study 3 was significant only for the Alum-mIgG group, and differences between groups were observed on days 0.25, 1, and 3 (see Table 42). The highest response was observed in the Alum-mIgG group, and it was significantly higher compared to the IO-mIgG and mIgG groups (see Figure 15). After day 3, the response in the Alum-mIgG group decreased and no further differences between the groups were observed.

The analysis of γδ T cell+ estimates in Study 3 found that the main effect of treatment was significant only for the Alum-mIgG group, and the differences between the groups were observed on days 0.25, 1, and 3 (see Table 43). The Alum-mIgG showed a significantly higher response compared to other groups, but no significant differences were observed between the IO-mIgG and mIgG groups (see Figure 16).

Table 41. *Treatment and day interaction effect estimates and main effects p-values of γδ T cell+ CD8-, Study 2*

Day	Treatment				
	Alum ($\bar{x} \pm SE$, n = 4)	**Alum-mIgG ($\bar{x} \pm SE$, n = 4)**	**IO ($\bar{x} \pm SE$, n = 4)**	**IO-mIgG ($\bar{x} \pm SE$, n = 4)**	**Day main effect P-value**
0	1.11 ± 0.42	0.52 ± 0.42	0.62 ± 0.42	1.32 ± 0.42	0.5005
0.25	4.78 ± 1.59	13.47 ± 1.59	1.69 ± 1.59	2.05 ± 1.59	0.0007
1	5.35 ± 1.51	15.48 ± 1.51	2.89 ± 1.51	2.71 ± 1.51	0.0002
2	7.96 ± 2.47	14.69 ± 2.47	1.85 ± 2.47	2.07 ± 2.47	0.0095
5	6.37 ± 1.91	4.59 ± 1.91	3.20 ± 1.91	2.74 ± 1.91	0.5525
7	2.73 ± 0.91	3.40 ± 0.91	1.52 ± 0.91	2.27 ± 0.91	0.5418
Treatment main effect P-value	0.0170	< 0.0001	0.0126	0.0583	

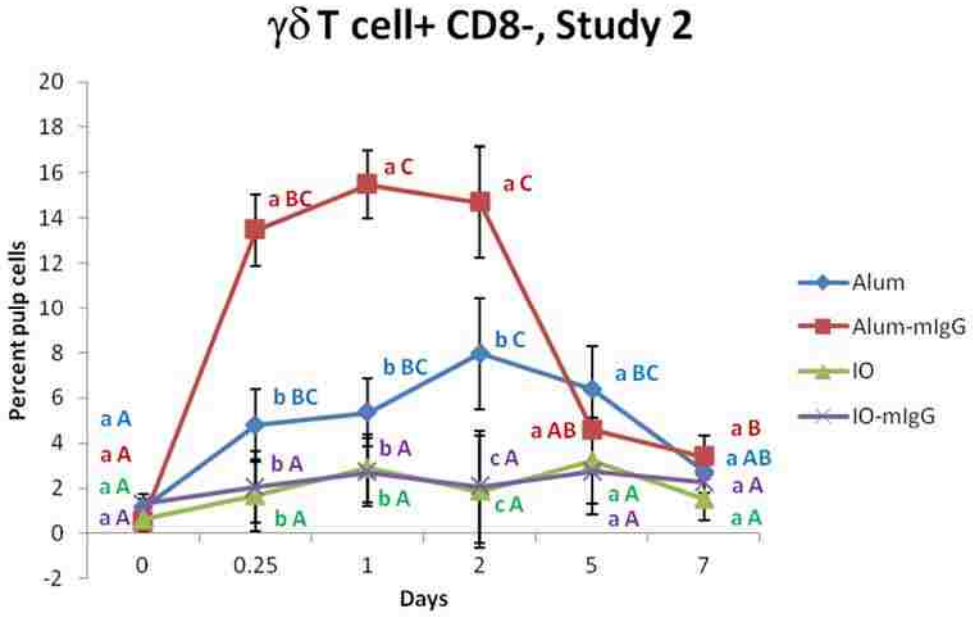

Figure 14. γδ T cell+ CD8- estimates by day and treatment group, Study 2.

The graph shows mean differences of the treatment*day effect, not the main effect. The highest response was observed in the Alum-mIgG group, followed by the Alum group. Different lowercase letters (a-c) within one time point represent significant differences ($p < 0.05$) between the treatment groups. Different uppercase letters (A-C) represent significant changes between days for each treatment group.

Table 42. *Treatment and day interaction effect estimates and main effects p-values of γδ T cell+ CD8-, Study 3*

Day	Treatment			Day main effect P-value
	Alum-mIgG ($\bar{x} \pm SE$, n = 4)	IO-mIgG ($\bar{x} \pm SE$, n = 4)	mIgG ($\bar{x} \pm SE$, n = 4)	
0	0.94 ± 0.41	1.73 ± 0.41	0.93 ± 0.41	0.3271
0.25	8.03 ± 0.92	1.98 ± 0.92	2.81 ± 0.92	0.0024
1	15.89 ± 3.22	2.99 ± 3.22	3.23 ± 3.22	0.0309
2	8.18 ± 2.20	5.27 ± 2.20	3.42 ± 2.20	0.3490
3	8.64 ± 0.93	4.67 ± 0.93	3.44 ± 0.93	0.0081
4	3.78 ± 0.99	4.01 ± 0.99	3.16 ± 0.99	0.8254
5	3.67 ± 0.55	2.83 ± 0.55	3.09 ± 0.55	0.5625
7	2.32 ± 0.91	4.12 ± 1.05	1.77 ± 0.91	0.2766
Treatment main effect P-value	< 0.0001	0.1044	0.1004	

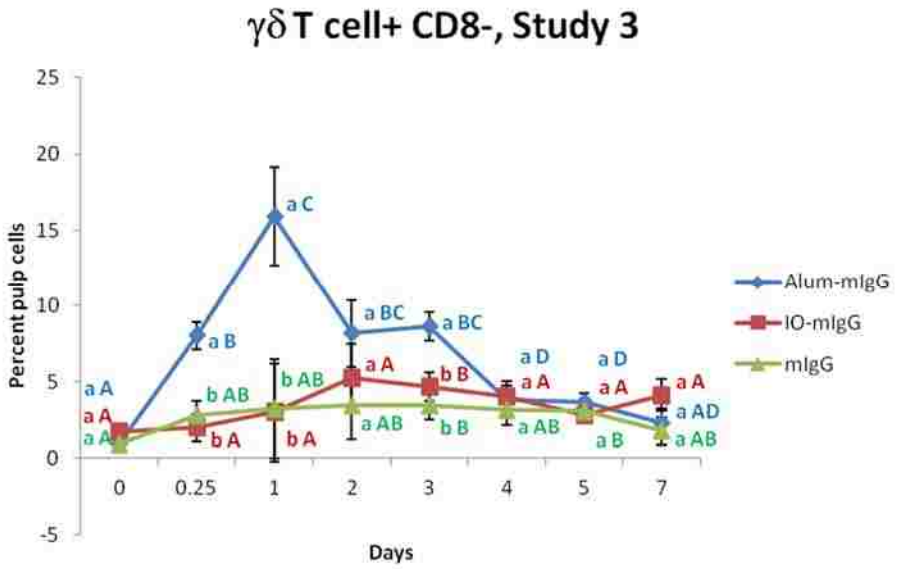

Figure 15. γδ T cell+ CD8- estimates by day and treatment group, Study 3.

The graph shows mean differences of the treatment*day effect, not the main effect. The response in the Alum-mIgG group was significantly higher compared to the other groups on days 0.25 and 1. Different lowercase letters (a-b) within one time point represent significant differences ($p < 0.05$) between the treatment groups. Different uppercase letters (A-D) represent significant changes between days for each treatment group.

Table 43. *Treatment and day interaction effect estimates and main effects p-values of γδ T cell+, Study 3*

Day	Treatment			Day main effect P-value
	Alum-mIgG ($\bar{x} \pm SE$, n = 4)	IO-mIgG ($\bar{x} \pm SE$, n = 4)	mIgG ($\bar{x} \pm SE$, n = 4)	
0	1.96 ± 0.53	2.77 ± 0.53	1.82 ± 0.53	0.4315
0.25	10.08 ± 1.00	3.05 ± 1.00	3.93 ± 1.00	0.0015
1	19.10 ± 3.34	4.87 ± 3.34	4.74 ± 3.34	0.0211
2	9.87 ± 2.41	6.83 ± 2.41	5.03 ± 2.41	0.3955
3	10.52 ± 0.92	6.00 ± 0.92	4.65 ± 0.92	0.0036
4	4.71 ± 1.27	5.38 ± 1.27	4.48 ± 1.27	0.8747
5	4.75 ± 0.55	4.30 ± 0.55	4.52 ± 0.55	0.8476
7	3.47 ± 1.09	6.16 ± 1.25	2.93 ± 1.09	0.1858
Treatment main effect P-value	< 0.0001	0.0839	0.0887	

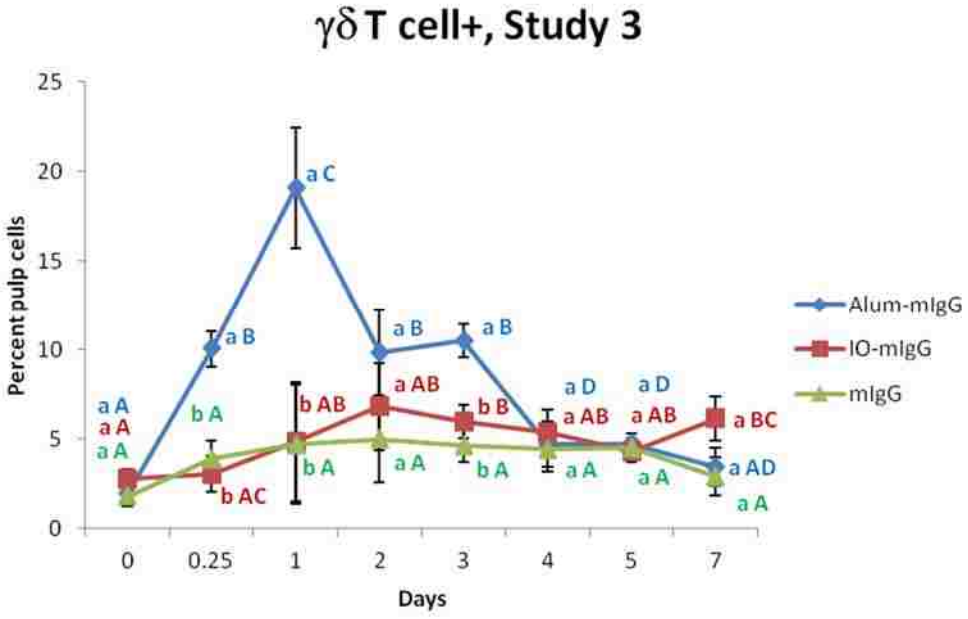

Figure 16. γδ T cell+ estimates by day and treatment group, Study 3.

The graph shows mean differences of the treatment*day effect, not the main effect. The response in the Alum-mIgG group was significantly higher compared to other groups on days 0.25 and 1. Different lowercase letters (a-b) within one time point represent significant differences ($p < 0.05$) between the treatment groups. Different uppercase letters (A-D) represent significant changes between days for each treatment group.

The αβ1 T cell+ estimates increased significantly by day 0.25 in Study 1, and they continued to increase by day 5 (see Table 44). On day 7, the estimates were still elevated compared to baseline estimates, but the difference was not statistically significant. The response in the IOEF group was significantly lower compared to the IO and the IO-mIgG groups.

Table 44. *αβ1 T cell+ estimates by day and treatment group, Study 1*

Response Factor	Fixed Effect	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
αβ1 T cell+	*Days*		
	0	0.33 ± 0.06	a
	0.25	0.89 ± 0.11	b
	1	1.33 ± 0.35	c
	2	2.25 ± 0.48	cd
	3	2.54 ± 0.56	cd
	4	2.64 ± 0.37	d
	5	3.08 ± 0.76	cd
	7	0.76 ± 0.22	ab
	Treatment		
	IO	2.44 ± 0.34	a
	IOEF	0.54 ± 0.38	b
	IO-mIgG	2.21 ± 0.34	a

Note: Different letters (a-d) represent significant differences ($p < 0.05$) between days and between treatment groups.

The αβ1 T cell+ estimates in Study 2 increased significantly by day 0.25 and continued increasing until day 5 (see Table 45). By day 7, the estimates decreased significantly compared to day 5, but they were still significantly elevated compared to baseline estimates. The highest response was observed in the IO group, but the response was not significantly higher compared to the IO-mIgG and Alum-mIgG groups. The lowest response was observed in the Alum group, and it was significantly lower compared to the other three groups.

Table 45. *αβ1 T cell+ estimates by day and treatment group, Study 2*

Response Factor	Fixed Effect	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
αβ1 T cell+	*Days*		
	0	1.32 ± 0.34	a
	0.25	2.87 ± 0.39	b
	1	4.75 ± 0.71	c
	2	4.89 ± 0.70	c
	5	10.37 ± 0.79	d
	7	5.83 ± 0.45	c
	Treatment		
	Alum	3.01 ± 0.50	a
	Alum-mIgG	5.60 ± 0.50	b
	IO	6.35 ± 0.50	b
	IO-mIgG	5.06 ± 0.50	b

Note: Different letters (a-d) represent significant differences ($p < 0.05$) between days and between treatment groups.

The αβ1 T cell+ CD4+ CD8+ estimates in Study 3 increased significantly by day 0.25 from baseline and continued increasing until day 3 (see Table 46). The estimates reduced on days 4, 5, and 7 compared to day 3, but the decrease was not statistically significant, so they remained elevated compared to estimates observed at baseline. The αβ1 T cell+ CD4- CD8- response factor decreased significantly by day 0.25 and returned to baseline on day 1 (see Table 42). However, the estimates reduced significantly again on day 2 and remained significantly lower compared to baseline until day 5. On day 7, the estimates were still lower compared to baseline, but the difference was not statistically significant.

Table 46. *αβ1 T cell+ CD4+ CD8+ and αβ1 T cell+ CD4- CD8- estimates by day, Study 3*

Response Factor	Fixed Effect	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
αβ1 T cell+	*Days*		
CD4+ CD8+	0	0.10 ± 0.03	a
	0.25	0.20 ± 0.03	b
	1	0.26 ± 0.12	ab
	2	0.39 ± 0.11	bd
	3	1.10 ± 0.30	c
	4	0.84 ± 0.27	cd
	5	0.96 ± 0.20	c
	7	0.99 ± 0.20	c
αβ1 T cell+	*Days*		
CD4- CD8-	0	2.19 ± 0.20	a
	0.25	0.84 ± 0.12	b
	1	2.46 ± 0.30	a
	2	0.93 ± 0.09	b
	3	0.90 ± 0.14	b
	4	0.48 ± 0.08	c
	5	0.83 ± 0.12	bc
	7	1.46 ± 0.41	ab

Note: Different letters (a-d) represent significant differences ($p < 0.05$) between days.

The effects of collection time and type of treatment, but not their interaction, were significant only for the αβ1 T cell+ CD4+ CD8- response factor in Study 3 (see Table 47). The estimates increased significantly by day 0.25 and continued increasing until day 3. The estimates remained consistent between days 3 and 5, after which they reduced significantly on day 7. However, they were still significantly higher compared to baseline estimates. The highest response was observed in the Alum-mIgG group, which was significantly higher compared to the mIgG group, but not compared to the IO-mIgG group.

Table 47. *αβ1 T cell+ CD4+ CD8- estimates by day and treatment group, Study 3*

Response Factor	Fixed Effect	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
αβ1 T cell+ CD4+ CD8-	*Days*		
	0	1.22 ± 0.27	a
	0.25	3.17 ± 0.36	b
	1	4.92 ± 0.88	b
	2	7.47 ± 0.74	c
	3	11.15 ± 1.04	d
	4	8.35 ± 1.75	cde
	5	9.21 ± 1.32	cd
	7	4.52 ± 0.82	be
	Treatment		
	Alum-mIgG	7.86 ± 0.62	a
	IO-mIgG	6.14 ± 0.62	ab
	mIgG	4.74 ± 0.62	b

Note: Different letters (a-e) represent significant differences ($p < 0.05$) between days and between treatment groups.

For the αβ1 T cell+ response factor in Study 3, the treatment main effect was significant for all groups, whereas the day main effect was significant on days 0.25, 1, and 3 (see Table 48). The highest response was observed in the Alum-mIgG group, and it was significantly higher compared to the other groups on day 0.25. On day 1, the response in the Alum-mIgG group was higher compared to the IO-mIgG group only. On days 2 and 3, the response in the Alum-mIgG group was higher compared to the mIgG group only. The response in IO-mIgG group was the highest compared to other groups on day seven, but it was significantly higher only compared to the mIgG group, not the Alum-mIgG group (see Figure 17).

Table 48. *Treatment and day interaction effect estimates and main effects p-values of αβ1 T cell+, Study 3*

Day	Treatment			Day main effect P-value
	Alum-mIgG ($\bar{x} \pm SE$, n = 4)	IO-mIgG ($\bar{x} \pm SE$, n = 4)	mIgG ($\bar{x} \pm SE$, n = 4)	
0	3.37 ± 0.59	4.21 ± 0.59	2.86 ± 0.59	0.3031
0.25	7.64 ± 0.72	3.72 ± 0.72	3.87 ± 0.72	0.0061
1	11.54 ± 1.47	5.25 ± 1.47	9.09 ± 1.47	0.0405
2	14.11 ± 1.59	10.79 ± 1.59	8.25 ± 1.59	0.0788
3	23.62 ± 2.74	16.64 ± 2.74	11.11 ± 2.74	0.0310
4	16.27 ± 4.27	11.38 ± 4.27	10.55 ± 4.27	0.6098
5	15.85 ± 2.96	15.33 ± 2.96	9.45 ± 2.96	0.2868
7	8.39 ± 1.67	11.06 ± 1.93	5.06 ± 1.67	0.1174
Treatment main effect P-value	< 0.0001	< 0.0001	0.0032	

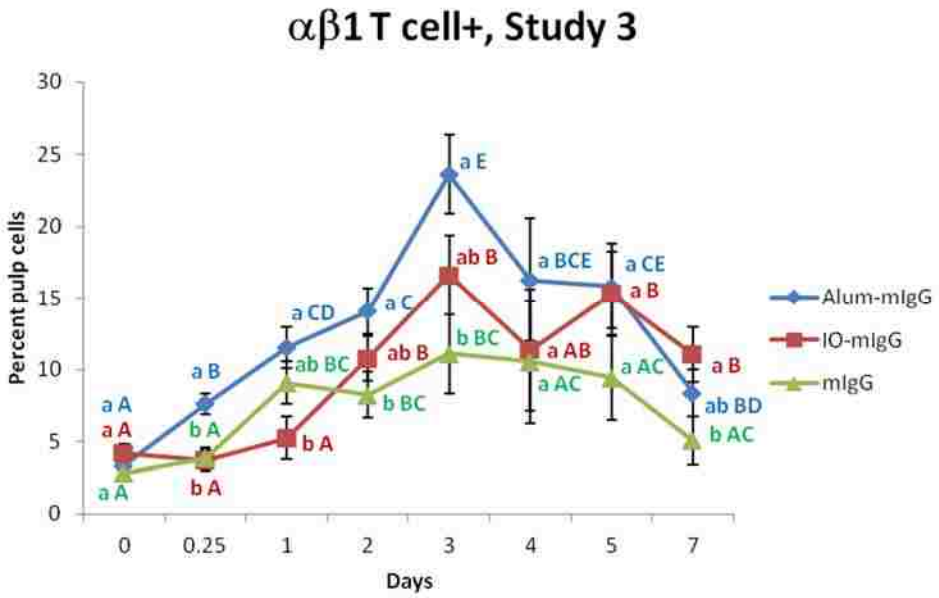

Figure 17. αβ1 T cell+ estimates by day and treatment group, Study 3.

The graph shows mean differences of the treatment*day effect, not the main effect. The highest response was observed in the Alum-mIgG group, which was significantly different compared to one or both groups until day 3. On day 7, the response was significantly higher in the IO-mIgG group than in the mIgG group. Different lowercase letters (a-b) within one time point represent significant differences ($p < 0.05$) between the treatment groups. Different uppercase letters (A-D) represent significant changes between days for each treatment group.

For the αβ1 T cell+ CD4- CD8+ response factor in Study 3, the treatment main effect was significant for all groups, whereas the day main effect was significant on days 0.25, 2, 3, and 7 (see Table 49). The response in the Alum-mIgG group was significantly higher compared to the IO-mIgG group on days 0.25 and 1. On days 2, 3, and 7, the response in the Alum-mIgG group was significantly higher compared to the mIgG group, but not compared to the IO-mIgG group (see Figure 18).

Table 49. *Treatment and day interaction effect estimates and main effects p-values of αβ1 T cell+ CD4- CD8+, Study 3*

Day	Treatment			Day main effect P-value
	Alum-mIgG ($\bar{x} \pm SE$, n = 4)	**IO-mIgG ($\bar{x} \pm SE$, n = 4)**	**mIgG ($\bar{x} \pm SE$, n = 4)**	
0	0.44 ± 0.09	0.47 ± 0.09	0.29 ± 0.09	0.3905
0.25	1.53 ± 0.19	0.57 ± 0.19	1.24 ± 0.19	0.0171
1	2.05 ± 0.32	0.82 ± 0.32	1.35 ± 0.32	0.0620
2	3.60 ± 0.41	2.61 ± 0.41	1.43 ± 0.41	0.0141
3	7.39 ± 0.81	3.75 ± 0.81	2.56 ± 0.81	0.0058
4	5.24 ± 1.02	3.23 ± 1.02	2.40 ± 1.02	0.1843
5	3.96 ± 0.67	2.94 ± 0.67	2.10 ± 0.67	0.2000
7	2.99 ± 0.43	1.59 ± 0.49	1.24 ± 0.43	0.0454
Treatment main effect P-value	< 0.0001	0.0001	0.0009	

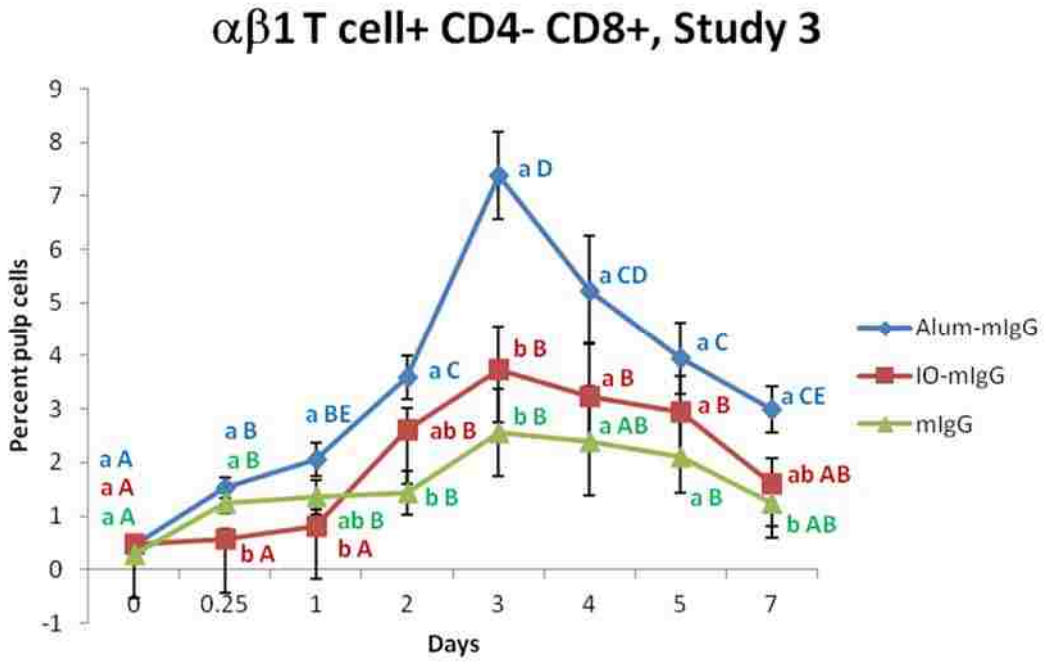

Figure 18. αβ1 T cell+ CD4- CD8+ estimates by day and treatment group, Study 3.

The graph shows mean differences of the treatment*day effect, not the main effect. The highest response was observed in the Alum-mIgG group, which was significantly different compared to one or both groups until day 3. On day 7, the response was significantly higher in the Alum-mIgG group than in the mIgG group. Different lowercase letters (a-b) within one time point represent significant differences ($p < 0.05$) between the treatment groups. Different uppercase letters (A-E) represent significant changes between days for each treatment group.

The αβ2 T cell+ response factor in Study 1 increased significantly by day 0.25 and continued to increase by until day 5 (see Table 50). On day 7, the estimates significantly decreased compared to the previous day and were similar to those observed at baseline. The response was significantly higher in the IO and IO-mIgG groups compared to the IOEF group.

The αβ2 T cell+ response factor in Study 2 increased significantly by day 0.25 and the highest estimates were observed on day 5 (see Table 51). The estimates reduced significantly from day 5 to day 7, but they were still significantly higher on day 7 compared to baseline. The

highest response was observed in the Alum-mIgG group, and it was significantly higher compared to the IO-mIgG group and the Alum group. The response in the IO-mIgG group was higher compared to the Alum group and lower compared to the IO group, but the differences were not statistically significant.

Table 50. *αβ2 T cell+ response by day and treatment group, Study 1*

Response Factor	Fixed Effect	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
αβ2 Tcell+	*Days*		
	0	0.15 ± 0.03	a
	0.25	0.40 ± 0.04	b
	1	0.49 ± 0.13	bc
	2	0.83 ± 0.15	cd
	3	0.76 ± 0.15	cd
	4	0.88 ± 0.13	d
	5	1.02 ± 0.26	cd
	7	0.21 ± 0.07	a
	Treatment		
	IO	0.80 ± 0.13	a
	IOEF	0.25 ± 0.14	b
	IO-mIgG	0.73 ± 0.12	a

Note: Different letters (a-d) represent significant differences ($p < 0.05$) between days and between treatment groups.

Table 51. *αβ2 T cell+ response by day and treatment group, Study 2*

Response Factor	Fixed Effect	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
αβ2 T cell+	*Days*		
	0	0.40 ± 0.10	a
	0.25	0.94 ± 0.17	b
	1	1.65 ± 0.26	c
	2	1.64 ± 0.21	c
	5	3.14 ± 0.21	d
	7	1.75 ± 0.14	c
	Treatment		
	Alum	0.95 ± 0.19	a
	Alum-mIgG	2.08 ± 0.19	b
	IO	1.85 ± 0.19	bc
	IO-mIgG	1.45 ± 0.19	ac

Note: Different letters (a-d) represent significant differences ($p < 0.05$) between days and between treatment groups.

In Study 3, the αβ2 T cell+ CD4- CD8- response factor remained consistent with baseline values until day 1 (see Table 52). On day 2, a significant decrease was observed compared to baseline. The decrease continued until day 4, after which the estimates started increasing, but they were still significantly lower on day 7 compared to baseline. The αβ2 T cell+ CD4+ CD8- response factor estimates increased significantly by day 0.25 and continued increasing until they peaked day 3 (see Table 52). Although the estimates decreased by day 7, they were still significantly higher compared to the estimates observed at baseline.

Table 52. *αβ2 T cell+ CD4- CD8- and αβ2 T cell+ CD4+ CD8- estimates by day, Study 3*

Response Factor	Fixed Effect	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
αβ2 T cell+	*Days*		
CD4- CD8-	0	1.05 ± 0.12	a
	0.25	1.29 ± 0.32	a
	1	0.93 ± 0.12	a
	2	0.53 ± 0.06	bd
	3	0.15 ± 0.02	c
	4	0.12 ± 0.02	c
	5	0.39 ± 0.04	b
	7	0.59 ± 0.06	d
αβ2 T cell+	*Days*		
CD4+ CD8-	0	0.26 ± 0.07	a
	0.25	0.87 ± 0.16	b
	1	1.37 ± 0.24	bd
	2	3.03 ± 0.57	c
	3	3.61 ± 0.42	c
	4	2.58 ± 0.59	cd
	5	2.90 ± 0.43	c
	7	1.36 ± 0.28	bd

Note: Different letters (a-d) represent significant differences ($p < 0.05$) between days for each response factor.

The effect of treatment was the only significant effect for αβ2 T cell+ CD4- CD8+ in Study 3. The strongest response was observed in the Alum-mIgG group, followed by the IO-mIgG group. The weakest response was observed in the mIgG group, and it was significantly lower compared to the response observed in the Alum-mIgG group, but not compared to the response observed in the IO-mIgG group (see Table 53).

Table 53. *αβ2 T cell+ CD4- CD8+ estimates by treatment, Study 3*

Response Factor	Treatment	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
αβ2 T cell+	Alum-mIgG	1.10 ± 0.12	a
CD4- CD8+	IO-mIgG	0.77 ± 0.12	ab
	mIgG	0.58 ± 0.12	b

Note: Different letters (a-b) represent significant differences ($p < 0.05$) between days and between treatment groups.

B cells. The B cell response in Study 1 changed significantly by time of collection and treatment (see Table 54). A significant response compared to baseline was observed on day 0.25, and the estimates peaked on day 4. On day 7, B cell estimates were no longer significantly higher compared to baseline estimates. A significantly higher response was observed in the IO group compared to the IOEF group and in the IO-mIgG group compared to the IOEF group.

Table 54. *B cell estimates by day and treatment, Study 1*

Response Factor	Fixed Effect	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
B cells	*Days*		
	0	0.12 ± 0.02	a
	0.25	0.28 ± 0.05	b
	1	0.90 ± 0.25	c
	2	1.74 ± 0.37	cd
	3	1.59 ± 0.42	cd
	4	1.85 ± 0.34	d
	5	1.61 ± 0.31	cd
	7	0.50 ± 0.18	ab
	Treatment		
	IO	1.42 ± 0.21	a
	IO-mIgG	1.49 ± 0.21	a
	IOEF	0.31 ± 0.24	b

Note: Different letters (a-d) represent significant differences ($p < 0.05$) between days and between treatment groups.

The effect of the interaction between days and treatment on B cells was significant in Study 2. There were no significant changes by day in the Alum treatment group, but estimates changed significantly by day in the IO group, IO-mIgG group, and the Alum-mIgG group (see Table 55). Significant differences between the groups by day were observed only after day 2, when the response in the Alum-mIgG group significantly increased compared to other groups (see Figure 19). On day 5, the B cell estimates in the Alum-mIgG group were still significantly higher compared to other groups, but the IO-mIgG and IO groups also demonstrated a significantly higher response compared to the Alum group. Because the mIgG group did not show a significant response, the B cell estimates in that group were significantly lower compared to other groups on day 7.

Table 55. *Treatment and day interaction effect estimates and main effects p-values of B cells, Study 2*

Day	Treatment				
	Alum ($\bar{x} \pm SE$, n = 4)	**Alum-mIgG ($\bar{x} \pm SE$, n = 4)**	**IO ($\bar{x} \pm SE$, n = 4)**	**IO-mIgG ($\bar{x} \pm SE$, n = 4)**	**Day main effect P-value**
0	0.25 ± 0.22	0.11 ± 0.22	0.21 ± 0.22	0.51 ± 0.22	0.6288
0.25	0.53 ± 0.19	0.90 ± 0.19	0.62 ± 0.19	0.37 ± 0.19	0.2897
1	2.06 ± 1.22	5.72 ± 1.22	2.47 ± 1.22	2.69 ± 1.22	0.1850
2	1.82 ± 1.89	11.44 ± 1.89	2.88 ± 1.89	3.81 ± 1.89	0.0144
5	1.27 ± 1.34	13.31 ± 1.34	5.47 ± 1.34	6.49 ± 1.34	0.0003
7	0.63 ± 0.99	4.30 ± 0.99	4.32 ± 0.99	5.89 ± 0.99	0.0168
Treatment main effect P-value	0.6105	0.0010	0.0398	0.0025	

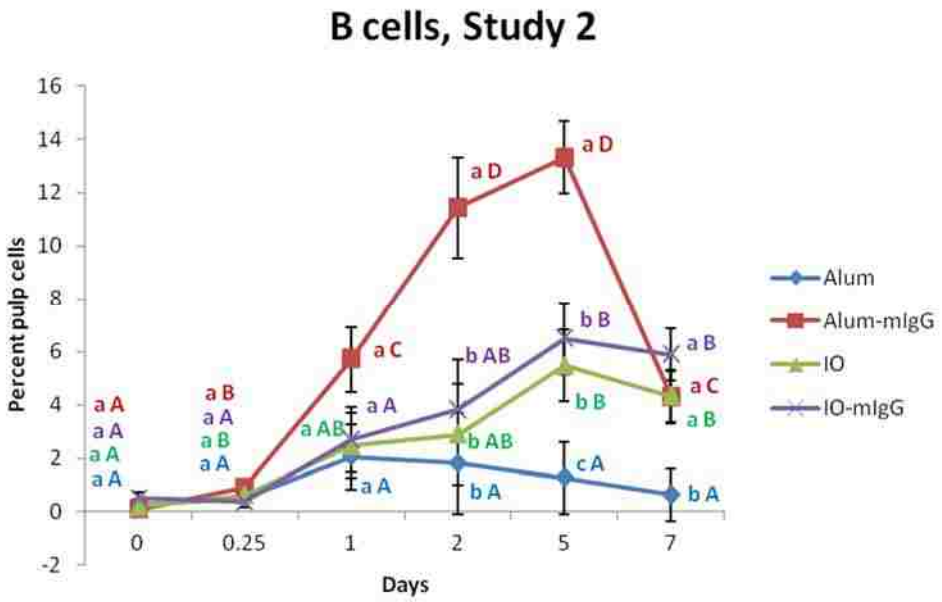

Figure 19. B cell estimates by day and treatment group, Study 2.

The graph shows mean differences of the treatment*day effect, not the main effect. The highest response was observed in the Alum-mIgG group, and differences between the groups were observed from day 1 until day 7. Different lowercase letters (a-c) within one time point represent significant differences ($p < 0.05$) between the treatment groups. Different uppercase letters (A-C) represent significant changes between days for each treatment group.

B cell receptors. The effect of collection time was significant for all B cell receptors in Study 3 (i.e., IgM+, IgM-, IgG+, and IgG-), whereas the type of treatment had a significant effect on IgM+ and IgG-. The interaction between time of collection and type of treatment was significant only for IgM-.

IgG+ increased significantly on day 2 compared to baseline and continued to increase until day 7 (see Table 56). However, because of the large standard errors on some days, the differences compared to baseline were not always significant. For example, the response was higher on day 4 compared to previous days, but it was not significantly higher compared to baseline estimates because of the large standard error.

Table 56. *B cell+ IgG+ estimates by day and treatment, Study 3*

Response Factor	Days	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
B cell+ IgG+	0	0.02 ± 0.01	a
	0.25	0.10 ± 0.07	abc
	1	0.06 ± 0.02	ab
	2	0.09 ± 0.02	b
	3	0.26 ± 0.07	c
	4	0.32 ± 0.18	abc
	5	0.67 ± 0.21	cd
	7	1.14 ± 0.33	e

Note: Different letters (a-e) represent significant differences ($p < 0.05$) between days.

IgG- increased significantly by day 1 and continued to increase until their estimates peaked on day 5. A decrease was observed on day 7 compared to the previous sample collection time, but the estimates were still significantly higher compared to baseline. Alum-mIgG elicited the highest response, which was significantly higher compared to mIgG alone, but not significantly higher compared to IO-mIgG (see Table 57).

IgM+ changed significantly by day and significant differences were observed between treatment groups (see Table 58). A significant response was observed on day 1. The response

peaked on day 5 and remained significantly elevated until day 7. Alum-mIgG elicited the highest response, which was significantly higher compared to mIgG, but not significantly higher compared to IO-mIgG. The response in the IO-mIgG group was also higher compared to the mIgG group.

Table 57. *B cell+ IgG- estimates by day and treatment, Study 3*

Response Factor	Fixed Effect	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
B cell+ IgG-	*Days*		
	0	0.38 ± 0.10	a
	0.25	1.14 ± 0.43	a
	1	3.12 ± 0.75	b
	2	8.46 ± 1.43	c
	3	8.11 ± 1.83	c
	4	7.43 ± 1.97	bc
	5	14.56 ± 3.28	c
	7	7.30 ± 2.30	bc
	Treatment		
	Alum-mIgG	8.97 ± 1.07	a
	IO-mIgG	5.93 ± 1.11	ab
	mIgG	4.04 ± 1.14	b

Note: Different letters (a-d) represent significant differences ($p < 0.05$) between days and treatment groups.

Table 58. *B cell+ IgM+ estimates by day and treatment, Study 3*

Response Factor	Fixed Effect	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
B cell+ IgM+	*Days*		
	0	0.37 ± 0.09	a
	0.25	0.99 ± 0.40	a
	1	2.63 ± 0.65	b
	2	6.67 ± 1.34	c
	3	8.52 ± 1.86	cd
	4	8.95 ± 2.10	cd
	5	13.61 ± 2.55	d
	7	6.66 ± 1.91	bc
	Treatment		
	Alum-mIgG	8.07 ± 0.97	a
	IO-mIgG	6.63 ± 0.99	a
	mIgG	3.45 ± 0.97	b

Note: Different letters (a-d) represent significant differences ($p < 0.05$) between days and treatment groups.

IgM- showed a significant effect of the interaction between type of treatment and time of collection. The treatment main effect was significant only for the Alum-mIgG group, and significant changes between the groups were observed on days 0.25 and 2 (see Table 59). IgM- in the IO-mIgG group initially declined, and a significant response was observed only on day 3 compared to baseline estimates. Alum-mIgG elicited a significant IgM- response by day 0.25, and IgM- estimates remained elevated compared to baseline on day 7. One significant difference between the groups was observed on day 0.25 because Alum-mIgG elicited a significantly higher response compared to IO-mIgG and mIgG treatments. The other significant difference between groups was observed on day 2 as both Alum and Alum-mIgG groups had significantly higher IgM- cell estimates compared to the IO-mIgG group (see Figure 20).

Table 59. *Treatment and day interaction effect estimates and main effects p-values of IgM-, Study 3*

Day	Treatment			Day main effect P-value
	Alum-mIgG ($\bar{x} \pm SE$, n = 4)	IO-mIgG ($\bar{x} \pm SE$, n = 4)	mIgG ($\bar{x} \pm SE$, n = 4)	
0	0.05 ± 0.04	0.11 ± 0.04	0.07 ± 0.04	0.6269
0.25	0.43 ± 0.06	0.03 ± 0.06	0.15 ± 0.06	0.0034
1	1.04 ± 0.33	0.36 ± 0.33	0.76 ± 0.33	0.3805
2	3.33 ± 0.62	1.30 ± 0.62	1.55 ± 0.62	0.0836
3	2.66 ± 0.58	1.38 ± 0.58	1.47 ± 0.58	0.2669
4	1.12 ± 0.57	0.94 ± 0.57	1.72 ± 0.57	0.6150
5	2.79 ± 0.69	1.18 ± 0.69	1.46 ± 0.69	0.2631
7	1.75 ± 0.70	1.36 ± 0.80	0.77 ± 0.70	0.6313
Treatment main effect P-value	<.0001	0.2436	0.1111	

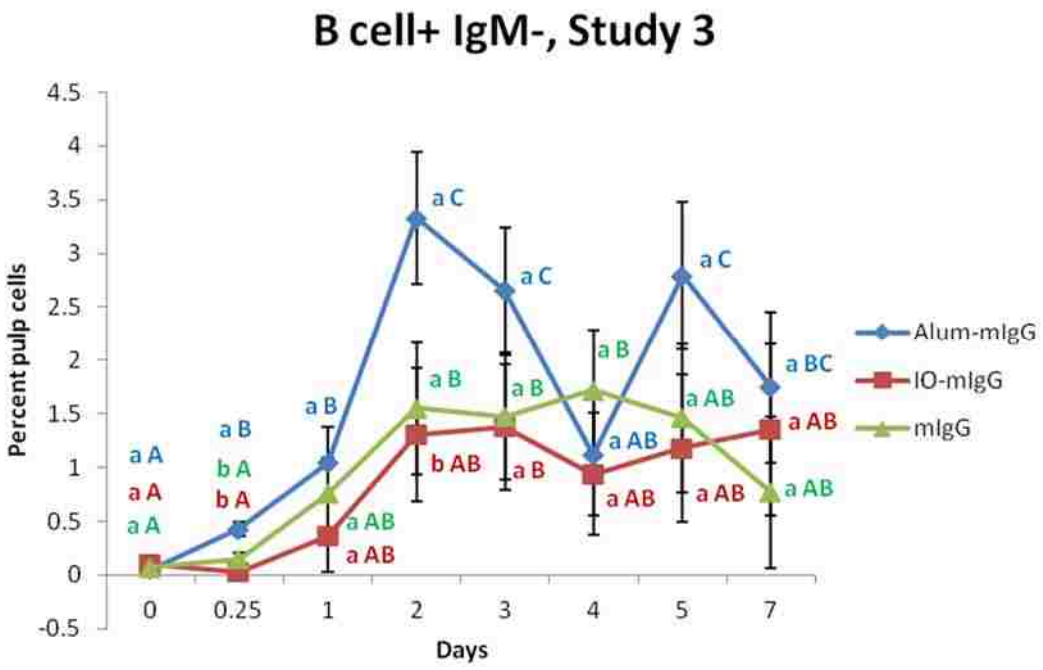

Figure 20. B cell+ IgM- estimates by day and treatment group, Study 3.

The graph shows mean differences of the treatment*day effect, not the main effect. The highest response was observed in the Alum-mIgG group, and it was significantly higher than both groups on day 0.25 and significantly higher than IO-mIgG on day 2. Different lowercase letters (a-c) within one time point represent significant differences ($p < 0.05$) between the treatment groups. Different uppercase letters (A-C) represent significant changes between days for each treatment group.

Summary. A total of 11 pulp cell responses in Study 1 changed significantly by day, whereas the differences between groups were observed in the following nine response factors: lymphocytes, CD45+, CD4- CD8+, CD4+ CD8+, CD4+ CD8-, γδ T cell+ CD8-, αβ2 T cell+, αβ1 T cell+, and MHCII+ B cell+ response factors. IO-mIgG elicited a significantly higher response compared to IOEF for all response factors, whereas IO elicited a significantly higher response compared to IOEF for most response factors, with the exception of CD4+ CD8+ and γδ

T cell+ CD8- responses. However, no significant differences were observed in the responses observed in the IO-mIgG and IO groups.

The interaction between time of collection and treatment in Study 1 was significant for live cells and γδ T cell+ CD8+ factors. However, the only significant difference in live cells between the groups was observed before the injections as the IO group had a significantly higher live cell count compared to the other groups. The initial response of the γδ T cell+ CD8+ factor was significant by day 0.25 and similar in all groups, but the response in the IO and IO-mIgG groups was significantly higher on day 4 compared to the IOEF group. The response remained elevated longest in the IO group, which had significantly higher live cell estimates compared to the other two groups on day 5.

In Study 2, a total of 11 response factors changed significantly by day, whereas the effect of treatment type was significant for the following three response factors: CD4+ CD8-, αβ2 T cell+, and αβ1 T cell+ response factors. Alum-mIgG and IO elicited significantly higher responses of all factors compared to the Alum treatment. IO-mIgG elicited a significantly higher response compared to Alum only for CD4+ CD8- and αβ1 T cell+ response factors.

The interaction between time of collection and treatment in Study 2 was significant for the following three response factors: CD45+, γδ T cell+ CD8-, and B cell response factors. A significantly higher response of all three factors was observed in the Alum-mIgG group compared to other groups. No significant differences were observed between the IO and IO-mIgG groups for those factors.

In Study 3, a total of seven response factors changed significantly only by day. The fixed effect of treatment was significant for the following six response factors: CD4- CD8+, MHCII+ macrophages, αβ1 T cell+ CD4+ CD8-, αβ2 T cell+ CD4- CD8+, B cell+ IgG-, and B cell+

IgM+ factors. Alum-mIgG and IO-mIgG elicited a significantly higher response of MHCII+ macrophages and B cell+ IgM+ factors compared to mIgG. Alum-mIgG elicited a significantly higher response of αβ1 T cell+ CD4+ CD8-, αβ2 T cell+ CD4- CD8+, and B cell+ IgG- factors compared to mIgG, but not compared to IO-mIgG. The CD4+ CD8- response was significantly higher in the Alum-mIgG group compared to other groups.

The interaction between time of collection and treatment in the 2014 studies was observed for the following ten response factors: CD45+, heterophils, γδ T cell+, γδ T cell+ CD4- CD8+, γδ T cell+ CD4- CD8-, αβ1 T cell+, αβ1 T cell+ CD4- CD8+, CD3+, CD4/CD8 ratio, and B cell+ IgM- factors. The highest response for all immune cells was observed in the Alum-mIgG group, with the exception of heterophils as their estimates were significantly higher in the IO-mIgG group compared to the other groups.

The difference of least squares means and the simple effect comparisons results for pulp cell responses observed in all three feather injection studies are included in Appendix F.

Discussion

The purpose of the feather injection studies was to monitor and assess local leukocytes infiltration into the pulp upon the administration of antigen-conjugated IONPs and IONPs using growing feather as a test site. It was hypothesized that the nature and extent of the leukocyte infiltration would depend on the treatment used. A total of 69 response factors in all three studies varied significantly by day only. However, the fixed effect of treatment was significant for a total of 24 response factors, whereas the interaction between treatment and type of collection was a significant fixed effect for a total of 23 response factors in all three studies. Therefore, a total of 24 response factors were dependant on the type of treatment used.

In Study 1, it was hypothesized that the immune reaction in the IOEF group would be significantly lower compared to the IO and IO-mIgG groups. Differences between treatment groups were observed for nine response factors in Study 1, and a significantly higher immune reaction was observed in the IO-mIgG group compared to the IOEF group. The immune reaction in the IO group was significantly higher compared to the IOEF group for seven response factors and similar to the IOEF group for CD4+ CD8+ and γδ T cell+ CD8- responses. The interaction between treatment and time of collection was significant for live cells and γδ T cell+ CD8+. The differences between groups for live cells were observed only prior to the injections on day 0, but the γδ T cell+ CD8+ factor was significantly lower in the IOEF group on day 4 compared to the other groups. Therefore, the hypothesis was confirmed as the IOEF group had significantly lower responses compared to other groups, so it was not included as one of the treatments in the 2013 IO vs. Alum and 2014 studies.

In Study 2, it was hypothesized that the leukocyte infiltration would be significantly lower in the IO group compared to the other groups. Although the Alum-mIgG elicited significantly higher responses of for CD45+, γδ T cell+ CD8-, and B cell factors compared to the IO group, no significant differences were observed between the IO and IO-mIgG treatment groups for those response factors. The Alum treatment elicited a significantly weaker CD45+ and B cell response compared to IO and IO-mIgG , but the γδ T cell+ CD8- response to Alum was significantly higher compared to the IO and IO-mIgG treatments. The CD4+ CD8-, αβ2 T cell+, and αβ1 T cell+ response factors showed a significant fixed effect of treatment, but no significant differences were observed between the IO and Alum-mIgG or between IO and IO-mIgG. These findings suggest that the immune responses were affected by the IONPs, not by the conjugated antigen.

The complete blood cell count test found that lymphocyte concentration in Study 2 was significantly lower in the IO and IO-mIgG groups compared to the Alum Alum-mIgG group. However, only the Alum-mIgG group elicited a stronger T cell and B cell responses compared to the IO and IO-mIgG groups, whereas Alum elicited a weaker adaptive immunity response. Therefore, it is possible the high lymphocyte concentration in the blood is not a good predictor of adaptive immune cell activity at the injection site.

In Study 3, the mIgG concentrations were matched to observe how the immune responses would behave when both Alum and IONP treatments have the same antigen concentration. Consistent with the findings from Study 2, the Alum-mIgG group in Study 3 elicited significantly higher responses compared to the other groups, whereas no significant differences were observed between IO and IO-mIgG groups. Although the mIgG concentrations were matched in Study 3, whereas the IO-mIgG groups in the 2013 studies had more than 20-fold lower mIgG concentration compared to the other groups, matching the concentrations did not appear to change the response patterns. For example, the IO-mIgG group's CD45+ response in Study 2 achieved a maximum mean value of 44.93 ± 5.15% pulp cells. In Study 3, the maximum mean value of the CD45+ response in the IO-mIgG was 43.15 ± 7.24% pulp cells.

The differences between the treatment groups were consistent in all studies. For most response factors that showed a significant fixed effect of treatment or interaction between treatment and sample collection time, Alum-mIgG elicited a stronger response compared to IO-mIgG and other groups. The exception was heterophils as their concentration increased significantly more in the IO-mIgG group compared to the Alum-mIgG group in the complete blood cell count of Study 2. In Study 3, the percentage of heterophils in all live pulp cells in the IO-mIgG group was also significantly higher compared to the Alum-mIgG and Alum groups.

These results suggest that matching the mIgG concentration in all treatment groups did not contribute to differences in immune responses between groups. However, it is possible that increasing the IO concentration from 0.2 mg/ml in the 2013 study to 1 mg/ml in Study 3 enhanced innate response to IONPs because heterophil infiltration into the growing feather pulp occurred only in Study 3.

The role of heterophils is to eliminate foreign substances by phagocytosis, so the IO-mIgG treatment did elicit a high innate immune response. However, it appears that the heterophils eliminated the IONPs from the pulp as their estimates peaked on days 0.25 and 1, after which they significantly reduced by day 2 and were similar to the estimates observed at baseline. Although IO-mIgG did show a significant increase of γδ and αβ1 T cells in the pulp 24-48 hours after the injection, the response was significantly higher in the Alum-mIgG group. The high estimates of heterophils observed in the IO-mIgG group could mean that the IONPs are taken up by the heterophils before they could elicit a strong adaptive immune response.

Based on the complete blood cell count, thrombocyte concentration in Study 3 was significantly higher in the Alum-mIgG group compared to the IO-mIgG group, but not compared to the mIgG group. Thrombocytes in chickens perform the same functions as platelets in mammals. The role of platelets in early innate responses has been well-established, but they also establish communication between innate and adaptive immunity through CD154 expression to signal early alterations to B cells and through inducing dendritic cell (DC) maturation to activate naïve T cells (Elzey et al., 2003). The role of thrombocytes in facilitating the adaptive immune response could explain why Alum-mIgG elicited stronger responses of T cells and B cells compared to the IO-mIgG and mIgG groups.

The IONPs demonstrated the ability to elicit an innate immune response, but failed to elicit a strong adaptive response. The conjugation of mIgG was important only for Alum because the Alum-mIgG treatment was always associated with higher immune responses compared to Alum and mIgG alone. According to Walls (1977), the injection of Alum without specific antigenic stimulation attracts eosinophils to the injection site, which leads to the depletion of T cells because they are not needed when antigen recognition is not required. Although eosinophils in the growing feather pulp were not measured in these studies, the T cells in the Alum group in Study 2 reduced after day 1, whereas they continued increasing in other groups until day 5. These findings are consistent with the idea that Alum alone depletes T cells from the injection site.

The lack of Alum also explains why mIgG alone did not elicit strong immune cell responses. Without an adjuvant, the antigen is simply taken up by lymph node DCs, so the antigen does not remain long enough in the system to elicit an adaptive immune response. It was previously thought that aluminum-based adjuvants deposited at the injection site and slowly released the antigens, so the prolonged exposure to the antigen was considered to activate antigen-specific lymphocytes (Coffman et al., 2010; McKee, Munks, & Marrack, 2007). However, it was found that aluminum-based adjuvants produce immunopotentiation even when the antigen is rapidly released (Iyer, HogenEsch, & Hem, 2003). It is currently thought that the injection of an aluminum-containing adjuvant and antigen causes the inflammatory monocytes to take up the antigen instead of the lymph node DCs (Kool et al., 2008). The monocytes then become inflammatory DCs and induce a Th2 response.

At the moment there is no definite mechanism that would explain why antigen conjugation does not improve the immunopotentiation activities of IONPs as it does for Alum.

One possible explanation is that IONPs are usually taken up quickly by macrophages and eliminated via the reticuloendothelial system, regardless of the antigen conjugation (Chao et al., 2012). In order to replicate the mechanism of Alum-mIgG uptake by immune cells and perhaps achieve a similar or higher response, surface modifications might be required to target monocytes so that they can take up the NPs and induce a Th2 response (Lartigue et al, 2012). Pre-treatment of IONPs with Intralipid proved to decrease initial uptake of particles by the reticuloendothelial system and increase blood monocyte labeling in rats (Liu et al., 2013).

The current results suggest that the leukocyte infiltration in the pulp of the growing feathers in LBL chickens is significantly higher in the Alum-mIgG group compared to other treatments used in these experiments. However, the properties of NPs also have to be considered to determine why the responses elicited by IONPs were lower compared to Alum-mIgG. Previous studies found that size and coating are some of the factors that determine how NPs behave *in vivo*. This study used 10 nm particles, which is consistent with previous findings that reported reduced clearance of NPs via the reticuloendothelial system for particles between 10 and 100 nm (Longmire et al., 2008). However, IONPs with different coating should be further investigated to determine which coatings can prevent phagocytosis elicit an adaptive immune response.

Although further research will be required to develop IONPs capable of eliciting immune responses comparable to aluminum-based adjuvants, the findings of these studies have two important implications. The first implication stems from the fact that IONPs proved to elicit a significantly higher heterophil response compared to other treatments. Because the heterophils in avian species are similar to neutrophils in humans, it is possible to expect that IONPs can increase neutrophil count in deficient. The lack of neutrophils is a condition referred to as

neutropenia. It is a common side-effect of medication, usually chemotherapy, and reduced neutrophil concentration can lead to severe risks of infections (Sullivan & Moreno, 2015). If IONPs can elicit neutrophil responses to protect patients with high infection risks, they will have important implications in clinical practice. Therefore, the potential of IONPs in practice as protective agents for neutropenia patients should be further investigated.

The second implication is that these studies establish the chicken model as a minimally invasive alternative to existing models, as well as a reliable model that allows periodic monitoring of responses to substances injected in growing feathers. The pattern of immune responses observed in the LBL chickens' growing feather pulp is consistent with the activation of different immune cells observed in previous studies. Chen, Cihak, Lösch, and Cooper (1988) reported that γδ T cells are activated first, whereas the αβ1 T cells increase after the frequency of γδ T cells diminishes. In Study 3, the γδ T cells peaked on day 1 in the Alum-mIgG group, after which they continued declining, whereas the αβ1 T cells peaked on day 3.

The TCR3 subpopulation in avian species was distinguished from TCR2 based on differences in peptide chains of their heterodimers (Chen et al., 1989). However, Lahti et al. (1991) found that both TCR2 and TCR3 are subsets of αβ cells and that their β chains are encoded by the same diversity, joining, and constant gene region segments in the TCRβ locus. In Study 3, the αβ2 T cell+ CD4- CD8- response decreased significantly on day 1 compared to baseline, whereas the αβ2 T cell+ CD4+ CD8- response peaked on day 3 as the γδ T cells started decreasing after day 1. These changes by day are consistent with the fact that TCR1 cells are generated first, whereas the TCR2 and TCR3 cells are generated once the frequency of TCR1 cells starts decreasing (Chen et al., 1988).

In Study 3, the CD4/CD8 ratio did not change significantly by day in the mIgG group, but it did decrease significantly in the IO-mIgG and Alum-mIgG groups. In humans, a low CD4/CD8 ratio indicates ongoing immune activation, so it is usually observed in HIV patients because of the permanently low CD4 count and permanently high CD8 count (Serrano-Villar et al., 2013). A low CD4/CD8 ratio in patients after resuscitation was also established as a poor prognostic factor (Syrjälä, Surcel, & Ilonen, 1991). In chickens, a 1:1 CD4/CD8 ratio was observed at the onset of Smyth line vitiligo, but the progression of the disorder was associated with an increase in CD8+ cells and the reduction of the ratio to 0.3 (Erf et al., 1995). However, the range of the CD4/CD8 ratio in Study 3 was between 1.91 ± 0.33 and 3.39 ± 0.54 in the Alum-mIgG group and between 1.94 ± 0.33 and 4.49 ± 0.54 in the IO-mIgG group. The minimum ratio was observed on day 4 in both groups, after which it started increasing. The observed decrease was consistent with the fact that immune activation is characterized by a low CD4/CD8 ratio, but the decrease was not permanent.

The first mature naïve B cells express IgM in response to an infection, whereas the IgG antibody is produced only after the B cells receive information about the antigen through interaction with T cells (Racine et al., 2011). The B cell receptor responses observed in Study 3 are consistent with this mechanism as the increase of IgM+ and IgM- cells was observed by day 0.25. In contrast, IgG+ and IgG- cells started increasing significantly after days 2 and 1, respectively.

The immune cell infiltration in the pulp of the growing feathers observed in these studies suggests that the interactions between injected substances and the immune system can be observed *in vivo* over time in one subject using the chicken model. The results are considered reliable as the patterns of responses are consistent with the sequences of immune cell activation

observed in previous studies. No other current animal model can offer a non-invasive method to achieve the same objective.

It is important to note that these experiments did not use a control group with PBS. This is not considered a limitation because previous studies showed that PBS has no significant effects on immune cell activities in chickens (Bowen, Erf, Chapman, & Wideman, 2007; Vandaveer, Erf, & Durdik, 2001). The staining results from the pilot study conducted prior to these experiments are shown in Figure 21, and they also support that PBS does not elicit immune responses.

Figure 21. PBS vs. IO-mIgG immune cell induction 6 hours after feather pulp injections.

Top: No immune cell induction was observed in the PBS group 6 hours after the injection into the feather pulp.

Bottom: The IO-mIgG treatment was associated with an infiltration of immune cells into the pulp 6 hours after the injections.

Even though tissue, organs, and plasma were collected during the experiments, the analysis of location trafficking and immune cell alterations is still an ongoing study. Prussian blue staining revealed that IONPs are present in the feather pulp (see Figure 22). This explains the innate immune response activities, but it is not possible to determine which cells took up the IONPs at the moment. Based on findings of previous studies, it is expected that macrophages took up the IONPs because they can easily take them up if polymer coatings are not used (Orlando et al., 2015). However, the high heterophil response observed in IO treatment groups also suggests that heterophils could have taken up IO. The ongoing study of cytokine gene expression based on the plasma samples will determine whether the cytokine expression was altered.

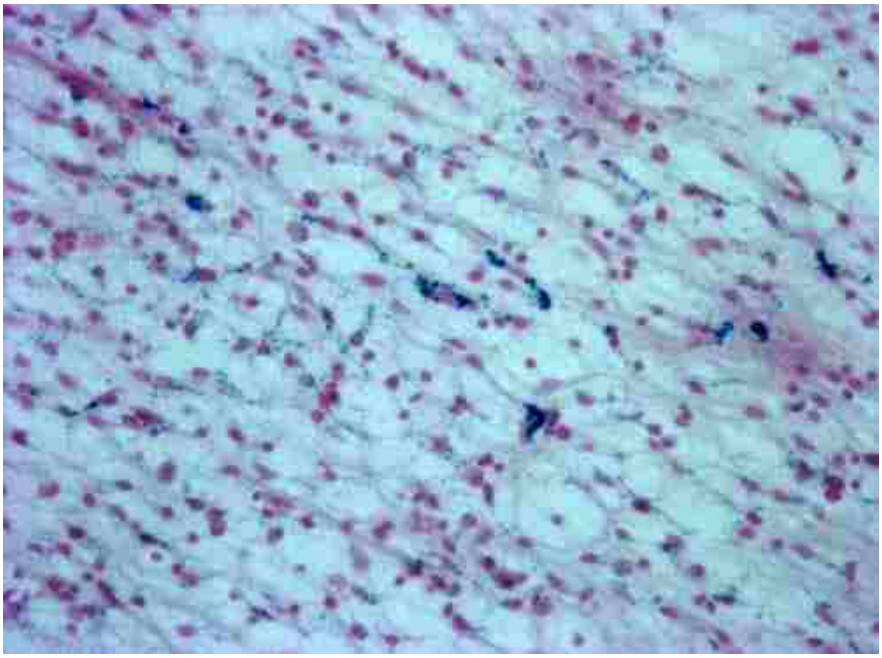

Figure 22. Prussian blue staining of IONPs 6 hours after feather pulp injections.

The blue stains show the position of IONPs in the feather pulp 6 hours after the injections. It is expected that innate immune cells took up the IONPs because the treatments did not enhance the adaptive immunity response, but ongoing studies will determine which innate immune cells took up the IONPs.

The findings of this study show that IONPs can be used to elicit immune responses, but further research is required to develop particle pre-treatment strategies for targeting monocytes and reducing the risk of clearance by macrophages. Using that approach should replicate the mechanism of immune responses aluminum-based adjuvants, thus improving the strength and longevity of the immune response. The LBL chickens used in the experiments support the effectiveness of the chicken model for observing the *in vivo* interactions between administered substances and physiological processes. The model has the advantage of allowing for minimally invasive measuring of physiological changes over time in one subject, which makes it unique compared to other contemporary animal models.

CHAPTER 4—THE EFFECTS OF INTRAMUSCULAR IONP-mIgG INJECTIONS ON PRIMARY AND SECONDARY IMMUNE RESPONSES

Abstract

The ability of nanoparticles to stimulate both humoral and cell-mediated immune responses makes them a promising vaccine adjuvant. However, current animal models are highly invasive and do not allow researchers to monitor trends in immune responses over time in one subject. The purpose of these studies is to test the utility of iron oxide nanoparticles (IONPs) as vaccine adjuvants by monitoring the primary and memory immune responses to intramuscular injections. In the primary and memory response investigations of Study A, a sample of 18 and 20 chickens was used, respectively. In the Study A primary response, the chickens were divided into three groups, and the following treatments were administered: Alum-mIgG (15%-5 mg/ml), IO-mIgG (1 mg/ml-0.26 mg/ml), and mIgG (5 mg/ml). In the Study A memory response, the chickens were divided into three treatment groups and a control group, and the following treatments were administered: Alum-mIgG (15%-5 mg/ml), IO-mIgG (1 mg/ml-0.26 mg/ml), mIgG (5 mg/ml), and PBS (1X). In the primary and memory response investigations of Study B, samples of 12 and 16 chickens were used, respectively. In the Study B primary response, the chickens were divided into three groups, and the following treatments were administered: Alum-mIgG (15%-0.26 mg/ml), IO-mIgG (1 mg/ml-0.26 mg/ml), and mIgG (0.26 mg/ml). In the Study B memory response study, the chickens were divided into three treatment groups and one control group, and the following treatments were administered: Alum-mIgG (15%-0.26 mg/ml), IO-mIgG (1 mg/ml-0.26 mg/ml), mIgG (0.26 mg/ml), and PBS (1X). The treatment preparations were injected in the breast muscle in the amount of 0.1 ml/chicken. In Study A, the mIgG concentration in the IONP group was 20-fold lower compared to the other groups, but IO-mIgG

elicited significantly higher responses compared to the other groups. IONPs elicited significantly higher MHCII+ B cell+, γδ T cell+ CD8+, and γδ T cell+ CD8- responses compared to the other groups. However, when the mIgG concentration for all treatment groups was matched in Study B, the IO-mIgG group elicited similar or significantly lower responses compared to the other groups. These findings suggest that growing feathers in chickens can be used to monitor immune responses to treatments periodically in one subject, even when the agents are not administered directly into the feather pulp. The clinical utility of IONPs as vaccine adjuvants is promising considering these findings, but further research is necessary to improve the knowledge of IONP biointeractions, toxicity levels, and the optimal concentration of conjugated antigen in the preparation.

Keywords: aluminum, IONP, memory response, vaccine adjuvant, primary response

Introduction

Most adjuvants improve vaccine efficiency and provide protection from pathogens by enhancing the humoral immune response (Plotkin, 2010). The humoral response involves B cells, which are responsible for producing memory cells that provide the body with future immunity against specific antigens. The antibody responses are usually sufficient to develop long-term immunity to most antigens and require no additional boosting (Amanna, Carlson, & Slifka, 2007). However, a major challenge for most adjuvants is eliciting a cell-mediated response that results in building effective and long-term T cell immunity (Coffman, Sher, & Seder, 2010). The ability to elicit protective T cell responses is critical for the development of vaccines for disease that could be prevented or regulated by T cells.

NPs are promising adjuvants, but their ability to stimulate both humoral and cell-mediated immune responses must be investigated using suitable *in vivo* test systems. The mouse model is not considered suitable for investigating immune responses because they usually have low transferability due to various differences compared to human immunity, such as differences in cellular expressions of pattern recognition receptors (Campbell et al., 2009) The use of non-human primates for researching adjuvants is recommended because they are more similar to humans compared to rats (Coffman et al., 2010). However, animal availability and high maintenance costs are often significant barriers to conducting studies using non-human primates.

The purpose of the intramuscular injection studies was to demonstrate the feasibility of the chicken model as an *in vivo* test system for monitoring humoral and cell-mediated immune responses. Compared to the mouse model, the chicken model is less invasive and offers better transferability of results due to the similarities between avian and mammalian immune systems. Compared to the non-human primate model, the chicken model is cost-effective and less

invasive. The chicken model is also the only model that allows for periodical monitoring of adaptive immune responses to injections when GF is used as a test site. These studies will also monitor differences in humoral and cell-mediated responses to different treatment preparations to determine whether NPs could address the identified limitations of conventional adjuvants. It is hypothesized that the antigen-conjugated NPs will stimulate a higher primary and memory response compared to antigen and antigen-conjugated Alum treatments.

Study A: In Vivo Monitoring of Primary and Memory Immune Responses to Intramuscular Injections of IONPs in Chickens, 2013

In the primary response study, time of collection was defined as a class of eight levels with the following values: 0, 0.25, 1, 2, 3, 4, 5, and 7 days. In the memory response study, time of collection was defined as a class of seven levels with the following values: 0, 0.25, 1, 2, 3, 4, and 5 days. Type of treatment was a class with three levels in both primary and memory studies, and it contained the following values: Alum-mIgG, IO-mIgG, and mIgG.

The PBS control group was used in the memory study. The observations in that group were analyzed using time of collection as the only fixed effect. Time of collection was defined as a class of eight levels with the following values: 0, 0.25, 1, 2, 3, 4, 5, and 7 days. The fixed effect of collection time was not significant for any of the response factors monitored, so the results for this group are not reported.

The concentration of mIgG in Study A was 20-fold lower in the IO-mIgG group compared to the other groups in which mIgG was used, whereas Study B matched mIgG concentrations in all treatment groups. The results of the type III test of fixed effects for the Study A primary response are shown in Appendix G. The results of the type III test of fixed effects for the Study A memory response are shown in Appendix I.

Leukocytes

For CD45+ cells in the primary response study, the changes in estimates were affected by time of collection only (see Table 60). The estimates increased significantly by day 0.25 and peaked on day 1, after which they continued declining. On day 7, the CD45+ estimates were lower compared to the baseline estimates, but the difference was not statistically significant.

In the memory response study, significant changes in CD45+ estimates were observed by day and treatment group, but not by their interaction (see Table 61). The CD45+ estimates increased significantly by day 0.25 and remained consistent until day 3, when they reduced significantly. However, the CD45+ estimates observed between days 3 and 5 were still significantly higher compared to baseline estimates. A significantly higher response was observed in the IO-mIgG group compared to the Alum-mIgG and mIgG groups.

Table 60. *All leukocytes by day, Study A primary response*

Response Factor	Days	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
CD45+	0	10.31 ± 0.85	a
	0.25	25.79 ± 1.36	b
	1	27.02 ± 1.26	b
	2	25.00 ± 1.50	b
	3	18.78 ± 1.04	c
	4	16.70 ± 1.17	c
	5	13.74 ± 0.85	d
	7	7.58 ± 0.81	a

Note: different letters (a-d) represent significant differences ($p < 0.05$) between days.

Table 61. *All leukocytes by day and treatment group, Study A memory response*

Response Factor	Fixed Effect	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
CD45+	*Days*		
	0	12.05 ± 0.88	a
	0.25	33.84 ± 2.57	b
	1	30.99 ± 2.87	b
	2	33.38 ± 2.73	b
	3	22.76 ± 2.52	c
	4	21.26 ± 2.48	c
	5	17.86 ± 1.05	c
	Treatment		
	Alum-mIgG	22.46 ± 1.45	a
	IO-mIgG	29.46 ± 1.45	b
	mIgG	21.85 ± 1.59	a

Note: different letters (a-c) represent significant differences ($p < 0.05$) between days and between treatment groups.

Major Histocompatibility Complex

MHCII+ macrophages were significantly affected by time of collection in the primary response study (see Table 62). Estimates increased significantly by day 0.25, after which they continued decreasing, but remained significantly elevated compared to baseline until day 5. On day 7, their estimates were significantly lower compared to baseline.

In the memory response study, the response for MHCII+ macrophages was significant on day 0.25, after which the estimates were significantly lower than the estimates observed at baseline on days 1, 2, and 5 and similar to estimates observed at baseline on days 3 and 4 (see Table 63).

Table 62. *MHCII+ macrophage estimates by day, Study A primary response*

Response Factor	Days	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
MHCII+	0	0.93 ± 0.08	a
Macrophages+	0.25	5.38 ± 0.52	b
	1	2.14 ± 0.16	cd
	2	2.48 ± 0.14	c
	3	1.78 ± 0.12	d
	4	1.86 ± 0.20	d
	5	1.45 ± 0.12	e
	7	0.46 ± 0.12	f

Note: different letters (a-f) represent significant differences ($p < 0.05$) between days for each response factor.

Table 63. *MHCII+ macrophage estimates by day, Study A memory response*

Response Factor	Days	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
MHCII+	0	0.51 ± 0.06	a
Macrophages+	0.25	1.66 ± 0.40	b
	1	0.38 ± 0.04	c
	2	0.19 ± 0.02	d
	3	0.38 ± 0.09	acd
	4	0.58 ± 0.07	a
	5	0.34 ± 0.04	c

Note: different letters (a-d) represent significant differences ($p < 0.05$) between days.

In the primary response study, MHCII+ B cells+ were significantly affected only by time of collection (see Table 64). The estimates increased significantly on day 0.25 and peaked on day 2. The estimates decreased significantly on day 3 compared to day 2, but they were still significantly higher on day 7 compared to baseline estimates. No significant fixed effects were observed for MHCII+ B cells+ in the memory response study.

Table 64. *MHCII+ B cell+ estimates by day, Study A primary response*

Response Factor	Days	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
MHCII+ B cell+	0	0.07 ± 0.02	a
	0.25	0.20 ± 0.03	b
	1	0.63 ± 0.05	c
	2	0.89 ± 0.12	c
	3	0.34 ± 0.04	d
	4	0.44 ± 0.08	d
	5	0.38 ± 0.08	d
	7	0.29 ± 0.06	bd

Note: different letters (a-d) represent significant differences ($p < 0.05$) between days.

T Cells

The CD4- CD8+ estimates increased significantly by day in both primary and memory studies. In the primary response study, the estimates increased significantly by day 0.25 and peaked on day 1 (see Table 65). The estimates continued reducing and returned to values similar to those observed at baseline on day 5. In the memory response study, the CD4- CD8+ estimates increased significantly by day 0.25 and peaked on day 3 (see Table 66). Despite the significant decrease after day 3, the estimates remained significantly higher on day 5 compared to baseline.

Table 65. *CD4- CD8+ estimates by day, Study A primary response*

Response Factor	Days	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
CD4- CD8+	0	0.34 ± 0.04	a
	0.25	2.59 ± 0.45	b
	1	3.25 ± 0.89	b
	2	2.43 ± 0.32	b
	3	1.02 ± 0.17	c
	4	0.71 ± 0.09	c
	5	0.36 ± 0.10	a
	7	0.31 ± 0.03	a

Note: different letters (a-c) represent significant differences ($p < 0.05$) between days.

Table 66. *CD4- CD8+ estimates by day, Study A memory response*

Response Factor	Days	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
CD4- CD8+	0	0.75 ± 0.07	a
	0.25	2.71 ± 0.39	bc
	1	3.28 ± 0.47	b
	2	3.15 ± 0.30	b
	3	3.50 ± 0.52	b
	4	1.94 ± 0.24	cd
	5	1.89 ± 0.18	d

Note: different letters (a-d) represent significant differences ($p < 0.05$) between days.

CD4+ CD8+ cells were significantly affected by the time of collection in the primary and memory response studies. In the primary response study, the estimates increased significantly by day 1 and peaked on day 2, after which they started declining. The CD4+ CD8+ estimates were no longer significantly higher compared to baseline on day 5 (see Table 67). In the memory response study, CD4+ CD8+ cells increased significantly on day 3 compared to baseline and remained significantly elevated on day 5 (see Table 68).

Table 67. *CD4+ CD8+ estimates by day, Study A primary response*

Response Factor	Days	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
CD4+ CD8+	0	0.09 ± 0.02	a
	0.25	0.23 ± 0.15	ab
	1	0.33 ± 0.03	b
	2	0.67 ± 0.17	c
	3	0.28 ± 0.08	bd
	4	0.23 ± 0.05	bd
	5	0.14 ± 0.03	ad
	7	0.09 ± 0.01	a

Note: different letters (a-d) represent significant differences ($p < 0.05$) between days.

Table 68. *CD4+ CD8+ estimates by day, Study A memory response*

Response Factor	Days	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
CD4+ CD8+	0	0.17 ± 0.02	a
	0.25	0.22 ± 0.02	a
	1	0.24 ± 0.03	a
	2	0.19 ± 0.02	a
	3	0.65 ± 0.09	b
	4	0.44 ± 0.08	c
	5	0.50 ± 0.06	bc

Note: different letters (a-c) represent significant differences ($p < 0.05$) between days.

Table 69. *Treatment and day interaction effect estimates and main effects p-values of CD4+ CD8-, Study A primary response*

Day	Treatment			Day main effect P-value
	Alum-mIgG ($\bar{x} \pm SE$, n = 6)	IO-mIgG ($\bar{x} \pm SE$, n = 6)	mIgG ($\bar{x} \pm SE$, n = 6)	
0	0.40 ± 0.13	0.55 ± 0.13	0.84 ± 0.13	0.0777
0.25	0.72 ± 0.27	1.16 ± 0.27	0.49 ± 0.27	0.2403
1	5.52 ± 0.67	6.99 ± 0.67	6.29 ± 0.67	0.3223
2	4.78 ± 0.82	6.65 ± 0.82	5.63 ± 0.82	0.3002
3	2.98 ± 0.61	3.69 ± 0.61	2.74 ± 0.61	0.5315
4	2.81 ± 0.69	3.22 ± 0.69	2.47 ± 0.69	0.7466
5	1.12 ± 0.32	1.60 ± 0.32	1.41 ± 0.32	0.5710
7	0.67 ± 0.16	0.56 ± 0.16	0.66 ± 0.16	0.8494
Treatment main effect P-value	0.0009	0.0004	< 0.0001	

In the primary response study, the treatment main effect was significant for CD4+ CD8- in all treatment groups (see Table 69). However, the day main effect was not significant for any time of collection because the only significant difference between the groups was observed on day 0 (see Figure 23). The CD4+ CD8- estimates in the Alum-mIgG group before the injections were significantly lower compared to the mIgG group. A significant response was observed in all groups by day 1, and the CD4+ CD8- estimates returned to baseline values in all groups on day 7.

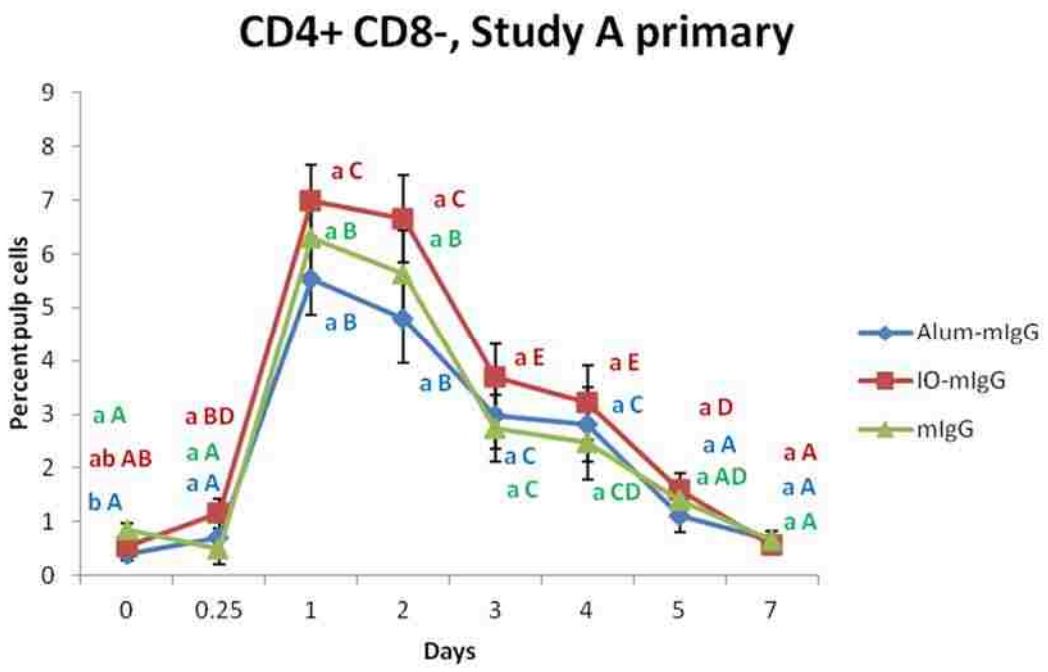

Figure 23. CD4+ CD8- estimates by day and treatment group, Study A primary response.

The graph shows mean differences of the treatment*day effect, not the main effect. The only significant difference between the groups was observed at baseline because the mIgG group had higher estimates compared to the Alum-mIgG group. Different lowercase letters (a-b) within one time point represent significant differences ($p < 0.05$) between the treatment groups. Different uppercase letters (A-E) represent significant changes between days for each treatment group.

In the memory response study, both time of collection and treatment, but not their interaction, were a significant fixed effect for CD4+ CD8- estimates (see Table 70). The estimates increased significantly by day 0.25 and peaked on day 1, after which they continued reducing. However, the estimates were still significantly higher on day 5 compared to baseline. The strongest response was observed in the IO-mIgG group, and it was significantly higher compared to the response observed in the Alum-mIgG and mIgG groups.

Table 70. *CD4+ CD8- estimates by day and treatment group, Study A memory response*

Response Factor	Fixed Effect	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
CD4+ CD8-	*Days*		
	0	2.92 ± 0.26	a
	0.25	6.00 ± 0.75	bd
	1	8.74 ± 1.10	bc
	2	8.33 ± 0.78	c
	3	5.79 ± 0.79	d
	4	4.47 ± 0.59	de
	5	3.98 ± 0.35	e
	Treatment		
	Alum-mIgG	5.17 ± 0.47	a
	IO-mIgG	6.97 ± 0.47	b
	mIgG	5.09 ± 0.52	a

Note: different letters (a-d) represent significant differences ($p < 0.05$) between days and between treatment groups.

For the CD25+ CD4+ and CD25+ CD4- response factors, the only the significant fixed effect was collection time in the primary response study (see Table 71). The CD25+ CD4+ estimates increased significantly by day 1, decreased significantly on day 2, and increased significantly again on day 4. No significant changes were observed after day 4, and the estimates remained significantly higher compared to baseline on day 7. The CD25+ CD4- estimates increased significantly by day 0.25 and increased until day 1, after which they started decreasing. The minimum value was observed on day 3, and it was significantly lower compared to baseline. However, the estimates started increasing again on day 4 and achieved the maximum value during the observation on day 7, which was significantly higher compared to all other collection times.

Table 71. *CD25+ CD4+ and CD25+ CD4- estimates by day, Study A primary response*

Response Factor	Days	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
CD25+ CD4+	0	0.04 ± 0.01	ab
	0.25	0.04 ± 0.01	b
	1	0.08 ± 0.01	c
	2	0.01 ± 0.01	a
	3	0.02 ± 0.01	ab
	4	0.09 ± 0.02	c
	5	0.08 ± 0.01	c
	7	0.08 ± 0.01	c
CD25+ CD4-	0	0.05 ± 0.01	a
	0.25	0.09 ± 0.02	b
	1	0.11 ± 0.02	b
	2	0.07 ± 0.02	ab
	3	0.02 ± 0.01	c
	4	0.04 ± 0.01	a
	5	0.05 ± 0.01	a
	7	0.23 ± 0.03	d

Note: different letters (a-d) represent significant differences ($p < 0.05$) between days for each response factor.

Table 72. *CD25+ CD4+ and CD25+ CD4- estimates by day, Study A memory response*

Response Factor	Days	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
CD25+ CD4+	0	0.15 ± 0.02	ab
	0.25	0.09 ± 0.02	b
	1	0.42 ± 0.08	c
	2	0.21 ± 0.03	ad
	3	0.29 ± 0.04	cd
	4	0.23 ± 0.04	cd
	5	0.24 ± 0.03	cd
CD25+ CD4-	0	0.58 ± 0.06	ab
	0.25	0.66 ± 0.07	b
	1	1.01 ± 0.09	c
	2	0.49 ± 0.05	abd
	3	0.40 ± 0.04	ad
	4	0.48 ± 0.03	ad
	5	0.44 ± 0.04	d

Note: different letters (a-d) represent significant differences ($p < 0.05$) between days for each response factor.

The CD25+ CD4+ and CD25+ CD4- response factors were affected by collection time in the memory response study as well (see Table 72). The CD25+ CD4+ estimates decreased significantly by day 0.25 compared to baseline, but they increased on day 1 and were significantly higher compared to baseline. On day 2, the CD25+ CD4+ estimates were similar to those observed at baseline and significantly higher compared to baseline on subsequent collection times. The CD25+ CD4- estimates increased significantly by day 1, after which they reduced significantly and were no longer significantly higher compared to baseline.

T cell receptors. Significant fixed effects of day and treatment, but not their interaction were observed for the γδ T cell+ CD8+ response in the primary response study (see Table 73). The increase was significant on day 1 compared to baseline. After day 1, the response started decreasing and the estimates were no longer significantly different from those observed at baseline after day 3. The estimates in the Alum-mIgG group were significantly higher compared to the IO-mIgG group, but not compared to the mIgG group.

The γδ T cell+ CD8+ estimates in the memory response study increased significantly by day 0.25 (see Table 74). The highest estimates were observed on days 0.25, 3, and 5. Between those collection times, the γδ T cell+ CD8+ estimates would decrease significantly only to increase again.

Table 73. *γδ T cell+ CD8+ estimates by day and treatment group, Study A primary response*

Response Factor	Fixed Effect	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
γδ T cell+ CD8+	*Days*		
	0	0.12 ± 0.02	ad
	0.25	0.15 ± 0.02	ad
	1	0.48 ± 0.04	b
	2	0.39 ± 0.04	c
	3	0.17 ± 0.02	a
	4	0.16 ± 0.02	ad
	5	0.10 ± 0.02	d
	7	0.13 ± 0.02	ad
	Treatment		
	Alum-mIgG	0.17 ± 0.02	a
	IO-mIgG	0.26 ± 0.02	b
	mIgG	0.20 ± 0.02	ab

Note: different letters (a-d) represent significant differences ($p < 0.05$) between days and between treatment groups.

Table 74. *γδ T cell+ CD8+ estimates by day, Study A memory response*

Response Factor	Days	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
γδ T cell+ CD8+	0	0.12 ± 0.02	a
	0.25	0.59 ± 0.06	b
	1	0.51 ± 0.07	bc
	2	0.41 ± 0.03	c
	3	0.72 ± 0.06	b
	4	0.32 ± 0.04	d
	5	0.49 ± 0.03	bc

Note: different letters (a-c) represent significant differences ($p < 0.05$) between days.

In the primary response study, the interaction between treatment and time of collection was a significant fixed effect for the γδ T cell+ CD8- response (see Table 75). The treatment main effect was significant for all groups, whereas the day main effect was significant only on day 2 because the response in the IO-mIgG group remained elevated longer compared to the Alum-mIgG and mIgG groups (see Figure 24).

Table 75. *Treatment and day interaction effect estimates and main effects p-values of γδ T cell+ CD8-, Study A primary response*

Day	Treatment			Day main effect P-value
	Alum-mIgG ($\bar{x} \pm SE$, n = 6)	IO-mIgG ($\bar{x} \pm SE$, n = 6)	mIgG ($\bar{x} \pm SE$, n = 6)	
0	0.58 ± 0.18	0.33 ± 0.18	0.42 ± 0.18	0.6110
0.25	3.20 ± 0.61	2.21 ± 0.61	1.93 ± 0.61	0.3224
1	2.66 ± 0.55	3.35 ± 0.55	2.58 ± 0.55	0.5609
2	0.99 ± 0.20	2.27 ± 0.20	1.15 ± 0.20	0.0006
3	0.55 ± 0.12	0.76 ± 0.12	0.40 ± 0.12	0.1182
4	0.27 ± 0.08	0.48 ± 0.08	0.30 ± 0.08	0.1811
5	0.18 ± 0.07	0.31 ± 0.07	0.25 ± 0.07	0.4137
7	0.30 ± 0.04	0.24 ± 0.04	0.21 ± 0.04	0.3870
Treatment main effect P-value	0.0060	0.0003	0.0455	

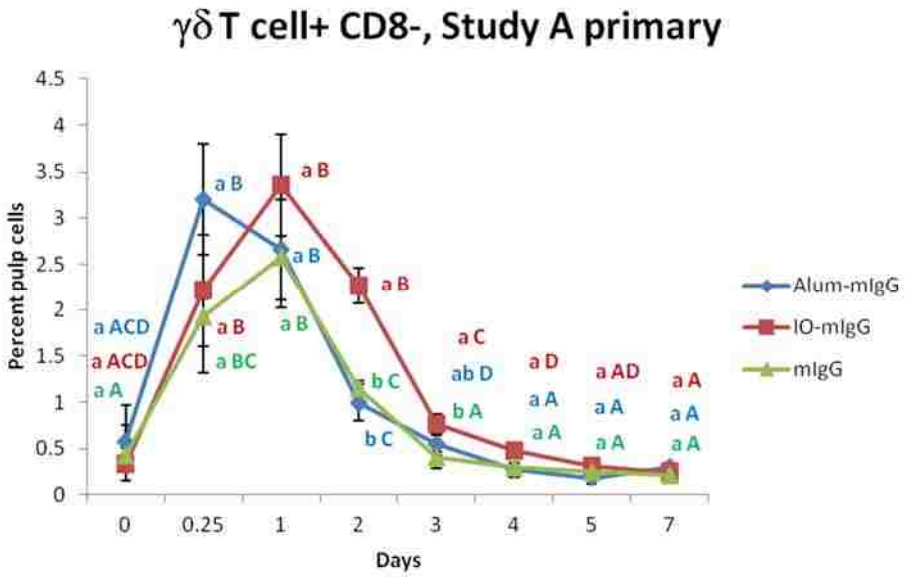

Figure 24. γδ T cell+ CD8- estimates by day and treatment group, Study A primary response.

The graph shows mean differences of the treatment*day effect, not the main effect. Significant differences between the groups were observed on days 2 and 3 because the IO-mIgG group elicited a longer response compared to the mIgG group and the Alum-mIgG group. Different lowercase letters (a-b) within one time point represent significant differences ($p < 0.05$) between the treatment groups. Different uppercase letters (A-D) represent significant changes between days for each treatment group.

In the memory response study, time of collection was the only significant fixed effect for γδ T cell+ CD8- estimates (see Table 76). The estimates increased significantly by day 0.25, after which they continued to reduce. However, the decreases observed after day 3 were not significant, so the response remained significantly higher compared to baseline.

Table 76. *γδ T cell+ CD8- estimates by day, Study A memory response*

Response Factor	Days	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
γδ T cell+ CD8-	0	0.46 ± 0.13	a
	0.25	5.13 ± 0.94	b
	1	3.53 ± 0.39	b
	2	1.58 ± 0.15	c
	3	1.27 ± 0.16	d
	4	0.88 ± 0.13	d
	5	0.95 ± 0.11	d

Note: different letters (a-c) represent significant differences ($p < 0.05$) between days.

The αβ1 T cell+ response factor estimates changed significantly by day only in the primary response study (see Table 77). The estimates increased significantly by day 0.25 and achieved the highest values on days 1 and 2, after which they continued decreasing. On day 7, the estimates were no longer significantly higher compared to baseline.

In the memory response study, the fixed effects of collection time and treatment, but not their interaction, were significant for the αβ1 T cell+ response factor (see Table 78). The estimates increased significantly by day 0.25, and the highest estimate was observed on day 1. The estimates started decreasing after day 1, but they were still significantly higher compared to baseline on day 5. The highest response was observed in the IO-mIgG group, and it was significantly higher compared to the other groups.

Table 77. *αβ1 T cell+ estimates by day, Study A primary response*

Response Factor	Days	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
αβ1 T cell+	0	0.40 ± 0.06	a
	0.25	1.24 ± 0.16	b
	1	4.97 ± 0.33	c
	2	4.84 ± 0.38	c
	3	2.54 ± 0.31	d
	4	2.25 ± 0.33	d
	5	1.34 ± 0.34	b
	7	0.28 ± 0.04	a

Note: different letters (a-d) represent significant differences ($p < 0.05$) between days.

Table 78. *αβ1 T cell+ estimates by day and treatment group, Study A memory response*

Response Factor	Fixed Effect	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
αβ1 T cell+	*Days*		
	0	0.57 ± 0.11	a
	0.25	3.97 ± 0.61	b
	1	6.32 ± 0.95	bc
	2	5.93 ± 0.62	c
	3	4.53 ± 0.70	bc
	4	2.26 ± 0.33	d
	5	2.45 ± 0.32	d
	Treatment		
	Alum-mIgG	3.23 ± 0.38	b
	IO-mIgG	4.78 ± 0.38	a
	mIgG	3.14 ± 0.42	b

Note: different letters (a-d) represent significant differences ($p < 0.05$) between days and between treatment groups.

The αβ2 T cell+ response factor estimates changed significantly only by day in the primary response study (see Table 79). The estimates increased significantly by day 0.25 and achieved the highest values on days 1 and 2. The estimates decreased significantly by day 3 and continued decreasing. On day 7, they were no longer significantly higher compared to baseline.

The αβ2 T cell+ response factor estimates also changed significantly only by day in the memory response study (see Table 80). The estimates increased significantly on day 0.25 and

remained elevated until day 3, after which they decreased significantly. However, the estimates on days 4 and 5 were still significantly higher compared to baseline.

Table 79. *αβ2 T cell+ estimates by day, Study A primary response*

Response Factor	Days	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
αβ2 T cell+	0	0.15 ± 0.02	a
	0.25	0.39 ± 0.05	b
	1	1.57 ± 0.10	c
	2	1.47 ± 0.11	c
	3	0.75 ± 0.09	d
	4	0.59 ± 0.08	bd
	5	0.37 ± 0.05	b
	7	0.11 ± 0.02	a

Note: different letters (a-d) represent significant differences ($p < 0.05$) between days.

Table 80. *αβ2 T cell+ estimates by day, Study A memory response*

Response Factor	Days	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
αβ2 T cell+	0	0.39 ± 0.06	a
	0.25	2.11 ± 0.36	b
	1	2.49 ± 0.33	b
	2	2.17 ± 0.24	b
	3	1.81 ± 0.24	b
	4	0.98 ± 0.12	c
	5	1.01 ± 0.12	c

Note: different letters (a-d) represent significant differences ($p < 0.05$) between days.

B Cells

B cells were significantly affected by the interaction between time of collection and treatment type in the primary response study (see Table 81). The main effect of treatment was significant for all treatment groups, whereas the day main effect was significant only for day 2. The only significant difference between the groups was observed on day 2 because the IO-mIgG treatment elicited a significantly stronger response compared to the Alum-mIgG and mIgG treatments (see Figure 25).

Table 81. *Treatment and day interaction effect estimates and main effects p-values of B cells, Study A primary response*

Day	Treatment			Day main effect P-value
	Alum-mIgG ($\bar{x} \pm SE$, n = 6)	IO-mIgG ($\bar{x} \pm SE$, n = 6)	mIgG ($\bar{x} \pm SE$, n = 6)	
0	0.08 ± 0.04	0.12 ± 0.04	0.17 ± 0.04	0.3940
0.25	0.34 ± 0.10	0.40 ± 0.10	0.16 ± 0.10	0.2539
1	2.65 ± 0.45	3.30 ± 0.45	2.27 ± 0.45	0.2954
2	2.26 ± 0.91	7.50 ± 0.91	2.99 ± 0.91	0.0020
3	1.70 ± 0.80	3.79 ± 0.80	1.53 ± 0.80	0.1147
4	1.01 ± 0.58	2.58 ± 0.58	1.32 ± 0.58	0.1625
5	0.57 ± 0.22	0.89 ± 0.22	0.81 ± 0.22	0.5553
7	0.17 ± 0.25	0.48 ± 0.25	0.16 ± 0.25	0.6066
Treatment main effect P-value	0.0026	< 0.0001	0.0006	

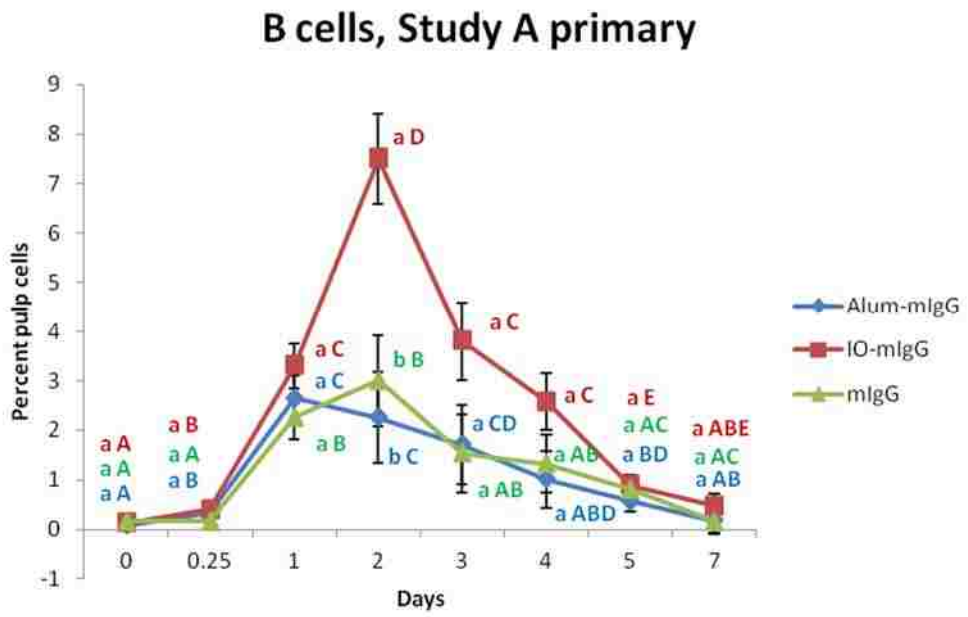

Figure 25. B cell estimates by day and treatment group, Study A primary response.

The graph shows mean differences of the treatment*day effect, not the main effect. The IO-mIgG treatment elicited a significantly higher response on day 2 compared to the other treatments. Different lowercase letters (a-b) within one time point represent significant differences ($p < 0.05$) between the treatment groups. Different uppercase letters (A-D) represent significant changes between days for each treatment group.

In the memory response study, the fixed effects of collection time and treatment, but not their interaction, were significant for the B cell response (see Table 82). A significant increase was observed on day 0.25, and the estimates continued increasing until they peaked on day 2. On day 5, the estimates were still significantly higher compared to baseline. The highest response was observed in the IO-mIgG group, and it was significantly higher compared to the Alum-mIgG group, but not compared to the mIgG group.

Table 82. *B cell estimates by day and treatment group, Study A memory response*

Response Factor	Fixed Effect	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
B cells	*Days*		
	0	0.23 ± 0.04	a
	0.25	1.11 ± 0.14	b
	1	4.56 ± 0.66	ce
	2	10.23 ± 1.29	d
	3	7.84 ± 1.36	de
	4	5.26 ± 0.94	c
	5	3.38 ± 0.37	c
	Treatment		
	Alum-mIgG	3.47 ± 0.65	b
	IO-mIgG	6.06 ± 0.65	a
	mIgG	4.44 ± 0.71	ab

Note: different letters (a-d) represent significant differences ($p < 0.05$) between days and between treatment groups.

Summary

In the primary response study, significant changes by day only were observed for 11 response factors. The fixed effect of treatment was significant only for the γδ T cell+ CD8+ response factor. The γδ T cell+ CD8+ estimates in the IO-mIgG group were significantly higher compared to the Alum-mIgG group, but not compared to the mIgG group.

The fixed effect of interaction between treatment and collection time was significant for the following three response factors: CD4+ CD8-, γδ T cell+ CD8-, and B cell response factors.

The response in the IO-mIgG group was significantly higher for γδ T cell+ CD8- and B cell response factors. The only difference between the groups for the CD4+ CD8- was observed prior to the injections because the Alum-mIgG group had lower baseline estimates compared to the mIgG group.

In the memory response study, significant changes by day only were observed for ten response factors. The fixed effect of treatment was significant for the following four response factors: CD45+, CD4+ CD8-, αβ1 T cell+, and B cell response factors. The highest responses of those four factors were observed in the IO-mIgG group, and they were significantly higher compared to the Alum-mIgG and mIgG groups. The significant effect of collection time and treatment type was not observed for any of the response factors measured in the 2013 memory response study.

The difference of least squares means and the simple effect comparisons results for the primary responses observed in Study A are included in Appendix H. The difference of least squares means and the simple effect comparisons results for the memory responses observed in Study A are included in Appendix J.

Study B: In Vivo Monitoring of Primary and Memory Immune Responses to Intramuscular Injections of IONPs in Chickens, 2014

In the primary response study, time of collection was defined as a class of eight levels with the following values: 0, 0.25, 1, 2, 3, 4, 5, and 7 days. In the memory response study, time of collection was defined as a class of seven levels with the following values: 0, 0.25, 1, 2, 3, 4, and 5 days. Type of treatment was a class with three levels in both primary and memory studies, and it contained the following values: Alum-mIgG, IO-mIgG, and mIgG. In the memory study,

a PBS control group was used. Time of collection was the only fixed effect in the analysis, and it was defined as a class of seven levels with the following values: 0, 0.25, 1, 2, 3, 4, and 5 days.

The concentration of mIgG in Study A was 20-fold lower in the IO-mIgG group compared to the other groups in which mIgG was used, whereas Study B matched mIgG concentrations in all treatment groups. The results of the type III test of fixed effects for the Study B primary response are shown in Appendix G. The results of the type III test of fixed effects for the Study B memory response are shown in Appendix I.

Live Cells

No significant fixed effects were observed for live cells in the primary response study. In the memory response study, time of collection was the only significant fixed effect for live cells (see Table 83). Their estimates increased significantly by day 0.25 and remained elevated until day 4, when they returned to values similar to those observed at baseline. In the PBS group, significant changes in live cell estimates by day were also observed, but only because the values increased significantly on days 2 and 4 compared to baseline (see Table 83).

Table 83. *Live cell estimates by day, Study B memory response*

Response Factor	Days	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
Live cells	0	53.54 ± 0.82	a
	0.25	63.20 ± 1.90	b
	1	67.08 ± 1.78	b
	2	65.73 ± 1.51	b
	3	65.14 ± 0.91	b
	4	53.73 ± 1.21	a
	5	56.49 ± 1.37	a
Live cells (PBS)	0	53.25 ± 0.93	a
	0.25	51.78 ± 1.37	a
	1	57.71 ± 3.18	ab
	2	61.07 ± 1.35	b
	3	58.55 ± 3.11	ab
	4	62.37 ± 1.20	b
	5	59.17 ± 5.99	ab

Note: different letters (a-b) represent significant differences ($p < 0.05$) between days.

Major Histocompatibility Complex

In the primary response study, the interaction between time of collection and treatment type had a significant effect on MHCII+ macrophages (see Table 84). The main effect of treatment was significant only for the Alum-mIgG study. The day main effect was not significant ($p < 0.05$) for any time of sample collection. Only one difference between the groups was observed on day 0.25 when MHCII+ macrophages significantly increased in the Alum-mIgG group and were significantly higher compared to the estimates observed in the IO-mIgG group (see Figure 26, top). However, a significant decrease occurred by day 1 and no further differences between the groups were observed.

In the memory response study, the control group showed no significant changes in MHCII+ macrophage estimates, but a significant fixed effect of the interaction between collection time and treatment was observed in the treatment groups (see Table 85). The treatment main effect was significant only in the Alum-mIgG group, and the day main effect was

significant only on day 2 because the response in the Alum-mIgG group lasted longer compared to the other groups (see Figure 26, bottom).

Table 84. *Treatment and day interaction effect estimates and main effects p-values of MHCII+ Macrophages, Study B primary response*

Day	Treatment			Day main effect P-value
	Alum-mIgG ($\bar{x} \pm SE$, n = 4)	**IO-mIgG ($\bar{x} \pm SE$, n = 4)**	**mIgG ($\bar{x} \pm SE$, n = 4)**	
0	2.58 ± 0.41	2.48 ± 0.41	2.23 ± 0.41	0.8244
0.25	8.83 ± 1.83	2.38 ± 1.83	4.64 ± 1.83	0.0892
1	3.06 ± 0.52	2.39 ± 0.52	2.65 ± 0.52	0.6696
2	2.99 ± 0.50	2.20 ± 0.50	2.49 ± 0.50	0.5477
3	3.77 ± 0.89	1.64 ± 0.89	1.72 ± 0.89	0.2137
4	1.84 ± 0.35	1.16 ± 0.35	1.28 ± 0.35	0.3845
5	1.76 ± 0.22	1.26 ± 0.22	1.67 ± 0.22	0.2993
7	2.02 ± 0.37	2.04 ± 0.37	1.75 ± 0.37	0.8278
Treatment main effect P-value	0.0033	0.6701	0.0641	

Table 85. *Treatment and day interaction effect estimates and main effects p-values of MHCII+ Macrophages+, Study B memory response*

Day	Treatment			Day main effect P-value
	Alum-mIgG ($\bar{x} \pm SE$, n = 4)	**IO-mIgG ($\bar{x} \pm SE$, n = 4)**	**mIgG ($\bar{x} \pm SE$, n = 4)**	
0	0.88 ± 0.14	1.33 ± 0.14	1.26 ± 0.14	0.1020
0.25	6.79 ± 2.03	5.46 ± 2.03	3.23 ± 2.03	0.4845
1	4.57 ± 0.85	2.37 ± 0.85	1.95 ± 0.85	0.1168
2	2.10 ± 0.26	0.92 ± 0.26	1.17 ± 0.26	0.0239
3	2.41 ± 0.36	1.96 ± 0.36	1.71 ± 0.36	0.4225
4	2.02 ± 0.74	2.91 ± 0.74	1.77 ± 0.74	0.5441
5	2.91 ± 0.68	2.33 ± 0.68	2.39 ± 0.68	0.8040
Treatment main effect P-value	0.0118	0.0875	0.5624	

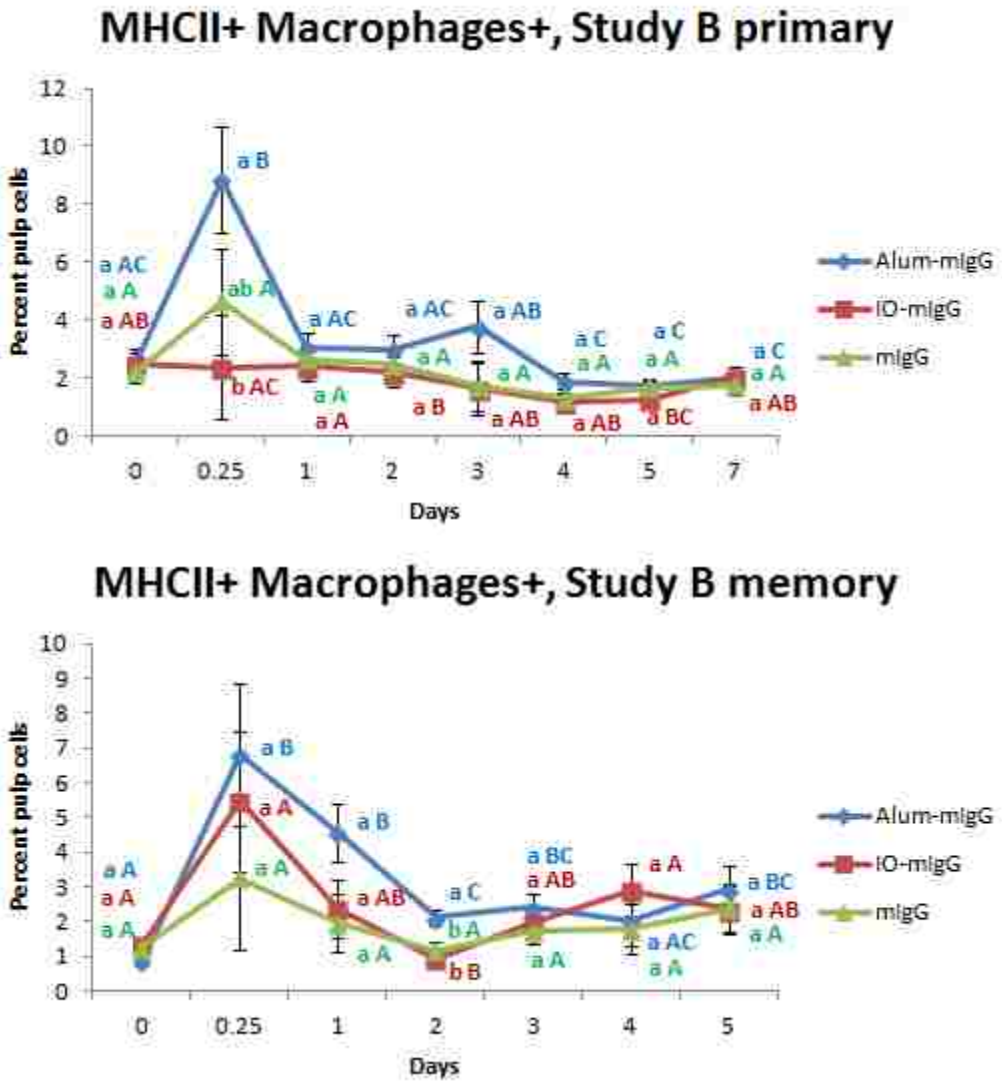

Figure 26. MHCII+ Macrophage+ estimates by day and treatment group, Study B.

The graph shows mean differences of the treatment*day effect, not the main effect. Different lowercase letters (a-b) within one time point represent significant differences ($p < 0.05$) between the treatment groups. Different uppercase letters (A-C) represent significant changes between days for each treatment group.

Top: The primary response in the Alum-mIgG group was significantly higher compared to the IO-mIgG group, but not compared to the mIgG group on day 0.25.

Bottom: The memory response in the Alum-mIgG group was significantly higher compared to the other groups on day 2.

In the primary response study, MHCII+ B cell+ estimates were significantly affected only by time of collection (see Table 86). The estimates increased significantly by day 1 and continued increasing until day 3, when the highest estimate of MHCII+ B cells+ was observed. Although the estimates started decreasing after day 3, MHCII+ B cells+ estimates were still significantly higher on day 7 compared to baseline.

In the memory response study, MHCII+ B cell+ estimates changed significantly only by day (see Table 87). The increase was significant by day 0.25, but the estimates continued increasing until day 3, when they peaked. On days 4 and 5, the estimates reduced significantly compared to day 3, but they remained significantly higher compared to baseline estimates. The PBS control group in the memory response study also showed significant changes by day (see Table 87), but a significant response was observed on day 1 and the estimates remained consistent until day 3. Further non-significant increases compared to days 1-3 were observed on days 4 and 5, when the estimates of MHCII+ B cells+ in the treatment groups were already declining toward baseline values.

Table 86. *MHCII+ B cell+ estimates by day, Study B primary response*

Response Factor	Days	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
MHCII+ B cell+	0	0.39 ± 0.11	a
	0.25	0.68 ± 0.16	a
	1	4.87 ± 0.57	b
	2	6.18 ± 1.63	bd
	3	11.86 ± 1.93	c
	4	9.53 ± 1.89	cd
	5	5.37 ± 0.59	b
	7	2.42 ± 0.35	e

Note: different letters (a-e) represent significant differences ($p < 0.05$) between days.

Table 87. *MHCII+ B cell+ estimates by day, Study B memory response*

Response Factor	Days	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
MHCII+ B cell+	0	0.51 ± 0.20	a
	0.25	1.77 ± 0.19	b
	1	12.69 ± 2.95	c
	2	12.55 ± 1.71	c
	3	13.18 ± 1.55	c
	4	3.05 ± 0.67	b
	5	2.27 ± 0.49	b
MHCII+ B cell+ (PBS)	0	0.42 ± 0.14	a
	0.25	0.38 ± 0.11	a
	1	5.07 ± 0.51	b
	2	4.19 ± 1.02	b
	3	5.58 ± 1.97	ab
	4	7.95 ± 1.07	b
	5	10.24 ± 3.67	ab

Note: different letters (a-c) represent significant differences ($p < 0.05$) between days.

T Cells

In the primary response study, the CD4- CD8+ estimates increased significantly by day 0.25 (see Table 88). The estimates peaked on day 2 and remained significantly higher compared to baseline on day 7. The CD4- CD8+ estimates changed significantly only by day in the memory response study treatment groups and the control group (see Table 89). In the treatment groups, a significant response was observed by day 0.25, and the estimates peaked on day 1. A

significant decrease was observed from day 1 to day 2, and the estimates continued decreasing until day 5, but they were still significantly higher compared to baseline estimates. In the memory response PBS control group, a similar response trend was observed, but a significant increase compared to baseline was observed on day 1, and the estimates on day 5 were no longer significantly higher compared to baseline (see Table 89).

Table 88. *CD4- CD8+ estimates by day, Study B primary response*

Response Factor	Days	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
CD4- CD8+	0	0.29 ± 0.04	a
	0.25	0.96 ± 0.17	b
	1	3.23 ± 0.57	c
	2	3.44 ± 0.52	c
	3	3.25 ± 0.81	c
	4	1.65 ± 0.27	cb
	5	1.16 ± 0.14	b
	7	1.33 ± 0.25	b

Note: different letters (a-c) represent significant differences ($p < 0.05$) between days.

Table 89. *CD4- CD8+ estimates by day, Study B memory response*

Response Factor	Days	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
CD4- CD8+	0	0.54 ± 0.08	a
	0.25	2.44 ± 0.37	b
	1	4.19 ± 0.47	c
	2	3.03 ± 0.42	b
	3	2.63 ± 0.32	b
	4	1.09 ± 0.13	d
	5	0.95 ± 0.13	d
CD4- CD8+ (PBS)	0	0.43 ± 0.07	a
	0.25	0.70 ± 0.15	a
	1	3.61 ± 0.82	b
	2	2.19 ± 0.42	b
	3	2.76 ± 0.88	ab
	4	2.55 ± 0.35	b
	5	1.71 ± 0.52	ab

Note: different letters (a-d) represent significant differences ($p < 0.05$) between days for each response factor.

Table 90. *Treatment and day interaction effect estimates and main effects p-values of CD4+ CD8+, Study B memory response*

Day	Treatment			Day main effect P-value
	Alum-mIgG ($\bar{x} \pm SE$, n = 4)	IO-mIgG ($\bar{x} \pm SE$, n = 4)	mIgG ($\bar{x} \pm SE$, n = 4)	
0	0.04 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.6898
0.25	0.39 ± 0.16	0.18 ± 0.16	0.47 ± 0.16	0.4678
1	0.84 ± 0.33	0.63 ± 0.33	0.66 ± 0.33	0.8906
2	1.34 ± 0.36	0.46 ± 0.36	0.63 ± 0.36	0.2314
3	0.64 ± 0.26	0.55 ± 0.26	0.57 ± 0.26	0.9672
4	0.42 ± 0.11	0.22 ± 0.11	0.09 ± 0.11	0.1492
5	0.29 ± 0.10	0.13 ± 0.10	0.08 ± 0.10	0.3480
Treatment main effect P-value	0.0029	0.6578	0.4893	

No significant changes in CD4+ CD8+ estimates were observed in the primary response study. However, in the memory response study, a significant fixed effect of the interaction between treatment and collection time was observed for CD4+ CD8+ estimates (see Table 90). The treatment main effect was significant only for Alum-mIgG, which elicited the highest response. However, the day main effect was not significant for any of the collection times as CD4+ CD8+ the estimates in the Alum-mIgG group were not significantly higher compared to the estimates in the other groups (see Figure 27).

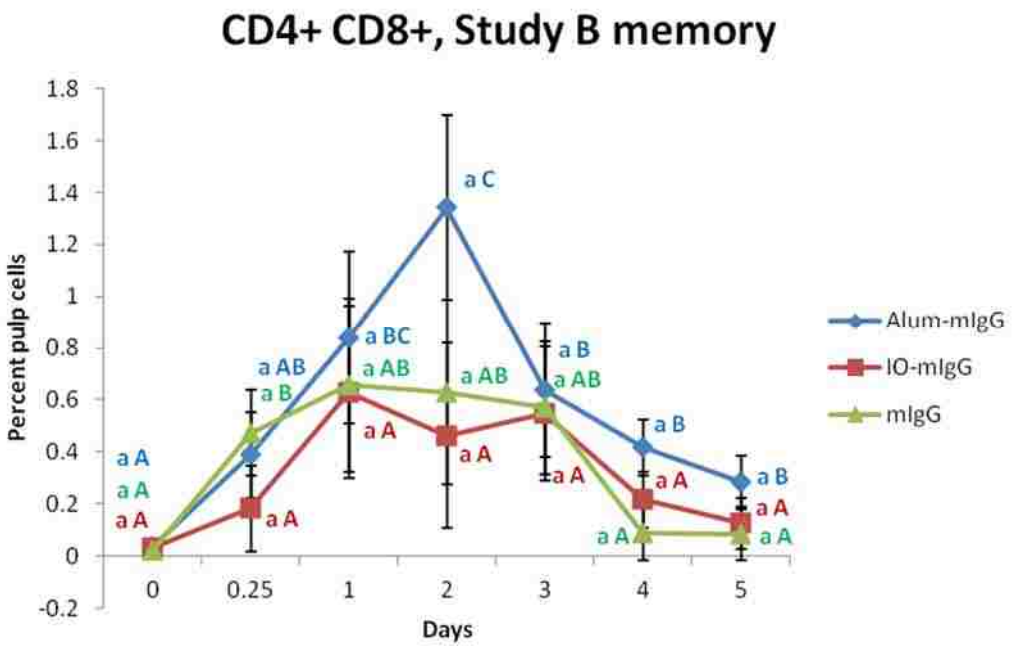

Figure 27. CD4+ CD8+ estimates by day and treatment group, Study B memory response.

The graph shows mean differences of the treatment*day effect, not the main effect. The Alum-mIgG treatment elicited the highest response on day 2 compared to the other treatments, but the difference between the groups was not statistically significant. Different lowercase letters (a-b) within one time point represent significant differences ($p < 0.05$) between the treatment groups. Different uppercase letters (A-C) represent significant changes between days for each treatment group.

In the PBS control group of the memory response study, a significant fixed effect of collection time was observed for CD4+ CD8+ estimates (see Table 91). The response was significant on days 1, 2, and 4 compared to baseline. However, no significant differences were observed between the collection times after day 1.

Table 91. *CD4+ CD8+ estimates by day, Study B memory response*

Response Factor	Days	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
CD4+ CD8+ (PBS)	0	0.06 ± 0.02	a
	0.25	0.07 ± 0.02	a
	1	0.85 ± 0.09	b
	2	0.63 ± 0.16	b
	3	1.08 ± 0.47	ab
	4	0.89 ± 0.15	b
	5	0.73 ± 0.39	ab

Note: different letters (a-d) represent significant differences ($p < 0.05$) between days for each response factor.

CD4+ CD8- cells were significantly affected by the time of collection in the primary response study. The CD4+ CD8- estimates in the primary response study increased significantly by day 0.25 and continued increasing until day 2 (see Table 92). Although they decreased significantly by day 7 compared to maximum observed values, the estimates were still significantly higher on day 7 compared to baseline.

The CD4+ CD8- estimates changed significantly by day in the memory response study as well (see Table 93). In the treatment groups, the response was significant by day 0.25 and the highest estimates were observed on day 1. The estimates started reducing significantly after day 1, but they were still higher compared to baseline on day 5. In the PBS control group, the response was significant on days 1, 2, and 4 compared to baseline. However, no significant differences were observed between the collection times after day 1.

Table 92. *CD4+ CD8- estimates by day, Study B primary response*

Response Factor	Days	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
CD4+ CD8-	0	1.06 ± 0.22	a
	0.25	2.85 ± 0.57	b
	1	8.60 ± 0.68	c
	2	8.73 ± 0.73	c
	3	7.40 ± 0.82	cd
	4	5.73 ± 0.75	d
	5	3.85 ± 0.62	b
	7	3.49 ± 0.61	b

Note: different letters (a-d) represent significant differences ($p < 0.05$) between days.

Table 93. *CD4+ CD8- estimates by day, Study B memory response*

Response Factor	Days	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
CD4+ CD8-	0	1.07 ± 0.26	a
	0.25	4.54 ± 0.48	b
	1	9.39 ± 1.04	c
	2	7.09 ± 0.84	d
	3	6.46 ± 0.56	d
	4	2.47 ± 0.36	e
	5	2.47 ± 0.48	e
CD4+ CD8- (PBS)	0	0.95 ± 0.17	a
	0.25	0.77 ± 0.16	a
	1	5.74 ± 0.61	b
	2	4.67 ± 0.73	b
	3	5.25 ± 2.06	ab
	4	4.62 ± 0.38	b
	5	3.89 ± 1.27	ab

Note: different letters (a-c) represent significant differences ($p < 0.05$) between days for each response factor.

T cell receptors. No significant fixed effects were observed for γδ T cell+ CD8+ in the primary response study, but a significant interaction of collection time and treatment was observed in the memory response study. The treatment main effect was significant for all treatment groups, but the day main effect was not significant for any of the collection times (see Table 94). The highest response was observed in the Alum-mIgG group, but the only difference between the groups was observed on day 0 because the γδ T cell+ CD8+ estimates were higher in the IO-mIgG group compared to the mIgG group (see Figure 28).

Table 94. *Treatment and day interaction effect estimates and main effects p-values of γδ T cell+ CD8+, Study B memory response*

Day	Treatment			Day main effect P-value
	Alum-mIgG ($\bar{x} \pm SE$, n = 4)	**IO-mIgG ($\bar{x} \pm SE$, n = 4)**	**mIgG ($\bar{x} \pm SE$, n = 4)**	
0	0.17 ± 0.06	0.33 ± 0.06	0.08 ± 0.06	0.0563
0.25	0.68 ± 0.14	0.50 ± 0.14	0.84 ± 0.14	0.2712
1	1.11 ± 0.27	1.89 ± 0.27	1.18 ± 0.27	0.1354
2	0.97 ± 0.15	1.06 ± 0.15	0.69 ± 0.15	0.2490
3	0.94 ± 0.22	1.38 ± 0.22	1.03 ± 0.22	0.3593
4	0.49 ± 0.13	0.55 ± 0.13	0.40 ± 0.13	0.7302
5	0.36 ± 0.08	0.51 ± 0.08	0.31 ± 0.08	0.2638
Treatment main effect P-value	0.0002	0.0012	0.0001	

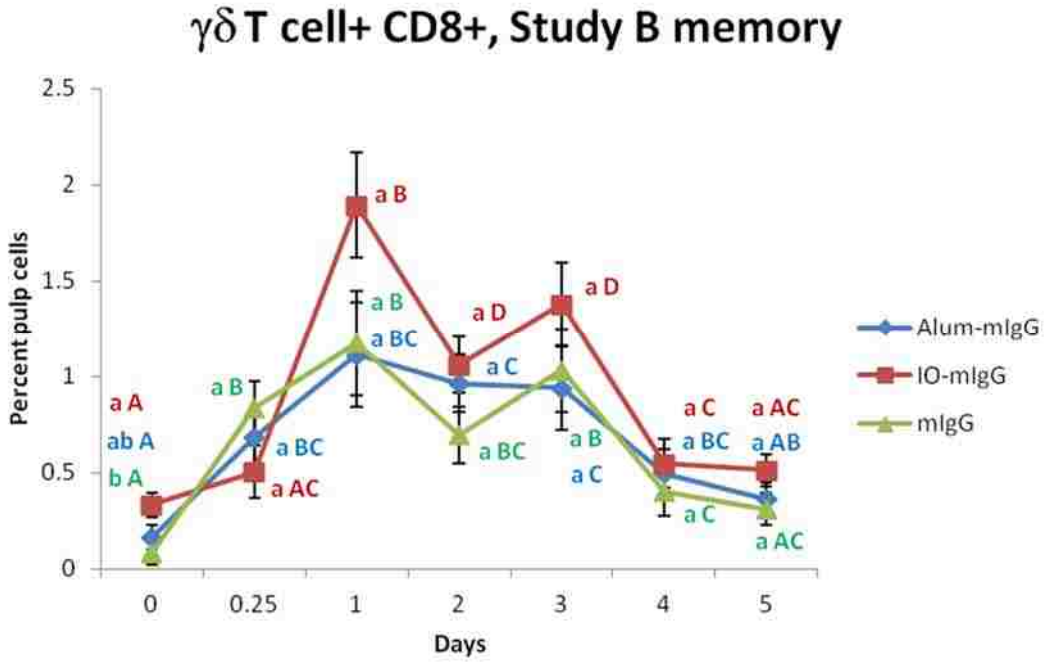

Figure 28. γδ T cell+ CD8+ estimates by day and treatment group, Study B memory response. The graph shows mean differences of the treatment*day effect, not the main effect. The IO-mIgG treatment elicited the highest response, but the only significant difference between the groups was observed at baseline because the IO-mIgG group had significantly higher estimates compared to the mIgG group. Different lowercase letters (a-b) within one time point represent significant differences ($p < 0.05$) between the treatment groups. Different uppercase letters (A-C) represent significant changes between days for each treatment group.

For the γδ T cell+ CD8- response factor, a significant fixed effect of collection time was observed in the primary response study (see Table 95). The estimates increased significantly by day 0.25 and peaked on day 1, but they decreased significantly already on day 2. However, the estimates were still significantly higher on day 7 compared to baseline.

In the memory response study, a significant effect of both collection time and treatment, but not their interaction, was observed of the γδ T cell+ CD8- response factor (see Table 96). The highest response was observed on day 0.25, and it started decreasing significantly after day 1. On day 5, the response was no longer significantly higher compared to baseline.

Table 95. *γδ T cell+ CD8- estimates by day, Study B primary response*

Response Factor	Days	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
γδ T cell+ CD8-	0	0.69 ± 0.14	a
	0.25	4.63 ± 1.47	b
	1	5.87 ± 0.90	c
	2	2.08 ± 0.23	b
	3	2.20 ± 0.46	bd
	4	1.66 ± 0.20	b
	5	1.32 ± 0.17	d
	7	1.31 ± 0.28	d

Note: different letters (a-d) represent significant differences ($p < 0.05$) between days.

Table 96. *γδ T cell+ CD8- estimates by day and treatment group, Study B memory response*

Response Factor	Fixed Effect	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
γδ T cell+ CD8-	*Days*		
	0	0.70 ± 0.18	a
	0.25	7.88 ± 1.48	b
	1	5.99 ± 0.65	b
	2	2.55 ± 0.25	c
	3	2.38 ± 0.26	c
	4	1.13 ± 0.15	d
	5	1.17 ± 0.25	ad
	Treatment		
	Alum-mIgG	3.88 ± 0.29	a
	IO-mIgG	2.78 ± 0.29	b
	mIgG	2.69 ± 0.29	b

Note: different letters (a-d) represent significant differences ($p < 0.05$) between days and between treatment groups.

In the memory response study, significant changes by day for both γδ T cell+ CD8+ and γδ T cell+ CD8- estimates were observed in the PBS control group (see Table 97). The γδ T cell+ CD8+ were significantly higher compared to baseline on days 1, 2, and 4. The γδ T cell+ CD8- estimates were significantly higher compared to baseline on days 1 and 2.

Table 97. *γδ T cell+ CD8+ and CD8- estimates by day, Study B memory response (PBS)*

Response Factor	Days	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
γδ T cell+ CD8+	0	0.15 ± 0.03	a
	0.25	0.27 ± 0.06	a
	1	1.01 ± 0.18	b
	2	0.62 ± 0.09	b
	3	0.77 ± 0.23	ab
	4	0.62 ± 0.08	b
	5	0.48 ± 0.15	ab
γδ T cell+ CD8-	0	0.65 ± 0.13	a
	0.25	1.96 ± 0.63	ab
	1	2.84 ± 0.28	b
	2	1.84 ± 0.35	b
	3	1.55 ± 0.52	ab
	4	1.29 ± 0.26	a
	5	1.54 ± 0.54	ab

Note: different letters (a-e) represent significant differences ($p < 0.05$) between days for each response factor.

The αβ1 T cell+ response factor estimates changed significantly only by day in the primary response study (see Table 98). The estimates increased significantly by day 0.25 and achieved the highest values on days 1 and 2, after which they continued decreasing. The estimates were still significantly higher on day 7 compared to baseline estimates.

In the memory response study treatment groups and the PBS control group, the αβ1 T cell+ response factor also changed significantly by day only (see Table 99). In the treatment groups, the response was significant by day 0.25 and peaked on day 1. The estimates continued declining, but they were still significantly higher compared to baseline estimates. In the control group, the estimates increased by day 1, and they remained similar until day 5, when they were still significantly higher compared to baseline values.

Table 98. *αβ1 T cell+ estimates by day, Study B primary response*

Response Factor	Days	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
αβ1 T cell+	0	2.20 ± 0.19	a
	0.25	3.95 ± 0.58	b
	1	10.63 ± 1.06	c
	2	10.18 ± 0.72	c
	3	7.33 ± 0.81	d
	4	6.61 ± 0.87	d
	5	5.17 ± 0.84	b
	7	4.60 ± 0.72	b

Note: different letters (a-d) represent significant differences ($p < 0.05$) between days.

Table 99. *αβ1 T cell+ estimates by day, Study B memory response*

Response Factor	Days	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
αβ1 T cell+	0	1.89 ± 0.21	a
	0.25	5.74 ± 0.51	b
	1	11.29 ± 0.85	c
	2	9.43 ± 0.81	cd
	3	8.09 ± 0.79	df
	4	4.22 ± 0.52	e
	5	6.19 ± 0.46	bf
αβ1 T cell+ (PBS)	0	1.99 ± 0.29	a
	0.25	2.04 ± 0.17	a
	1	8.76 ± 1.20	b
	2	6.99 ± 1.06	b
	3	8.07 ± 2.94	ab
	4	6.49 ± 0.74	b
	5	7.04 ± 1.05	b

Note: different letters (a-f) represent significant differences ($p < 0.05$) between days for each response factor.

In the primary response study, no significant fixed effects were observed for αβ1 T cell+ CD4+ CD8+, whereas the αβ1 T cell+ CD4- CD8+, αβ1 T cell+ CD4- CD8-, and αβ1 T cell+ CD4+ CD8- response factors all changed significantly only by day (see Table 100). The αβ1 T cell+ CD4- CD8+ estimates increased significantly by day 0.25 and achieved the highest values on days 1 and 2. By day 3, the estimates decreased significantly compared to day 2, but they were still significantly higher compared to baseline on day 7. The αβ1 T cell+ CD4- CD8- estimates also increased significantly by day 0.25, but they achieved their highest values on day

3. Although their estimates decreased significantly after day 3, the estimates were still significantly higher on day 7 compared to baseline. The αβ1 T cell+ CD4+ CD8- estimates increased significantly by day 0.25 and continued to increase until they peaked on days 1 and 2. Although they started decreasing significantly after day 2, their estimates were significantly higher on day 7 compared to baseline.

Table 100. *αβ1 T cell+ CD4- CD8+, CD4- CD8-, and CD4+ CD8- estimates by day, Study B primary response*

Response Factor	Days	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
αβ1 T cell+ CD4- CD8+	0	0.34 ± 0.09	a
	0.25	1.04 ± 0.09	b
	1	2.45 ± 0.35	c
	2	2.52 ± 0.31	cd
	3	1.64 ± 0.20	d
	4	1.08 ± 0.17	b
	5	1.25 ± 0.16	bd
	7	1.31 ± 0.19	bd
αβ1 T cell+ CD4- CD8-	0	0.03 ± 0.01	a
	0.25	0.23 ± 0.05	b
	1	0.26 ± 0.03	b
	2	0.22 ± 0.03	b
	3	0.80 ± 0.11	c
	4	0.24 ± 0.04	b
	5	0.27 ± 0.03	b
	7	0.29 ± 0.04	b
αβ1 T cell+ CD4+ CD8-	0	0.97 ± 0.19	a
	0.25	2.44 ± 0.44	b
	1	7.15 ± 0.60	c
	2	7.03 ± 0.50	c
	3	4.59 ± 0.56	de
	4	4.93 ± 0.66	e
	5	3.32 ± 0.58	bd
	7	2.74 ± 0.46	b

Note: different letters (a-e) represent significant differences ($p < 0.05$) between days for each response factor.

In the memory response study, αβ1 T cell+ CD4- CD8+, αβ1 T cell+ CD4+ CD8+, and αβ1 T cell+ CD4+ CD8- estimates changed significantly by day in the treatment groups (see Table 101). The αβ1 T cell+ CD4- CD8+ estimates increased significantly by day 0.25 and returned to values similar to those observed at baseline on day 4, but they increased significantly again on day 5. The αβ1 T cell+ CD4+ CD8+ estimates increased significantly by day 1. The estimates were no longer significantly higher compared to baseline on day 4, but they increased significantly again on day 5. The αβ1 T cell+ CD4+ CD8- estimates increased significantly by day 0.25 and peaked on day 1, after which they started decreasing. However, they were still significantly higher on day 5 compared to baseline estimates. Although a high αβ1 T cell+ response was observed in the PBS control group, only the αβ1 T cell+ CD4+ CD8- changed significantly by day (see Table 101). A significant increase compared to baseline occurred on day 1, and no significant changes were observed after day 1. The response was significantly higher compared to baseline only on days 1, 2, and 4.

Table 101. *αβ1 T cell+ CD4- CD8+, CD4+ CD8+, and CD4+ CD8- estimates by day, Study B memory response*

Response Factor	Days	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
αβ1 T cell+	0	0.82 ± 0.16	a
CD4- CD8+	0.25	1.64 ± 0.23	b
	1	2.91 ± 0.29	c
	2	2.39 ± 0.19	cd
	3	1.88 ± 0.18	bd
	4	1.05 ± 0.15	a
	5	2.75 ± 0.26	c
αβ1 T cell+	0	0.28 ± 0.03	a
CD4+ CD8+	0.25	0.30 ± 0.05	a
	1	0.82 ± 0.17	bc
	2	0.83 ± 0.13	bc
	3	0.75 ± 0.17	bc
	4	0.59 ± 0.15	ac
	5	1.15 ± 0.13	b
αβ1 T cell+	0	0.72 ± 0.17	a
CD4+ CD8-	0.25	3.76 ± 0.36	b
	1	7.46 ± 0.79	c
	2	6.25 ± 0.71	ce
	3	5.02 ± 0.52	be
	4	2.08 ± 0.29	d
	5	2.31 ± 0.47	d
αβ1 T cell+	0	0.73 ± 0.13	a
CD4+ CD8- (PBS)	0.25	0.79 ± 0.16	a
	1	4.95 ± 0.57	b
	2	4.17 ± 0.64	b
	3	4.22 ± 1.80	ab
	4	4.07 ± 0.42	b
	5	3.47 ± 1.14	ab

Note: different letters (a-e) represent significant differences ($p < 0.05$) between days for each response factor.

For the αβ1 T cell+ CD4- CD8- response in the memory response study, a significant interaction between treatment and time of collection was observed (see Table 102). The treatment main effect was significant for the Alum-mIgG and the IO-mIgG group, but not for the mIgG group. The day main effect was not significant for any collection time, and the only difference observed between the groups was at baseline because the IO-mIgG group had significantly higher αβ1 T cell+ CD4- CD8- estimates compared to the Alum-mIgG group (see Figure 29).

Table 102. *Treatment and day interaction effect estimates and main effects p-values of αβ1 T cell+ CD4- CD8-, Study B memory response*

Day	Treatment			Day main effect P-value
	Alum-mIgG ($\bar{x} \pm SE$, n = 4)	**IO-mIgG ($\bar{x} \pm SE$, n = 4)**	**mIgG ($\bar{x} \pm SE$, n = 4)**	
0	0.24 ± 0.05	0.42 ± 0.05	0.30 ± 0.05	0.0590
0.25	0.25 ± 0.05	0.14 ± 0.05	0.28 ± 0.05	0.1268
1	0.53 ± 0.15	0.37 ± 0.15	0.36 ± 0.15	0.6931
2	0.53 ± 0.11	0.35 ± 0.11	0.36 ± 0.11	0.4917
3	0.92 ± 0.13	0.59 ± 0.13	0.53 ± 0.13	0.1321
4	0.64 ± 0.23	0.86 ± 0.23	0.59 ± 0.23	0.6874
5	0.91 ± 0.18	0.74 ± 0.18	0.68 ± 0.18	0.6413
Treatment main effect P-value	0.0076	0.0195	0.2109	

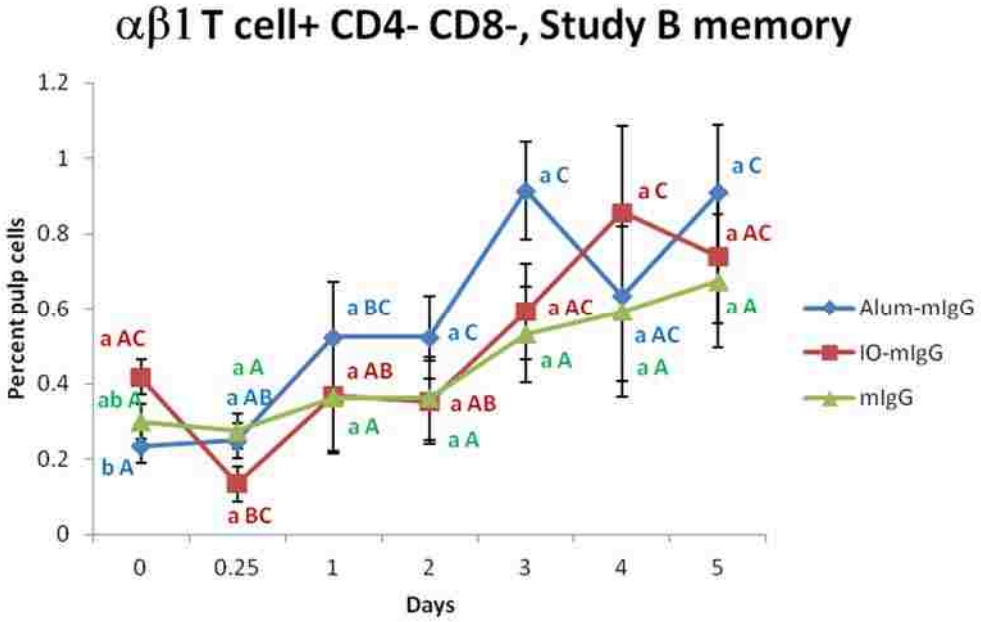

Figure 29. αβ1 T cell+ CD4- CD8- estimates by day and treatment group, Study B memory response.

The graph shows mean differences of the treatment*day effect, not the main effect. The Alum-mIgG treatment elicited the highest response, but the only significant difference between the groups was observed at baseline because the IO-mIgG group had significantly higher estimates compared to the Alum-mIgG group. Different lowercase letters (a-b) within one time point represent significant differences ($p < 0.05$) between the treatment groups. Different uppercase letters (A-C) represent significant changes between days for each treatment group.

In the primary response study, the αβ2 T cell+ response factor estimates changed significantly by day only (see Table 103). In Study 3, the αβ2 T cell+ estimates increased significantly by day 0.25 and peaked on day 1. The estimates started decreasing significantly after day 2, but they were still significantly higher on day 7 compared to baseline.

In the memory response study, the αβ2 T cell+ response factor estimates also changed significantly only by day in the treatment groups and the PBS control group (see Table 104). In

the treatment groups, the response was significant by day 0.25 and the estimates peaked on day 1. The decrease after day 1 continued until day 4, and the estimates increased significantly again on day 5. In the PBS control group, the estimates increased significantly by day 0.25, and no significant differences between collection times were observed after day 1.

Table 103. *αβ2 T cell+ estimates by day, Study B primary response*

Response Factor	Days	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
αβ2 T cell+	0	0.98 ± 0.06	a
	0.25	2.07 ± 0.27	b
	1	4.61 ± 0.46	c
	2	3.67 ± 0.32	cd
	3	2.93 ± 0.33	e
	4	3.31 ± 0.52	de
	5	1.71 ± 0.28	b
	7	1.81 ± 0.30	b

Note: different letters (a-d) represent significant differences ($p < 0.05$) between days for each response factor.

Table 104. *αβ2 T cell+ estimates by day, Study B memory response*

Response Factor	Days	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
αβ2 T cell+	0	0.75 ± 0.11	a
	0.25	2.87 ± 0.28	b
	1	4.63 ± 0.53	c
	2	3.81 ± 0.21	c
	3	3.63 ± 0.31	bc
	4	1.96 ± 0.22	d
	5	4.28 ± 0.21	c
αβ2 T cell+ (PBS)	0	0.69 ± 0.10	a
	0.25	1.39 ± 0.14	b
	1	2.94 ± 0.73	abc
	2	2.72 ± 0.56	bc
	3	2.57 ± 0.67	bc
	4	2.10 ± 0.10	c
	5	3.40 ± 0.18	c

Note: different letters (a-d) represent significant differences ($p < 0.05$) between days for each response factor.

In the primary response study, no significant changes were observed in αβ2 T cell+ CD4- CD8- estimates, but a significant fixed effect of collection time was observed in the memory response study treatment groups (see Table 105). The increase was significant by day 0.25, after which the estimates decreased significantly on day 2. However, further significant increases were observed after day 2 and the αβ2 T cell+ CD4- CD8- estimates on day 5 were significantly higher compared to the previous days.

Table 105. *αβ2 T cell+ CD4- CD8- estimates by day, Study B memory response*

Response Factor	Days	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
αβ2 T cell+ CD4- CD8-	0	0.10 ± 0.01	a
	0.25	0.49 ± 0.08	b
	1	0.32 ± 0.10	bc
	2	0.22 ± 0.03	c
	3	0.31 ± 0.03	b
	4	0.36 ± 0.05	b
	5	0.79 ± 0.11	d

Note: different letters (a-d) represent significant differences ($p < 0.05$) between days.

The αβ2 T cell+ CD4- CD8+ estimates changed significantly only by day in the primary response study (see Table 106) and in the memory response study treatment groups (see Table 107). In the primary response study, αβ2 T cell+ CD4- CD8+ estimates increased significantly by day 0.25 and peaked on day 1, after which they started decreasing significantly. However, the estimates on day 7 were still significantly higher compared to baseline. In the memory response study, a significant increase in αβ2 T cell+ CD4- CD8+ estimates was observed by day 0.25, and the response remained significantly higher compared to baseline on day 5.

Table 106. *αβ2 T cell+ CD4- CD8+ estimates by day, Study B primary response*

Response Factor	Days	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
αβ2 T cell+	0	0.22 ± 0.04	a
CD4- CD8+	0.25	0.78 ± 0.08	b
	1	1.49 ± 0.21	c
	2	1.02 ± 0.14	b
	3	0.68 ± 0.10	d
	4	1.03 ± 0.15	bd
	5	0.42 ± 0.07	e
	7	0.48 ± 0.09	e

Note: different letters (a-d) represent significant differences ($p < 0.05$) between days for each response factor.

Table 107. *αβ2 T cell+ CD4- CD8+ estimates by day, Study B memory response*

Response Factor	Days	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
αβ2 T cell+	0	0.18 ± 0.02	a
CD4- CD8+	0.25	0.99 ± 0.12	bc
	1	1.33 ± 0.14	ce
	2	1.17 ± 0.11	bc
	3	0.91 ± 0.09	b
	4	0.59 ± 0.08	d
	5	1.84 ± 0.24	e

Note: different letters (a-d) represent significant differences ($p < 0.05$) between days for each response factor.

The αβ2 T cell+ CD4+ CD8- estimates in the primary response study showed a significant fixed effect of the interaction between treatment and time of collection. The treatment main effect was significant for all treatment groups, and the day main effect was significant only on day 1 (see Table 108). The only significant difference between the groups was observed on day 1 as the mIgG group elicited a significantly higher response compared to the other groups (see Figure 30).

Table 108. *Treatment and day interaction effect estimates and main effects p-values of αβ2 T cell+ CD4+ CD8-, Study B primary response*

Day	Treatment			Day main effect P-value
	Alum-mIgG ($\bar{x} \pm SE$, n = 4)	IO-mIgG ($\bar{x} \pm SE$, n = 4)	mIgG ($\bar{x} \pm SE$, n = 4)	
0	0.26 ± 0.10	0.24 ± 0.10	0.33 ± 0.10	0.8288
0.25	1.05 ± 0.26	1.06 ± 0.26	0.45 ± 0.26	0.2326
1	2.25 ± 0.35	1.70 ± 0.35	3.55 ± 0.35	0.0121
2	2.30 ± 0.39	2.03 ± 0.39	2.49 ± 0.39	0.7114
3	2.12 ± 0.38	1.29 ± 0.38	2.18 ± 0.38	0.2291
4	2.25 ± 0.65	2.20 ± 0.65	1.23 ± 0.65	0.4817
5	1.03 ± 0.27	0.88 ± 0.27	0.89 ± 0.27	0.9038
7	0.94 ± 0.31	1.19 ± 0.31	0.63 ± 0.31	0.4731
Treatment main effect P-value	0.0002	0.0003	0.0001	

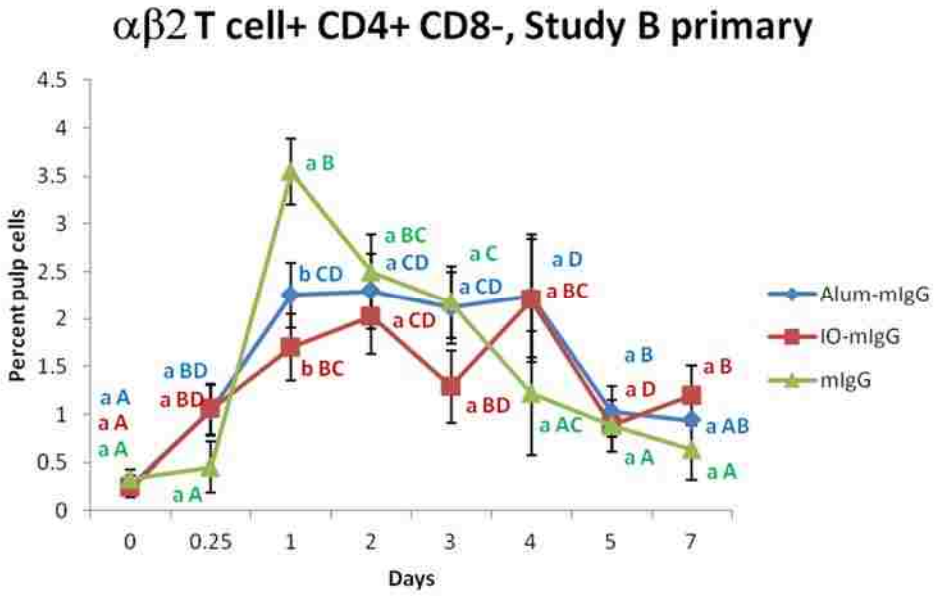

Figure 30. αβ2 T cell+ CD4+ CD8- estimates by day and treatment group, Study B primary response.

The graph shows mean differences of the treatment*day effect, not the main effect. The mIgG treatment elicited the highest response, and it was significantly higher compared to the other groups on day 1. Different lowercase letters (a-b) within one time point represent significant differences ($p < 0.05$) between the treatment groups. Different uppercase letters (A-C) represent significant changes between days for each treatment group.

The αβ2 T cell+ CD4+ CD8- estimates in the treatment and control groups of the memory response study changed significantly only by day (see Table 109). In the treatment groups, the response increased significantly by day 0.25 and peaked on day 1, after which it started decreasing. The estimates on day 5 were still significantly higher compared to baseline. In the control group, the estimates increased significantly compared to baseline on day 1. No significant changes in estimates were observed after day 1, and they were no longer significantly higher compared to baseline on day 5.

Table 109. *αβ2 T cell+ CD4+ CD8- estimates by day, Study B memory response*

Response Factor	Days	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
αβ2 T cell+	0	0.33 ± 0.09	a
CD4+ CD8-	0.25	1.37 ± 0.19	b
	1	2.68 ± 0.43	c
	2	2.22 ± 0.26	c
	3	1.85 ± 0.19	b
	4	0.68 ± 0.12	d
	5	0.84 ± 0.15	d
αβ2 T cell+	0	0.20 ± 0.03	a
CD4+ CD8- (PBS)	0.25	0.18 ± 0.04	a
	1	1.47 ± 0.29	b
	2	1.21 ± 0.24	b
	3	1.11 ± 0.50	ab
	4	1.15 ± 0.09	b
	5	1.13 ± 0.38	ab

Note: different letters (a-d) represent significant differences ($p < 0.05$) between days for each response factor.

A significant fixed effect of the interaction between treatment and collection time was observed for the αβ2 T cell+ CD4+ CD8+ response in the primary response study (see Table 110). The main effect of treatment was significant in the Alum-mIgG and IO-mIgG groups, but not in the mIgG group. The day main effect was not significant for any time of sample collection as no significant differences between the groups were observed by day (see Figure 31).

Table 110. *Treatment and day interaction effect estimates and main effects p-values of αβ2 T cell+ CD4+ CD8+, Study B primary response*

Day	Treatment			Day main effect P-value
	Alum-mIgG ($\bar{x} \pm SE$, n = 4)	IO-mIgG ($\bar{x} \pm SE$, n = 4)	mIgG ($\bar{x} \pm SE$, n = 4)	
0	0.88 ± 0.15	0.84 ± 0.15	0.71 ± 0.15	0.7376
0.25	0.24 ± 0.15	0.35 ± 0.15	0.56 ± 0.15	0.3362
1	0.68 ± 0.25	0.72 ± 0.25	0.58 ± 0.25	0.9183
2	0.55 ± 0.13	0.41 ± 0.13	0.31 ± 0.13	0.4754
3	0.43 ± 0.13	0.39 ± 0.13	0.29 ± 0.13	0.7355
4	0.49 ± 0.11	0.36 ± 0.11	0.30 ± 0.11	0.5144
5	0.44 ± 0.12	0.26 ± 0.12	0.40 ± 0.12	0.5699
7	0.42 ± 0.14	0.47 ± 0.14	0.35 ± 0.14	0.8231
Treatment main effect P-value	0.0045	0.0198	0.7336	

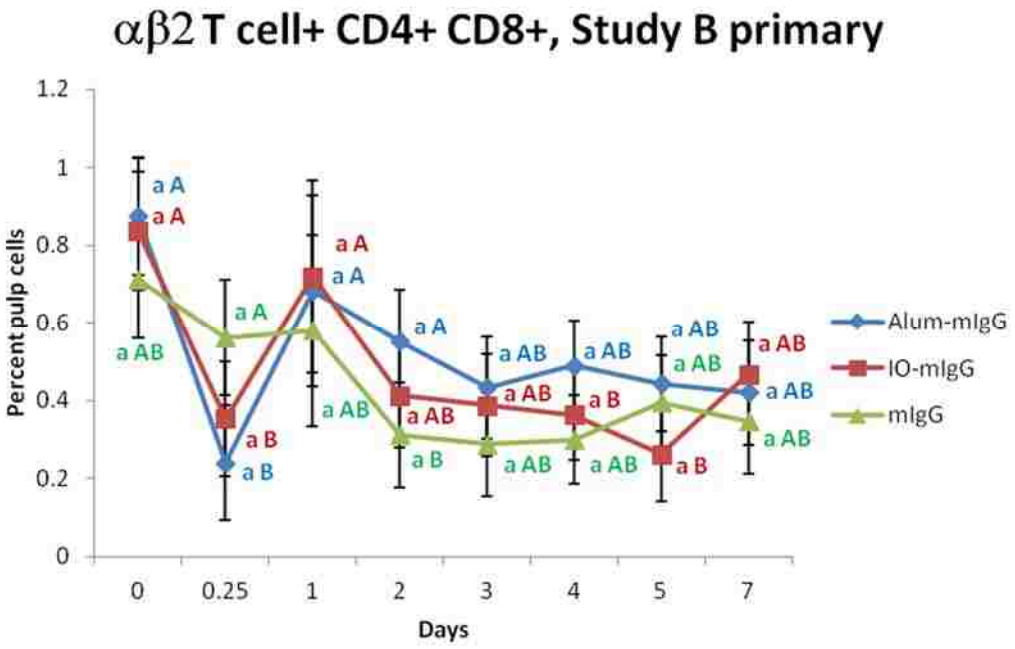

Figure 31. αβ2 T cell+ CD4+ CD8+ estimates by day and treatment group, Study B primary response.

The graph shows mean differences of the treatment*day effect, not the main effect. No significant differences were observed between the groups. Different lowercase letters (a-b) within one time point represent significant differences ($p < 0.05$) between the treatment groups. Different uppercase letters (A-C) represent significant changes between days for each treatment group.

In the memory response study, the αβ2 T cell+ CD4+ CD8+ response changed significantly by day, but only in the treatment groups (see Table 111). The estimates increased significantly by day 1 and remained consistent until day 4. On day 5, another significant increase compared to previous days was observed.

Table 111. *αβ2 T cell+ CD4+ CD8+ estimates by day, Study B memory response*

Response Factor	Days	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
αβ2 T cell+	0	0.21 ± 0.03	a
CD4+ CD8+	0.25	0.18 ± 0.01	a
	1	0.37 ± 0.07	b
	2	0.42 ± 0.06	b
	3	0.45 ± 0.09	b
	4	0.42 ± 0.13	ab
	5	1.21 ± 0.18	c

Note: different letters (a-d) represent significant differences ($p < 0.05$) between days for each response factor.

B Cell Receptors

In the primary response study, the effect of collection time was significant for B cell+ IgM+, which increased significantly by day 1 and continued to increase until their estimates peaked on day 3 (see Table 112). Although the estimates started decreasing significantly after day 3, the estimates on day 7 were still significantly higher compared to the estimates at baseline.

Table 112. *B cell+ IgM+ estimates by day, Study B primary response*

Response Factor	Days	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
B cell+ IgM+	0	0.26 ± 0.10	a
	0.25	0.52 ± 0.13	a
	1	3.67 ± 0.44	b
	2	8.16 ± 1.28	c
	3	11.14 ± 1.70	d
	4	9.47 ± 1.54	cd
	5	4.75 ± 0.63	b
	7	1.66 ± 0.28	e

Note: different letters (a-e) represent significant differences ($p < 0.05$) between days.

In the memory response study, a significant effect of the interaction between collection time and treatment was observed for the B cell+ IgM+ response factor. The treatment main effect was significant in IO-mIgG and mIgG groups, and the collection time main effect was significant only on day 3 (see Table 113). The only significant difference between the groups was observed on day 3 as the response was significantly higher in the IO-mIgG group compared to the Alum-mIgG group, but not compared to the mIgG group (see Figure 32).

Table 113. *Treatment and day interaction effect estimates and main effects p-values of B cell+ IgM+ cells, Study B memory response*

Day	Treatment			Day main effect P-value
	Alum-mIgG ($\bar{x} \pm SE$, n = 4)	IO-mIgG ($\bar{x} \pm SE$, n = 4)	mIgG ($\bar{x} \pm SE$, n = 4)	
0	0.16 ± 0.10	0.34 ± 0.10	0.06 ± 0.10	0.1897
0.25	0.93 ± 0.25	1.17 ± 0.25	1.30 ± 0.25	0.5915
1	11.99 ± 4.96	13.11 ± 4.96	8.15 ± 4.96	0.7656
2	9.73 ± 2.52	13.18 ± 2.52	10.21 ± 2.52	0.5962
3	6.52 ± 2.02	15.79 ± 2.02	11.05 ± 2.02	0.0307
4	2.80 ± 0.99	3.30 ± 0.99	0.98 ± 0.99	0.2663
5	1.41 ± 0.81	3.35 ± 0.81	1.88 ± 0.81	0.2642
Treatment main effect P-value	0.0904	0.0084	0.0020	

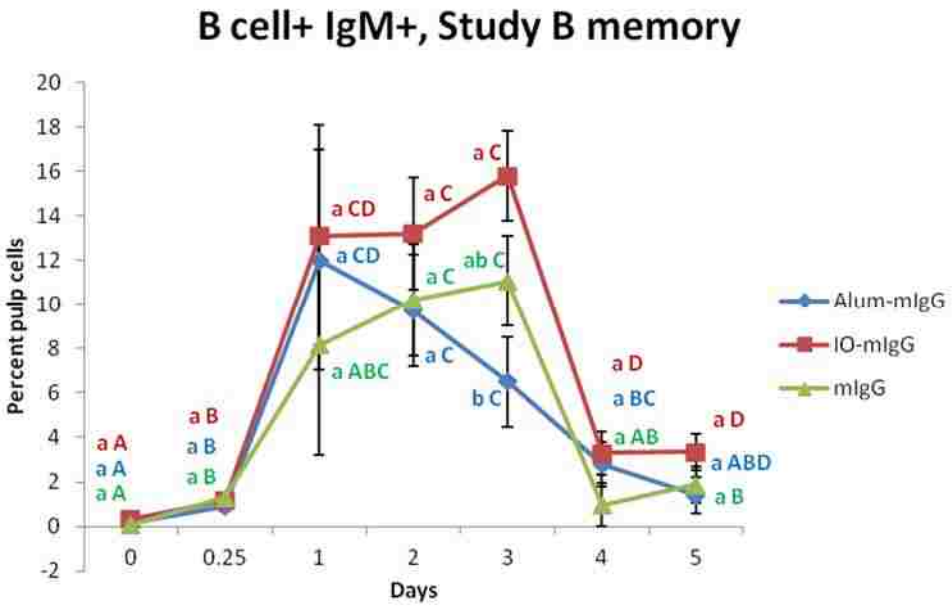

Figure 32. B cell+ IgM+ estimates by day and treatment group, Study B memory response.

The graph shows mean differences of the treatment*day effect, not the main effect. IO-mIgG elicited the strongest response, and it was significantly higher compared to the Alum-mIgG group on day 3, but not compared to the mIgG group. Different lowercase letters (a-b) within one time point represent significant differences ($p < 0.05$) between the treatment groups. Different uppercase letters (A-D) represent significant changes between days for each treatment group.

The B cell+ IgM+ estimates also changed significantly by day in the PBS group (see Table 114). Significant differences compared to baseline were observed on days 1, 2, and 4. Unlike the response in the treatment groups, which showed decreases in IgM+ estimates after day 1 or 3, the response in the control group increased progressively until day 5.

Table 114. *B cell+ IgM+ estimates by day, Study B memory response (PBS)*

Response Factor	Days	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
B cell+ IgM+	0	0.16 ± 0.05	a
	0.25	0.18 ± 0.06	a
	1	3.85 ± 0.36	b
	2	3.69 ± 0.91	b
	3	4.37 ± 1.58	ab
	4	7.05 ± 1.05	b
	5	9.47 ± 3.43	ab

Note: different letters (a-e) represent significant differences ($p < 0.05$) between days.

B cell+ IgM- cells were significantly affected by both collection time and treatment type in the primary response study, but not by their interaction (see Table 115). Their estimates increased significantly by day 0.25, and continued increasing until they peaked on day 2. The B cell+ IgM- estimates remained consistent until day 7, when they reduced significantly compared to day 5, but they were still significantly higher compared to baseline estimates. The strongest response was observed in the mIgG group, and it was significantly higher compared to response observed in the Alum-mIgG group, but not compared to the IO-mIgG group.

In the memory response study, B cell+ IgM- estimates changed significantly by day in the treatment groups and the control group (see Table 116). In the treatment groups, the response increased significantly by day 1 and remained elevated until day 4, when a significant decrease was observed. The estimates were no longer significantly higher on day 5 compared to baseline estimates. In the control group, significantly higher estimates compared to baseline were observed on days 1, 2, and 4. Unlike the response observed in the treatment groups, the response in the control group progressively increased from baseline until day 5.

Table 115. *B cell+ IgM- estimates by day and treatment group, Study B primary response*

Response Factor	Fixed Effect	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
B cell+ IgM-	*Days*		
	0	0.05 ± 0.01	a
	0.25	0.16 ± 0.04	b
	1	0.95 ± 0.12	c
	2	1.96 ± 0.40	d
	3	1.58 ± 0.26	cd
	4	1.66 ± 0.26	d
	5	1.29 ± 0.17	cd
	7	0.52 ± 0.10	e
	Treatment		
	Alum-mIgG	0.69 ± 0.18	a
	IO-mIgG	0.95 ± 0.18	ab
	mIgG	1.43 ± 0.18	b

Note: different letters (a-e) represent significant differences ($p < 0.05$) between days and between treatment groups.

Table 116. *B cell+ IgM- estimates by day, Study B memory response*

Response Factor	Days	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
B cell+ IgM-	0	0.28 ± 0.06	ac
	0.25	0.23 ± 0.04	a
	1	2.57 ± 0.59	b
	2	1.79 ± 0.43	b
	3	2.21 ± 0.41	b
	4	0.61 ± 0.14	c
	5	0.42 ± 0.13	ac
B cell+ IgM- (PBS)	0	0.10 ± 0.04	a
	0.25	0.03 ± 0.01	a
	1	0.72 ± 0.13	b
	2	0.68 ± 0.12	b
	3	0.89 ± 0.36	ab
	4	1.01 ± 0.18	b
	5	1.27 ± 0.48	ab

Note: different letters (a-e) represent significant differences ($p < 0.05$) between days.

In the primary response study, no significant changes were observed in B cell+ IgG+ estimates, but a significant fixed effect of collection time was observed in the treatment groups of the memory response study (see Table 117). The response was significant by day 0.25, and the estimates remained significantly higher compared to baseline estimates on subsequent sample collection times.

Table 117. *B cell+ IgG+ estimates by day, Study B memory response*

Response Factor	Days	Estimate ($\bar{x} \pm SE$)	Difference of Least Squares Means
B cell+ IgG+	0	0.01 ± 0.002	a
	0.25	0.11 ± 0.04	b
	1	0.40 ± 0.12	bcd
	2	0.22 ± 0.03	c
	3	0.47 ± 0.08	d
	4	0.22 ± 0.05	c
	5	0.15 ± 0.04	bc

Note: different letters (a-e) represent significant differences ($p < 0.05$) between days.

The interaction between time of collection and treatment type was significant for B cell+ IgG- cells in the primary response study (see Table 118). The main effect of treatment was significant for all treatment groups, whereas the main effect of collection time was significant only on day 5. However, significant differences between the groups were observed between days 3 and 5. On day 3, the estimates were significantly higher in the mIgG group compared to the Alum-mIgG group, but not compared to the IO-mIgG group. On day 4, the estimates were significantly higher on day 4 in the IO-mIgG group compared to the Alum-mIgG group, but not compared to the mIgG group. On day 5, both IO-mIgG and mIgG groups had significantly higher IgG- estimates compared to the Alum-mIgG group (see Figure 33).

Table 118. *Treatment and day interaction effect estimates and main effects p-values of B cell+ IgG- cells, Study B primary response*

Day	Treatment			Day main effect P-value
	Alum-mIgG ($\bar{x} \pm SE$, n = 4)	IO-mIgG ($\bar{x} \pm SE$, n = 4)	mIgG ($\bar{x} \pm SE$, n = 4)	
0	0.30 ± 0.18	0.31 ± 0.18	0.37 ± 0.18	0.9595
0.25	0.59 ± 0.28	0.86 ± 0.28	0.33 ± 0.28	0.4245
1	4.52 ± 0.99	3.52 ± 0.99	6.19 ± 0.99	0.2091
2	8.12 ± 2.75	9.29 ± 2.75	12.76 ± 2.75	0.4922
3	10.05 ± 3.63	10.75 ± 3.63	21.94 ± 3.63	0.0809
4	6.40 ± 2.95	16.59 ± 2.95	8.47 ± 2.95	0.0824
5	2.00 ± 1.12	8.01 ± 1.12	5.59 ± 1.12	0.0129
7	2.21 ± 0.53	2.86 ± 0.53	1.44 ± 0.53	0.2199
Treatment main effect P-value	0.0394	0.0137	0.0012	

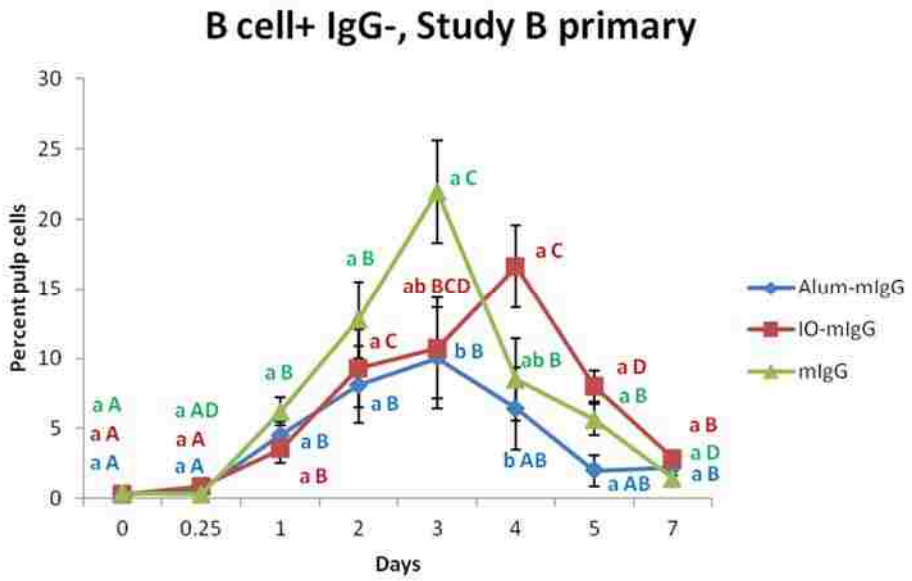

Figure 33. B cell+ IgG- estimates by day and treatment group, Study B primary response.

The graph shows mean differences of the treatment*day effect, not the main effect. The mIgG treatment elicited a stronger response on day 3 compared to the Alum-mIgG treatment, and the IO-mIgG treatment elicited a stronger response on day 4 compared to the Alum-mIgG treatment. Different lowercase letters (a-b) within one time point represent significant differences ($p < 0.05$) between the treatment groups. Different uppercase letters (A-D) represent significant changes between days for each treatment group.

In the memory response study treatment groups and the control group, significant changes by day were observed in the B cell+ IgG- estimates by day (see Table 119). In the treatment group, a significant response was observed by day 0.25, and the highest values were observed on days 1-3. A significant decrease was observed on days 4 and 5, but the estimates were still significantly higher on those days compared to baseline estimates. In the control group, significantly higher estimates compared to baseline were observed on days 1, 2, and 4. Unlike the response observed in the treatment groups, the response in the control group progressively increased from baseline until day 5.

Table 119. *B cell+ IgG- estimates by day, Study B memory response*

Response Factor	**Days**	**Estimate ($\bar{x} \pm SE$)**	**Difference of Least Squares Means**
B cell+ IgG-	0	0.41 ± 0.16	a
	0.25	1.42 ± 0.19	b
	1	12.12 ± 2.84	c
	2	12.88 ± 1.69	c
	3	12.19 ± 1.46	c
	4	2.83 ± 0.61	b
	5	2.24 ± 0.49	b
B cell+ IgG- (PBS)	0	0.38 ± 0.12	a
	0.25	0.16 ± 0.07	a
	1	4.70 ± 0.67	b
	2	4.26 ± 1.14	b
	3	5.12 ± 1.89	ab
	4	8.03 ± 0.98	b
	5	8.07 ± 2.82	ab

Note: different letters (a-e) represent significant differences ($p < 0.05$) between days.

Summary

In the primary response study, significant changes by day only were observed for 13 response factors. The fixed effect of treatment was significant only for the IgM- response factor because the estimates were significantly higher in the mIgG group compared to the Alum-mIgG group, but not compared to the IO-mIgG group.

The fixed effect of interaction between treatment and collection time was significant for the following three response factors: MHCII+ macrophages, αβ2 T cells+ CD4+ CD8+, and IgG-. However, no significant differences between the groups were observed for the αβ2 T cells+ CD4+ CD8+ response factor. The MHCII+ macrophage response was significantly higher in the Alum-mIgG group compared to the IO-mIgG group, but not compared to the mIgG group. The IgG- response was significantly higher in the IO-mIgG and mIgG groups compared to the Alum-mIgG group.

In the memory response study, time of collection was the only significant fixed effect for a total of 16 response factors. The fixed effect of treatment was significant only for the γδ T cell+ CD8- response factor. The highest estimates were observed in the Alum-mIgG group, and they were significantly higher compared to the other groups.

The interaction between treatment and time of collection in the memory response study was a significant fixed effect for the following 5 factors: MHCII+ macrophages, CD4+ CD8+, γδ T cell+ CD8+, αβ1 T cell+ CD4- CD8-, and B cell+ IgM+ response factors. However, no differences were observed in CD4+ CD8+ estimates between the groups, and the only differences observed for γδ T cell+ CD4- CD8+ and αβ1 T cell+ CD4- CD8- estimates were at baseline, not after the injections. Alum-mIgG elicited a significantly higher MHCII+

macrophage response compared to the other groups, but IO-mIgG and mIgG elicited a significantly higher IgM+ response compared to Alum-mIgG.

In the PBS control group of the memory response study, a total of 15 response factors changed significantly by day. The PBS group was not statistically differnet compared to the treatment groups, but the estimates observed indicate that the response in the control group was weaker, slower, and shorter in duration compared to the treatment groups. For most factors, significant changes compared to baseline were observed only on days 1, 2, and 4, whereas the treatment groups would typically elicit a strong response by day 0.25.

The difference of least squares means and the simple effect comparisons results are included in Appendix H for the primary response studies and Appendix J for the memory response studies.

Discussion

The purpose of the intramuscular injection studies was to demonstrate the feasibility of the chicken model as an *in vivo* test system for monitoring humoral and cell-mediated immune responses. The treatment preparations were injected into the chicken breast muscles, followed by feather injections of the recall Ag at the height of the primary immune response 10 days post-primary intramuscular immunization and at the heights of the secondary immune response 5 days post-secondary intramuscular immunization. The recall antigen injections recruited the immune cells to the growing feather pulp, so it was possible to monitor the immune responses periodically in each subject without using invasive methods to obtain samples for the analysis. Therefore, the growing feather in chickens is a suitable *in vivo* test site for observing the interactions between NPs and the immune system.

The second purpose of these studies was to compare the strength and duration of immune responses among Alum-mIgG, IO-mIgG, and mIgG treatment preparations. In Study A, the concentration of mIgG in the IONP group was 0.26 mg/ml, whereas a concentration of 5 mg/ml was used in the mIgG and Alum-mIgG groups. The treatment concentrations for all groups were matched in Study B by reducing the mIgG and Alum-mIgG treatment concentrations to 0. 26 mg/ml. It was hypothesized that IONPs would elicit a significantly higher immune response compared to the other groups.

In the Study A primary response, significant changes by day only were observed for 11 response factors, and the fixed effect of treatment was significant only for the γδ T cell+ CD8+ response factor. The γδ T cell+ CD8+ estimates in the IO-mIgG group were significantly higher compared to the Alum-mIgG group, but not compared to the mIgG group. The CD4+ CD8-, γδ T cell+ CD8-, and MHCII+ B cell+ response factors showed a significant fixed effect of the interaction. For the CD4+ CD8- estimates, the only significant difference was observed at baseline between the groups, but IO-mIgG elicited a significantly higher response for γδ T cell+ CD8- and MHCII+ B cell+ factors compared to other treatment preparations.

In the Study A memory response, significant changes by day only were observed for ten response factors. The fixed effect of treatment was significant for CD45+, CD4+ CD8-, αβ1 T cell+, and MHCII+ B cell+ response factors. The highest responses were observed in the IO-mIgG group, and they were significantly higher compared to the Alum-mIgG and mIgG groups.

These results indicate that IONPs are more efficient adjuvants compared to an aluminum-based adjuvant and antigen preparations alone for eliciting both primary and memory immune responses. However, the results of Study B were not consistent with the results observed in Study A. In the Study B primary response, significant changes by day only were observed for 13

response factors. The fixed effect of treatment was significant only for the IgM- response factor because the estimates were significantly higher in the mIgG group compared to the Alum-mIgG group, but not compared to the IO-mIgG group.

The fixed effect of interaction between treatment and collection time in the Study B primary response was significant for MHCII+ macrophages, αβ2 T cells+ CD4+ CD8+, and IgG- response factors. However, no significant differences between the groups were observed for the αβ2 T cells+ CD4+ CD8+ response factor. The MHCII+ macrophage response was significantly higher in the Alum-mIgG group compared to the IO-mIgG group, but not compared to the mIgG group. The B cell+ IgG- response was significantly higher in the IO-mIgG and mIgG groups compared to the Alum-mIgG group.

In the Study B memory response, time of collection was the only significant fixed effect for a total of 16 response factors. The fixed effect of treatment was significant only for the γδ T cell+ CD8- response factor. The highest estimates were observed in the Alum-mIgG group, and they were significantly higher compared to the other groups. The interaction between treatment and time of collection in the 2014 memory response study was a significant fixed effect for MHCII+ macrophages, CD4+ CD8+, γδ T cell+ CD8+, αβ1 T cell+ CD4- CD8-, and B cell+ IgM+ response factors. No differences were observed in CD4+ CD8+ estimates between the groups, and the only differences observed for γδ T cell+ CD8+ and αβ1 T cell+ CD4- CD8- estimates were at baseline, not after the injections. Alum-mIgG elicited a significantly higher MHCII+ macrophage response compared to the other groups, but IO-mIgG and mIgG elicited a significantly higher IgM+ response compared to Alum-mIgG.

The PBS memory response control group in Study A did not display significant changes by day in the observed response factors. However, the fixed effect of day was statistically

significant for 15 response factors in the PBS memory response control group. The PBS group was not statistically different compared to the treatment groups, but the estimates observed indicate that the response in the control group was not associated with the treatment preparation. For most factors, significant changes compared to baseline were observed only on days 1, 2, and 4, whereas the treatment groups would typically elicit a strong response by day 0.25. Strong responses in the PBS group usually occurred in a progressive trend, whereas the response would usually decrease after days 0.25 or 1 in the treatment groups.

The differences between Study A and Study B were not statistically analyzed, so it is not possible to determine whether the changes in mIgG concentration increased the responses in the mIgG and Alum-mIgG groups significantly. However, the reduction of mIgG concentration in the mIgG and Alum-mIgG groups from 5 mg/ml to 0.26 mg/ml could explain the differences between the two studies. It is possible that the high mIgG concentration in Study A resulted in low avidity because of the excess antigen, so it was easy for the antigen to dissociate from the antibodies and fail to elicit a significant immune response. Therefore, antigen excess in the Alum-mIgG and mIgG groups of Study A could explain why IO-mIgG elicitied significantly higher responses compared to the other groups. Once the mIgG concentrations were matched in Study B, it is possible that the immune responses for Alum-mIgG and mIgG treatments increased because antigen excess was no longer an issue.

Antigen disassociation is also possible in NPs, but conjugation was used in both studies to achieve a strong interaction between IONPs and mIgG. Conjugation is a surface modification achieved when an antigen is chemically cross-linked to the NPs. These types of links result in a strong interaction between the antigen and NPs, and they are less likely to disassociate *in vivo* compared to other NP modification methods, such as adsorption (Zhao et al., 2014). Once an

immune cell takes up an NP with a conjugated antigen, the NP dissolves and releases the antigen, which the innate immune cells can then present to the adaptive immune cells.

If the differences in elicited responses between Study A and Study B Alum-mIgG and mIgG groups are not statistically significant, it is possible that the different adaptive immune system activation pathways of IONPs could account for the differences observed between the studies. The IO-mIgG treatment in the feather injection studies demonstrated a high innate response, but failed to elicit a high adaptive response. In Study A, IO-mIgG elicited did not elicit higher innate responses compared to the other groups, and Study B demonstrated that no significant changes were observed in MHCII+ macrophage+ estimates in the primary response to IO-mIgG. The IO-mIgG treatment elicited significantly higher T cell responses compared to the other groups in Study A, but not in Study B.

Although IO-mIgG did not elicit significantly higher innate and T cell responses compared to the other groups in Study B, it elicited significantly higher B cell, B cell+ IgM+, and B cell+ IgG- responses compared to the other treatments. In the primary response of Study A, the B cell response was significantly higher on day 2 in the IO-mIgG groups compared to the other groups. In the primary response of Study B, the B cell+ IgG- response increased significantly in all groups. However, the response in the IO-mIgG group was still increasing and significantly higher compared to the Alum-mIgG group on day 4, when the response in the other groups was already decreasing. In the memory response of Study B, the B cell+ IgM+ response was significantly higher on day 4 compared to the Alum-mIgG group, but not compared to the mIgG group.

The membrane bound IgM and IgG antibodies were not analyzed in the sample used in Study A, but their plasma concentration was measured and analyzed by Wilson (2014). After the

primary immunization, IgG and IgM levels in the plasma increased in the mIgG and Alum-mIgG groups between days 7-10, and they were significantly higher compared to antibody levels of the IO-mIgG group. However, increased plasma levels of antibodies in the IO-mIgG group were observed after day 10, and they were significantly higher compared to the concentrations of other groups, which started declining after day 7 or 10. After the secondary immunizations, the plasma levels of IgG and IgM increased in all groups, but the increase was significantly higher in the IO-mIgG group compared to the other groups as the concentrations consistently increased until day 10.

The possibility of direct humoral immunity stimulation is expected of small nanoparticles (< 30 nm) because their small size enhances tissue penetration and allows them to reach the mural lymph nodes without the assistance of dendritic cells (Smith, Simon, & Baker Jr, 2013). Perhaps the 10 nm IONPs used in these studies were trapped in the feather pulp tissue when injected into the feathers, so they had to be cleared by macrophages or heterophils. However, the IONPs injected into the breast muscles were probably able to penetrate tissues because of their small size and reach the mural lymph nodes even without the interference of dendritic cells. This could explain why IONPs could activate B cells and B cell receptors more efficiently than the other treatments without causing significant increases of innate immune cells.

Another possible explanation of these findings is the spatial organization of antigen on the surface of IONPs. When spherical NPs are used, the conjugation of antigen to their surface results in a repetitive antigen display (Smith et al., 2013). This could elicit a high B cell receptor response because polyvalent antigens increase the avidity of interaction between antibody and antigen, allowing them to form large immune complexes and initiate inflammatory reactions.

These findings suggest that IO-mIgG can elicit an efficient and direct humoral immune response, but further research is necessary to optimize IONP-antigen treatment preparations for better results. The size of the IONPs used in this study (10 nm) is consistent with previous findings that suggest smaller particles allow the dendritic cells to take them up and present the antigen to the T and B cells in the mural lymph nodes (Xiang et al., 2006). Even if the dendritic cells fail to take up the small IONPs, the small size of the particles allows them to penetrate tissue membranes and reach the mural lymph nodes alone (Smith et al., 2013). However, drug delivery studies using IONPs demonstrated that the majority of the payload is released immediately upon *in vivo* injections (Mahmoudi, Sant, Wang, Laurent, & Sen, 2011). Perhaps the conjugated antigen can also be released if the concentration is too high relative to the size of the IONPs used or incompatible with the surface materials.

In the memory response of Study A, the PBS did not affect any of the monitored response factors, but a total of 15 significant responses were observed in the PBS group in the memory response of Study B. This difference between the two studies could be attributed to a confounding variable. Blalock (2005) demonstrated that the PBS injection alone is a stressor that can trigger the immune system, but injecting negligible amounts of corticotropin-releasing hormone after the injections amplified the immune responses significantly. The immune cells in the PBS control group in Study B did not behave like immune cells in the treatment groups, so it is possible that significant increases in immune cell estimates could be attributed to differences between the samples used in Study A and Study B PBS control groups rather than the injections. Specifically, elevated levels of stress response neurotransmitters in the Study A control could have affected the results. Future studies need to consider that immune responses to PBS are not

necessarily a placebo effect, and biological stress response factors should be considered as a possible confounding factor when interpreting findings.

An important limitation of these studies is that the doses were not calibrated for individual subjects. As demonstrated by Teeguarden et al. (2014), the toxicity levels of IONPs depend on the volume of the targeted organs. For the use of IONPs as adjuvants, it will be important to investigate various factors before clinical implementations are researched in humans, such as subject bodyweight and age.

These studies have important implications for future research and for guiding the development of clinical guidelines regarding the use of IONPs as vaccine adjuvants. The findings of these studies suggest that IONPs are promising adjuvants because they elicited a significantly higher humoral immune response compared to the other treatments. The majority of studies focused on determining how shape, size, surface modifications, and charge affect immune responses to IONPs (Gregory, Titball, & Williamson, 2013). However, none of the studies considered antigen concentration as a factor that determines the efficiency of IONPs as adjuvants. Before clinical applications and guidelines for the use of IONPs as adjuvants are developed, future studies need to determine how the size of the NPs determine optimal antigen concentrations so that the antigen can reach the mural lymph nodes and trigger an adaptive immune response. Other methods of achieving interactions between NPs and antigen should also be explored in future studies because it is possible that encapsulation could prove to be a more effective antigen delivery method compared to conjugation.

The tissue targeting ability of NPs also needs to be investigated in future studies. At the moment, the attempts to elicit an immune response using IONPs usually rely on uptake by macrophages or dendritic cells, which should then present the conjugated antigen in the mural

lymph nodes. However, the ability to target specific tissues and evade the innate immune cells would enable IONPs to reach the mural lymph nodes and present the antigen to T and B cells without the involvement of the innate response cells. Conventional adjuvants cannot use those methods to achieve the same objective, so the main advantage of IONPs is to trigger a faster adaptive response by directly presenting the antigen to the immune cells in the mural lymph nodes.

CONCLUSION

The feather injection studies demonstrated the advantages of the chicken model for studying IONP bioactivities *in vivo*. Injections of treatment preparations into the growing feather pulp resulted in an infiltration of leukocytes that was then monitored periodically for each individual subject. The model was minimally invasive as the samples for analyses can be obtained by plucking the growing feathers. The intramuscular injection studies also demonstrated that direct administrations of treatment preparations into the feather pulp are not required to monitor the responses using the growing feather pulp as tissue samples.

In the feather injection studies, IO-mIgG did not elicit a strong adaptive immune response, but heterophil blood concentration and MHCII+ macrophage pulp infiltration was significantly higher in the IO-mIgG group compared to the other groups. It is expected that those innate cells took up and cleared the IONPs without presenting the antigen to T or B cells. The CD4/CD8 ratio remained within normal ranges and returned to baseline values on day 4 in the IO-mIgG group of Study 3. Therefore, the application of IONPs could be considered safe, but more research will be required to better understand IONP clearance, degradation, and long-term toxicity *in vivo*.

In the intramuscular injection studies, the IO-mIgG treatment elicited a stronger immune response compared to the other treatment groups in Study A. However, the results of Study B were not consistent with the results observed in Study A. It is possible that the concentration of antigen in the Alum-mIgG and the mIgG groups was too high in Study A and could have resulted in antigen excess and low immune responses. Another possibility is that IO-mIgG was able to elicit a significant humoral immune response directly because the repetitive spatial organization of antigens on their surface increased the avidity of antibody and antigen

interactions. This could explain how IO-mIgG elicited a significantly higher IgG- and IgM+ responses in Study B compared to the Alum-mIgG group without eliciting significantly higher responses of other factors measured. The 10 nm IONPs used in these studies could also penetrate tissue because of their small size and reach the mural lymph nodes directly to deliver the antigen to antigen presenting cells. These results suggest that IONPs have the ability to elicit an adaptive response, but their efficacy can depend on various factors, including surface modifications, size, and antigen concentration.

The clinical utility of IONPs as vaccine adjuvants is promising, but needs to be further investigated. Future studies need to explore how antigen concentration relative to the size of the IONPs determines the efficacy of immune response elicitation so that the zone of equivalence can be reached *in vivo*. Surface modifications to prevent immediate releases of the conjugated antigen and other methods for establishing interactions between the IONPs and antigen should also be investigated to evaluate their efficacy. Although IONPs are already considered safe, longitudinal research will be necessary to monitor the long-term effects of IONP administration on individual subjects, as well as their effects on genetic traits of future generations.

References

Abbas, A. K., Lichtman, A. H. H., & Pillai, S. (2015). *Cellular and molecular immunology* (8th ed.). Philadelphia, PA: Saunders.

Abdul-Careem, M. F., Hunter, B. D., Sarson, A. J., Parvizi, P., Haghighi, H. R., Read, L., ... & Sharif, S. (2008). Host responses are induced in feathers of chickens infected with Marek's disease virus. *Virology*, *370*(2), 323-332.

Aguilar, Z. P., Aguilar, Y., Xu, H., Jones, B., Dixon, J., Xu, H., & Wang, Y. A. (2010). Nanomaterials in medicine. *ECS Transactions*, *33*(8), 69-74.

Alaamri, O., Byrne, K., Falcon, D., & Erf, G. (2014). In vivo monitoring of the immune response to mIgG conjugated to iron oxide nanoparticles using the pulp (dermis) of growing feathers as a test-tissue in chickens, (IRC8P. 495). *The Journal of Immunology*, *192*(S1), 190-23.

Albanese, A., Tang, P. S., & Chan, W. C. (2012). The effect of nanoparticle size, shape, and surface chemistry on biological systems. *Annual Review of Biomedical Engineering*, *14*, 1-16.

Amanna, I. J., Carlson, N. E., & Slifka, M. K. (2007). Duration of humoral immunity to common viral and vaccine antigens. *New England Journal of Medicine*, *357*(19), 1903-1915.

Batista-Duharte, A., Lindblad, E. B., & Oviedo-Orta, E. (2011). Progress in understanding adjuvant immunotoxicity mechanisms. *Toxicology Letters*, *203*(2), 97-105.

Blalock, J. E. (2005). The immune system as the sixth sense. *Journal of Internal Medicine*, *257*(2), 126-138.

Bourdon, J. A., Saber, A. T., Jacobsen, N. R., Jensen, K. A., Madsen, A. M., Lamson, J. S., ... & Vogel, U. B. (2012). Carbon black nanoparticle instillation induces sustained inflammation and genotoxicity in mouse lung and liver. *Particle and Fibre Toxicology*, *9*(5): 1-14.

Bowen, O. T., Erf, G. F., Chapman, M. E., & Wideman, R. F. (2007). Plasma nitric oxide concentrations in broilers after intravenous injections of lipopolysaccharide or microparticles. *Poultry Science*, *86*(12), 2550-2554.

Bridle, B. W., Julian, R., Shewen, P. E., Vaillancourt, J. P., & Kaushik, A. K. (2006). T lymphocyte subpopulations diverge in commercially raised chickens. *Canadian Journal of Veterinary Research*, *70*(3), 183.

Campbell, J. D., Cho, Y., Foster, M. L., Kanzler, H., Kachura, M. A., Lum, J. A., … Hessel, E. M. (2009). CpG-containing immunostimulatory DNA sequences elicit TNF-α–dependent

toxicity in rodents but not in humans. *The Journal of Clinical Investigation*, *119*(9), 2564–2576. doi:10.1172/JCI38294

Casella, C. R., & Mitchell, T. C. (2008). Putting endotoxin to work for us: monophosphoryl lipid A as a safe and effective vaccine adjuvant. *Cellular and Molecular Life Sciences*, *65*(20), 3231-3240.

Chao, Y., Makale, M., Karmali, P. P., Sharikov, Y., Tsigelny, I., Merkulov, S., ... & Simberg, D. (2012). Recognition of dextran–superparamagnetic iron oxide nanoparticle conjugates (Feridex) via macrophage scavenger receptor charged domains. *Bioconjugate Chemistry*, *23*(5), 1003-1009.

Chen, C. I. H., Cihak, J., Lösch, U., & Cooper, M. D. (1988). Differential expression of two T cell receptors, TcR1 and TcR2, on chicken lymphocytes. *European Journal of Immunology*, *18*(4), 539-544.

Chen, W. H., Kozlovsky, B. F., Effros, R. B., Grubeck-Loebenstein, B., Edelman, R., & Sztein, M. B. (2009). Vaccination in the elderly: An immunological perspective. *Trends in Immunology*, *30*(7), 351–359.

Chen, C. H., Sowder, J. T., Lahti, J. M., Cihak, J., Lösch, U., & Cooper, M. D. (1989). TCR3: a third T-cell receptor in the chicken. *Proceedings of the National Academy of Sciences*, *86*(7), 2351-2355.

Chen, H., Zhen, Z., Todd, T., Chu, P. K., & Xie, J. (2013). Nanoparticles for improving cancer diagnosis. *Materials Science and Engineering: R: Reports*, *74*(3), 35-69.

Chithrani, B. D., Ghazani, A. A., & Chan, W. C. (2006). Determining the size and shape dependence of gold nanoparticle uptake into mammalian cells. *Nano Letters*, *6*(4), 662-668.

Cho, W. S., Duffin, R., Poland, C. A., Duschl, A., Oostingh, G. J., MacNee, W., ... & Donaldson, K. (2012). Differential pro-inflammatory effects of metal oxide nanoparticles and their soluble ions in vitro and in vivo; zinc and copper nanoparticles, but not their ions, recruit eosinophils to the lungs. *Nanotoxicology*, *6*(1), 22-35.

Coffman, R. L., Sher, A., & Seder, R. A. (2010). Vaccine adjuvants: Putting innate immunity to work. *Immunity*, *33*(4), 492–503. doi:10.1016/j.immuni.2010.10.002

Cognet, L., Tardin, C., Boyer, D., Choquet, D., Tamarat, P., & Lounis, B. (2003). Single metallic nanoparticle imaging for protein detection in cells. *Proceedings of the National Academy of Sciences*, *100*(20), 11350-11355.

Degl'Innocenti, E., Grioni, M., Boni, A., Camporeale, A., Bertilaccio, M. T., Freschi, M., ... & Bellone, M. (2005). Peripheral T cell tolerance occurs early during spontaneous prostate cancer development and can be rescued by dendritic cell immunization. *European journal of immunology*, *35*(1), 66-75.

Diwan, M., Elamanchili, P., Lane, H., Gainer, A., & Samuel, J. (2003). Biodegradable nanoparticle mediated antigen delivery to human cord blood derived dendritic cells for induction of primary T cell responses. *Journal of Drug Targeting*, *11*(8-10), 495-507.

Dobrovolskaia, M. A., & McNeil, S. E. (2007). Immunological properties of engineered nanomaterials. *Nature Nanotechnology*, *2*(8), 469-478.

Drachenberg, K. J., Wheeler, A. W., Stuebner, P., & Horak, F. (2001). A well-tolerated grass pollen-specific allergy vaccine containing a novel adjuvant, monophosphoryl lipid A, reduces allergic symptoms after only four preseasonal injections. *Allergy*, *56*(6), 498-505.

Edelstein, R. L., Tamanaha, C. R., Sheehan, P. E., Miller, M. M., Baselt, D. R., Whitman, L., & Colton, R. J. (2000). The BARC biosensor applied to the detection of biological warfare agents. *Biosensors and Bioelectronics*, *14*(10), 805-813.

Elzey, B. D., Tian, J., Jensen, R. J., Swanson, A. K., Lees, J. R., Lentz, S. R., ... & Ratliff, T. L. (2003). Platelet-mediated modulation of adaptive immunity: a communication link between innate and adaptive immune compartments. *Immunity*, *19*(1), 9-19.

Erf, G. F. (2004). Cell-mediated immunity in poultry. *Poultry science*, *83*(4), 580-590.

Erf, G. F. (2010). Animal Models. In M. Picardo & A. Taieb (Eds.), *Vitiligo* (pp. 205-218). New York, NY: Springer.

Erf, G. F. (2012). *U.S. Patent No. 8,216,551*. Washington, DC: U.S. Patent and Trademark Office.

Erf, G. F., Trejo-Skalli, A. V., & Smyth, J. R. (1995). T cells in regenerating feathers of Smyth line chickens with vitiligo. *Clinical Immunology and Immunopathology*, *76*(2), 120-126.

Fair, J. M., Taylor-McCabe, K. J., Shou, Y., & Marrone, B. L. (2008). Immunophenotyping of chicken peripheral blood lymphocyte subpopulations: Individual variability and repeatability. *Veterinary Immunology and Immunopathology*, *125*(3), 268-273.

Figuerola, A., Di Corato, R., Manna, L., & Pellegrino, T. (2010). From iron oxide nanoparticles towards advanced iron-based inorganic materials designed for biomedical applications. *Pharmacological Research*, *62*(2), 126-143.

Glazer, E. S., Zhu, C., Massey, K. L., Thompson, C. S., Kaluarachchi, W. D., Hamir, A. N., & Curley, S. A. (2010). Noninvasive radiofrequency field destruction of pancreatic adenocarcinoma xenografts treated with targeted gold nanoparticles. *Clinical Cancer Research*, *16*(23), 5712-5721.

Gobin, A. M., Lee, M. H., Halas, N. J., James, W. D., Drezek, R. A., & West, J. L. (2007). Near-infrared resonant nanoshells for combined optical imaging and photothermal cancer therapy. *Nano Letters*, *7*(7), 1929-1934.

Gopee, N. V., Roberts, D. W., Webb, P., Cozart, C. R., Siitonen, P. H., Latendresse, J. R., ... & Howard, P. C. (2009). Quantitative determination of skin penetration of PEG-coated CdSe quantum dots in dermabraded but not intact SKH-1 hairless mouse skin. *Toxicological Sciences*, *111*(1), 37-48.

Gratton, S. E., Ropp, P. A., Pohlhaus, P. D., Luft, J. C., Madden, V. J., Napier, M. E., & DeSimone, J. M. (2008). The effect of particle design on cellular internalization pathways. *Proceedings of the National Academy of Sciences*, *105*(33), 11613-11618.

Gregory, A. E., Titball, R., & Williamson, D. (2013). Vaccine delivery using nanoparticles. *Frontiers in Cellular and Infection Microbiology*, *3*. doi:10.3389/fcimb.2013.00013

Grossmann, M. E., Davila, E., & Celis, E. (2001). Avoiding tolerance against prostatic antigens with subdominant peptide epitopes. *Journal of Immunotherapy*, *24*(3), 237-241.

Grzegorzewska, A. E. (2014). Hepatitis B vaccination in chronic kidney disease patients: A call for novel vaccines. *Expert Review of Vaccines*, *13*(11), 1317-1326.

Hadjipanayis, C. G., Machaidze, R., Kaluzova, M., Wang, L., Schuette, A. J., Chen, H., ... & Mao, H. (2010). EGFRvIII antibody–conjugated iron oxide nanoparticles for magnetic resonance imaging–guided convection-enhanced delivery and targeted therapy of glioblastoma. *Cancer Research*, *70*(15), 6303-6312.

Hamal, K. R., Wideman, R. F., Anthony, N. B., & Erf, G. F. (2010). Differential expression of vasoactive mediators in microparticle-challenged lungs of chickens that differ in susceptibility to pulmonary arterial hypertension. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *298*(1), R235-R242.

Harisinghani, M. G., Barentsz, J., Hahn, P. F., Deserno, W. M., Tabatabaei, S., van de Kaa, C. H., ... & Weissleder, R. (2003). Noninvasive detection of clinically occult lymph-node metastases in prostate cancer. *New England Journal of Medicine*, *348*(25), 2491-2499.

Harrell, M. I., Iritani, B. M., & Ruddell, A. (2008). Lymph node mapping in the mouse. *Journal of Immunological Methods*, *332*(1), 170-174.

Huang, X., & El-Sayed, M. A. (2010). Gold nanoparticles: Optical properties and implementations in cancer diagnosis and photothermal therapy. *Journal of Advanced Research*, *1*(1), 13-28.

Hussain, S., Vanoirbeek, J. A., & Hoet, P. H. (2012). Interactions of nanomaterials with the immune system. *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology*, *4*(2), 169-183.

Ignatowicz, L., Kappler, J., & Marrack, P. (1996). The repertoire of T cells shaped by a single MHC/peptide ligand. *Cell*, *84*(4), 521-529.

Iyer, S., HogenEsch, H., & Hem, S. L. (2003). Relationship between the degree of antigen adsorption to aluminum hydroxide adjuvant in interstitial fluid and antibody production. *Vaccine*, *21*(11), 1219-1223.

Jain, T. K., Reddy, M. K., Morales, M. A., Leslie-Pelecky, D. L., & Labhasetwar, V. (2008). Biodistribution, clearance, and biocompatibility of iron oxide magnetic nanoparticles in rats. *Molecular Pharmaceutics*, *5*(2), 316-327.

Jain, S., Hirst, D. G., & O'Sullivan, J. M. (2012). Gold nanoparticles as novel agents for cancer therapy. *The British Journal of Radiology, 85*(1010), 101-113.

Johnston, H. J., Hutchison, G., Christensen, F. M., Peters, S., Hankin, S., & Stone, V. (2010). A review of the in vivo and in vitro toxicity of silver and gold particulates: particle attributes and biological mechanisms responsible for the observed toxicity. *Critical Reviews in Toxicology*, *40*(4), 328-346.

Karmali, P. P., Chao, Y., Park, J. H., Sailor, M. J., Ruoslahti, E., Esener, S. C., & Simberg, D. (2012). Different effect of hydrogelation on antifouling and circulation properties of dextran–iron oxide nanoparticles. *Molecular Pharmaceutics*, *9*(3), 539-545.

Keith, L. S., Jones, D. E., Chou, C. H. (2002). Aluminum toxicokinetics regarding infrant diet and vaccinations. *Vaccine, 20*(S3), 513-517.

Kelsoe, G. (2000). Studies of the humoral immune response. *Immunologic Research*, *22*(2-3), 199-210.

Kievit, F. M., & Zhang, M. (2011). Surface engineering of iron oxide nanoparticles for targeted cancer therapy. *Accounts of Chemical Research*, *44*(10), 853-862.

Koller, L. D. (1982). In vitro assessment of humoral immunity following exposure to heavy metals. *Environmental Health Perspectives*, *43*, 37-39.

Kool, M., Soullié, T., van Nimwegen, M., Willart, M. A., Muskens, F., Jung, S., ... & Lambrecht, B. N. (2008). Alum adjuvant boosts adaptive immunity by inducing uric acid and activating inflammatory dendritic cells. *The Journal of Experimental Medicine*, *205*(4), 869-882.

Lahti, J. M., Chen, C. L., Tjoelker, L. W., Pickel, J. M., Schat, K. A., Calnek, B. W., ... & Cooper, M. D. (1991). Two distinct alpha beta T-cell lineages can be distinguished by the differential usage of T-cell receptor V beta gene segments. *Proceedings of the National Academy of Sciences*, *88*(23), 10956-10960.

Lartigue, L., Wilhelm, C., Servais, J., Factor, C., Dencausse, A., Bacri, J. C., ... & Gazeau, F. (2012). Nanomagnetic sensing of blood plasma protein interactions with iron oxide nanoparticles: impact on macrophage uptake. *ACS Nano*, *6*(3), 2665-2678.

Lévy, F., & Colombetti, S. (2006). Promises and limitations of murine models in the development of anticancer T-cell vaccines. *International Reviews of Immunology*, *25*(5-6), 269-295.

Lim, E. K., Huh, Y. M., Yang, J., Lee, K., Suh, J. S., & Haam, S. (2011). pH-Triggered Drug-Releasing Magnetic Nanoparticles for Cancer Therapy Guided by Molecular Imaging by MRI. *Advanced Materials*, *23*(21), 2436-2442.

Limaye, S. (2010). Tests for cell-mediated immunity. *Australian Prescriber*, *33*(3), 84-87.

Liu, L., Hitchens, T. K., Ye, Q., Wu, Y., Barbe, B., Prior, D. E., ... & Ho, C. (2013). Decreased reticuloendothelial system clearance and increased blood half-life and immune cell labeling for nano-and micron-sized superparamagnetic iron-oxide particles upon pre-treatment with Intralipid. *Biochimica et Biophysica Acta (BBA)-General Subjects*, *1830*(6), 3447-3453.

Llevot, A., & Astruc, D. (2012). Applications of vectorized gold nanoparticles to the diagnosis and therapy of cancer. *Chemical Society Reviews*, *41*(1), 242-257.

Lockhart, B. R., & Erf, G. F. (2004). Oxidative stress and antioxidant levels in Feather-derived and Embryo-derived Melanocytes from Vitiligo-Prone Smyth line and normally pigmented chickens. *Pigment Cell Research*, *17*(4), 440-440.

Longmire, M., Choyke, P. L., & Kobayashi, H. (2008). Clearance properties of nano-sized particles and molecules as imaging agents: Considerations and caveats. *Nanomedicine, 3*(5), 703-717.

Lucas, A. M., & Stettenheim, P. R. (1971). Avian anatomy-integument. Washington, DC: Department of Agriculture.

Mahmoudi, M., Sant, S., Wang, B., Laurent, S., & Sen, T. (2011). Superparamagnetic iron oxide nanoparticles (SPIONs): Development, surface modification and applications in chemotherapy. *Advanced Drug Delivery Reviews*, *63*(1), 24-46.

McKee, A. S., Munks, M. W., & Marrack, P. (2007). How do adjuvants work? Important considerations for new generation adjuvants. *Immunity*, *27*(5), 687-690.

Medzhitov, R., & Janeway, C. A. (2002). Decoding the patterns of self and nonself by the innate immune system. *Science*, *296*(5566), 298-300.

Medzhitov, R. (2007). Recognition of microorganisms and activation of the immune response. *Nature*, *449*(7164), 819-826.

Mempel, T. R., Scimone, M. L., Mora, J. R., & von Andrian, U. H. (2004). In vivo imaging of leukocyte trafficking in blood vessels and tissues. *Current Opinion in Immunology*, *16*(4), 406-417.

Messaoudi, I., Estep, R., Robinson, B., & Wong, S. W. (2011). Nonhuman primate models of human immunology. *Antioxidants & Redox Signaling*, *14*(2), 261-273.

Mestas, J., & Hughes, C. C. (2004). Of mice and not men: differences between mouse and human immunology. *The Journal of Immunology*, *172*(5), 2731-2738.

Mok, H., Veiseh, O., Fang, C., Kievit, F. M., Wang, F. Y., Park, J. O., & Zhang, M. (2010). pH-Sensitive siRNA nanovector for targeted gene silencing and cytotoxic effect in cancer cells. *Molecular Pharmaceutics*, *7*(6), 1930-1939.

Moon, J. J., Suh, H., Polhemus, M. E., Ockenhouse, C. F., Yadava, A., & Irvine, D. J. (2012). Antigen-displaying lipid-enveloped PLGA nanoparticles as delivery agents for a Plasmodium vivax malaria vaccine. *PLoS One*, *7*(2), e31472.

Mortensen, L. J., Oberdörster, G., Pentland, A. P., & DeLouise, L. A. (2008). In vivo skin penetration of quantum dot nanoparticles in the murine model: the effect of UVR. *Nano letters*, *8*(9), 2779-2787.

Mulens-Arias, V., Rojas, J. M., Pérez-Yagüe, S., Morales, M. P., & Barber, D. F. (2015). Polyethylenimine-coated SPIONs trigger macrophage activation through TLR-4 signaling and ROS production and modulate podosome dynamics. *Biomaterials, 52*, 494-506.

Müller, K., Skepper, J. N., Posfai, M., Trivedi, R., Howarth, S., Corot, C., ... & Gillard, J. H. (2007). Effect of ultrasmall superparamagnetic iron oxide nanoparticles (Ferumoxtran-10) on human monocyte-macrophages in vitro. *Biomaterials*, *28*(9), 1629-1642.

Nakanishi, Y., Henson, P. M., & Shiratsuchi, A. (2009). Pattern recognition in phagocytic clearance of altered self. *Advances in Experimental Medicine and Biology*, *653*, 129-138.

Nam, J. M., Thaxton, C. S., & Mirkin, C. A. (2003). Nanoparticle-based bio-bar codes for the ultrasensitive detection of proteins. *Science*, *301*(5641), 1884-1886.

National Research Council. (2012). A research strategy for environmental, health, and safety aspects of engineered nanomaterials. Washington, DC: The National Academies Press. Retrieved from http://www.nap.edu/catalog/13347/a-research-strategy-for-environmental-health-and-safety-aspects-of-engineered-nanomaterials

Norup, L. R., & Juul-Madsen, H. R. (2007). An assay for measuring the mannan-binding lectin pathway of complement activation in chickens. *Poultry Science*, *86*(11), 2322-2326.

Ogutu, B. R., Apollo, O. J., McKinney, D., Okoth, W., Siangla, J., Dubovsky, F., ... & MSP-1 Malaria Vaccine Working Group. (2009). Blood stage malaria vaccine eliciting high antigen-specific antibody concentrations confers no protection to young children in Western Kenya. *PLoS One*, *4*(3), e4708.

Oostingh, G. J., Casals, E., Italiani, P., Colognato, R., Stritzinger, R., Ponti, J., ... & Boraschi, D. (2011). Problems and challenges in the development and validation of human cell-based

assays to determine nanoparticle-induced immunomodulatory effects. *Particle and Fibre Toxicology*, *8*(1), 8.

Orlando, A., Colombo, M., Prosperi, D., Gregori, M., Panariti, A., Rivolta, I., ... & Cazzaniga, E. (2015). Iron oxide nanoparticles surface coating and cell uptake affect biocompatibility and inflammatory responses of endothelial cells and macrophages. *Journal of Nanoparticle Research*, *17*(9), 1-13.

Pan, Y., Neuss, S., Leifert, A., Fischler, M., Wen, F., Simon, U., ... & Jahnen-Dechent, W. (2007). Size-dependent cytotoxicity of gold nanoparticles. *Small*, *3*(11), 1941-1949.

Parmentier, H. K., Baelmans, R., Savelkoul, H. F., Dorny, P., Demey, F., & Berkvens, D. (2004). Serum haemolytic complement activities in 11 different MHC (B) typed chicken lines. *Veterinary Immunology and Immunopathology*, *100*(1), 25-32.

Plotkin, S. A. (2010). Correlates of protection induced by vaccination. *Clinical and Vaccine Immunology : CVI*, *17*(7), 1055–1065. doi:10.1128/CVI.00131-10

Pradhan, P., Giri, J., Banerjee, R., Bellare, J., & Bahadur, D. (2007). Cellular interactions of lauric acid and dextran-coated magnetite nanoparticles. *Journal of Magnetism and Magnetic Materials*, *311*(1), 282-287.

Pusic, K., Xu, H., Stridiron, A., Aguilar, Z., Wang, A., & Hui, G. (2011). Blood stage merozoite surface protein conjugated to nanoparticles induce potent parasite inhibitory antibodies. *Vaccine*, *29*(48), 8898-8908.

Pusic, K., Aguilar, Z., McLoughlin, J., Kobuch, S., Xu, H., Tsang, M., ... & Hui, G. (2013). Iron oxide nanoparticles as a clinically acceptable delivery platform for a recombinant blood-stage human malaria vaccine. *The FASEB Journal*, *27*(3), 1153-1166.

Racine, R., McLaughlin, M., Jones, D. D., Wittmer, S. T., MacNamara, K. C., Woodland, D. L., & Winslow, G. M. (2011). IgM production by bone marrow plasmablasts contributes to long-term protection against intracellular bacterial infection. *The Journal of Immunology*, *186*(2), 1011-1021.

Reiter, L. T., Potocki, L., Chien, S., Gribskov, M., & Bier, E. (2001). A systematic analysis of human disease-associated gene sequences in *Drosophila melanogaster*. *Genome Research*, *11*(6), 1114–1125.

Roco, M. C. (2005). Environmentally responsible development of nanotechnology. *Environmental Science & Technology*, *39*(5), 106A-112A.

Ryoo, S. R., Jang, H., Kim, K. S., Lee, B., Kim, K. B., Kim, Y. K., ... & Min, D. H. (2012). Functional delivery of DNAzyme with iron oxide nanoparticles for hepatitis C virus gene knockdown. *Biomaterials*, *33*(9), 2754-2761.

Salata, O. V. (2004). Applications of nanoparticles in biology and medicine. *Journal of Nanobiotechnology*, *2*(1), 3. doi:10.1186/1477-3155-2-3

Schat, K. A., Kaspers, B., & Kaiser, P. (Eds.). (2014). *Avian immunology*. San Diego, CA: Elsevier.

Serrano-Villar, S., Gutiérrez, C., Vallejo, A., Hernandez-Novoa, B., Díaz, L., Fernández, M. A., ... & Moreno, S. (2013). The CD4/CD8 ratio in HIV-infected subjects is independently associated with T-cell activation despite long-term viral suppression. *Journal of Infection*, *66*(1), 57-66.

Shen, C. C., Liang, H. J., Wang, C. C., Liao, M. H., & Jan, T. R. (2011). A role of cellular glutathione in the differential effects of iron oxide nanoparticles on antigen-specific T cell cytokine expression. *International Journal of Nanomedicine*, *6*, 2791-2798.

Shi, F., & Erf, G. F. (2012). IFN-γ, IL-21, and IL-10 co-expression in evolving autoimmune vitiligo lesions of Smyth line chickens. *Journal of Investigative Dermatology*, *132*, 642-649.

Shrestaa, S., Smyth, J. R., & Erf, G. F. (1997). Profiles of pulp infiltrating lymphocytes at various times throughout feather regeneration in Smyth line chickens with vitiligo. *Autoimmunity*, *25*(4), 193-201.

Sim, R. B., & Wallis, R. (2011). Surface properties: Immune attack on nanoparticles. *Nature nanotechnology*, *6*(2), 80-81.

Singh, M., Chakrapani, A., & O'Hagan, D. (2007). Nanoparticles and microparticles as vaccine-delivery systems. *Expert Review of Vaccines, 6*(5), 797-808.

Smijs, T. G., & Bouwstra, J. A. (2010). Focus on skin as a possible port of entry for solid nanoparticles and the toxicological impact. *Journal of Biomedical Nanotechnology*, *6*(5), 469-484.

Smith, D. M., Simon, J. K., & Baker Jr, J. R. (2013). Applications of nanotechnology for immunology. *Nature Reviews Immunology*, *13*(8), 592-605.

Sreekumar, G. P., Smyth, J. R., Ambady, S., & de Leon, F. A. P. (2000). Analysis of the effect of endogenous viral genes in the Smyth line chicken model for autoimmune vitiligo. *The American Journal of Pathology*, *156*(3), 1099-1107.

Stepicheva, N., Liyanage, R., Lay, J., Dienglewicz, R., & Erf, G. (2010). Abnormal morphology of melanosomes in the autoimmune vitiligo-prone Smyth line chicken does not appear to be due to alteration in lipid composition. *The Journal of Immunology*, *184*(S1), 83-16.

Sullivan, P. S., & Moreno, C. (2015). A multidisciplinary approach to perianal and intra-abdominal infections in the neutropenic cancer patient. *Oncology*, *29*(8), 581-590.

Syrjälä, H., Surcel, H. M., & Ilonen, J. (1991). Low CD4/CD8 T lymphocyte ratio in acute myocardial infarction. *Clinical and Experimental Immunology*, *83*(2), 326-328.

Tavaré, R., Sagoo, P., Varama, G., Tanriver, Y., Warely, A., Diebold, S. S., ... & Mullen, G. E. (2011). Monitoring of in vivo function of superparamagnetic iron oxide labelled murine dendritic cells during anti-tumour vaccination. *PloS One*, *6*(5), e19662.

Teeguarden, J. G., Mikheev, V. B., Minard, K. R., Forsythe, W. C., Wang, W., Sharma, G., ... & Thrall, B. D. (2014). Comparative iron oxide nanoparticle cellular dosimetry and response in mice by the inhalation and liquid cell culture exposure routes. *Particle and Fibre Toxicology, 11*(1), 1-18.

Thukkaram, M., Sitaram, S., Kannaiyan, S. K., & Subbiahdoss, G. (2014). Antibacterial efficacy of iron-oxide nanoparticles against biofilms on different biomaterial surfaces. *International Journal of Biomaterials, 2014*. doi:10.1155/2014/716080

Tinkle, S. S., Antonini, J. M., Rich, B. A., Roberts, J. R., Salmen, R., DePree, K., & Adkins, E. J. (2003). Skin as a route of exposure and sensitization in chronic beryllium disease. *Environmental Health Perspectives*, *111*(9), 1202.

Ulrich, J. T., & Myers, K. R. (1995). Monophosphoryl lipid A as an adjuvant. In M. F. Powell & M. J. Newman (Eds.)., *Vaccine design* (pp. 495-524). New York, NY: Springer.

van Zijverden, M., & Granum, B. (2000). Adjuvant activity of particulate pollutants in different mouse models. *Toxicology*, *152*(1), 69-77.

Vandaveer, S. S., Erf, G. F., & Durdik, J. M. (2001). Avian T helper one/two immune response balance can be shifted toward inflammation by antigen delivery to scavenger receptors. *Poultry Science*, *80*(2), 172-181.

Vecchio, G., Galeone, A., Brunetti, V., Maiorano, G., Sabella, S., Cingolani, R., & Pompa, P. P. (2012). Concentration-dependent, size-independent toxicity of citrate capped AuNPs in Drosophila melanogaster. *PLoS One*, *7*(1), e29980.

Vivier, E., Raulet, D. H., Moretta, A., Caligiuri, M. A., Zitvogel, L., Lanier, L. L., ... & Ugolini, S. (2011). Innate or adaptive immunity? The example of natural killer cells. *Science*, *331*(6013), 44-49.

Walls, R. S. (1977). Eosinophil response to alum adjuvants: involvement of T cells in non-antigen-dependent mechanisms. *Experimental Biology and Medicine*, *156*(3), 431-435.

Wang, X., & Erf, G. F. (2004). Apoptosis in feathers of Smyth line chickens with autoimmune vitiligo. *Journal of Autoimmunity*, *22*(1), 21-30.

Wilson, M. (2014). *Differences in humoral immune responses to nanoparticle conjugated protein antigen compared to immunization with conventional antigen adjuvant formulations* (Unpublished honors thesis). University of Arkansas, Fayetteville.

Xiang, S. D., Scholzen, A., Minigo, G., David, C., Apostolopoulos, V., Mottram, P. L., & Plebanski, M. (2006). Pathogen recognition and development of particulate vaccines: does size matter? *Methods*, *40*(1), 1-9.

Ye, L., Yong, K. T., Liu, L., Roy, I., Hu, R., Zhu, J., ... & Prasad, P. N. (2012). A pilot study in non-human primates shows no adverse response to intravenous injection of quantum dots. *Nature Nanotechnology*, *7*(7), 453-458.

Yigit, M. V., Moore, A., & Medarova, Z. (2012). Magnetic nanoparticles for cancer diagnosis and therapy. *Pharmaceutical Research*, *29*(5), 1180-1188.

Yoo, J. W., Chambers, E., & Mitragotri, S. (2010). Factors that control the circulation time of nanoparticles in blood: Challenges, solutions and future prospects. *Current Pharmaceutical Design*, *16*(21), 2298-2307.

Yu, M. K., Park, J., & Jon, S. (2012). Targeting strategies for multifunctional nanoparticles in cancer imaging and therapy. *Theranostics*, *2*(1), 3-44.

Yue, H., Wei, W., Fan, B., Yue, Z., Wang, L., Ma, G., & Su, Z. (2012). The orchestration of cellular and humoral responses is facilitated by divergent intracellular antigen trafficking in nanoparticle-based therapeutic vaccine. *Pharmacological Research*, *65*(2), 189-197.

Zhao, L., Seth, A., Wibowo, N., Zhao, C. X., Mitter, N., Yu, C., & Middelberg, A. P. (2014). Nanoparticle vaccines. *Vaccine*, *32*(3), 327-337.

Zhao, F., Zhao, Y., Liu, Y., Chang, X., Chen, C., & Zhao, Y. (2011). Cellular uptake, intracellular trafficking, and cytotoxicity of nanomaterials. *Small*, *7*(10), 1322-1337.

APPENDIX A

IACUC Protocol Approval Letter

UNIVERSITY OF ARKANSAS

Office of Research Compliance

MEMORANDUM

TO: Gisela Erf

FROM: Craig N. Coon, Chairman
Institutional Animal Care
And Use Committee

DATE: November 7, 2011

SUBJECT: IACUC PROTOCOL **APPROVAL**
Expiration date : **November 03, 2014**

The Institutional Animal Care and Use Committee (IACUC) has **APPROVED** Protocol **#12013-"MONITORING NANOPARTICLE BIOLOGY IN VIVO: UNIQUE OPPORTUNITIES IN THE AVIAN SYSTEM"**. You may begin this study immediately.

The IACUC encourages you to make sure that you are also in compliance with other UAF committees such as Biosafety, Toxic Substances and/or Radiation Safety if your project has components that fall under their purview.

In granting its approval, the IACUC has approved only the protocol provided. Should there be any changes in the protocol during the research, please notify the IACUC in writing [Modification Request form] **prior** to initiating the changes. If the study period is expected to extend beyond **11-03-2014,** you must submit a new protocol. By policy the IACUC cannot approve a study for more than 3 years at a time.

The IACUC appreciates your cooperation in complying with University and Federal guidelines for research involving animal subjects.

cnc/car

cc: Animal Welfare Veterinarian

Administration Building 210 • 1 University of Arkansas • Fayetteville, AR 72701-1201 • 479-575-4572
Fax: 479-575-3846 • http://vpred.uark.edu/199

APPENDIX B

Response Factors by Study

Table B1. *Response factors by study*

Response Factor	Feather Injection Studies			Intramuscular Injection Studies			
	1	2	3	Primary (A)	Memory (A)	Primary (B)	Memory (B)
Blood cells							
WBCs (K/ul)	+	+	+	-	-	-	-
Heterophils (K/ul)	+	+	+	-	-	-	-
Heterophils (%)	+	+	+	-	-	-	-
Lymphocyte (K/ul)	+	+	+	-	-	-	-
Lymphocyte (%)	+	+	+	-	-	-	-
Monocytes (K/ul)	+	+	+	-	-	-	-
Monocytes (%)	+	+	+	-	-	-	-
Eosinophils (K/ul)	+	+	+	-	-	-	-
Eosinophils (%)	+	+	+	-	-	-	-
Basophils (K/ul)	+	+	+	-	-	-	-
Basophil (%)	+	+	+	-	-	-	-
RBCs (K/ul)	+	+	+	-	-	-	-
Thrombocytes (K/ul)	+	+	+	-	-	-	-
Cell types							
Live cells	+	-	+	-	-	+	+
CD45+	+	+	+	+	+	-	-
Macrophages	+	+	-	-	-	-	-
Heterophils	-	+	+	-	-	-	-
Lymphocytes	+	-	-	-	-	-	-
MHCII+							
Macrophages+	+	+	+	+	+	+	+
B cell+	+	+	+	+	+	+	+
T cells							
CD3+	-	-	+	-	+	+	+
CD4- CD8+	+	+	+	+	+	+	+
CD4+ CD8+	+	+	+	+	+	+	+
CD4+ CD8-	+	+	+	+	+	+	+
CD25+ CD4+	-	-	-	+	+	-	-

Table B1. *Response factors by study* (Cont.)

Response Factor	Feather Injection Studies			Intramuscular Injection Studies			
	2013 IO	2013 IO vs. Alum	2014	Primary 2013	Memory 2013	Primary 2014	Memory 2014
γδ T cell+	-	-	+	-	-	+	+
γδ T cell+ CD8+	+	+	+	+	+	+	+
γδ T cell+ CD8-	+	+	+	+	+	+	+
αβ1 Tcell+	-	-	+	-	-	+	+
αβ2 Tcell+	+	+	-	+	+	-	-
αβ1 Tcell+	+	+	-	+	+	-	-
αβ1 T cell+ CD4-CD8+	-	-	+	-	-	+	+
αβ1 T cell+ CD4+CD8+	-	-	+	-	-	+	+
αβ1 T cell+ CD4-CD8-	-	-	+	-	-	+	+
αβ1 T cell+ CD4+CD8-	-	-	+	-	-	+	+
αβ2 T cell+	-	-	+	-	-	+	+
αβ2 T cell+ CD4-CD8+	-	-	+	-	-	+	+
αβ2 T cell+ CD4+CD8+	-	-	+	-	-	+	+
αβ2 T cell+ CD4-CD8-	-	-	+	-	-	+	+
αβ2 T cell+ CD4+CD8-	-	-	+	-	-	+	+
CD4/CD8 ratio	+	+	+	-	-	-	-
B cells							
B cell	+	+	-	+	+	-	-
B cell+ IgM+	-	-	+	-	-	+	+
B cell+ IgM-	-	-	+	-	-	+	+
B cell+ IgG+	-	-	+	-	-	+	+
B cell+ IgG-	-	-	+	-	-	+	+

APPENDIX C

Type III Test of Fixed Effects Results for Blood Cell Responses

		Study 1		Study 2		Study 3	
Response Factor	**Fixed Effect**	**F**	**p**	**F**	**p**	**F**	**p**
WBCs (K/uL)	Day	3.37	NS	6.42	0.0142	3.79	NS
	Treatment	0.37	NS	1.94	NS	2.06	NS
	Treatment*Day	0.51	NS	1.53	NS	0.42	NS
Heterophils (K/uL)	Day	42.08	< 0.0001	208.21	< 0.0001	9.61	0.0075
	Treatment	0.08	NS	0.54	NS	0.44	NS
	Treatment*Day	2.02	NS	4.93	0.0078	0.65	NS
Heterophils (%)	Day	67.05	< 0.0001	18.02	0.0003	29.34	0.0002
	Treatment	0.70	NS	1.60	NS	0.96	NS
	Treatment*Day	1.52	NS	0.79	NS	2.56	NS
Lymphocytes (K/uL)	Day	25.78	0.0001	1.03	NS	28.97	0.0002
	Treatment	0.21	NS	3.52	0.0488	2.29	NS
	Treatment*Day	0.39	NS	1.36	NS	3.21	NS
Lymphocytes (%)	Day	21.37	0.0002	72.87	< 0.0001	20.76	0.0007
	Treatment	0.23	NS	3.06	NS	0.93	NS
	Treatment*Day	0.60	NS	1.63	NS	1.66	NS
Monocytes (K/uL)	Day	1.69	NS	5.36	0.0237	15.64	0.0017
	Treatment	0.91	NS	1.44	NS	0.71	NS
	Treatment*Day	0.62	NS	0.64	NS	0.67	NS
Monocytes (%)	Day	5.93	0.02	5.20	0.0258	5.90	0.0267
	Treatment	0.25	NS	1.82	NS	0.02	NS
	Treatment*Day	0.46	NS	0.24	NS	0.47	NS
Eosinophils (K/uL)	Day	0.79	NS	2.99	NS	1.40	NS
	Treatment	0.88	NS	0.82	NS	2.16	NS
	Treatment*Day	0.53	NS	1.14	NS	0.11	NS
Eosinophils (%)	Day	1.06	NS	2.93	NS	4.75	0.0438
	Treatment	0.25	NS	0.91	NS	0.44	NS
	Treatment*Day	0.48	NS	1.06	NS	0.60	NS
Basophils (K/uL)	Day	1.35	NS	12.57	0.0014	5.21	0.0356
	Treatment	3.62	NS	1.41	NS	1.83	NS
	Treatment*Day	1.90	NS	1.82	NS	0.24	NS
Basophils (%)	Day	0.34	NS	17.92	0.0003	0.74	NS
	Treatment	3.75	NS	1.81	NS	0.72	NS
	Treatment*Day	1.03	NS	1.65	NS	0.18	NS
RBCs (K/uL)	Day	0.34	NS	8.95	0.0049	2.52	NS
	Treatment	0.54	NS	0.68	NS	0.93	NS
	Treatment*Day	0.51	NS	1.03	NS	0.07	NS
Thrombocytes (K/uL)	Day	15.75	0.0008	1.91	NS	4.60	0.0469
	Treatment	2.80	NS	3.06	NS	4.44	0.0455
	Treatment*Day	1.01	NS	2.03	NS	0.52	NS

APPENDIX D

Difference of Least Squares Means Results for Blood Cell Responses

Table D1. *Difference of least squares means for blood cell responses by day*

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
Study 1							
Heterophil (K/uL)	0	0.25	-3.6740	0.4023	11	-9.13	<.0001
	0	7	-1.2360	0.3327	11	-3.72	0.0034
	0.25	7	2.4380	0.3110	11	7.84	<.0001
Heterophil (%)	0	0.25	-17.2593	1.4849	11	-11.62	<.0001
	0	7	-10.8093	4.0198	11	-2.69	0.0211
	0.25	7	6.4500	3.5297	11	1.83	0.0949
Lymphocyte (K/uL)	0	0.25	8.1347	1.0876	11	7.48	<.0001
	0	7	4.7645	1.6416	11	2.90	0.0144
	0.25	7	-3.3702	1.4579	11	-2.31	0.0412
Lymphocyte (%)	0	0.25	24.4017	3.5894	11	6.80	<.0001
	0	7	11.0933	4.3271	11	2.56	0.0263
	0.25	7	-13.3083	4.8842	11	-2.72	0.0198
Monocyte (%)	0	0.25	-3.8332	1.0905	11	-3.52	0.0048
	0	7	0.03917	0.6331	11	0.06	0.9518
	0.25	7	3.8723	1.1381	11	3.40	0.0059
Thrombocyte (K/uL)	0	0.25	-7.9300	1.3850	11	-5.73	0.0001
	0	7	-2.4600	0.9623	11	-2.56	0.0267
	0.25	7	5.4700	1.5083	11	3.63	0.0040
Study 2							
WBC (K/uL)	0	0.25	-4.4188	1.2304	12	-3.59	0.0037
	0	7	-0.5937	2.4175	12	-0.25	0.8101
	0.25	7	3.8250	2.9408	12	1.30	0.2178
Heterophil (%)	0	0.25	-11.6062	1.8923	12	-6.13	<.0001
	0	7	1.1900	1.4368	12	0.83	0.4237
	0.25	7	12.7963	2.2863	12	5.60	0.0001
Lymphocyte (%)	0	0.25	13.2312	1.6245	12	8.14	<.0001
	0	7	5.2250	3.9693	12	1.32	0.2126
	0.25	7	-8.0062	5.2264	12	-1.53	0.1515
Monocyte (K/uL)	0	0.25	-1.2388	0.3707	12	-3.34	0.0059
	0	7	-0.8013	0.4708	12	-1.70	0.1145
	0.25	7	0.4375	0.5032	12	0.87	0.4017
Monocyte (%)	0	0.25	-2.9069	0.9049	12	-3.21	0.0075
	0	7	-2.4206	2.2450	12	-1.08	0.3021
	0.25	7	0.4863	2.4020	12	0.20	0.8430
Basophil (K/uL)	0	0.25	0.2031	0.1452	12	1.40	0.1873
	0	7	-1.2112	0.3197	12	-3.79	0.0026
	0.25	7	-1.4144	0.2819	12	-5.02	0.0003

Table D1. *Difference of least squares means for blood cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
Basophil (%)	0	0.25	1.3031	0.4250	12	3.07	0.0098
	0	7	-3.8531	1.3517	12	-2.85	0.0146
	0.25	7	-5.1562	1.1720	12	-4.40	0.0009
RBC (K/uL)	0	0.25	0.09000	0.02271	12	3.96	0.0019
	0	7	0.01937	0.03162	12	0.61	0.5514
	0.25	7	-0.07063	0.02660	12	-2.66	0.0210
Study 3							
Heterophil (K/uL)	0	0.25	-4.6167	1.1709	9	-3.94	0.0034
	0	7	1.3642	0.7121	9	1.92	0.0877
	0.25	7	5.9808	1.2868	9	4.65	0.0012
Heterophil (%)	0	0.25	-21.0083	2.7745	9	-7.57	<.0001
	0	7	0.2583	1.4962	9	0.17	0.8667
	0.25	7	21.2667	2.6626	9	7.99	<.0001
Lymphocyte (K/uL)	0	0.25	8.9658	1.1304	9	7.93	<.0001
	0	7	1.2208	1.7853	9	0.68	0.5113
	0.25	7	-7.7450	1.8378	9	-4.21	0.0023
Lymphocyte (%)	0	0.25	24.3017	3.7266	9	6.52	0.0001
	0	7	-2.8083	1.8419	9	-1.52	0.1617
	0.25	7	-27.1100	4.0247	9	-6.74	<.0001
Monocyte (K/uL)	0	0.25	-0.5817	0.4410	9	-1.32	0.2198
	0	7	0.9332	0.1604	9	5.82	0.0003
	0.25	7	1.5148	0.4735	9	3.20	0.0108
Monocyte (%)	0	0.25	-3.7408	1.6604	9	-2.25	0.0508
	0	7	2.1950	0.6583	9	3.33	0.0087
	0.25	7	5.9358	1.9331	9	3.07	0.0133
Eosinophil (%)	0	0.25	-0.08917	0.03684	9	-2.42	0.0386
	0	7	-0.09467	0.03739	9	-2.53	0.0321
	0.25	7	-0.00550	0.04842	9	-0.11	0.9121
Basophil (K/uL)	0	0.25	0.4102	0.2564	9	1.60	0.1440
	0	7	0.3754	0.1167	9	3.22	0.0105
	0.25	7	-0.03483	0.2666	9	-0.13	0.8989
Thrombocyte (K/uL)	0	0.25	4.3583	1.4054	9	3.10	0.0127
	0	7	3.3750	1.6863	9	2.00	0.0764
	0.25	7	-0.9833	1.7193	9	-0.57	0.5814

Table D2. *Difference of least squares means for blood cell responses by Treatment*

Study/Factor	Treatment	Treatment	Estimate	Std. Error	DF	t Value	Pr > |t|
Study 2							
Lymphocyte (K/ul)	Alum	Alum-mIgG	1.3000	2.6443	12	0.49	0.6318
	Alum	IO	7.6400	2.6443	12	2.89	0.0136
	Alum	IO-mIgG	5.1458	2.6443	12	1.95	0.0754
	Alum-mIgG	IO	6.3400	2.6443	12	2.40	0.0337
	Alum-mIgG	IO-mIgG	3.8458	2.6443	12	1.45	0.1715
	IO	IO-mIgG	-2.4942	2.6443	12	-0.94	0.3642
Study 3							
Thrombocyte (K/uL)	Alum-mIgG	IO-mIgG	4.2417	1.4997	9	2.83	0.0198
	Alum-mIgG	mIgG	3.3417	1.4997	9	2.23	0.0529
	IO-mIgG	mIgG	-0.9000	1.4997	9	-0.60	0.5632

Table D3. *Simple effect comparisons of treatment*day least squares means by treatment for heterophil concentration, Study 2*

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
Alum	0	0.25	-4.3125	0.7650	12	-5.64	0.0001
Alum	0	7	0.09500	0.7489	12	0.13	0.9012
Alum	0.25	7	4.4075	0.4638	12	9.50	<.0001
Alum-mIgG	0	0.25	-3.2825	0.7650	12	-4.29	0.0010
Alum-mIgG	0	7	-0.1125	0.7489	12	-0.15	0.8831
Alum-mIgG	0.25	7	3.1700	0.4638	12	6.84	<.0001
IO	0	0.25	-4.0775	0.7650	12	-5.33	0.0002
IO	0	7	0.7925	0.7489	12	1.06	0.3108
IO	0.25	7	4.8700	0.4638	12	10.50	<.0001
IO-mIgG	0	0.25	-7.0625	0.7650	12	-9.23	<.0001
IO-mIgG	0	7	-0.4450	0.7489	12	-0.59	0.5634
IO-mIgG	0.25	7	6.6175	0.4638	12	14.27	<.0001

Table D4. *Simple effect comparisons of treatment*day least squares means by say for heterophil concentration, Study 2*

Simple Effect Level	Treatment	Treatment	Estimate	Std. Error	DF	t Value	Pr > |t|
0	Alum	Alum-mIgG	-0.05500	0.7436	12	-0.07	0.9423
0	Alum	IO	-0.6050	0.7436	12	-0.81	0.4317
0	Alum	IO-mIgG	0.2225	0.7436	12	0.30	0.7699
0	Alum-mIgG	IO	-0.5500	0.7436	12	-0.74	0.4737
0	Alum-mIgG	IO-mIgG	0.2775	0.7436	12	0.37	0.7155
0	IO	IO-mIgG	0.8275	0.7436	12	1.11	0.2876
0.25	Alum	Alum-mIgG	0.9750	1.2906	12	0.76	0.4646
0.25	Alum	IO	-0.3700	1.2906	12	-0.29	0.7792
0.25	Alum	IO-mIgG	-2.5275	1.2906	12	-1.96	0.0738
0.25	Alum-mIgG	IO	-1.3450	1.2906	12	-1.04	0.3179
0.25	Alum-mIgG	IO-mIgG	-3.5025	1.2906	12	-2.71	0.0188
0.25	IO	IO-mIgG	-2.1575	1.2906	12	-1.67	0.1204
7	Alum	Alum-mIgG	-0.2625	1.0794	12	-0.24	0.8120
7	Alum	IO	0.09250	1.0794	12	0.09	0.9331
7	Alum	IO-mIgG	-0.3175	1.0794	12	-0.29	0.7737
7	Alum-mIgG	IO	0.3550	1.0794	12	0.33	0.7479
7	Alum-mIgG	IO-mIgG	-0.05500	1.0794	12	-0.05	0.9602
7	IO	IO-mIgG	-0.4100	1.0794	12	-0.38	0.7107

APPENDIX E

Type III Test of Fixed Effects Results for

Pulp Cell Response in Feather Injection Studies

Table E1. *Type III test of fixed effects results for pulp cell response in feather injection studies*

		Study 1		Study 2		Study 3	
Response Factor	**Fixed Effect**	**F Value**	**P Value**	**F Value**	**P Value**	**F Value**	**P Value**
Live cells	Day	6.95	0.0243	-	-	3.66	NS
	Treatment	2.34	NS	-	-	2.45	NS
	Treatment*Day	11.29	0.0040	-	-	0.45	NS
CD45+	Day	2.20	NS	73.12	<0.0001	31.09	<0.0001
	Treatment	5.90	0.0182	10.96	0.0009	15.52	<0.0001
	Treatment*Day	0.67	NS	2.78	0.0482	2.77	0.0102
Macrophages	Day	5.50	0.0394	12.77	0.0012	-	-
	Treatment	0.21	NS	1.47	NS	-	-
	Treatment*Day	1.49	NS	0.75	NS	-	-
Heterophils	Day	-	-	9.72	0.0030	15.56	<0.0001
	Treatment	-	-	0.91	NS	22.64	<0.0001
	Treatment*Day	-	-	0.82	NS	4.16	0.0006
Lymphocytes	Day	10.56	0.0098	-	-	-	-
	Treatment	8.26	0.0065	-	-	-	-
	Treatment*Day	2.68	NS	-	-	-	-
MHCII+	Day	14.54	0.0047	5.05	0.0219	31.47	0.0084
Macrophages+	Treatment	0.09	NS	3.44	NS	15.59	0.0011
	Treatment*Day	0.79	NS	1.24	NS	4.50	NS
MHCII+	Day	13.83	0.0053	20.13	0.0002	83.44	NS
B cells+	Treatment	3.41	NS	0.23	NS	3.30	NS
	Treatment*Day	1.31	NS	0.89	NS	15.29	NS
CD3+	Day	-	-	-	-	23.96	<0.0001
	Treatment	-	-	-	-	13.73	<0.0001
	Treatment*Day	-	-	-	-	4.01	0.0008
CD4- CD8+	Day	12.53	0.0067	22.96	<0.0001	51.70	0.0040
	Treatment	6.26	0.0153	0.27	NS	5.05	0.0330
	Treatment*Day	1.80	NS	0.91	NS	6.06	NS
CD4+ CD8+	Day	10.07	0.0109	5.68	0.0107	2.00	NS
	Treatment	4.81	0.0316	3.15	NS	1.06	NS
	Treatment*Day	1.15	NS	1.34	NS	1.22	NS
CD4+ CD8-	Day	15.03	0.0044	9.68	0.0017	15.86	0.0107
	Treatment	6.88	0.0115	4.51	0.0307	3.94	NS
	Treatment*Day	2.12	NS	0.98	NS	0.74	NS
γδ T cell+	Day	-	-	-	-	11.52	<0.0001
	Treatment	-	-	-	-	12.97	0.0001
	Treatment*Day	-	-	-	-	3.60	0.0016

Table E1. *Type III test of fixed effects results for pulp cell response in feather injection studies* (Cont.)

		Study 1		Study 2		Study 3	
Response Factor	**Fixed Effect**	**F Value**	**P Value**	**F Value**	**P Value**	**F Value**	**P Value**
γδ T cell+ CD8+	Day	4.60	NS	25.70	<0.0001	9.24	<0.0001
	Treatment	2.36	NS	0.87	NS	0.28	NS
	Treatment*Day	5.28	0.0273	2.07	NS	2.47	0.0207
γδ T cell+ CD8-	Day	9.34	0.0128	36.61	<0.0001	11.79	<0.0001
	Treatment	4.11	0.0464	16.96	0.0001	12.79	0.0002
	Treatment*Day	0.81	NS	5.70	0.0033	3.15	0.0042
αβ1 Tcell+	Day	-	-	-	-	22.09	<0.0001
	Treatment	-	-	-	-	9.62	0.0005
	Treatment*Day	-	-	-	-	2.27	0.0300
αβ2 Tcell+	Day	4.94	0.0488	31.03	<0.0001	-	-
	Treatment	4.85	0.0309	6.88	0.0060	-	-
	Treatment*Day	1.82	NS	2.74	NS	-	-
αβ1 Tcell+	Day	5.48	0.0396	23.26	0.0001	-	-
	Treatment	7.89	0.0075	8.15	0.0032	-	-
	Treatment*Day	2.23	NS	2.52	NS	-	-
αβ1 T cell+	Day	-	-	-	-	29.91	<0.0001
CD4-CD8+	Treatment	-	-	-	-	18.20	0.0002
	Treatment*Day	-	-	-	-	3.27	0.0042
αβ1 T cell+	Day	-	-	-	-	4.94	0.0019
CD4+CD8+	Treatment	-	-	-	-	2.40	NS
	Treatment*Day	-	-	-	-	1.02	NS
αβ1 T cell+	Day	-	-	-	-	413.04	0.0025
CD4-CD8-	Treatment	-	-	-	-	0.29	NS
	Treatment*Day	-	-	-	-	0.92	NS
αβ1 T cell+	Day	-	-	-	-	11.39	0.0411
CD4+CD8-	Treatment	-	-	-	-	6.30	0.0204
	Treatment*Day	-	-	-	-	2.07	NS
αβ2 T cell+	Day	-	-	-	-	4.99	NS
	Treatment	-	-	-	-	2.22	NS
	Treatment*Day	-	-	-	-	0.62	NS
αβ2 T cell+	Day	-	-	-	-	8.76	NS
CD4-CD8+	Treatment	-	-	-	-	4.82	0.0377
	Treatment*Day	-	-	-	-	0.49	NS
αβ2 T cell+	Day	-	-	-	-	2.14	NS
CD4+CD8+	Treatment	-	-	-	-	2.90	NS
	Treatment*Day	-	-	-	-	0.47	NS
αβ2 T cell+	Day	-	-	-	-	26.25	<0.0001
CD4-CD8-	Treatment	-	-	-	-	0.91	NS
	Treatment*Day	-	-	-	-	1.67	NS
αβ2 T cell+	Day	-	-	-	-	14.42	<0.0001
CD4+CD8-	Treatment	-	-	-	-	1.75	NS
	Treatment*Day	-	-	-	-	1.69	NS
CD4/CD8 ratio	Day	5.40	0.0409	11.70	0.0016	18.53	0.0069
	Treatment	0.29	NS	0.01	NS	3.26	NS

Table E1. *Type III test of fixed effects results for pulp cell response in feather injection studies* (Cont.)

		Study 1		Study 2		Study 3	
Response Factor	**Fixed Effect**	**F Value**	**P Value**	**F Value**	**P Value**	**F Value**	**P Value**
	Treatment*Day	2.67	NS	1.58	NS	7.48	0.0253
B cells	Day	8.61	0.0153	15.23	0.0063	-	-
	Treatment	8.34	0.0062	6.78	0.0007	-	-
	Treatment*Day	2.25	NS	4.12	0.0121	-	-
B cell+ IgM+	Day	-	-	-	-	13.12	<0.0001
	Treatment	-	-	-	-	5.95	0.0054
	Treatment*Day	-	-	-	-	1.35	NS
B cell+ IgM-	Day	-	-	-	-	7.49	0.0001
	Treatment	-	-	-	-	2.43	NS
	Treatment*Day	-	-	-	-	2.13	0.0473
B cell+ IgG+	Day	-	-	-	-	3.86	0.0073
	Treatment	-	-	-	-	2.69	NS
	Treatment*Day	-	-	-	-	1.16	NS
B cell+ IgG-	Day	-	-	-	-	12.60	<0.0001
	Treatment	-	-	-	-	5.09	0.0121
	Treatment*Day	-	-	-	-	1.25	NS

APPENDIX F

Difference of Least Squares Means Results for Pulp Cell Responses in Feather Injection Studies

Table F1. *Difference of least squares means for pulp cell responses by day*

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
Study 1							
Macrophages	0	0.25	0.02433	0.2704	11	0.09	0.9299
	0	1	0.3032	0.1854	11	1.64	0.1302
	0	2	0.8433	0.2578	11	3.27	0.0074
	0	3	0.5138	0.1914	11	2.68	0.0212
	0	4	0.2427	0.4489	11	0.54	0.5996
	0	5	0.8063	0.2375	11	3.40	0.0060
	0	7	0.6568	0.1773	11	3.70	0.0035
	0.25	1	0.2788	0.1931	11	1.44	0.1765
	0.25	2	0.8190	0.3097	11	2.64	0.0228
	0.25	3	0.4895	0.2578	11	1.90	0.0842
	0.25	4	0.2183	0.4807	11	0.45	0.6585
	0.25	5	0.7820	0.2623	11	2.98	0.0125
	0.25	7	0.6325	0.2364	11	2.68	0.0216
	1	2	0.5402	0.2965	11	1.82	0.0958
	1	3	0.2107	0.2126	11	0.99	0.3431
	1	4	-0.06050	0.4790	11	-0.13	0.9018
	1	5	0.5032	0.2502	11	2.01	0.0695
	1	7	0.3537	0.2137	11	1.66	0.1261
	2	3	-0.3295	0.2033	11	-1.62	0.1333
	2	4	-0.6007	0.5189	11	-1.16	0.2716
	2	5	-0.03700	0.2913	11	-0.13	0.9012
	2	7	-0.1865	0.2838	11	-0.66	0.5246
	3	4	-0.2712	0.4939	11	-0.55	0.5940
	3	5	0.2925	0.2238	11	1.31	0.2180
	3	7	0.1430	0.1829	11	0.78	0.4508
	4	5	0.5637	0.2886	11	1.95	0.0767
	4	7	0.4142	0.4692	11	0.88	0.3962
	5	7	-0.1495	0.2387	11	-0.63	0.5439
Lymphocytes	0	0.25	-2.1047	0.7578	11	-2.78	0.0180
	0	1	-4.1497	0.8561	11	-4.85	0.0005
	0	2	-6.8680	1.1955	11	-5.74	0.0001
	0	3	-6.2333	1.3201	11	-4.72	0.0006
	0	4	-6.0937	0.8910	11	-6.84	<.0001
	0	5	-5.3493	1.4201	11	-3.77	0.0031
	0	7	-0.3483	0.8249	11	-0.42	0.6810
	0.25	1	-2.0450	0.9838	11	-2.08	0.0618

Table F1. *Difference of least squares means for pulp cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
	0.25	2	-4.7633	1.3090	11	-3.64	0.0039
	0.25	3	-4.1287	1.3266	11	-3.11	0.0099
	0.25	4	-3.9890	1.1634	11	-3.43	0.0056
	0.25	5	-3.2447	1.4316	11	-2.27	0.0446
	0.25	7	1.7563	1.0417	11	1.69	0.1199
	1	2	-2.7183	1.3847	11	-1.96	0.0754
	1	3	-2.0837	1.6161	11	-1.29	0.2237
	1	4	-1.9440	0.8671	11	-2.24	0.0465
	1	5	-1.1997	1.6850	11	-0.71	0.4913
	1	7	3.8013	0.9425	11	4.03	0.0020
	2	3	0.6347	1.4879	11	0.43	0.6779
	2	4	0.7743	1.0034	11	0.77	0.4565
	2	5	1.5187	1.1883	11	1.28	0.2276
	2	7	6.5197	1.2599	11	5.17	0.0003
	3	4	0.1397	1.8495	11	0.08	0.9412
	3	5	0.8840	1.4406	11	0.61	0.5519
	3	7	5.8850	1.3267	11	4.44	0.0010
	4	5	0.7443	1.7069	11	0.44	0.6712
	4	7	5.7453	1.2600	11	4.56	0.0008
	5	7	5.0010	1.1474	11	4.36	0.0011
CD4- CD8+	0	0.25	-0.4038	0.07093	11	-5.69	0.0001
	0	1	-0.5285	0.09797	11	-5.39	0.0002
	0	2	-0.8775	0.1567	11	-5.60	0.0002
	0	3	-0.6535	0.1237	11	-5.28	0.0003
	0	4	-0.8855	0.1097	11	-8.07	<.0001
	0	5	-0.8162	0.1895	11	-4.31	0.0012
	0	7	-0.1628	0.08139	11	-2.00	0.0707
	0.25	1	-0.1247	0.1144	11	-1.09	0.2989
	0.25	2	-0.4737	0.1777	11	-2.67	0.0220
	0.25	3	-0.2497	0.1360	11	-1.84	0.0936
	0.25	4	-0.4817	0.1336	11	-3.61	0.0041
	0.25	5	-0.4123	0.2046	11	-2.01	0.0690
	0.25	7	0.2410	0.1162	11	2.07	0.0624
	1	2	-0.3490	0.2063	11	-1.69	0.1188
	1	3	-0.1250	0.1856	11	-0.67	0.5146
	1	4	-0.3570	0.1063	11	-3.36	0.0064
	1	5	-0.2877	0.2106	11	-1.37	0.1992
	1	7	0.3657	0.1211	11	3.02	0.0117
	2	3	0.2240	0.1800	11	1.24	0.2392
	2	4	-0.00800	0.1659	11	-0.05	0.9624
	2	5	0.06133	0.1661	11	0.37	0.7190
	2	7	0.7147	0.1615	11	4.42	0.0010
	3	4	-0.2320	0.1910	11	-1.21	0.2498
	3	5	-0.1627	0.1755	11	-0.93	0.3739

Table F1. *Difference of least squares means for pulp cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
	3	7	0.4907	0.1186	11	4.14	0.0016
	4	5	0.06933	0.2255	11	0.31	0.7642
	4	7	0.7227	0.1472	11	4.91	0.0005
	5	7	0.6533	0.1357	11	4.81	0.0005
CD4+ CD8+	0	0.25	-0.1912	0.1002	11	-1.91	0.0830
	0	1	-0.1082	0.03454	11	-3.13	0.0096
	0	2	-0.2722	0.05121	11	-5.31	0.0002
	0	3	-0.1992	0.04076	11	-4.89	0.0005
	0	4	-0.1252	0.04424	11	-2.83	0.0164
	0	5	-0.02417	0.05052	11	-0.48	0.6418
	0	7	0.02017	0.02907	11	0.69	0.5022
	0.25	1	0.08300	0.1052	11	0.79	0.4467
	0.25	2	-0.08100	0.1184	11	-0.68	0.5079
	0.25	3	-0.00800	0.1063	11	-0.08	0.9413
	0.25	4	0.06600	0.1117	11	0.59	0.5665
	0.25	5	0.1670	0.1076	11	1.55	0.1489
	0.25	7	0.2113	0.09805	11	2.16	0.0541
	1	2	-0.1640	0.05839	11	-2.81	0.0170
	1	3	-0.09100	0.04883	11	-1.86	0.0893
	1	4	-0.01700	0.03810	11	-0.45	0.6641
	1	5	0.08400	0.06244	11	1.35	0.2056
	1	7	0.1283	0.03072	11	4.18	0.0015
	2	3	0.07300	0.04686	11	1.56	0.1476
	2	4	0.1470	0.04501	11	3.27	0.0075
	2	5	0.2480	0.04294	11	5.78	0.0001
	2	7	0.2923	0.05156	11	5.67	0.0001
	3	4	0.07400	0.05229	11	1.42	0.1847
	3	5	0.1750	0.04236	11	4.13	0.0017
	3	7	0.2193	0.02796	11	7.85	<.0001
	4	5	0.1010	0.05276	11	1.91	0.0819
	4	7	0.1453	0.04204	11	3.46	0.0054
	5	7	0.04433	0.04330	11	1.02	0.3279
CD4+ CD8-	0	0.25	-0.7853	0.1777	11	-4.42	0.0010
	0	1	-1.4153	0.3964	11	-3.57	0.0044
	0	2	-2.7940	0.5237	11	-5.33	0.0002
	0	3	-3.0877	0.5935	11	-5.20	0.0003
	0	4	-3.0917	0.4254	11	-7.27	<.0001
	0	5	-3.0313	0.8556	11	-3.54	0.0046
	0	7	-0.5870	0.4620	11	-1.27	0.2301
	0.25	1	-0.6300	0.4049	11	-1.56	0.1481
	0.25	2	-2.0087	0.4899	11	-4.10	0.0018
	0.25	3	-2.3023	0.5680	11	-4.05	0.0019
	0.25	4	-2.3063	0.4368	11	-5.28	0.0003
	0.25	5	-2.2460	0.8112	11	-2.77	0.0183
	0.25	7	0.1983	0.4349	11	0.46	0.6573
	1	2	-1.3787	0.6468	11	-2.13	0.0564

Table F1. *Difference of least squares means for pulp cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
	1	3	-1.6723	0.7391	11	-2.26	0.0449
	1	4	-1.6763	0.5152	11	-3.25	0.0077
	1	5	-1.6160	0.9801	11	-1.65	0.1274
	1	7	0.8283	0.4649	11	1.78	0.1024
	2	3	-0.2937	0.6247	11	-0.47	0.6475
	2	4	-0.2977	0.4686	11	-0.64	0.5383
	2	5	-0.2373	0.5325	11	-0.45	0.6645
	2	7	2.2070	0.4708	11	4.69	0.0007
	3	4	-0.00400	0.8589	11	-0.00	0.9964
	3	5	0.05633	0.8373	11	0.07	0.9476
	3	7	2.5007	0.6280	11	3.98	0.0022
	4	5	0.06033	0.8041	11	0.08	0.9415
	4	7	2.5047	0.5262	11	4.76	0.0006
	5	7	2.4443	0.6992	11	3.50	0.0050
γδ T cell+ CD8-	0	0.25	-0.1453	0.08891	11	-1.63	0.1304
	0	1	-0.2657	0.1232	11	-2.16	0.0541
	0	2	-0.3270	0.09458	11	-3.46	0.0054
	0	3	-0.1333	0.08913	11	-1.50	0.1628
	0	4	-0.1903	0.09137	11	-2.08	0.0614
	0	5	-0.02500	0.08972	11	-0.28	0.7857
	0	7	0.05800	0.07772	11	0.75	0.4711
	0.25	1	-0.1203	0.09191	11	-1.31	0.2172
	0.25	2	-0.1817	0.06577	11	-2.76	0.0185
	0.25	3	0.01200	0.05722	11	0.21	0.8377
	0.25	4	-0.04500	0.05741	11	-0.78	0.4497
	0.25	5	0.1203	0.05931	11	2.03	0.0674
	0.25	7	0.2033	0.04404	11	4.62	0.0007
	1	2	-0.06133	0.1074	11	-0.57	0.5793
	1	3	0.1323	0.1053	11	1.26	0.2348
	1	4	0.07533	0.07998	11	0.94	0.3665
	1	5	0.2407	0.09509	11	2.53	0.0279
	1	7	0.3237	0.08626	11	3.75	0.0032
	2	3	0.1937	0.04000	11	4.84	0.0005
	2	4	0.1367	0.07132	11	1.92	0.0817
	2	5	0.3020	0.04292	11	7.04	<.0001
	2	7	0.3850	0.04908	11	7.84	<.0001
	3	4	-0.05700	0.06497	11	-0.88	0.3990
	3	5	0.1083	0.03410	11	3.18	0.0088
	3	7	0.1913	0.04131	11	4.63	0.0007
	4	5	0.1653	0.05817	11	2.84	0.0160
	4	7	0.2483	0.04267	11	5.82	0.0001
	5	7	0.08300	0.03199	11	2.59	0.0249
αβ2 T cell+	0	0.25	-0.2460	0.05456	11	-4.51	0.0009
	0	1	-0.3390	0.1408	11	-2.41	0.0347
	0	2	-0.6753	0.1630	11	-4.14	0.0016
	0	3	-0.6097	0.1649	11	-3.70	0.0035

Table F1. *Difference of least squares means for pulp cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
	0	4	-0.7330	0.1319	11	-5.56	0.0002
	0	5	-0.8683	0.2735	11	-3.17	0.0088
	0	7	-0.06000	0.08047	11	-0.75	0.4715
	0.25	1	-0.09300	0.1327	11	-0.70	0.4978
	0.25	2	-0.4293	0.1596	11	-2.69	0.0210
	0.25	3	-0.3637	0.1520	11	-2.39	0.0357
	0.25	4	-0.4870	0.1357	11	-3.59	0.0043
	0.25	5	-0.6223	0.2628	11	-2.37	0.0373
	0.25	7	0.1860	0.08319	11	2.24	0.0470
	1	2	-0.3363	0.1877	11	-1.79	0.1007
	1	3	-0.2707	0.1866	11	-1.45	0.1749
	1	4	-0.3940	0.1438	11	-2.74	0.0192
	1	5	-0.5293	0.2838	11	-1.87	0.0890
	1	7	0.2790	0.1320	11	2.11	0.0582
	2	3	0.06567	0.1827	11	0.36	0.7261
	2	4	-0.05767	0.1163	11	-0.50	0.6298
	2	5	-0.1930	0.1927	11	-1.00	0.3380
	2	7	0.6153	0.1209	11	5.09	0.0004
	3	4	-0.1233	0.2166	11	-0.57	0.5805
	3	5	-0.2587	0.2265	11	-1.14	0.2776
	3	7	0.5497	0.1379	11	3.99	0.0021
	4	5	-0.1353	0.2529	11	-0.54	0.6033
	4	7	0.6730	0.1310	11	5.14	0.0003
	5	7	0.8083	0.2095	11	3.86	0.0027
αβ1 T cell+	0	0.25	-0.5560	0.1301	11	-4.27	0.0013
	0	1	-0.9967	0.3764	11	-2.65	0.0227
	0	2	-1.9190	0.5203	11	-3.69	0.0036
	0	3	-2.2140	0.5878	11	-3.77	0.0031
	0	4	-2.3107	0.4057	11	-5.69	0.0001
	0	5	-2.7563	0.7875	11	-3.50	0.0050
	0	7	-0.4330	0.2448	11	-1.77	0.1046
	0.25	1	-0.4407	0.3560	11	-1.24	0.2416
	0.25	2	-1.3630	0.4939	11	-2.76	0.0186
	0.25	3	-1.6580	0.5398	11	-3.07	0.0106
	0.25	4	-1.7547	0.3902	11	-4.50	0.0009
	0.25	5	-2.2003	0.7619	11	-2.89	0.0148
	0.25	7	0.1230	0.2520	11	0.49	0.6350
	1	2	-0.9223	0.6492	11	-1.42	0.1831
	1	3	-1.2173	0.6844	11	-1.78	0.1029
	1	4	-1.3140	0.4239	11	-3.10	0.0101
	1	5	-1.7597	0.8459	11	-2.08	0.0617
	1	7	0.5637	0.3582	11	1.57	0.1439
	2	3	-0.2950	0.6884	11	-0.43	0.6765
	2	4	-0.3917	0.4369	11	-0.90	0.3892
	2	5	-0.8373	0.4906	11	-1.71	0.1159
	2	7	1.4860	0.4634	11	3.21	0.0084

Table F1. *Difference of least squares means for pulp cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
	3	4	-0.09667	0.8026	11	-0.12	0.9063
	3	5	-0.5423	0.7858	11	-0.69	0.5044
	3	7	1.7810	0.5434	11	3.28	0.0074
	4	5	-0.4457	0.7712	11	-0.58	0.5750
	4	7	1.8777	0.4418	11	4.25	0.0014
	5	7	2.3233	0.6373	11	3.65	0.0038
CD4/CD8 ratio	0	0.25	3.2477	1.2033	11	2.70	0.0207
	0	1	3.0793	1.3409	11	2.30	0.0423
	0	2	2.6628	1.2342	11	2.16	0.0539
	0	3	1.4751	1.3225	11	1.12	0.2885
	0	4	2.0945	1.3660	11	1.53	0.1534
	0	5	2.6561	1.1187	11	2.37	0.0369
	0	7	1.9216	1.4294	11	1.34	0.2059
	0.25	1	-0.1683	0.3325	11	-0.51	0.6226
	0.25	2	-0.5848	0.4506	11	-1.30	0.2209
	0.25	3	-1.7726	0.4579	11	-3.87	0.0026
	0.25	4	-1.1532	0.3952	11	-2.92	0.0140
	0.25	5	-0.5916	0.4073	11	-1.45	0.1743
	0.25	7	-1.3260	0.4841	11	-2.74	0.0193
	1	2	-0.4165	0.3276	11	-1.27	0.2298
	1	3	-1.6042	0.3479	11	-4.61	0.0008
	1	4	-0.9848	0.4660	11	-2.11	0.0582
	1	5	-0.4233	0.4347	11	-0.97	0.3512
	1	7	-1.1577	0.3781	11	-3.06	0.0108
	2	3	-1.1877	0.3587	11	-3.31	0.0069
	2	4	-0.5684	0.5491	11	-1.04	0.3229
	2	5	-0.00678	0.4525	11	-0.01	0.9883
	2	7	-0.7412	0.5991	11	-1.24	0.2418
	3	4	0.6194	0.5594	11	1.11	0.2918
	3	5	1.1809	0.4351	11	2.71	0.0201
	3	7	0.4465	0.4095	11	1.09	0.2988
	4	5	0.5616	0.5361	11	1.05	0.3173
	4	7	-0.1729	0.6383	11	-0.27	0.7915
	5	7	-0.7344	0.4670	11	-1.57	0.1441
MHCII+ B cell+	0	0.25	-0.2627	0.07720	11	-3.40	0.0059
	0	1	-0.5190	0.08780	11	-5.91	0.0001
	0	2	-0.9333	0.1192	11	-7.83	<.0001
	0	3	-0.5817	0.06813	11	-8.54	<.0001
	0	4	-0.6207	0.06594	11	-9.41	<.0001
	0	5	-0.2887	0.1776	11	-1.63	0.1324
	0	7	-0.2943	0.1196	11	-2.46	0.0316
	0.25	1	-0.2563	0.1057	11	-2.43	0.0336
	0.25	2	-0.6707	0.1322	11	-5.07	0.0004
	0.25	3	-0.3190	0.08462	11	-3.77	0.0031
	0.25	4	-0.3580	0.07066	11	-5.07	0.0004
	0.25	5	-0.02600	0.1898	11	-0.14	0.8935

Table F1. *Difference of least squares means for pulp cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
	0.25	7	-0.03167	0.1316	11	-0.24	0.8142
	1	2	-0.4143	0.1116	11	-3.71	0.0034
	1	3	-0.06267	0.09649	11	-0.65	0.5294
	1	4	-0.1017	0.08940	11	-1.14	0.2796
	1	5	0.2303	0.1573	11	1.46	0.1710
	1	7	0.2247	0.1153	11	1.95	0.0773
	2	3	0.3517	0.09834	11	3.58	0.0043
	2	4	0.3127	0.1346	11	2.32	0.0403
	2	5	0.6447	0.1640	11	3.93	0.0023
	2	7	0.6390	0.1111	11	5.75	0.0001
	3	4	-0.03900	0.09519	11	-0.41	0.6899
	3	5	0.2930	0.1896	11	1.55	0.1506
	3	7	0.2873	0.1122	11	2.56	0.0264
	4	5	0.3320	0.1946	11	1.71	0.1161
	4	7	0.3263	0.1451	11	2.25	0.0459
	5	7	-0.00567	0.1256	11	-0.05	0.9648
B cells	0	0.25	-0.1620	0.04327	11	-3.74	0.0032
	0	1	-0.7797	0.2455	11	-3.18	0.0088
	0	2	-1.6153	0.3548	11	-4.55	0.0008
	0	3	-1.4667	0.4158	11	-3.53	0.0047
	0	4	-1.7297	0.3325	11	-5.20	0.0003
	0	5	-1.4910	0.3018	11	-4.94	0.0004
	0	7	-0.3753	0.1793	11	-2.09	0.0602
	0.25	1	-0.6177	0.2385	11	-2.59	0.0252
	0.25	2	-1.4533	0.3627	11	-4.01	0.0021
	0.25	3	-1.3047	0.3938	11	-3.31	0.0069
	0.25	4	-1.5677	0.3375	11	-4.64	0.0007
	0.25	5	-1.3290	0.3030	11	-4.39	0.0011
	0.25	7	-0.2133	0.1851	11	-1.15	0.2735
	1	2	-0.8357	0.4026	11	-2.08	0.0622
	1	3	-0.6870	0.4720	11	-1.46	0.1735
	1	4	-0.9500	0.2466	11	-3.85	0.0027
	1	5	-0.7113	0.4313	11	-1.65	0.1273
	1	7	0.4043	0.2511	11	1.61	0.1357
	2	3	0.1487	0.5472	11	0.27	0.7909
	2	4	-0.1143	0.3231	11	-0.35	0.7301
	2	5	0.1243	0.4589	11	0.27	0.7914
	2	7	1.2400	0.4128	11	3.00	0.0120
	3	4	-0.2630	0.6106	11	-0.43	0.6750
	3	5	-0.02433	0.4090	11	-0.06	0.9536
	3	7	1.0913	0.3811	11	2.86	0.0154
	4	5	0.2387	0.5316	11	0.45	0.6622
	4	7	1.3543	0.4074	11	3.32	0.0068
	5	7	1.1157	0.2862	11	3.90	0.0025
Study 2							
Heterophils	0	0.25	-15.1825	1.9600	12	-7.75	<.0001

Table F1. *Difference of least squares means for pulp cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
	0	1	-7.2706	1.3297	12	-5.47	0.0001
	0	2	-1.4650	0.4219	12	-3.47	0.0046
	0	5	-0.2950	0.1563	12	-1.89	0.0835
	0	7	-0.1069	0.1259	12.01	-0.85	0.4125
	0.25	1	7.9119	1.9163	12	4.13	0.0014
	0.25	2	13.7175	1.9663	12	6.98	<.0001
	0.25	5	14.8875	1.9251	12	7.73	<.0001
	0.25	7	15.0756	1.9305	12	7.81	<.0001
	1	2	5.8056	1.0947	12	5.30	0.0002
	1	5	6.9756	1.2364	12	5.64	0.0001
	1	7	7.1637	1.3604	12	5.27	0.0002
	2	5	1.1700	0.4265	12	2.74	0.0178
	2	7	1.3581	0.4645	12	2.92	0.0127
	5	7	0.1881	0.2017	12	0.93	0.3693
Macrophages	0	0.25	-2.4781	0.4285	12	-5.78	<.0001
	0	1	-1.6975	0.3259	12	-5.21	0.0002
	0	2	-0.6231	0.2665	12	-2.34	0.0375
	0	5	-1.2394	0.1965	12	-6.31	<.0001
	0	7	-0.7100	0.1596	12	-4.45	0.0008
	0.25	1	0.7806	0.5541	12	1.41	0.1843
	0.25	2	1.8550	0.4788	12	3.87	0.0022
	0.25	5	1.2387	0.3518	12	3.52	0.0042
	0.25	7	1.7681	0.3825	12	4.62	0.0006
	1	2	1.0744	0.3590	12	2.99	0.0112
	1	5	0.4581	0.4119	12	1.11	0.2878
	1	7	0.9875	0.3901	12	2.53	0.0264
	2	5	-0.6163	0.2626	12	-2.35	0.0369
	2	7	-0.08688	0.2720	12	-0.32	0.7549
	5	7	0.5294	0.1597	12	3.32	0.0062
CD4- CD8+	0	0.25	-0.5606	0.1348	12	-4.16	0.0013
	0	1	-1.4844	0.3498	12	-4.24	0.0011
	0	2	-1.0687	0.2125	12	-5.03	0.0003
	0	5	-1.8631	0.1871	12	-9.96	<.0001
	0	7	-0.9963	0.1684	12	-5.91	<.0001
	0.25	1	-0.9237	0.3758	12	-2.46	0.0302
	0.25	2	-0.5081	0.2315	12	-2.20	0.0485
	0.25	5	-1.3025	0.2413	12	-5.40	0.0002
	0.25	7	-0.4356	0.2003	12	-2.18	0.0503
	1	2	0.4156	0.3282	12	1.27	0.2293
	1	5	-0.3788	0.3972	12	-0.95	0.3591
	1	7	0.4881	0.3810	12	1.28	0.2244
	2	5	-0.7944	0.2377	12	-3.34	0.0059
	2	7	0.07250	0.2415	12	0.30	0.7692
	5	7	0.8669	0.1603	12	5.41	0.0002
CD4+ CD8+	0	0.25	-0.07875	0.03430	12	-2.30	0.0405
	0	1	-0.2519	0.1003	12	-2.51	0.0273

Table F1. *Difference of least squares means for pulp cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
	0	2	-0.4981	0.1454	12	-3.43	0.0050
	0	5	-0.8681	0.1549	12	-5.61	0.0001
	0	7	-0.3219	0.05490	12	-5.86	<.0001
	0.25	1	-0.1731	0.08953	12	-1.93	0.0771
	0.25	2	-0.4194	0.1508	12	-2.78	0.0166
	0.25	5	-0.7894	0.1621	12	-4.87	0.0004
	0.25	7	-0.2431	0.05051	12	-4.81	0.0004
	1	2	-0.2462	0.1031	12	-2.39	0.0342
	1	5	-0.6162	0.1370	12	-4.50	0.0007
	1	7	-0.07000	0.08662	12	-0.81	0.4348
	2	5	-0.3700	0.1157	12	-3.20	0.0076
	2	7	0.1763	0.1297	12	1.36	0.1991
	5	7	0.5463	0.1262	12	4.33	0.0010
CD4+ CD8-	0	0.25	-1.5612	0.4783	12	-3.26	0.0068
	0	1	-3.3306	0.7174	12	-4.64	0.0006
	0	2	-3.7250	0.8360	12	-4.46	0.0008
	0	5	-10.2862	1.0583	12	-9.72	<.0001
	0	7	-5.2675	0.5991	12	-8.79	<.0001
	0.25	1	-1.7694	0.7261	12	-2.44	0.0313
	0.25	2	-2.1637	0.7140	12	-3.03	0.0105
	0.25	5	-8.7250	1.1575	12	-7.54	<.0001
	0.25	7	-3.7063	0.6261	12	-5.92	<.0001
	1	2	-0.3944	0.7878	12	-0.50	0.6257
	1	5	-6.9556	1.2488	12	-5.57	0.0001
	1	7	-1.9369	0.8771	12	-2.21	0.0474
	2	5	-6.5613	1.0519	12	-6.24	<.0001
	2	7	-1.5425	0.9954	12	-1.55	0.1472
	5	7	5.0187	1.0882	12	4.61	0.0006
γδ T cell+ CD8+	0	0.25	-0.1994	0.04450	12	-4.48	0.0008
	0	1	-0.3894	0.08341	12	-4.67	0.0005
	0	2	-0.4088	0.1652	12	-2.48	0.0292
	0	5	-0.4869	0.04295	12	-11.34	<.0001
	0	7	-0.2331	0.04223	12	-5.52	0.0001
	0.25	1	-0.1900	0.09573	12	-1.98	0.0705
	0.25	2	-0.2094	0.1899	12	-1.10	0.2918
	0.25	5	-0.2875	0.06164	12	-4.66	0.0005
	0.25	7	-0.03375	0.05528	12	-0.61	0.5529
	1	2	-0.01937	0.1752	12	-0.11	0.9138
	1	5	-0.09750	0.06250	12	-1.56	0.1447
	1	7	0.1562	0.06788	12	2.30	0.0401
	2	5	-0.07813	0.1688	12	-0.46	0.6518
	2	7	0.1756	0.1718	12	1.02	0.3267
	5	7	0.2537	0.03757	12	6.75	<.0001
αβ2 T cell+	0	0.25	-0.5431	0.1335	12	-4.07	0.0016
	0	1	-1.2550	0.2580	12	-4.86	0.0004
	0	2	-1.2400	0.2372	12	-5.23	0.0002

Table F1. *Difference of least squares means for pulp cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
	0	5	-2.7450	0.2341	12	-11.73	<.0001
	0	7	-1.3569	0.1592	12	-8.52	<.0001
	0.25	1	-0.7119	0.2767	12	-2.57	0.0244
	0.25	2	-0.6969	0.2218	12	-3.14	0.0085
	0.25	5	-2.2019	0.2893	12	-7.61	<.0001
	0.25	7	-0.8138	0.1957	12	-4.16	0.0013
	1	2	0.01500	0.3014	12	0.05	0.9611
	1	5	-1.4900	0.3812	12	-3.91	0.0021
	1	7	-0.1019	0.2640	12	-0.39	0.7064
	2	5	-1.5050	0.2941	12	-5.12	0.0003
	2	7	-0.1169	0.2668	12	-0.44	0.6691
	5	7	1.3881	0.2092	12	6.64	<.0001
αβ1 T cell+	0	0.25	-1.5481	0.3841	12	-4.03	0.0017
	0	1	-3.4250	0.7235	12	-4.73	0.0005
	0	2	-3.5700	0.7827	12	-4.56	0.0007
	0	5	-9.0469	0.8950	12	-10.11	<.0001
	0	7	-4.5044	0.5083	12	-8.86	<.0001
	0.25	1	-1.8769	0.7252	12	-2.59	0.0237
	0.25	2	-2.0219	0.7557	12	-2.68	0.0202
	0.25	5	-7.4988	1.0223	12	-7.33	<.0001
	0.25	7	-2.9563	0.5304	12	-5.57	0.0001
	1	2	-0.1450	0.8384	12	-0.17	0.8656
	1	5	-5.6219	1.1827	12	-4.75	0.0005
	1	7	-1.0794	0.8176	12	-1.32	0.2114
	2	5	-5.4769	0.9748	12	-5.62	0.0001
	2	7	-0.9344	0.9489	12	-0.98	0.3442
	5	7	4.5425	0.9339	12	4.86	0.0004
CD4/CD8 ratio	0	0.25	-0.3601	0.3380	12	-1.07	0.3077
	0	1	-0.2501	0.3130	12	-0.80	0.4399
	0	2	-1.0330	0.4122	12	-2.51	0.0276
	0	5	-2.6131	0.4913	12	-5.32	0.0002
	0	7	-2.0471	0.5568	12	-3.68	0.0032
	0.25	1	0.1101	0.3529	12	0.31	0.7605
	0.25	2	-0.6729	0.2220	12	-3.03	0.0104
	0.25	5	-2.2530	0.3754	12	-6.00	<.0001
	0.25	7	-1.6870	0.3827	12	-4.41	0.0009
	1	2	-0.7830	0.4114	12	-1.90	0.0813
	1	5	-2.3630	0.3817	12	-6.19	<.0001
	1	7	-1.7971	0.5065	12	-3.55	0.0040
	2	5	-1.5801	0.4702	12	-3.36	0.0057
	2	7	-1.0141	0.2854	12	-3.55	0.0040
	5	7	0.5659	0.5632	12	1.00	0.3348
B cells	0	0.25	0.04500	0.02942	12	1.53	0.1520
	0	1	-0.1281	0.07539	12	-1.70	0.1150
	0	2	-0.2469	0.05280	12	-4.68	0.0005
	0	5	-0.6681	0.1508	12	-4.43	0.0008

Table F1. *Difference of least squares means for pulp cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
	0	7	-0.4662	0.07020	12	-6.64	<.0001
	0.25	1	-0.1731	0.06693	12	-2.59	0.0238
	0.25	2	-0.2919	0.04712	12	-6.19	<.0001
	0.25	5	-0.7131	0.1362	12	-5.23	0.0002
	0.25	7	-0.5112	0.06959	12	-7.35	<.0001
	1	2	-0.1188	0.09245	12	-1.28	0.2232
	1	5	-0.5400	0.1205	12	-4.48	0.0008
	1	7	-0.3381	0.08821	12	-3.83	0.0024
	2	5	-0.4212	0.1522	12	-2.77	0.0170
	2	7	-0.2194	0.09171	12	-2.39	0.0340
	5	7	0.2019	0.1440	12	1.40	0.1864
MHCII+	0	0.25	-0.04125	0.04590	12	-0.90	0.3865
Macrophages+	0	1	-0.8131	0.1788	12	-4.55	0.0007
	0	2	-0.3213	0.1219	12	-2.64	0.0218
	0	5	-0.2844	0.1249	12	-2.28	0.0419
	0	7	0.02125	0.1144	12	0.19	0.8558
	0.25	1	-0.7719	0.1851	12	-4.17	0.0013
	0.25	2	-0.2800	0.1257	12	-2.23	0.0458
	0.25	5	-0.2431	0.1208	12	-2.01	0.0672
	0.25	7	0.06250	0.1080	12	0.58	0.5736
	1	2	0.4919	0.1811	12	2.72	0.0188
	1	5	0.5288	0.1070	12	4.94	0.0003
	1	7	0.8344	0.2507	12	3.33	0.0060
	2	5	0.03687	0.1604	12	0.23	0.8221
	2	7	0.3425	0.1698	12	2.02	0.0666
	5	7	0.3056	0.1835	12	1.67	0.1216
Study 3							
MHCII+	0	0.25	-2.6608	0.4894	8.945	-5.44	0.0004
Macrophages+	0	1	-2.4108	0.4057	9.076	-5.94	0.0002
	0	2	0.7417	0.3968	9.016	1.87	0.0944
	0	3	0.5708	0.4165	8.978	1.37	0.2038
	0	4	0.9117	0.3140	9.039	2.90	0.0174
	0	5	0.5408	0.3668	9.069	1.47	0.1742
	0	7	-0.08077	0.3708	9.14	-0.22	0.8324
	0.25	1	0.2500	0.4277	9.004	0.58	0.5733
	0.25	2	3.4025	0.2760	8.937	12.33	<.0001
	0.25	3	3.2317	0.3533	8.929	9.15	<.0001
	0.25	4	3.5725	0.3726	8.937	9.59	<.0001
	0.25	5	3.2017	0.3815	8.859	8.39	<.0001
	0.25	7	2.5801	0.3850	8.939	6.70	<.0001
	1	2	3.1525	0.3013	9.108	10.46	<.0001
	1	3	2.9817	0.2475	8.993	12.05	<.0001
	1	4	3.3225	0.3440	9.1	9.66	<.0001
	1	5	2.9517	0.3272	9.228	9.02	<.0001
	1	7	2.3301	0.4261	9.178	5.47	0.0004
	2	3	-0.1708	0.2914	8.975	-0.59	0.5722

Table F1. *Difference of least squares means for pulp cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
	2	4	0.1700	0.2177	8.982	0.78	0.4550
	2	5	-0.2008	0.2430	8.955	-0.83	0.4300
	2	7	-0.8224	0.3068	9.039	-2.68	0.0251
	3	4	0.3408	0.3325	8.965	1.02	0.3323
	3	5	-0.03000	0.2321	9.129	-0.13	0.9000
	3	7	-0.6516	0.3280	9.147	-1.99	0.0777
	4	5	-0.3708	0.2687	8.975	-1.38	0.2009
	4	7	-0.9924	0.2364	9.123	-4.20	0.0022
	5	7	-0.6216	0.1768	8.807	-3.52	0.0068
CD4- CD8+	0	0.25	-0.5925	0.1782	9.234	-3.32	0.0086
	0	1	-2.0492	0.4079	9.052	-5.02	0.0007
	0	2	-2.8083	0.5620	9.382	-5.00	0.0007
	0	3	-5.6525	0.8421	9.191	-6.71	<.0001
	0	4	-4.2633	1.2086	8.946	-3.53	0.0065
	0	5	-3.6483	0.6389	8.92	-5.71	0.0003
	0	7	-0.7350	0.5765	8.691	-1.27	0.2354
	0.25	1	-1.4567	0.4587	9.279	-3.18	0.0108
	0.25	2	-2.2158	0.7182	9.374	-3.09	0.0124
	0.25	3	-5.0600	0.8333	9.239	-6.07	0.0002
	0.25	4	-3.6708	1.2271	9.018	-2.99	0.0151
	0.25	5	-3.0558	0.5898	8.916	-5.18	0.0006
	0.25	7	-0.1425	0.6355	8.872	-0.22	0.8276
	1	2	-0.7592	0.5681	8.957	-1.34	0.2144
	1	3	-3.6033	0.7409	9.172	-4.86	0.0008
	1	4	-2.2142	0.9913	8.984	-2.23	0.0524
	1	5	-1.5992	0.8157	8.971	-1.96	0.0817
	1	7	1.3142	0.6355	8.641	2.07	0.0699
	2	3	-2.8442	0.9541	9.202	-2.98	0.0151
	2	4	-1.4550	1.1977	8.863	-1.21	0.2558
	2	5	-0.8400	0.9843	9.108	-0.85	0.4153
	2	7	2.0733	0.5333	8.918	3.89	0.0038
	3	4	1.3892	0.9183	9.229	1.51	0.1638
	3	5	2.0042	1.0655	9.095	1.88	0.0923
	3	7	4.9175	0.8835	9.026	5.57	0.0003
	4	5	0.6150	1.5060	8.936	0.41	0.6926
	4	7	3.5283	1.3984	8.938	2.52	0.0328
	5	7	2.9133	0.8366	8.922	3.48	0.0070
CD4+ CD8-	0.25	1	-1.8475	1.2304	8.984	-1.50	0.1675
	0.25	2	-4.7867	1.3817	8.907	-3.46	0.0072
	0.25	3	-11.8367	2.4415	8.989	-4.85	0.0009
	0.25	4	-6.9533	2.5689	8.98	-2.71	0.0242
	0.25	5	-7.6925	1.4422	9.028	-5.33	0.0005
	0.25	7	-1.6815	1.2868	9.153	-1.31	0.2232
	1	2	-2.9392	1.0116	9.042	-2.91	0.0174
	1	3	-9.9892	2.0590	9	-4.85	0.0009
	1	4	-5.1058	2.0447	8.999	-2.50	0.0340

Table F1. *Difference of least squares means for pulp cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
	1	5	-5.8450	2.4106	9.013	-2.42	0.0383
	1	7	0.1660	1.3937	9.222	0.12	0.9077
	2	3	-7.0500	2.2396	9.042	-3.15	0.0117
	2	4	-2.1667	2.3235	9.059	-0.93	0.3753
	2	5	-2.9058	2.3247	8.948	-1.25	0.2430
	2	7	3.1052	0.9497	8.168	3.27	0.0110
	3	4	4.8833	2.3506	9.008	2.08	0.0675
	3	5	4.1442	3.4117	9.02	1.21	0.2553
	3	7	10.1552	2.4099	9.096	4.21	0.0022
	4	5	-0.7392	3.3181	9.022	-0.22	0.8287
	4	7	5.2719	2.4882	9.014	2.12	0.0631
	5	7	6.0110	1.8400	9.051	3.27	0.0097
αβ1 T cell+	0	0.25	-0.09833	0.02915	19.36	-3.37	0.0031
CD4+ CD8+	0	1	-0.1533	0.1085	6.68	-1.41	0.2023
	0	2	-0.2908	0.1008	10.8	-2.89	0.0151
	0	3	-0.9992	0.2917	10.83	-3.42	0.0058
	0	4	-0.7333	0.2634	10.43	-2.78	0.0186
	0	5	-0.8592	0.1944	10.47	-4.42	0.0012
	0	7	-0.8869	0.1968	9.242	-4.51	0.0014
	0.25	1	-0.05500	0.1079	6.731	-0.51	0.6266
	0.25	2	-0.1925	0.1003	10.95	-1.92	0.0815
	0.25	3	-0.9008	0.2909	10.84	-3.10	0.0103
	0.25	4	-0.6350	0.2626	10.43	-2.42	0.0352
	0.25	5	-0.7608	0.1937	10.48	-3.93	0.0026
	0.25	7	-0.7886	0.1961	9.278	-4.02	0.0028
	1	2	-0.1375	0.1236	18.56	-1.11	0.2801
	1	3	-0.8458	0.2768	12.19	-3.06	0.0098
	1	4	-0.5800	0.2508	12.32	-2.31	0.0388
	1	5	-0.7058	0.1903	14.85	-3.71	0.0021
	1	7	-0.7336	0.1937	13	-3.79	0.0023
	2	3	-0.7083	0.2767	11.96	-2.56	0.0251
	2	4	-0.4425	0.2503	11.96	-1.77	0.1026
	2	5	-0.5683	0.1887	13.85	-3.01	0.0094
	2	7	-0.5961	0.1920	12.14	-3.10	0.0090
	3	4	0.2658	0.3166	19.92	0.84	0.4111
	3	5	0.1400	0.2892	16.7	0.48	0.6346
	3	7	0.1123	0.2928	16.93	0.38	0.7062
	4	5	-0.1258	0.2682	17.74	-0.47	0.6446
	4	7	-0.1536	0.2718	17.79	-0.57	0.5791
	5	7	-0.02774	0.2273	19.18	-0.12	0.9041
αβ1 T cell+	0	0.25	1.3458	0.2163	9.179	6.22	0.0001
CD4- CD8-	0	1	-0.2667	0.4443	9.33	-0.60	0.5627
	0	2	1.2617	0.1727	9.29	7.31	<.0001
	0	3	1.2883	0.2876	9.334	4.48	0.0014
	0	4	1.7058	0.1946	9.281	8.76	<.0001
	0	5	1.3600	0.1951	9.264	6.97	<.0001

Table F1. *Difference of least squares means for pulp cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
	0	7	0.7328	0.3916	7.473	1.87	0.1008
	0.25	1	-1.6125	0.3491	9.158	-4.62	0.0012
	0.25	2	-0.08417	0.1258	8.963	-0.67	0.5202
	0.25	3	-0.05750	0.1886	9.105	-0.30	0.7673
	0.25	4	0.3600	0.1282	8.982	2.81	0.0205
	0.25	5	0.01417	0.1912	8.905	0.07	0.9426
	0.25	7	-0.6131	0.4219	7.709	-1.45	0.1856
	1	2	1.5283	0.3061	9.268	4.99	0.0007
	1	3	1.5550	0.2437	9.105	6.38	0.0001
	1	4	1.9725	0.3196	9.192	6.17	0.0002
	1	5	1.6267	0.2865	9.289	5.68	0.0003
	1	7	0.9994	0.5542	8.768	1.80	0.1057
	2	3	0.02667	0.1439	9.249	0.19	0.8570
	2	4	0.4442	0.1032	9.022	4.30	0.0020
	2	5	0.09833	0.1282	8.954	0.77	0.4628
	2	7	-0.5289	0.3525	7.187	-1.50	0.1761
	3	4	0.4175	0.1441	9.178	2.90	0.0173
	3	5	0.07167	0.1530	9.138	0.47	0.6504
	3	7	-0.5556	0.4461	7.928	-1.25	0.2485
	4	5	-0.3458	0.1575	8.923	-2.20	0.0560
	4	7	-0.9731	0.4180	7.665	-2.33	0.0497
	5	7	-0.6272	0.4482	7.624	-1.40	0.2010
αβ1 T cell+	0	0.25	-1.9442	0.4520	8.261	-4.30	0.0024
CD4+ CD8-	0	1	-3.6917	0.8116	8.983	-4.55	0.0014
	0	2	-6.2458	0.6893	8.779	-9.06	<.0001
	0	3	-9.9267	1.1128	8.991	-8.92	<.0001
	0	4	-7.1233	1.8998	8.99	-3.75	0.0046
	0	5	-7.9817	1.4053	8.995	-5.68	0.0003
	0	7	-3.2920	0.8616	7.759	-3.82	0.0054
	0.25	1	-1.7475	1.0581	8.865	-1.65	0.1335
	0.25	2	-4.3017	0.9212	8.123	-4.67	0.0015
	0.25	3	-7.9825	1.1210	8.709	-7.12	<.0001
	0.25	4	-5.1792	1.9607	8.992	-2.64	0.0269
	0.25	5	-6.0375	1.1737	8.729	-5.14	0.0007
	0.25	7	-1.3479	1.0917	7.535	-1.23	0.2541
	1	2	-2.5542	1.0302	8.831	-2.48	0.0355
	1	3	-6.2350	1.1032	8.989	-5.65	0.0003
	1	4	-3.4317	1.5226	8.986	-2.25	0.0507
	1	5	-4.2900	1.9387	8.99	-2.21	0.0542
	1	7	0.3996	1.2530	8.327	0.32	0.7576
	2	3	-3.6808	1.0646	8.916	-3.46	0.0073
	2	4	-0.8775	1.9258	8.945	-0.46	0.6595
	2	5	-1.7358	1.4578	8.999	-1.19	0.2642
	2	7	2.9538	0.7946	8.918	3.72	0.0049
	3	4	2.8033	1.7744	8.977	1.58	0.1487
	3	5	1.9450	1.7415	9.003	1.12	0.2930

Table F1. *Difference of least squares means for pulp cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
	3	7	6.6346	1.3061	8.643	5.08	0.0008
	4	5	-0.8583	2.5978	8.983	-0.33	0.7487
	4	7	3.8313	1.8839	8.501	2.03	0.0743
	5	7	4.6896	1.4262	8.879	3.29	0.0096
αβ2 T cell+	0	0.25	-0.2367	0.3410	11.32	-0.69	0.5016
CD4- CD8-	0	1	0.1150	0.1628	17.94	0.71	0.4890
	0	2	0.5175	0.1334	13.97	3.88	0.0017
	0	3	0.8958	0.1185	9.495	7.56	<.0001
	0	4	0.9308	0.1183	9.467	7.87	<.0001
	0	5	0.6617	0.1230	10.95	5.38	0.0002
	0	7	0.4605	0.1340	13.95	3.44	0.0040
	0.25	1	0.3517	0.3398	11.18	1.04	0.3225
	0.25	2	0.7542	0.3266	9.722	2.31	0.0443
	0.25	3	1.1325	0.3208	9.064	3.53	0.0063
	0.25	4	1.1675	0.3207	9.06	3.64	0.0053
	0.25	5	0.8983	0.3225	9.26	2.79	0.0207
	0.25	7	0.6971	0.3268	9.747	2.13	0.0594
	1	2	0.4025	0.1302	14.22	3.09	0.0079
	1	3	0.7808	0.1150	9.532	6.79	<.0001
	1	4	0.8158	0.1148	9.503	7.11	<.0001
	1	5	0.5467	0.1196	11.08	4.57	0.0008
	1	7	0.3455	0.1309	14.19	2.64	0.0193
	2	3	0.3783	0.06754	10.57	5.60	0.0002
	2	4	0.4133	0.06730	10.43	6.14	<.0001
	2	5	0.1442	0.07518	14.17	1.92	0.0755
	2	7	-0.05703	0.09208	15.72	-0.62	0.5446
	3	4	0.03500	0.02806	16.5	1.25	0.2297
	3	5	-0.2342	0.04370	13.27	-5.36	0.0001
	3	7	-0.4354	0.06891	9.276	-6.32	0.0001
	4	5	-0.2692	0.04336	12.85	-6.21	<.0001
	4	7	-0.4704	0.06867	9.148	-6.85	<.0001
	5	7	-0.2012	0.07640	12.5	-2.63	0.0212
αβ2 T cell+	0	0.25	-0.6167	0.1603	11.06	-3.85	0.0027
CD4+ CD8-	0	1	-1.1100	0.2384	9.989	-4.66	0.0009
	0	2	-2.7750	0.5591	9.8	-4.96	0.0006
	0	3	-3.3533	0.4151	10.03	-8.08	<.0001
	0	4	-2.3217	0.5831	9.236	-3.98	0.0030
	0	5	-2.6450	0.4284	8.659	-6.17	0.0002
	0	7	-1.1034	0.2742	8.766	-4.02	0.0032
	0.25	1	-0.4933	0.2656	15.18	-1.86	0.0828
	0.25	2	-2.1583	0.5634	10.52	-3.83	0.0030
	0.25	3	-2.7367	0.4255	11.49	-6.43	<.0001
	0.25	4	-1.7050	0.5869	9.982	-2.91	0.0157
	0.25	5	-2.0283	0.4386	10.23	-4.63	0.0009
	0.25	7	-0.4868	0.2970	12.53	-1.64	0.1261
	1	2	-1.6650	0.5794	11.98	-2.87	0.0140

Table F1. *Difference of least squares means for pulp cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
	1	3	-2.2433	0.4506	14.01	-4.98	0.0002
	1	4	-1.2117	0.6019	11.34	-2.01	0.0685
	1	5	-1.5350	0.4629	13.12	-3.32	0.0055
	1	7	0.006552	0.3382	16.24	0.02	0.9848
	2	3	-0.5783	0.6502	15.86	-0.89	0.3870
	2	4	0.4533	0.7522	17.43	0.60	0.5545
	2	5	0.1300	0.6587	16.75	0.20	0.8459
	2	7	1.6716	0.5919	12.79	2.82	0.0146
	3	4	1.0317	0.6695	15.6	1.54	0.1434
	3	5	0.7083	0.5562	17.88	1.27	0.2192
	3	7	2.2499	0.4677	15.08	4.81	0.0002
	4	5	-0.3233	0.6779	16.49	-0.48	0.6397
	4	7	1.2182	0.6139	12.2	1.98	0.0702
	5	7	1.5416	0.4796	14.14	3.21	0.0062
B cell+ IgM+	0	0.25	-0.6300	0.4144	9.876	-1.52	0.1598
	0	1	-2.2617	0.6533	9.343	-3.46	0.0067
	0	2	-6.2983	1.3446	9.08	-4.68	0.0011
	0	3	-8.1500	1.8621	9.042	-4.38	0.0018
	0	4	-8.5842	2.0969	9.033	-4.09	0.0027
	0	5	-13.2467	2.5518	9.022	-5.19	0.0006
	0	7	-6.2942	1.9084	8.035	-3.30	0.0108
	0.25	1	-1.6317	0.7632	15.1	-2.14	0.0493
	0.25	2	-5.6683	1.4014	10.62	-4.04	0.0021
	0.25	3	-7.5200	1.9035	9.85	-3.95	0.0028
	0.25	4	-7.9542	2.1338	9.67	-3.73	0.0042
	0.25	5	-12.6167	2.5822	9.453	-4.89	0.0007
	0.25	7	-5.6642	1.9488	8.721	-2.91	0.0180
	1	2	-4.0367	1.4896	12.97	-2.71	0.0179
	1	3	-5.8883	1.9693	11.15	-2.99	0.0121
	1	4	-6.3225	2.1927	10.7	-2.88	0.0153
	1	5	-10.9850	2.6311	10.15	-4.18	0.0018
	1	7	-4.0325	2.0132	9.834	-2.00	0.0735
	2	3	-1.8517	2.2934	16.37	-0.81	0.4310
	2	4	-2.2858	2.4878	15.32	-0.92	0.3724
	2	5	-6.9483	2.8817	13.63	-2.41	0.0306
	2	7	0.004167	2.3311	14.69	0.00	0.9986
	3	4	-0.4342	2.8015	17.75	-0.15	0.8786
	3	5	-5.0967	3.1565	16.46	-1.61	0.1254
	3	7	1.8558	2.6634	16.88	0.70	0.4954
	4	5	-4.6625	3.3005	17.35	-1.41	0.1754
	4	7	2.2900	2.8326	16.98	0.81	0.4300
	5	7	6.9525	3.1840	16.18	2.18	0.0441
B cell+ IgG+	0	0.25	-0.07583	0.06696	8.102	-1.13	0.2898
	0	1	-0.03333	0.01597	13.54	-2.09	0.0563
	0	2	-0.06500	0.01799	13.18	-3.61	0.0031
	0	3	-0.2375	0.07256	10.14	-3.27	0.0082

Table F1. *Difference of least squares means for pulp cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
	0	4	-0.2933	0.1775	10.17	-1.65	0.1289
	0	5	-0.6432	0.2061	9.288	-3.12	0.0118
	0	7	-1.1126	0.3330	7.921	-3.34	0.0104
	0.25	1	0.04250	0.06678	8.506	0.64	0.5413
	0.25	2	0.01083	0.06681	8.742	0.16	0.8749
	0.25	3	-0.1617	0.08962	18.42	-1.80	0.0876
	0.25	4	-0.2175	0.1780	11.72	-1.22	0.2459
	0.25	5	-0.5674	0.2054	10.15	-2.76	0.0198
	0.25	7	-1.0368	0.3284	8.232	-3.16	0.0130
	1	2	-0.03167	0.02070	18.06	-1.53	0.1434
	1	3	-0.2042	0.07226	10.36	-2.83	0.0174
	1	4	-0.2600	0.1766	10.15	-1.47	0.1712
	1	5	-0.6099	0.2051	9.251	-2.97	0.0152
	1	7	-1.0793	0.3320	7.913	-3.25	0.0119
	2	3	-0.1725	0.07224	10.51	-2.39	0.0370
	2	4	-0.2283	0.1762	10.16	-1.30	0.2238
	2	5	-0.5782	0.2048	9.246	-2.82	0.0194
	2	7	-1.0476	0.3316	7.91	-3.16	0.0136
	3	4	-0.05583	0.1788	11.93	-0.31	0.7602
	3	5	-0.4057	0.2058	10.3	-1.97	0.0762
	3	7	-0.8751	0.3281	8.312	-2.67	0.0275
	4	5	-0.3499	0.2453	16	-1.43	0.1730
	4	7	-0.8193	0.3468	11.51	-2.36	0.0367
	5	7	-0.4694	0.3587	12.76	-1.31	0.2138
B cell+ IgG-	0	0.25	-0.7575	0.4441	9.868	-1.71	0.1193
	0	1	-2.7400	0.7608	9.287	-3.60	0.0054
	0	2	-8.0858	1.4296	9.08	-5.66	0.0003
	0	3	-7.7333	1.8370	9.049	-4.21	0.0022
	0	4	-7.0542	1.9738	9.042	-3.57	0.0059
	0	5	-14.1856	3.2850	8.013	-4.32	0.0025
	0	7	-6.9181	2.3010	8.028	-3.01	0.0168
	0.25	1	-1.9825	0.8706	14.36	-2.28	0.0386
	0.25	2	-7.3283	1.4909	10.65	-4.92	0.0005
	0.25	3	-6.9758	1.8851	10	-3.70	0.0041
	0.25	4	-6.2967	2.0186	9.869	-3.12	0.0111
	0.25	5	-13.4281	3.3121	8.279	-4.05	0.0034
	0.25	7	-6.1606	2.3396	8.57	-2.63	0.0283
	1	2	-5.3458	1.6138	13.67	-3.31	0.0053
	1	3	-4.9933	1.9838	11.96	-2.52	0.0271
	1	4	-4.3142	2.1110	11.58	-2.04	0.0644
	1	5	-11.4456	3.3692	8.846	-3.40	0.0081
	1	7	-4.1781	2.4197	9.717	-1.73	0.1158
	2	3	0.3525	2.3238	16.97	0.15	0.8812
	2	4	1.0317	2.4334	16.4	0.42	0.6771
	2	5	-6.0997	3.5800	10.96	-1.70	0.1166
	2	7	1.1678	2.7056	13.56	0.43	0.6728

Table F1. *Difference of least squares means for pulp cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
	3	4	0.6792	2.6930	17.91	0.25	0.8038
	3	5	-6.4522	3.7613	12.68	-1.72	0.1106
	3	7	0.8153	2.9413	15.75	0.28	0.7852
	4	5	-7.1314	3.8300	13.27	-1.86	0.0849
	4	7	0.1361	3.0285	16.27	0.04	0.9647
	5	7	7.2675	4.0084	14.32	1.81	0.0908

Table F2. *Difference of least squares means for pulp cell responses by treatment*

Study/Factor	Treatment	Treatment	Estimate	Std. Error	DF	t Value	Pr > |t|
Study 1							
Lymphocytes	IO	IOEF	4.3924	1.1438	11	3.84	0.0027
	IO	IOmIgG	0.6855	1.0784	11	0.64	0.5380
	IOEF	IOmIgG	-3.7069	1.1438	11	-3.24	0.0079
CD45+	IO	IOEF	5.1513	1.5922	11	3.24	0.0079
	IO	IOmIgG	0.7647	1.5011	11	0.51	0.6205
	IOEF	IOmIgG	-4.3865	1.5922	11	-2.75	0.0187
CD4- CD8+	IO	IOEF	0.5871	0.1705	11	3.44	0.0055
	IO	IOmIgG	0.1440	0.1608	11	0.90	0.3897
	IOEF	IOmIgG	-0.4431	0.1705	11	-2.60	0.0248
CD4+ CD8+	IO	IOEF	0.06256	0.03695	11	1.69	0.1185
	IO	IOmIgG	-0.05200	0.03484	11	-1.49	0.1636
	IOEF	IOmIgG	-0.1146	0.03695	11	-3.10	0.0101
CD4+ CD8-	IO	IOEF	2.1476	0.6070	11	3.54	0.0046
	IO	IOmIgG	0.3913	0.5723	11	0.68	0.5083
	IOEF	IOmIgG	-1.7564	0.6070	11	-2.89	0.0146
γδ T cell+ CD8-	IO	IOEF	0.1433	0.06846	11	2.09	0.0604
	IO	IOmIgG	-0.04800	0.06454	11	-0.74	0.4727
	IOEF	IOmIgG	-0.1913	0.06846	11	-2.79	0.0175
αβ2 T cell+	IO	IOEF	0.5582	0.1911	11	2.92	0.0139
	IO	IOmIgG	0.07600	0.1802	11	0.42	0.6813
	IOEF	IOmIgG	-0.4823	0.1911	11	-2.52	0.0283
αβ1 T cell+	IO	IOEF	1.8976	0.5125	11	3.70	0.0035
	IO	IOmIgG	0.2300	0.4834	11	0.48	0.6435
	IOEF	IOmIgG	-1.6676	0.5126	11	-3.25	0.0077
B cells	IO	IOEF	1.1184	0.3198	11	3.50	0.0050
	IO	IOmIgG	-0.06375	0.3014	11	-0.21	0.8364
	IOEF	IOmIgG	-1.1821	0.3197	11	-3.70	0.0035

Table F2. *Difference of least squares means for pulp cell responses by treatment* (Cont.)

Study/Factor	Treatment	Treatment	Estimate	Std. Error	DF	t Value	Pr > |t|
Study 2							
CD4+ CD8-	Alu	AlumIgG	-2.8712	0.6657	12	-4.31	0.0010
	Alu	IO	-3.3167	0.6657	12	-4.98	0.0003
	Alu	IOmIgG	-1.8825	0.6657	12	-2.83	0.0152
	AlumIgG	IO	-0.4454	0.6657	12	-0.67	0.5161
	AlumIgG	IOmIgG	0.9887	0.6657	12	1.49	0.1632
	IO	IOmIgG	1.4342	0.6657	12	2.15	0.0522
αβ2 T cell+	Alu	AlumIgG	-1.1321	0.2672	12	-4.24	0.0012
	Alu	IO	-0.8963	0.2672	12	-3.35	0.0057
	Alu	IOmIgG	-0.5017	0.2672	12	-1.88	0.0849
	AlumIgG	IO	0.2358	0.2672	12	0.88	0.3947
	AlumIgG	IOmIgG	0.6304	0.2672	12	2.36	0.0361
	IO	IOmIgG	0.3946	0.2672	12	1.48	0.1654
αβ1 T cell+	Alu	AlumIgG	-2.5963	0.7097	12	-3.66	0.0033
	Alu	IO	-3.3429	0.7097	12	-4.71	0.0005
	Alu	IOmIgG	-2.0554	0.7097	12	-2.90	0.0134
	AlumIgG	IO	-0.7467	0.7097	12	-1.05	0.3135
	AlumIgG	IOmIgG	0.5408	0.7097	12	0.76	0.4608
	IO	IOmIgG	1.2875	0.7097	12	1.81	0.0947
Study 3							
MHCII+ Macrophages	AlumIgG	IOmIgG	-0.2456	0.1925	9.208	-1.28	0.2333
	AlumIgG	mIgG	0.7834	0.1924	9.202	4.07	0.0027
	IOmIgG	mIgG	1.0290	0.1925	9.208	5.35	0.0004
CD4- CD8+	AlumIgG	IOmIgG	2.0691	0.8063	9.213	2.57	0.0298
	AlumIgG	mIgG	2.3219	0.8169	9.211	2.84	0.0189
	IOmIgG	mIgG	0.2528	0.8449	9.213	0.30	0.7714
αβ1 T cell+	AlumIgG	IOmIgG	1.7167	0.8814	8.686	1.95	0.0844
CD4+ CD8-	AlumIgG	mIgG	3.1162	0.8795	8.659	3.54	0.0067
	IOmIgG	mIgG	1.3995	0.8814	8.686	1.59	0.1480
αβ2 T cell+	AlumIgG	IOmIgG	0.3374	0.1718	9.049	1.96	0.0810
CD4- CD8+	AlumIgG	mIgG	0.5256	0.1716	9.036	3.06	0.0135
	IOmIgG	mIgG	0.1883	0.1718	9.049	1.10	0.3016
B cell+ IgM+	AlumIgG	IOmIgG	1.4409	1.3883	40.75	1.04	0.3054
	AlumIgG	mIgG	4.6216	1.3698	40.5	3.37	0.0016
	IOmIgG	mIgG	3.1806	1.3883	40.75	2.29	0.0272
B cell+ IgG-	AlumIgG	IOmIgG	3.0376	1.5403	33.61	1.97	0.0569
	AlumIgG	mIgG	4.9223	1.5652	30.64	3.14	0.0037
	IOmIgG	mIgG	1.8847	1.5888	31.1	1.19	0.2445

Table F3. *Simple effect comparisons of treatment*day least squares means by treatment for live cells, Study 1*

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
IO	0	0.25	1.5700	3.3976	11	0.46	0.6530
IO	0	1	-7.4260	2.9533	11	-2.51	0.0288
IO	0	2	-1.4700	3.6770	11	-0.40	0.6970
IO	0	3	1.7360	3.5521	11	0.49	0.6346
IO	0	4	4.5640	3.7216	11	1.23	0.2457
IO	0	5	4.8140	2.5530	11	1.89	0.0860
IO	0	7	4.6420	2.0164	11	2.30	0.0419
IO	0.25	1	-8.9960	3.4578	11	-2.60	0.0246
IO	0.25	2	-3.0400	4.3254	11	-0.70	0.4968
IO	0.25	3	0.1660	3.5301	11	0.05	0.9633
IO	0.25	4	2.9940	3.7872	11	0.79	0.4459
IO	0.25	5	3.2440	2.1090	11	1.54	0.1523
IO	0.25	7	3.0720	3.0351	11	1.01	0.3332
IO	1	2	5.9560	2.9456	11	2.02	0.0682
IO	1	3	9.1620	2.6092	11	3.51	0.0049
IO	1	4	11.9900	3.2606	11	3.68	0.0036
IO	1	5	12.2400	2.3706	11	5.16	0.0003
IO	1	7	12.0680	2.8051	11	4.30	0.0013
IO	2	3	3.2060	2.3325	11	1.37	0.1966
IO	2	4	6.0340	3.3450	11	1.80	0.0987
IO	2	5	6.2840	3.4997	11	1.80	0.1001
IO	2	7	6.1120	2.9518	11	2.07	0.0627
IO	3	4	2.8280	3.0870	11	0.92	0.3793
IO	3	5	3.0780	2.4661	11	1.25	0.2379
IO	3	7	2.9060	3.4177	11	0.85	0.4133
IO	4	5	0.2500	2.3033	11	0.11	0.9155
IO	4	7	0.07800	3.9250	11	0.02	0.9845
IO	5	7	-0.1720	3.0093	11	-0.06	0.9554
IOEF	0	0.25	2.1325	3.7986	11	0.56	0.5858
IOEF	0	1	-15.2625	3.3019	11	-4.62	0.0007
IOEF	0	2	-8.1675	4.1111	11	-1.99	0.0724
IOEF	0	3	-5.2250	3.9714	11	-1.32	0.2150
IOEF	0	4	1.0625	4.1609	11	0.26	0.8032
IOEF	0	5	-2.3725	2.8544	11	-0.83	0.4235
IOEF	0	7	1.7275	2.2545	11	0.77	0.4596
IOEF	0.25	1	-17.3950	3.8659	11	-4.50	0.0009
IOEF	0.25	2	-10.3000	4.8360	11	-2.13	0.0566
IOEF	0.25	3	-7.3575	3.9467	11	-1.86	0.0892
IOEF	0.25	4	-1.0700	4.2343	11	-0.25	0.8052
IOEF	0.25	5	-4.5050	2.3579	11	-1.91	0.0825
IOEF	0.25	7	-0.4050	3.3933	11	-0.12	0.9071

Table F3. *Simple effect comparisons of treatment*day least squares means by treatment for live cells, Study 1* (Cont.)

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
IOEF	1	2	7.0950	3.2933	11	2.15	0.0542
IOEF	1	3	10.0375	2.9171	11	3.44	0.0055
IOEF	1	4	16.3250	3.6454	11	4.48	0.0009
IOEF	1	5	12.8900	2.6504	11	4.86	0.0005
IOEF	1	7	16.9900	3.1362	11	5.42	0.0002
IOEF	2	3	2.9425	2.6078	11	1.13	0.2832
IOEF	2	4	9.2300	3.7399	11	2.47	0.0312
IOEF	2	5	5.7950	3.9128	11	1.48	0.1667
IOEF	2	7	9.8950	3.3002	11	3.00	0.0121
IOEF	3	4	6.2875	3.4514	11	1.82	0.0958
IOEF	3	5	2.8525	2.7572	11	1.03	0.3231
IOEF	3	7	6.9525	3.8211	11	1.82	0.0961
IOEF	4	5	-3.4350	2.5752	11	-1.33	0.2092
IOEF	4	7	0.6650	4.3883	11	0.15	0.8823
IOEF	5	7	4.1000	3.3645	11	1.22	0.2485
IOmIgG	0	0.25	0.9220	3.3976	11	0.27	0.7911
IOmIgG	0	1	-12.1980	2.9533	11	-4.13	0.0017
IOmIgG	0	2	-4.4780	3.6770	11	-1.22	0.2488
IOmIgG	0	3	-7.3000	3.5521	11	-2.06	0.0644
IOmIgG	0	4	-5.8280	3.7216	11	-1.57	0.1456
IOmIgG	0	5	-6.9020	2.5530	11	-2.70	0.0205
IOmIgG	0	7	-3.1380	2.0164	11	-1.56	0.1479
IOmIgG	0.25	1	-13.1200	3.4578	11	-3.79	0.0030
IOmIgG	0.25	2	-5.4000	4.3254	11	-1.25	0.2378
IOmIgG	0.25	3	-8.2220	3.5301	11	-2.33	0.0399
IOmIgG	0.25	4	-6.7500	3.7872	11	-1.78	0.1023
IOmIgG	0.25	5	-7.8240	2.1090	11	-3.71	0.0034
IOmIgG	0.25	7	-4.0600	3.0351	11	-1.34	0.2080
IOmIgG	1	2	7.7200	2.9456	11	2.62	0.0238
IOmIgG	1	3	4.8980	2.6092	11	1.88	0.0872
IOmIgG	1	4	6.3700	3.2606	11	1.95	0.0766
IOmIgG	1	5	5.2960	2.3706	11	2.23	0.0472
IOmIgG	1	7	9.0600	2.8051	11	3.23	0.0080
IOmIgG	2	3	-2.8220	2.3325	11	-1.21	0.2517
IOmIgG	2	4	-1.3500	3.3450	11	-0.40	0.6943
IOmIgG	2	5	-2.4240	3.4997	11	-0.69	0.5029
IOmIgG	2	7	1.3400	2.9518	11	0.45	0.6587
IOmIgG	3	4	1.4720	3.0870	11	0.48	0.6428
IOmIgG	3	5	0.3980	2.4661	11	0.16	0.8747
IOmIgG	3	7	4.1620	3.4177	11	1.22	0.2488

Table F3. *Simple effect comparisons of treatment*day least squares means by treatment for live cells, Study 1* (Cont.)

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
IOmIgG	4	5	-1.0740	2.3033	11	-0.47	0.6501
IOmIgG	4	7	2.6900	3.9250	11	0.69	0.5073
IOmIgG	5	7	3.7640	3.0093	11	1.25	0.2370

Table F4. *Simple effect comparisons of treatment*day least squares means by day for live cells, Study 1*

Simple Effect Level	Treatment	Treatment	Estimate	Std. Error	DF	t Value	Pr > |t|
day 0	IO	IOEF	7.1235	2.6895	11	2.65	0.0226
day 0	IO	IOmIgG	8.8360	2.5357	11	3.48	0.0051
day 0	IOEF	IOmIgG	1.7125	2.6895	11	0.64	0.5373
day 0.25	IO	IOEF	7.6860	3.8270	11	2.01	0.0698
day 0.25	IO	IOmIgG	8.1880	3.6081	11	2.27	0.0444
day 0.25	IOEF	IOmIgG	0.5020	3.8270	11	0.13	0.8980
day 1	IO	IOEF	-0.7130	3.3221	11	-0.21	0.8340
day 1	IO	IOmIgG	4.0640	3.1321	11	1.30	0.2210
day 1	IOEF	IOmIgG	4.7770	3.3221	11	1.44	0.1783
day 2	IO	IOEF	0.4260	4.4206	11	0.10	0.9250
day 2	IO	IOmIgG	5.8280	4.1678	11	1.40	0.1896
day 2	IOEF	IOmIgG	5.4020	4.4206	11	1.22	0.2472
day 3	IO	IOEF	0.1625	3.4697	11	0.05	0.9635
day 3	IO	IOmIgG	-0.2000	3.2713	11	-0.06	0.9523
day 3	IOEF	IOmIgG	-0.3625	3.4697	11	-0.10	0.9187
day 4	IO	IOEF	3.6220	4.1736	11	0.87	0.4040
day 4	IO	IOmIgG	-1.5560	3.9349	11	-0.40	0.7001
day 4	IOEF	IOmIgG	-5.1780	4.1736	11	-1.24	0.2405
day 5	IO	IOEF	-0.06300	2.3296	11	-0.03	0.9789
day 5	IO	IOmIgG	-2.8800	2.1964	11	-1.31	0.2165
day 5	IOEF	IOmIgG	-2.8170	2.3296	11	-1.21	0.2519
day 7	IO	IOEF	4.2090	3.2763	11	1.28	0.2253
day 7	IO	IOmIgG	1.0560	3.0889	11	0.34	0.7389
day 7	IOEF	IOmIgG	-3.1530	3.2763	11	-0.96	0.3565

Table F5. *Simple effect comparisons of treatment*day least squares means by treatment for γδ T cell+ CD8+, Study 1*

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
IO	0	0.25	-1.6840	0.8049	11	-2.09	0.0604
IO	0	1	-0.9840	0.3557	11	-2.77	0.0184
IO	0	2	-1.3160	0.3629	11	-3.63	0.0040
IO	0	3	-1.2520	0.3346	11	-3.74	0.0033
IO	0	4	-0.9120	0.1942	11	-4.70	0.0007
IO	0	5	-1.2800	0.2877	11	-4.45	0.0010
IO	0	7	-0.5720	0.2191	11	-2.61	0.0242
IO	0.25	1	0.7000	0.7601	11	0.92	0.3768
IO	0.25	2	0.3680	0.7668	11	0.48	0.6407
IO	0.25	3	0.4320	0.7283	11	0.59	0.5651
IO	0.25	4	0.7720	0.7284	11	1.06	0.3119
IO	0.25	5	0.4040	0.8036	11	0.50	0.6250
IO	0.25	7	1.1120	0.8151	11	1.36	0.1997
IO	1	2	-0.3320	0.5073	11	-0.65	0.5263
IO	1	3	-0.2680	0.4895	11	-0.55	0.5950
IO	1	4	0.07200	0.3715	11	0.19	0.8499
IO	1	5	-0.2960	0.4507	11	-0.66	0.5249
IO	1	7	0.4120	0.3884	11	1.06	0.3116
IO	2	3	0.06400	0.2788	11	0.23	0.8226
IO	2	4	0.4040	0.2491	11	1.62	0.1332
IO	2	5	0.03600	0.2260	11	0.16	0.8763
IO	2	7	0.7440	0.2808	11	2.65	0.0226
IO	3	4	0.3400	0.3059	11	1.11	0.2901
IO	3	5	-0.02800	0.2533	11	-0.11	0.9140
IO	3	7	0.6800	0.2412	11	2.82	0.0167
IO	4	5	-0.3680	0.3039	11	-1.21	0.2513
IO	4	7	0.3400	0.2520	11	1.35	0.2043
IO	5	7	0.7080	0.2010	11	3.52	0.0048
IOEF	0	0.25	-1.7000	0.8999	11	-1.89	0.0855
IOEF	0	1	-0.5400	0.3977	11	-1.36	0.2018
IOEF	0	2	-0.4600	0.4057	11	-1.13	0.2810
IOEF	0	3	-0.4100	0.3741	11	-1.10	0.2965
IOEF	0	4	-0.1450	0.2172	11	-0.67	0.5181
IOEF	0	5	-0.1050	0.3217	11	-0.33	0.7502
IOEF	0	7	-0.1350	0.2449	11	-0.55	0.5926
IOEF	0.25	1	1.1600	0.8498	11	1.37	0.1995
IOEF	0.25	2	1.2400	0.8573	11	1.45	0.1760
IOEF	0.25	3	1.2900	0.8143	11	1.58	0.1414
IOEF	0.25	4	1.5550	0.8144	11	1.91	0.0826
IOEF	0.25	5	1.5950	0.8984	11	1.78	0.1035
IOEF	0.25	7	1.5650	0.9113	11	1.72	0.1139
IOEF	1	2	0.08000	0.5672	11	0.14	0.8904
IOEF	1	3	0.1300	0.5473	11	0.24	0.8166
IOEF	1	4	0.3950	0.4153	11	0.95	0.3620
IOEF	1	5	0.4350	0.5040	11	0.86	0.4065

Table F5. *Simple effect comparisons of treatment*day least squares means by treatment for γδ T cell+ CD8+, Study 1*

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
IOEF	1	7	0.4050	0.4343	11	0.93	0.3711
IOEF	2	3	0.05000	0.3117	11	0.16	0.8755
IOEF	2	4	0.3150	0.2785	11	1.13	0.2822
IOEF	2	5	0.3550	0.2527	11	1.40	0.1877
IOEF	2	7	0.3250	0.3140	11	1.04	0.3228
IOEF	3	4	0.2650	0.3420	11	0.77	0.4548
IOEF	3	5	0.3050	0.2832	11	1.08	0.3046
IOEF	3	7	0.2750	0.2697	11	1.02	0.3298
IOEF	4	5	0.04000	0.3398	11	0.12	0.9084
IOEF	4	7	0.01000	0.2817	11	0.04	0.9723
IOEF	5	7	-0.03000	0.2247	11	-0.13	0.8962
IOmIgG	0	0.25	-0.9120	0.8049	11	-1.13	0.2813
IOmIgG	0	1	-1.0760	0.3557	11	-3.02	0.0116
IOmIgG	0	2	-1.4000	0.3629	11	-3.86	0.0027
IOmIgG	0	3	-0.7280	0.3346	11	-2.18	0.0523
IOmIgG	0	4	-1.0520	0.1942	11	-5.42	0.0002
IOmIgG	0	5	-0.3600	0.2877	11	-1.25	0.2368
IOmIgG	0	7	0.04400	0.2191	11	0.20	0.8445
IOmIgG	0.25	1	-0.1640	0.7601	11	-0.22	0.8331
IOmIgG	0.25	2	-0.4880	0.7668	11	-0.64	0.5375
IOmIgG	0.25	3	0.1840	0.7283	11	0.25	0.8052
IOmIgG	0.25	4	-0.1400	0.7284	11	-0.19	0.8511
IOmIgG	0.25	5	0.5520	0.8036	11	0.69	0.5063
IOmIgG	0.25	7	0.9560	0.8151	11	1.17	0.2656
IOmIgG	1	2	-0.3240	0.5073	11	-0.64	0.5361
IOmIgG	1	3	0.3480	0.4895	11	0.71	0.4919
IOmIgG	1	4	0.02400	0.3715	11	0.06	0.9496
IOmIgG	1	5	0.7160	0.4507	11	1.59	0.1405
IOmIgG	1	7	1.1200	0.3884	11	2.88	0.0149
IOmIgG	2	3	0.6720	0.2788	11	2.41	0.0346
IOmIgG	2	4	0.3480	0.2491	11	1.40	0.1900
IOmIgG	2	5	1.0400	0.2260	11	4.60	0.0008
IOmIgG	2	7	1.4440	0.2808	11	5.14	0.0003
IOmIgG	3	4	-0.3240	0.3059	11	-1.06	0.3123
IOmIgG	3	5	0.3680	0.2533	11	1.45	0.1742
IOmIgG	3	7	0.7720	0.2412	11	3.20	0.0084
IOmIgG	4	5	0.6920	0.3039	11	2.28	0.0438
IOmIgG	4	7	1.0960	0.2520	11	4.35	0.0012
IOmIgG	5	7	0.4040	0.2010	11	2.01	0.0696

Table F6. *Simple effect comparisons of treatment*day least squares means by day for γδ T cell+ CD8+, Study 1*

Simple Effect Level	Treatment	Treatment	Estimate	Std. Error	DF	t Value	Pr > |t|
day 0	IO	IOEF	0.07500	0.1341	11	0.56	0.5871
day 0	IO	IOmIgG	-0.06000	0.1264	11	-0.47	0.6443
day 0	IOEF	IOmIgG	-0.1350	0.1341	11	-1.01	0.3357
day 0.25	IO	IOEF	0.05900	1.1717	11	0.05	0.9607
day 0.25	IO	IOmIgG	0.7120	1.1047	11	0.64	0.5324
day 0.25	IOEF	IOmIgG	0.6530	1.1717	11	0.56	0.5885
day 1	IO	IOEF	0.5190	0.5950	11	0.87	0.4017
day 1	IO	IOmIgG	-0.1520	0.5610	11	-0.27	0.7914
day 1	IOEF	IOmIgG	-0.6710	0.5950	11	-1.13	0.2834
day 2	IO	IOEF	0.9310	0.5047	11	1.84	0.0922
day 2	IO	IOmIgG	-0.1440	0.4759	11	-0.30	0.7678
day 2	IOEF	IOmIgG	-1.0750	0.5047	11	-2.13	0.0566
day 3	IO	IOEF	0.9170	0.4027	11	2.28	0.0438
day 3	IO	IOmIgG	0.4640	0.3797	11	1.22	0.2472
day 3	IOEF	IOmIgG	-0.4530	0.4027	11	-1.12	0.2846
day 4	IO	IOEF	0.8420	0.3001	11	2.81	0.0171
day 4	IO	IOmIgG	-0.2000	0.2830	11	-0.71	0.4944
day 4	IOEF	IOmIgG	-1.0420	0.3001	11	-3.47	0.0052
day 5	IO	IOEF	1.2500	0.3907	11	3.20	0.0085
day 5	IO	IOmIgG	0.8600	0.3683	11	2.33	0.0395
day 5	IOEF	IOmIgG	-0.3900	0.3907	11	-1.00	0.3396
day 7	IO	IOEF	0.5120	0.2929	11	1.75	0.1083
day 7	IO	IOmIgG	0.5560	0.2761	11	2.01	0.0692
day 7	IOEF	IOmIgG	0.04400	0.2929	11	0.15	0.8833

Table F7. *Simple effect comparisons of treatment*day least squares means by treatment for CD45+, Study 2*

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
Alu	0	0.25	-30.0875	7.3676	12	-4.08	0.0015
Alu	0	1	-25.8525	5.4672	12	-4.73	0.0005
Alu	0	2	-17.8300	5.8121	12	-3.07	0.0098
Alu	0	5	-25.0625	5.7467	12	-4.36	0.0009
Alu	0	7	-9.6550	4.9311	12	-1.96	0.0739
Alu	0.25	1	4.2350	6.8437	12	0.62	0.5476
Alu	0.25	2	12.2575	8.3297	12	1.47	0.1669
Alu	0.25	5	5.0250	11.0718	12	0.45	0.6580
Alu	0.25	7	20.4325	8.1559	12	2.51	0.0276
Alu	1	2	8.0225	5.6309	12	1.42	0.1797
Alu	1	5	0.7900	8.5602	12	0.09	0.9280
Alu	1	7	16.1975	6.7747	12	2.39	0.0341
Alu	2	5	-7.2325	6.6833	12	-1.08	0.3004

Table F7. *Simple effect comparisons of treatment*day least squares means by treatment for CD45+, Study 2* (Cont.)

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
Alu	2	7	8.1750	5.3888	12	1.52	0.1552
Alu	5	7	15.4075	6.3612	12	2.42	0.0322
AlumIgG	0	0.25	-43.4925	7.3676	12	-5.90	<.0001
AlumIgG	0	1	-53.5800	5.4672	12	-9.80	<.0001
AlumIgG	0	2	-48.4400	5.8121	12	-8.33	<.0001
AlumIgG	0	5	-57.5500	5.7467	12	-10.01	<.0001
AlumIgG	0	7	-34.4200	4.9311	12	-6.98	<.0001
AlumIgG	0.25	1	-10.0875	6.8437	12	-1.47	0.1662
AlumIgG	0.25	2	-4.9475	8.3297	12	-0.59	0.5636
AlumIgG	0.25	5	-14.0575	11.0718	12	-1.27	0.2283
AlumIgG	0.25	7	9.0725	8.1559	12	1.11	0.2878
AlumIgG	1	2	5.1400	5.6309	12	0.91	0.3793
AlumIgG	1	5	-3.9700	8.5602	12	-0.46	0.6511
AlumIgG	1	7	19.1600	6.7747	12	2.83	0.0152
AlumIgG	2	5	-9.1100	6.6833	12	-1.36	0.1979
AlumIgG	2	7	14.0200	5.3888	12	2.60	0.0232
AlumIgG	5	7	23.1300	6.3612	12	3.64	0.0034
IO	0	0.25	-22.4025	7.3676	12	-3.04	0.0103
IO	0	1	-16.6825	5.4672	12	-3.05	0.0101
IO	0	2	-8.0100	5.8121	12	-1.38	0.1933
IO	0	5	-36.7900	5.7467	12	-6.40	<.0001
IO	0	7	-25.8825	4.9311	12	-5.25	0.0002
IO	0.25	1	5.7200	6.8437	12	0.84	0.4196
IO	0.25	2	14.3925	8.3297	12	1.73	0.1096
IO	0.25	5	-14.3875	11.0718	12	-1.30	0.2182
IO	0.25	7	-3.4800	8.1559	12	-0.43	0.6772
IO	1	2	8.6725	5.6309	12	1.54	0.1495
IO	1	5	-20.1075	8.5602	12	-2.35	0.0368
IO	1	7	-9.2000	6.7747	12	-1.36	0.1995
IO	2	5	-28.7800	6.6833	12	-4.31	0.0010
IO	2	7	-17.8725	5.3888	12	-3.32	0.0061
IO	5	7	10.9075	6.3612	12	1.71	0.1121
IOmIgG	0	0.25	-30.0825	7.3676	12	-4.08	0.0015
IOmIgG	0	1	-20.4400	5.4672	12	-3.74	0.0028
IOmIgG	0	2	-14.1325	5.8121	12	-2.43	0.0316
IOmIgG	0	5	-35.4200	5.7467	12	-6.16	<.0001
IOmIgG	0	7	-23.1750	4.9311	12	-4.70	0.0005
IOmIgG	0.25	1	9.6425	6.8437	12	1.41	0.1842
IOmIgG	0.25	2	15.9500	8.3297	12	1.91	0.0797
IOmIgG	0.25	5	-5.3375	11.0718	12	-0.48	0.6384
IOmIgG	0.25	7	6.9075	8.1559	12	0.85	0.4136
IOmIgG	1	2	6.3075	5.6309	12	1.12	0.2846
IOmIgG	1	5	-14.9800	8.5602	12	-1.75	0.1056

Table F7. *Simple effect comparisons of treatment*day least squares means by treatment for CD45+, Study 2* (Cont.)

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
IOmIgG	1	7	-2.7350	6.7747	12	-0.40	0.6935
IOmIgG	2	5	-21.2875	6.6833	12	-3.19	0.0078
IOmIgG	2	7	-9.0425	5.3888	12	-1.68	0.1192
IOmIgG	5	7	12.2450	6.3612	12	1.92	0.0783

Table F8. *Simple effect comparisons of treatment*day least squares means by day for CD45+, Study 2*

Simple Effect Level	Treatment	Treatment	Estimate	Std. Error	DF	t Value	Pr > |t|
day 0	Alu	AlumIgG	2.5425	3.6585	12	0.69	0.5003
day 0	Alu	IO	0.7850	3.6585	12	0.21	0.8337
day 0	Alu	IOmIgG	-0.1000	3.6585	12	-0.03	0.9786
day 0	AlumIgG	IO	-1.7575	3.6585	12	-0.48	0.6396
day 0	AlumIgG	IOmIgG	-2.6425	3.6585	12	-0.72	0.4840
day 0	IO	IOmIgG	-0.8850	3.6585	12	-0.24	0.8129
day 0.25	Alu	AlumIgG	-10.8625	10.4830	12	-1.04	0.3205
day 0.25	Alu	IO	8.4700	10.4830	12	0.81	0.4348
day 0.25	Alu	IOmIgG	-0.09500	10.4830	12	-0.01	0.9929
day 0.25	AlumIgG	IO	19.3325	10.4830	12	1.84	0.0900
day 0.25	AlumIgG	IOmIgG	10.7675	10.4830	12	1.03	0.3246
day 0.25	IO	IOmIgG	-8.5650	10.4830	12	-0.82	0.4298
day 1	Alu	AlumIgG	-25.1850	8.6075	12	-2.93	0.0127
day 1	Alu	IO	9.9550	8.6075	12	1.16	0.2700
day 1	Alu	IOmIgG	5.3125	8.6075	12	0.62	0.5486
day 1	AlumIgG	IO	35.1400	8.6075	12	4.08	0.0015
day 1	AlumIgG	IOmIgG	30.4975	8.6075	12	3.54	0.0040
day 1	IO	IOmIgG	-4.6425	8.6075	12	-0.54	0.5995
day 2	Alu	AlumIgG	-28.0675	7.5646	12	-3.71	0.0030
day 2	Alu	IO	10.6050	7.5646	12	1.40	0.1863
day 2	Alu	IOmIgG	3.5975	7.5646	12	0.48	0.6429
day 2	AlumIgG	IO	38.6725	7.5646	12	5.11	0.0003
day 2	AlumIgG	IOmIgG	31.6650	7.5646	12	4.19	0.0013
day 2	IO	IOmIgG	-7.0075	7.5646	12	-0.93	0.3725
day 5	Alu	AlumIgG	-29.9450	7.2893	12	-4.11	0.0015
day 5	Alu	IO	-10.9425	7.2893	12	-1.50	0.1592
day 5	Alu	IOmIgG	-10.4575	7.2893	12	-1.43	0.1769
day 5	AlumIgG	IO	19.0025	7.2893	12	2.61	0.0229
day 5	AlumIgG	IOmIgG	19.4875	7.2893	12	2.67	0.0203
day 5	IO	IOmIgG	0.4850	7.2893	12	0.07	0.9480
day 7	Alu	AlumIgG	-22.2225	5.8717	12	-3.78	0.0026
day 7	Alu	IO	-15.4425	5.8717	12	-2.63	0.0220
day 7	Alu	IOmIgG	-13.6200	5.8717	12	-2.32	0.0388
day 7	AlumIgG	IO	6.7800	5.8717	12	1.15	0.2707
day 7	AlumIgG	IOmIgG	8.6025	5.8717	12	1.47	0.1686
day 7	IO	IOmIgG	1.8225	5.8717	12	0.31	0.7616

Table F9. *Simple effect comparisons of treatment*day least squares means by treatment for B cells, Study 2*

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
Alu	0	0.25	-0.2850	0.2457	12	-1.16	0.2686
Alu	0	1	-1.8150	1.0986	12	-1.65	0.1244
Alu	0	2	-1.5750	1.8760	12	-0.84	0.4176
Alu	0	5	-1.0275	1.3653	12	-0.75	0.4662
Alu	0	7	-0.3850	1.0612	12	-0.36	0.7231
Alu	0.25	1	-1.5300	1.0807	12	-1.42	0.1823
Alu	0.25	2	-1.2900	1.7777	12	-0.73	0.4820
Alu	0.25	5	-0.7425	1.2676	12	-0.59	0.5689
Alu	0.25	7	-0.10000	0.9034	12	-0.11	0.9137
Alu	1	2	0.2400	1.3576	12	0.18	0.8626
Alu	1	5	0.7875	1.4158	12	0.56	0.5883
Alu	1	7	1.4300	1.1872	12	1.20	0.2516
Alu	2	5	0.5475	1.5652	12	0.35	0.7326
Alu	2	7	1.1900	1.4639	12	0.81	0.4321
Alu	5	7	0.6425	1.1854	12	0.54	0.5977
AlumIgG	0	0.25	-0.7950	0.2457	12	-3.24	0.0071
AlumIgG	0	1	-5.6150	1.0986	12	-5.11	0.0003
AlumIgG	0	2	-11.3300	1.8760	12	-6.04	<.0001
AlumIgG	0	5	-13.2075	1.3653	12	-9.67	<.0001
AlumIgG	0	7	-4.1900	1.0612	12	-3.95	0.0019
AlumIgG	0.25	1	-4.8200	1.0807	12	-4.46	0.0008
AlumIgG	0.25	2	-10.5350	1.7777	12	-5.93	<.0001
AlumIgG	0.25	5	-12.4125	1.2676	12	-9.79	<.0001
AlumIgG	0.25	7	-3.3950	0.9034	12	-3.76	0.0027
AlumIgG	1	2	-5.7150	1.3576	12	-4.21	0.0012
AlumIgG	1	5	-7.5925	1.4158	12	-5.36	0.0002
AlumIgG	1	7	1.4250	1.1872	12	1.20	0.2532
AlumIgG	2	5	-1.8775	1.5652	12	-1.20	0.2535
AlumIgG	2	7	7.1400	1.4639	12	4.88	0.0004
AlumIgG	5	7	9.0175	1.1854	12	7.61	<.0001
IO	0	0.25	-0.4075	0.2457	12	-1.66	0.1231
IO	0	1	-2.2550	1.0986	12	-2.05	0.0626
IO	0	2	-2.6625	1.8760	12	-1.42	0.1813
IO	0	5	-5.2600	1.3653	12	-3.85	0.0023
IO	0	7	-4.1075	1.0612	12	-3.87	0.0022
IO	0.25	1	-1.8475	1.0807	12	-1.71	0.1130
IO	0.25	2	-2.2550	1.7777	12	-1.27	0.2287
IO	0.25	5	-4.8525	1.2676	12	-3.83	0.0024
IO	0.25	7	-3.7000	0.9034	12	-4.10	0.0015
IO	1	2	-0.4075	1.3576	12	-0.30	0.7692
IO	1	5	-3.0050	1.4158	12	-2.12	0.0553
IO	1	7	-1.8525	1.1872	12	-1.56	0.1447
IO	2	5	-2.5975	1.5652	12	-1.66	0.1229
IO	2	7	-1.4450	1.4639	12	-0.99	0.3431
IO	5	7	1.1525	1.1854	12	0.97	0.3501

Table F9. *Simple effect comparisons of treatment*day least squares means by treatment for B cells, Study 2* (Cont.)

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
IOmIgG	0	0.25	0.1425	0.2457	12	0.58	0.5727
IOmIgG	0	1	-2.1775	1.0986	12	-1.98	0.0708
IOmIgG	0	2	-3.3025	1.8760	12	-1.76	0.1038
IOmIgG	0	5	-5.9750	1.3653	12	-4.38	0.0009
IOmIgG	0	7	-5.3800	1.0612	12	-5.07	0.0003
IOmIgG	0.25	1	-2.3200	1.0807	12	-2.15	0.0529
IOmIgG	0.25	2	-3.4450	1.7777	12	-1.94	0.0765
IOmIgG	0.25	5	-6.1175	1.2676	12	-4.83	0.0004
IOmIgG	0.25	7	-5.5225	0.9034	12	-6.11	<.0001
IOmIgG	1	2	-1.1250	1.3576	12	-0.83	0.4235
IOmIgG	1	5	-3.7975	1.4158	12	-2.68	0.0200
IOmIgG	1	7	-3.2025	1.1872	12	-2.70	0.0194
IOmIgG	2	5	-2.6725	1.5652	12	-1.71	0.1135
IOmIgG	2	7	-2.0775	1.4639	12	-1.42	0.1813
IOmIgG	5	7	0.5950	1.1854	12	0.50	0.6248

Table F10. *Simple effect comparisons of treatment*day least squares means by day for B cells, Study 2*

Simple Effect Level	Treatment	Treatment	Estimate	Std. Error	DF	t Value	Pr > |t|
day 0	Alu	AlumIgG	0.1400	0.3147	12	0.44	0.6643
day 0	Alu	IO	0.03250	0.3147	12	0.10	0.9195
day 0	Alu	IOmIgG	-0.2650	0.3147	12	-0.84	0.4162
day 0	AlumIgG	IO	-0.1075	0.3147	12	-0.34	0.7386
day 0	AlumIgG	IOmIgG	-0.4050	0.3147	12	-1.29	0.2224
day 0	IO	IOmIgG	-0.2975	0.3147	12	-0.95	0.3631
day 0.25	Alu	AlumIgG	-0.3700	0.2663	12	-1.39	0.1900
day 0.25	Alu	IO	-0.09000	0.2663	12	-0.34	0.7412
day 0.25	Alu	IOmIgG	0.1625	0.2663	12	0.61	0.5531
day 0.25	AlumIgG	IO	0.2800	0.2663	12	1.05	0.3138
day 0.25	AlumIgG	IOmIgG	0.5325	0.2663	12	2.00	0.0687
day 0.25	IO	IOmIgG	0.2525	0.2663	12	0.95	0.3618
day 1	Alu	AlumIgG	-3.6600	1.7256	12	-2.12	0.0554
day 1	Alu	IO	-0.4075	1.7256	12	-0.24	0.8173
day 1	Alu	IOmIgG	-0.6275	1.7256	12	-0.36	0.7225
day 1	AlumIgG	IO	3.2525	1.7256	12	1.88	0.0839
day 1	AlumIgG	IOmIgG	3.0325	1.7256	12	1.76	0.1043
day 1	IO	IOmIgG	-0.2200	1.7256	12	-0.13	0.9007
day 2	Alu	AlumIgG	-9.6150	2.6783	12	-3.59	0.0037
day 2	Alu	IO	-1.0550	2.6783	12	-0.39	0.7006
day 2	Alu	IOmIgG	-1.9925	2.6783	12	-0.74	0.4712
day 2	AlumIgG	IO	8.5600	2.6783	12	3.20	0.0077
day 2	AlumIgG	IOmIgG	7.6225	2.6783	12	2.85	0.0147
day 2	IO	IOmIgG	-0.9375	2.6783	12	-0.35	0.7324
day 5	Alu	AlumIgG	-12.0400	1.8997	12	-6.34	<.0001
day 5	Alu	IO	-4.2000	1.8997	12	-2.21	0.0472
day 5	Alu	IOmIgG	-5.2125	1.8997	12	-2.74	0.0178
day 5	AlumIgG	IO	7.8400	1.8997	12	4.13	0.0014
day 5	AlumIgG	IOmIgG	6.8275	1.8997	12	3.59	0.0037
day 5	IO	IOmIgG	-1.0125	1.8997	12	-0.53	0.6038
day 7	Alu	AlumIgG	-3.6650	1.3986	12	-2.62	0.0224
day 7	Alu	IO	-3.6900	1.3986	12	-2.64	0.0216
day 7	Alu	IOmIgG	-5.2600	1.3986	12	-3.76	0.0027
day 7	AlumIgG	IO	-0.02500	1.3986	12	-0.02	0.9860
day 7	AlumIgG	IOmIgG	-1.5950	1.3986	12	-1.14	0.2764
day 7	IO	IOmIgG	-1.5700	1.3986	12	-1.12	0.2836

Table F11. *Simple effect comparisons of treatment*day least squares means by treatment for γδ T cell+ CD8+, Study 2*

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
Alu	0	0.25	-3.6675	1.5883	12	-2.31	0.0395
Alu	0	1	-4.2400	1.5915	12	-2.66	0.0206
Alu	0	2	-6.8500	2.6142	12	-2.62	0.0224
Alu	0	5	-5.2575	1.9844	12	-2.65	0.0212
Alu	0	7	-1.6125	0.9373	12	-1.72	0.1110
Alu	0.25	1	-0.5725	2.4147	12	-0.24	0.8166
Alu	0.25	2	-3.1825	3.2150	12	-0.99	0.3418
Alu	0.25	5	-1.5900	2.8479	12	-0.56	0.5869
Alu	0.25	7	2.0550	1.4513	12	1.42	0.1822
Alu	1	2	-2.6100	2.0713	12	-1.26	0.2316
Alu	1	5	-1.0175	2.6704	12	-0.38	0.7098
Alu	1	7	2.6275	1.8828	12	1.40	0.1881
Alu	2	5	1.5925	2.3712	12	0.67	0.5146
Alu	2	7	5.2375	2.2102	12	2.37	0.0354
Alu	5	7	3.6450	1.9919	12	1.83	0.0922
AlumIgG	0	0.25	-12.9425	1.5883	12	-8.15	<.0001
AlumIgG	0	1	-14.9575	1.5915	12	-9.40	<.0001
AlumIgG	0	2	-14.1700	2.6142	12	-5.42	0.0002
AlumIgG	0	5	-4.0650	1.9844	12	-2.05	0.0630
AlumIgG	0	7	-2.8775	0.9373	12	-3.07	0.0097
AlumIgG	0.25	1	-2.0150	2.4147	12	-0.83	0.4203
AlumIgG	0.25	2	-1.2275	3.2150	12	-0.38	0.7093
AlumIgG	0.25	5	8.8775	2.8479	12	3.12	0.0089
AlumIgG	0.25	7	10.0650	1.4513	12	6.94	<.0001
AlumIgG	1	2	0.7875	2.0713	12	0.38	0.7104
AlumIgG	1	5	10.8925	2.6704	12	4.08	0.0015
AlumIgG	1	7	12.0800	1.8828	12	6.42	<.0001
AlumIgG	2	5	10.1050	2.3712	12	4.26	0.0011
AlumIgG	2	7	11.2925	2.2102	12	5.11	0.0003
AlumIgG	5	7	1.1875	1.9919	12	0.60	0.5622
IO	0	0.25	-1.0725	1.5883	12	-0.68	0.5123
IO	0	1	-2.2700	1.5915	12	-1.43	0.1793
IO	0	2	-1.2350	2.6142	12	-0.47	0.6451
IO	0	5	-2.5875	1.9844	12	-1.30	0.2167
IO	0	7	-0.9000	0.9373	12	-0.96	0.3559
IO	0.25	1	-1.1975	2.4147	12	-0.50	0.6289
IO	0.25	2	-0.1625	3.2150	12	-0.05	0.9605
IO	0.25	5	-1.5150	2.8479	12	-0.53	0.6045
IO	0.25	7	0.1725	1.4513	12	0.12	0.9074
IO	1	2	1.0350	2.0713	12	0.50	0.6263
IO	1	5	-0.3175	2.6704	12	-0.12	0.9073
IO	1	7	1.3700	1.8828	12	0.73	0.4808
IO	2	5	-1.3525	2.3712	12	-0.57	0.5789
IO	2	7	0.3350	2.2102	12	0.15	0.8820
IO	5	7	1.6875	1.9919	12	0.85	0.4135

Table F11. *Simple effect comparisons of treatment*day least squares means by treatment for γδ T cell+ CD8+, 2013 IO vs. Alum* (Cont.)

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
IOmIgG	0	0.25	-0.7325	1.5883	12	-0.46	0.6529
IOmIgG	0	1	-1.3925	1.5915	12	-0.87	0.3988
IOmIgG	0	2	-0.7500	2.6142	12	-0.29	0.7791
IOmIgG	0	5	-1.4225	1.9844	12	-0.72	0.4872
IOmIgG	0	7	-0.9500	0.9373	12	-1.01	0.3308
IOmIgG	0.25	1	-0.6600	2.4147	12	-0.27	0.7892
IOmIgG	0.25	2	-0.01750	3.2150	12	-0.01	0.9957
IOmIgG	0.25	5	-0.6900	2.8479	12	-0.24	0.8127
IOmIgG	0.25	7	-0.2175	1.4513	12	-0.15	0.8834
IOmIgG	1	2	0.6425	2.0713	12	0.31	0.7617
IOmIgG	1	5	-0.03000	2.6704	12	-0.01	0.9912
IOmIgG	1	7	0.4425	1.8828	12	0.24	0.8182
IOmIgG	2	5	-0.6725	2.3712	12	-0.28	0.7815
IOmIgG	2	7	-0.2000	2.2102	12	-0.09	0.9294
IOmIgG	5	7	0.4725	1.9919	12	0.24	0.8165

Table F12. *Simple effect comparisons of treatment*day least squares means by day for γδ T cell+ CD8+, Study 2*

Simple Effect Level	Treatment	Treatment	Estimate	Std. Error	DF	t Value	Pr > |t|
day 0	Alu	AlumIgG	0.5900	0.5963	12	0.99	0.3420
day 0	Alu	IO	0.4975	0.5963	12	0.83	0.4204
day 0	Alu	IOmIgG	-0.2075	0.5963	12	-0.35	0.7339
day 0	AlumIgG	IO	-0.09250	0.5963	12	-0.16	0.8793
day 0	AlumIgG	IOmIgG	-0.7975	0.5963	12	-1.34	0.2059
day 0	IO	IOmIgG	-0.7050	0.5963	12	-1.18	0.2600
day 0.25	Alu	AlumIgG	-8.6850	2.2508	12	-3.86	0.0023
day 0.25	Alu	IO	3.0925	2.2508	12	1.37	0.1946
day 0.25	Alu	IOmIgG	2.7275	2.2508	12	1.21	0.2489
day 0.25	AlumIgG	IO	11.7775	2.2508	12	5.23	0.0002
day 0.25	AlumIgG	IOmIgG	11.4125	2.2508	12	5.07	0.0003
day 0.25	IO	IOmIgG	-0.3650	2.2508	12	-0.16	0.8739
day 1	Alu	AlumIgG	-10.1275	2.1379	12	-4.74	0.0005
day 1	Alu	IO	2.4675	2.1379	12	1.15	0.2709
day 1	Alu	IOmIgG	2.6400	2.1379	12	1.23	0.2405
day 1	AlumIgG	IO	12.5950	2.1379	12	5.89	<.0001
day 1	AlumIgG	IOmIgG	12.7675	2.1379	12	5.97	<.0001
day 1	IO	IOmIgG	0.1725	2.1379	12	0.08	0.9370
day 2	Alu	AlumIgG	-6.7300	3.4891	12	-1.93	0.0777
day 2	Alu	IO	6.1125	3.4891	12	1.75	0.1053
day 2	Alu	IOmIgG	5.8925	3.4891	12	1.69	0.1170
day 2	AlumIgG	IO	12.8425	3.4891	12	3.68	0.0031
day 2	AlumIgG	IOmIgG	12.6225	3.4891	12	3.62	0.0035
day 2	IO	IOmIgG	-0.2200	3.4891	12	-0.06	0.9508
day 5	Alu	AlumIgG	1.7825	2.6949	12	0.66	0.5208
day 5	Alu	IO	3.1675	2.6949	12	1.18	0.2626
day 5	Alu	IOmIgG	3.6275	2.6949	12	1.35	0.2032
day 5	AlumIgG	IO	1.3850	2.6949	12	0.51	0.6166
day 5	AlumIgG	IOmIgG	1.8450	2.6949	12	0.68	0.5066
day 5	IO	IOmIgG	0.4600	2.6949	12	0.17	0.8673
day 7	Alu	AlumIgG	-0.6750	1.2912	12	-0.52	0.6106
day 7	Alu	IO	1.2100	1.2912	12	0.94	0.3672
day 7	Alu	IOmIgG	0.4550	1.2912	12	0.35	0.7307
day 7	AlumIgG	IO	1.8850	1.2912	12	1.46	0.1700
day 7	AlumIgG	IOmIgG	1.1300	1.2912	12	0.88	0.3987
day 7	IO	IOmIgG	-0.7550	1.2912	12	-0.58	0.5696

Table F13. *Simple effect comparisons of treatment*day least squares means by treatment for CD45+, Study 3*

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
Alum-mIGg	0	0.25	-27.2200	3.2916	15.09	-8.27	<.0001
Alum-mIGg	0	1	-42.6175	8.8510	9.726	-4.81	0.0008
Alum-mIGg	0	2	-39.7025	5.4876	11	-7.23	<.0001
Alum-mIGg	0	3	-50.2900	7.4431	10.04	-6.76	<.0001
Alum-mIGg	0	4	-29.9200	12.1563	9.378	-2.46	0.0351
Alum-mIGg	0	5	-48.3750	9.6487	9.607	-5.01	0.0006
Alum-mIGg	0	7	-21.3125	8.2382	8.751	-2.59	0.0300
Alum-mIGg	0.25	1	-15.3975	9.1156	10.84	-1.69	0.1197
Alum-mIGg	0.25	2	-12.4825	5.9049	13.79	-2.11	0.0532
Alum-mIGg	0.25	3	-23.0700	7.7559	11.62	-2.97	0.0120
Alum-mIGg	0.25	4	-2.7000	12.3503	9.967	-0.22	0.8314
Alum-mIGg	0.25	5	-21.1550	9.8920	10.55	-2.14	0.0568
Alum-mIGg	0.25	7	5.9075	8.5218	9.912	0.69	0.5041
Alum-mIGg	1	2	2.9150	10.1180	14.73	0.29	0.7773
Alum-mIGg	1	3	-7.6725	11.2987	17.44	-0.68	0.5060
Alum-mIGg	1	4	12.6975	14.8336	16.37	0.86	0.4044
Alum-mIGg	1	5	-5.7575	12.8591	17.86	-0.45	0.6597
Alum-mIGg	1	7	21.3050	11.8375	17	1.80	0.0897
Alum-mIGg	2	3	-10.5875	8.9126	16.34	-1.19	0.2518
Alum-mIGg	2	4	9.7825	13.1076	12.25	0.75	0.4696
Alum-mIGg	2	5	-8.6725	10.8227	13.96	-0.80	0.4364
Alum-mIGg	2	7	18.3900	9.5865	13.92	1.92	0.0758
Alum-mIGg	3	4	20.3700	14.0391	14.76	1.45	0.1677
Alum-mIGg	3	5	1.9150	11.9338	16.82	0.16	0.8744
Alum-mIGg	3	7	28.9775	10.8253	16.55	2.68	0.0162
Alum-mIGg	4	5	-18.4550	15.3230	17.07	-1.20	0.2449
Alum-mIGg	4	7	8.6075	14.4763	15.39	0.59	0.5608
Alum-mIGg	5	7	27.0625	12.4452	16.82	2.17	0.0442
IO-mIgG	0	0.25	-29.8275	3.2916	15.09	-9.06	<.0001
IO-mIgG	0	1	-26.0575	8.8510	9.726	-2.94	0.0151
IO-mIgG	0	2	-24.0600	5.4876	11	-4.38	0.0011
IO-mIgG	0	3	-31.7900	7.4431	10.04	-4.27	0.0016
IO-mIgG	0	4	-24.3075	12.1563	9.378	-2.00	0.0753
IO-mIgG	0	5	-28.4100	9.6487	9.607	-2.94	0.0153
IO-mIgG	0	7	-26.1342	9.4592	8.563	-2.76	0.0230
IO-mIgG	0.25	1	3.7700	9.1156	10.84	0.41	0.6872
IO-mIgG	0.25	2	5.7675	5.9049	13.79	0.98	0.3455
IO-mIgG	0.25	3	-1.9625	7.7559	11.62	-0.25	0.8047
IO-mIgG	0.25	4	5.5200	12.3503	9.967	0.45	0.6645
IO-mIgG	0.25	5	1.4175	9.8920	10.55	0.14	0.8888
IO-mIgG	0.25	7	3.6933	9.7072	9.44	0.38	0.7120
IO-mIgG	1	2	1.9975	10.1180	14.73	0.20	0.8462
IO-mIgG	1	3	-5.7325	11.2987	17.44	-0.51	0.6183
IO-mIgG	1	4	1.7500	14.8336	16.37	0.12	0.9075
IO-mIgG	1	5	-2.3525	12.8591	17.86	-0.18	0.8569

Table F13. *Simple effect comparisons of treatment*day least squares means by treatment for CD45+, Study 3* (Cont.)

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
IO-mIgG	1	7	-0.07667	12.7176	16.73	-0.01	0.9953
IO-mIgG	2	3	-7.7300	8.9126	16.34	-0.87	0.3983
IO-mIgG	2	4	-0.2475	13.1076	12.25	-0.02	0.9852
IO-mIgG	2	5	-4.3500	10.8227	13.96	-0.40	0.6938
IO-mIgG	2	7	-2.0742	10.6541	12.69	-0.19	0.8487
IO-mIgG	3	4	7.4825	14.0391	14.76	0.53	0.6020
IO-mIgG	3	5	3.3800	11.9338	16.82	0.28	0.7805
IO-mIgG	3	7	5.6558	11.7812	15.55	0.48	0.6379
IO-mIgG	4	5	-4.1025	15.3230	17.07	-0.27	0.7921
IO-mIgG	4	7	-1.8267	15.2043	16.39	-0.12	0.9058
IO-mIgG	5	7	2.2758	13.2850	16.98	0.17	0.8660
mIgG	0	0.25	-8.2550	3.2916	15.09	-2.51	0.0240
mIgG	0	1	-17.7050	8.8510	9.726	-2.00	0.0742
mIgG	0	2	-13.4250	5.4876	11	-2.45	0.0325
mIgG	0	3	-18.4025	7.4431	10.04	-2.47	0.0329
mIgG	0	4	-23.6325	12.1563	9.378	-1.94	0.0825
mIgG	0	5	-22.8650	9.6487	9.607	-2.37	0.0403
mIgG	0	7	-9.2100	8.2382	8.751	-1.12	0.2933
mIgG	0.25	1	-9.4500	9.1156	10.84	-1.04	0.3224
mIgG	0.25	2	-5.1700	5.9049	13.79	-0.88	0.3963
mIgG	0.25	3	-10.1475	7.7559	11.62	-1.31	0.2160
mIgG	0.25	4	-15.3775	12.3503	9.967	-1.25	0.2416
mIgG	0.25	5	-14.6100	9.8920	10.55	-1.48	0.1689
mIgG	0.25	7	-0.9550	8.5218	9.912	-0.11	0.9130
mIgG	1	2	4.2800	10.1180	14.73	0.42	0.6784
mIgG	1	3	-0.6975	11.2987	17.44	-0.06	0.9515
mIgG	1	4	-5.9275	14.8336	16.37	-0.40	0.6946
mIgG	1	5	-5.1600	12.8591	17.86	-0.40	0.6930
mIgG	1	7	8.4950	11.8375	17	0.72	0.4827
mIgG	2	3	-4.9775	8.9126	16.34	-0.56	0.5841
mIgG	2	4	-10.2075	13.1076	12.25	-0.78	0.4509
mIgG	2	5	-9.4400	10.8227	13.96	-0.87	0.3978
mIgG	2	7	4.2150	9.5865	13.92	0.44	0.6669
mIgG	3	4	-5.2300	14.0391	14.76	-0.37	0.7148
mIgG	3	5	-4.4625	11.9338	16.82	-0.37	0.7131
mIgG	3	7	9.1925	10.8253	16.55	0.85	0.4079
mIgG	4	5	0.7675	15.3230	17.07	0.05	0.9606
mIgG	4	7	14.4225	14.4763	15.39	1.00	0.3345
mIgG	5	7	13.6550	12.4452	16.82	1.10	0.2880

Table F14. *Simple effect comparisons of treatment*day least squares means by day for CD45+, Study 3*

Simple Effect Level	Treatment	Treatment	Estimate	Std. Error	DF	t Value	Pr > |t|
day 0	Alum-mIGg	IO-mIgG	-0.8275	2.4659	9	-0.34	0.7449
day 0	Alum-mIGg	mIgG	2.7750	2.4659	9	1.13	0.2896
day 0	IO-mIgG	mIgG	3.6025	2.4659	9	1.46	0.1780
day 0.25	Alum-mIGg	IO-mIgG	-3.4350	3.9482	9	-0.87	0.4069
day 0.25	Alum-mIGg	mIgG	21.7400	3.9482	9	5.51	0.0004
day 0.25	IO-mIgG	mIgG	25.1750	3.9482	9	6.38	0.0001
day 1	Alum-mIGg	IO-mIgG	15.7325	12.2719	9	1.28	0.2319
day 1	Alum-mIGg	mIgG	27.6875	12.2719	9	2.26	0.0505
day 1	IO-mIgG	mIgG	11.9550	12.2719	9	0.97	0.3554
day 2	Alum-mIGg	IO-mIgG	14.8150	7.3585	9	2.01	0.0749
day 2	Alum-mIGg	mIgG	29.0525	7.3585	9	3.95	0.0034
day 2	IO-mIgG	mIgG	14.2375	7.3585	9	1.93	0.0850
day 3	Alum-mIGg	IO-mIgG	17.6725	10.2333	9	1.73	0.1182
day 3	Alum-mIGg	mIgG	34.6625	10.2333	9	3.39	0.0080
day 3	IO-mIgG	mIgG	16.9900	10.2333	9	1.66	0.1312
day 4	Alum-mIGg	IO-mIgG	4.7850	17.0139	9	0.28	0.7849
day 4	Alum-mIGg	mIgG	9.0625	17.0139	9	0.53	0.6072
day 4	IO-mIgG	mIgG	4.2775	17.0139	9	0.25	0.8071
day 5	Alum-mIGg	IO-mIgG	19.1375	13.4206	9	1.43	0.1876
day 5	Alum-mIGg	mIgG	28.2850	13.4206	9	2.11	0.0643
day 5	IO-mIgG	mIgG	9.1475	13.4206	9	0.68	0.5126
day 7	Alum-mIGg	IO-mIgG	-5.6492	12.2989	8	-0.46	0.6582
day 7	Alum-mIGg	mIgG	14.8775	11.3866	8	1.31	0.2277
day 7	IO-mIgG	mIgG	20.5267	12.2989	8	1.67	0.1337

Table F15. *Simple effect comparisons of treatment*day least squares means by treatment for heterophils, Study 3*

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
Alum-mIGg	0	0.25	-5.4200	1.7332	11.29	-3.13	0.0094
Alum-mIGg	0	1	-1.4550	1.8898	10.67	-0.77	0.4580
Alum-mIGg	0	2	1.0800	0.6638	11.24	1.63	0.1314
Alum-mIGg	0	3	1.1100	0.7161	14.27	1.55	0.1430
Alum-mIGg	0	4	0.6975	0.7184	14.2	0.97	0.3478
Alum-mIGg	0	5	1.3625	0.6839	12.49	1.99	0.0687
Alum-mIGg	0	7	0.4250	0.8033	16.73	0.53	0.6037
Alum-mIGg	0.25	1	3.9650	2.3762	17.36	1.67	0.1131
Alum-mIGg	0.25	2	6.5000	1.6595	9.453	3.92	0.0032
Alum-mIGg	0.25	3	6.5300	1.6751	9.875	3.90	0.0030
Alum-mIGg	0.25	4	6.1175	1.6755	9.886	3.65	0.0045
Alum-mIGg	0.25	5	6.7825	1.6656	9.607	4.07	0.0024
Alum-mIGg	0.25	7	5.8450	1.7075	10.66	3.42	0.0059

Table F15. *Simple effect comparisons of treatment*day least squares means by treatment for heterophils, Study 3* (Cont.)

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
Alum-mIGg	1	2	2.5350	1.8239	9.051	1.39	0.1978
Alum-mIGg	1	3	2.5650	1.8376	9.451	1.40	0.1947
Alum-mIGg	1	4	2.1525	1.8379	9.469	1.17	0.2701
Alum-mIGg	1	5	2.8175	1.8293	9.199	1.54	0.1572
Alum-mIGg	1	7	1.8800	1.8665	10.15	1.01	0.3372
Alum-mIGg	2	3	0.03000	0.4395	14.77	0.07	0.9465
Alum-mIGg	2	4	-0.3825	0.4440	14.48	-0.86	0.4030
Alum-mIGg	2	5	0.2825	0.3787	16.84	0.75	0.4660
Alum-mIGg	2	7	-0.6550	0.5775	10.83	-1.13	0.2812
Alum-mIGg	3	4	-0.4125	0.5231	17.04	-0.79	0.4412
Alum-mIGg	3	5	0.2525	0.4710	16.61	0.54	0.5990
Alum-mIGg	3	7	-0.6850	0.6383	14.24	-1.07	0.3011
Alum-mIGg	4	5	0.6650	0.4750	16.56	1.40	0.1800
Alum-mIGg	4	7	-0.2725	0.6410	14.41	-0.43	0.6770
Alum-mIGg	5	7	-0.9375	0.6010	12.24	-1.56	0.1443
IO-mIgG	0	0.25	-16.9125	1.7332	11.29	-9.76	<.0001
IO-mIgG	0	1	-13.0075	1.8898	10.67	-6.88	<.0001
IO-mIgG	0	2	0.4625	0.6638	11.24	0.70	0.5001
IO-mIgG	0	3	0.3525	0.7161	14.27	0.49	0.6300
IO-mIgG	0	4	0.8750	0.7184	14.2	1.22	0.2431
IO-mIgG	0	5	0.2250	0.6839	12.49	0.33	0.7476
IO-mIgG	0	7	0.1432	0.8629	16.98	0.17	0.8702
IO-mIgG	0.25	1	3.9050	2.3762	17.36	1.64	0.1183
IO-mIgG	0.25	2	17.3750	1.6595	9.453	10.47	<.0001
IO-mIgG	0.25	3	17.2650	1.6751	9.875	10.31	<.0001
IO-mIgG	0.25	4	17.7875	1.6755	9.886	10.62	<.0001
IO-mIgG	0.25	5	17.1375	1.6656	9.607	10.29	<.0001
IO-mIgG	0.25	7	17.0557	1.7363	11.27	9.82	<.0001
IO-mIgG	1	2	13.4700	1.8239	9.051	7.39	<.0001
IO-mIgG	1	3	13.3600	1.8376	9.451	7.27	<.0001
IO-mIgG	1	4	13.8825	1.8379	9.469	7.55	<.0001
IO-mIgG	1	5	13.2325	1.8293	9.199	7.23	<.0001
IO-mIgG	1	7	13.1507	1.8929	10.66	6.95	<.0001
IO-mIgG	2	3	-0.1100	0.4395	14.77	-0.25	0.8058
IO-mIgG	2	4	0.4125	0.4440	14.48	0.93	0.3680
IO-mIgG	2	5	-0.2375	0.3787	16.84	-0.63	0.5390
IO-mIgG	2	7	-0.3193	0.6579	10.14	-0.49	0.6377
IO-mIgG	3	4	0.5225	0.5231	17.04	1.00	0.3318
IO-mIgG	3	5	-0.1275	0.4710	16.61	-0.27	0.7900
IO-mIgG	3	7	-0.2093	0.7119	13.03	-0.29	0.7734
IO-mIgG	4	5	-0.6500	0.4750	16.56	-1.37	0.1895
IO-mIgG	4	7	-0.7318	0.7142	13.2	-1.02	0.3239
IO-mIgG	5	7	-0.08182	0.6786	11.27	-0.12	0.9062

Table F15. *Simple effect comparisons of treatment*day least squares means by treatment for heterophils, Study 3* (Cont.)

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
mIgG	0	0.25	-2.1975	1.7332	11.29	-1.27	0.2304
mIgG	0	1	-1.9775	1.8898	10.67	-1.05	0.3185
mIgG	0	2	0.9150	0.6638	11.24	1.38	0.1949
mIgG	0	3	0.7275	0.7161	14.27	1.02	0.3266
mIgG	0	4	1.4000	0.7184	14.2	1.95	0.0714
mIgG	0	5	1.2700	0.6839	12.49	1.86	0.0870
mIgG	0	7	0.4850	0.8033	16.73	0.60	0.5541
mIgG	0.25	1	0.2200	2.3762	17.36	0.09	0.9273
mIgG	0.25	2	3.1125	1.6595	9.453	1.88	0.0919
mIgG	0.25	3	2.9250	1.6751	9.875	1.75	0.1118
mIgG	0.25	4	3.5975	1.6755	9.886	2.15	0.0577
mIgG	0.25	5	3.4675	1.6656	9.607	2.08	0.0651
mIgG	0.25	7	2.6825	1.7075	10.66	1.57	0.1454
mIgG	1	2	2.8925	1.8239	9.051	1.59	0.1470
mIgG	1	3	2.7050	1.8376	9.451	1.47	0.1735
mIgG	1	4	3.3775	1.8379	9.469	1.84	0.0976
mIgG	1	5	3.2475	1.8293	9.199	1.78	0.1089
mIgG	1	7	2.4625	1.8665	10.15	1.32	0.2161
mIgG	2	3	-0.1875	0.4395	14.77	-0.43	0.6758
mIgG	2	4	0.4850	0.4440	14.48	1.09	0.2925
mIgG	2	5	0.3550	0.3787	16.84	0.94	0.3619
mIgG	2	7	-0.4300	0.5775	10.83	-0.74	0.4724
mIgG	3	4	0.6725	0.5231	17.04	1.29	0.2158
mIgG	3	5	0.5425	0.4710	16.61	1.15	0.2657
mIgG	3	7	-0.2425	0.6383	14.24	-0.38	0.7096
mIgG	4	5	-0.1300	0.4750	16.56	-0.27	0.7877
mIgG	4	7	-0.9150	0.6410	14.41	-1.43	0.1747
mIgG	5	7	-0.7850	0.6010	12.24	-1.31	0.2155

Table F16. *Simple effect comparisons of treatment*day least squares means by day for heterophils, Study 3*

Simple Effect Level	Treatment	Treatment	Estimate	Std. Error	DF	t Value	Pr > |t|
day 0	Alum-mIGg	IO-mIgG	0.2150	0.8965	8.807	0.24	0.8160
day 0	Alum-mIGg	mIgG	-0.05750	0.8965	8.807	-0.06	0.9503
day 0	IO-mIgG	mIgG	-0.2725	0.8965	8.807	-0.30	0.7682
day 0.25	Alum-mIGg	IO-mIgG	-11.2775	2.3470	9.262	-4.80	0.0009
day 0.25	Alum-mIGg	mIgG	3.1650	2.3470	9.262	1.35	0.2095
day 0.25	IO-mIgG	mIgG	14.4425	2.3470	9.262	6.15	0.0001
day 1	Alum-mIGg	IO-mIgG	-11.3375	2.5810	8.802	-4.39	0.0018
day 1	Alum-mIGg	mIgG	-0.5800	2.5810	8.802	-0.22	0.8273
day 1	IO-mIgG	mIgG	10.7575	2.5810	8.802	4.17	0.0025
day 2	Alum-mIGg	IO-mIgG	-0.4025	0.3511	9.265	-1.15	0.2804
day 2	Alum-mIGg	mIgG	-0.2225	0.3511	9.265	-0.63	0.5416
day 2	IO-mIgG	mIgG	0.1800	0.3511	9.265	0.51	0.6202
day 3	Alum-mIGg	IO-mIgG	-0.5425	0.5397	9.154	-1.01	0.3407
day 3	Alum-mIGg	mIgG	-0.4400	0.5397	9.154	-0.82	0.4356
day 3	IO-mIgG	mIgG	0.1025	0.5397	9.154	0.19	0.8535
day 4	Alum-mIGg	IO-mIgG	0.3925	0.5477	9.31	0.72	0.4912
day 4	Alum-mIGg	mIgG	0.6450	0.5477	9.31	1.18	0.2682
day 4	IO-mIgG	mIgG	0.2525	0.5477	9.31	0.46	0.6554
day 5	Alum-mIGg	IO-mIgG	-0.9225	0.4307	8.767	-2.14	0.0616
day 5	Alum-mIGg	mIgG	-0.1500	0.4307	8.767	-0.35	0.7359
day 5	IO-mIgG	mIgG	0.7725	0.4307	8.767	1.79	0.1074
day 7	Alum-mIGg	IO-mIgG	-0.06682	0.8259	7.897	-0.08	0.9375
day 7	Alum-mIGg	mIgG	0.002500	0.7635	7.87	0.00	0.9975
day 7	IO-mIgG	mIgG	0.06932	0.8259	7.897	0.08	0.9352

Table F17. *Simple effect comparisons of treatment*day least squares means by treatment for γδ T cell+, Study 3*

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
Alum-mIGg	0	0.25	-8.1175	1.1321	13.75	-7.17	<.0001
Alum-mIGg	0	1	-17.1350	3.3816	9.459	-5.07	0.0006
Alum-mIGg	0	2	-7.9050	2.4679	9.88	-3.20	0.0096
Alum-mIGg	0	3	-8.5600	1.0627	14.45	-8.05	<.0001
Alum-mIGg	0	4	-2.7450	1.3797	12.07	-1.99	0.0698
Alum-mIGg	0	5	-2.7925	0.7673	17.98	-3.64	0.0019
Alum-mIGg	0	7	-1.5075	1.2098	11.72	-1.25	0.2371
Alum-mIGg	0.25	1	-9.0175	3.4853	10.6	-2.59	0.0259
Alum-mIGg	0.25	2	0.2125	2.6082	12	0.08	0.9364
Alum-mIGg	0.25	3	-0.4425	1.3571	17.88	-0.33	0.7482
Alum-mIGg	0.25	4	5.3725	1.6174	17.04	3.32	0.0040
Alum-mIGg	0.25	5	5.3250	1.1406	14.02	4.67	0.0004
Alum-mIGg	0.25	7	6.6100	1.4751	16.66	4.48	0.0003

Table F17. *Simple effect comparisons of treatment*day least squares means by treatment for γδ T cell+, Study 3* (Cont.)

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
Alum-mIGg	1	2	9.2300	4.1178	16.37	2.24	0.0392
Alum-mIGg	1	3	8.5750	3.4634	10.36	2.48	0.0320
Alum-mIGg	1	4	14.3900	3.5735	11.56	4.03	0.0018
Alum-mIGg	1	5	14.3425	3.3845	9.49	4.24	0.0019
Alum-mIGg	1	7	15.6275	3.5114	10.87	4.45	0.0010
Alum-mIGg	2	3	-0.6550	2.5788	11.56	-0.25	0.8040
Alum-mIGg	2	4	5.1600	2.7248	13.66	1.89	0.0796
Alum-mIGg	2	5	5.1125	2.4718	9.94	2.07	0.0656
Alum-mIGg	2	7	6.3975	2.6428	12.45	2.42	0.0316
Alum-mIGg	3	4	5.8150	1.5696	16.38	3.70	0.0019
Alum-mIGg	3	5	5.7675	1.0718	14.74	5.38	<.0001
Alum-mIGg	3	7	7.0525	1.4225	16.19	4.96	0.0001
Alum-mIGg	4	5	-0.04750	1.3867	12.27	-0.03	0.9732
Alum-mIGg	4	7	1.2375	1.6727	16.84	0.74	0.4696
Alum-mIGg	5	7	1.2850	1.2178	11.95	1.06	0.3122
IO-mIgG	0	0.25	-0.2800	1.1321	13.75	-0.25	0.8083
IO-mIgG	0	1	-2.1025	3.3816	9.459	-0.62	0.5488
IO-mIgG	0	2	-4.0625	2.4679	9.88	-1.65	0.1311
IO-mIgG	0	3	-3.2325	1.0627	14.45	-3.04	0.0085
IO-mIgG	0	4	-2.6075	1.3797	12.07	-1.89	0.0830
IO-mIgG	0	5	-1.5300	0.7673	17.98	-1.99	0.0615
IO-mIgG	0	7	-3.3933	1.3625	10.84	-2.49	0.0303
IO-mIgG	0.25	1	-1.8225	3.4853	10.6	-0.52	0.6118
IO-mIgG	0.25	2	-3.7825	2.6082	12	-1.45	0.1726
IO-mIgG	0.25	3	-2.9525	1.3571	17.88	-2.18	0.0432
IO-mIgG	0.25	4	-2.3275	1.6174	17.04	-1.44	0.1683
IO-mIgG	0.25	5	-1.2500	1.1406	14.02	-1.10	0.2916
IO-mIgG	0.25	7	-3.1133	1.6027	15.74	-1.94	0.0702
IO-mIgG	1	2	-1.9600	4.1178	16.37	-0.48	0.6404
IO-mIgG	1	3	-1.1300	3.4634	10.36	-0.33	0.7507
IO-mIgG	1	4	-0.5050	3.5735	11.56	-0.14	0.8901
IO-mIgG	1	5	0.5725	3.3845	9.49	0.17	0.8692
IO-mIgG	1	7	-1.2908	3.5669	11.46	-0.36	0.7240
IO-mIgG	2	3	0.8300	2.5788	11.56	0.32	0.7533
IO-mIgG	2	4	1.4550	2.7248	13.66	0.53	0.6019
IO-mIgG	2	5	2.5325	2.4718	9.94	1.02	0.3299
IO-mIgG	2	7	0.6692	2.7162	13.43	0.25	0.8091
IO-mIgG	3	4	0.6250	1.5696	16.38	0.40	0.6956
IO-mIgG	3	5	1.7025	1.0718	14.74	1.59	0.1334
IO-mIgG	3	7	-0.1608	1.5545	15.05	-0.10	0.9190
IO-mIgG	4	5	1.0775	1.3867	12.27	0.78	0.4519
IO-mIgG	4	7	-0.7858	1.7863	16.97	-0.44	0.6655
IO-mIgG	5	7	-1.8633	1.3696	11.03	-1.36	0.2008

Table F17. *Simple effect comparisons of treatment*day least squares means by treatment for γδ T cell+, Study 3* (Cont.)

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
mIgG	0	0.25	-2.1050	1.1321	13.75	-1.86	0.0845
mIgG	0	1	-2.9225	3.3816	9.459	-0.86	0.4088
mIgG	0	2	-3.2050	2.4679	9.88	-1.30	0.2235
mIgG	0	3	-2.8325	1.0627	14.45	-2.67	0.0181
mIgG	0	4	-2.6550	1.3797	12.07	-1.92	0.0782
mIgG	0	5	-2.7025	0.7673	17.98	-3.52	0.0024
mIgG	0	7	-1.1125	1.2098	11.72	-0.92	0.3763
mIgG	0.25	1	-0.8175	3.4853	10.6	-0.23	0.8190
mIgG	0.25	2	-1.1000	2.6082	12	-0.42	0.6807
mIgG	0.25	3	-0.7275	1.3571	17.88	-0.54	0.5985
mIgG	0.25	4	-0.5500	1.6174	17.04	-0.34	0.7380
mIgG	0.25	5	-0.5975	1.1406	14.02	-0.52	0.6086
mIgG	0.25	7	0.9925	1.4751	16.66	0.67	0.5103
mIgG	1	2	-0.2825	4.1178	16.37	-0.07	0.9461
mIgG	1	3	0.09000	3.4634	10.36	0.03	0.9798
mIgG	1	4	0.2675	3.5735	11.56	0.07	0.9416
mIgG	1	5	0.2200	3.3845	9.49	0.07	0.9495
mIgG	1	7	1.8100	3.5114	10.87	0.52	0.6165
mIgG	2	3	0.3725	2.5788	11.56	0.14	0.8876
mIgG	2	4	0.5500	2.7248	13.66	0.20	0.8430
mIgG	2	5	0.5025	2.4718	9.94	0.20	0.8430
mIgG	2	7	2.0925	2.6428	12.45	0.79	0.4433
mIgG	3	4	0.1775	1.5696	16.38	0.11	0.9113
mIgG	3	5	0.1300	1.0718	14.74	0.12	0.9051
mIgG	3	7	1.7200	1.4225	16.19	1.21	0.2440
mIgG	4	5	-0.04750	1.3867	12.27	-0.03	0.9732
mIgG	4	7	1.5425	1.6727	16.84	0.92	0.3695
mIgG	5	7	1.5900	1.2178	11.95	1.31	0.2162

Table F18. *Simple effect comparisons of treatment*day least squares means by day for γδ T cell+, Study 3*

Simple Effect Level	Treatment	Treatment	Estimate	Std. Error	DF	t Value	Pr > |t|
day 0	Alum-mIGg	IO-mIgG	-0.8100	0.7546	9	-1.07	0.3110
day 0	Alum-mIGg	mIgG	0.1400	0.7546	9	0.19	0.8569
day 0	IO-mIgG	mIgG	0.9500	0.7546	9	1.26	0.2397
day 0.25	Alum-mIGg	IO-mIgG	7.0275	1.4120	9	4.98	0.0008
day 0.25	Alum-mIGg	mIgG	6.1525	1.4120	9	4.36	0.0018
day 0.25	IO-mIgG	mIgG	-0.8750	1.4120	9	-0.62	0.5508
day 1	Alum-mIGg	IO-mIgG	14.2225	4.7224	9	3.01	0.0147
day 1	Alum-mIGg	mIgG	14.3525	4.7224	9	3.04	0.0140
day 1	IO-mIgG	mIgG	0.1300	4.7224	9	0.03	0.9786
day 2	Alum-mIGg	IO-mIgG	3.0325	3.4076	9	0.89	0.3967
day 2	Alum-mIGg	mIgG	4.8400	3.4076	9	1.42	0.1892
day 2	IO-mIgG	mIgG	1.8075	3.4076	9	0.53	0.6086
day 3	Alum-mIGg	IO-mIgG	4.5175	1.2998	9	3.48	0.0070
day 3	Alum-mIGg	mIgG	5.8675	1.2998	9	4.51	0.0015
day 3	IO-mIgG	mIgG	1.3500	1.2998	9	1.04	0.3261
day 4	Alum-mIGg	IO-mIgG	-0.6725	1.7994	9	-0.37	0.7173
day 4	Alum-mIGg	mIgG	0.2300	1.7994	9	0.13	0.9011
day 4	IO-mIgG	mIgG	0.9025	1.7994	9	0.50	0.6280
day 5	Alum-mIGg	IO-mIgG	0.4525	0.7798	9	0.58	0.5760
day 5	Alum-mIGg	mIgG	0.2300	0.7798	9	0.29	0.7747
day 5	IO-mIgG	mIgG	-0.2225	0.7798	9	-0.29	0.7818
day 7	Alum-mIGg	IO-mIgG	-2.6958	1.6585	8	-1.63	0.1427
day 7	Alum-mIGg	mIgG	0.5350	1.5355	8	0.35	0.7365
day 7	IO-mIgG	mIgG	3.2308	1.6585	8	1.95	0.0873

Table F19. *Simple effect comparisons of treatment*day least squares means by treatment for γδ T cell+ CD8+, Study 3*

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
Alum-mIGg	0	0.25	-0.5350	0.1015	18.87	-5.27	<.0001
Alum-mIGg	0	1	-1.1500	0.2228	11.34	-5.16	0.0003
Alum-mIGg	0	2	-0.7350	0.1859	10.3	-3.95	0.0026
Alum-mIGg	0	3	-1.0500	0.1772	11.72	-5.93	<.0001
Alum-mIGg	0	4	-0.4475	0.2554	10.58	-1.75	0.1086
Alum-mIGg	0	5	-0.4800	0.1669	12.45	-2.88	0.0135
Alum-mIGg	0	7	-0.1775	0.1985	8.35	-0.89	0.3964
Alum-mIGg	0.25	1	-0.6150	0.2217	12.25	-2.77	0.0165
Alum-mIGg	0.25	2	-0.2000	0.1864	11.72	-1.07	0.3049
Alum-mIGg	0.25	3	-0.5150	0.1782	13.37	-2.89	0.0123
Alum-mIGg	0.25	4	0.08750	0.2532	11.23	0.35	0.7360
Alum-mIGg	0.25	5	0.05500	0.1686	14.37	0.33	0.7490
Alum-mIGg	0.25	7	0.3575	0.1984	9.338	1.80	0.1038

Table F19. *Simple effect comparisons of treatment*day least squares means by treatment for γδ T cell+ CD8+, Study 3* (Cont.)

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
Alum-mIGg	1	2	0.4150	0.2431	20.2	1.71	0.1031
Alum-mIGg	1	3	0.1000	0.2393	19.77	0.42	0.6805
Alum-mIGg	1	4	0.7025	0.2817	20.71	2.49	0.0212
Alum-mIGg	1	5	0.6700	0.2352	18.74	2.85	0.0104
Alum-mIGg	1	7	0.9725	0.2488	20.25	3.91	0.0009
Alum-mIGg	2	3	-0.3150	0.2147	20.37	-1.47	0.1576
Alum-mIGg	2	4	0.2875	0.2665	17.97	1.08	0.2950
Alum-mIGg	2	5	0.2550	0.2091	20.42	1.22	0.2365
Alum-mIGg	2	7	0.5575	0.2269	18.89	2.46	0.0239
Alum-mIGg	3	4	0.6025	0.2637	17.08	2.29	0.0354
Alum-mIGg	3	5	0.5700	0.2035	21.43	2.80	0.0106
Alum-mIGg	3	7	0.8725	0.2224	18.39	3.92	0.0010
Alum-mIGg	4	5	-0.03250	0.2605	16.29	-0.12	0.9023
Alum-mIGg	4	7	0.2700	0.2709	18.88	1.00	0.3316
Alum-mIGg	5	7	0.3025	0.2176	16.84	1.39	0.1825
IO-mIgG	0	0.25	0.06500	0.1015	18.87	0.64	0.5295
IO-mIgG	0	1	-0.3150	0.2228	11.34	-1.41	0.1844
IO-mIgG	0	2	-0.4650	0.1859	10.3	-2.50	0.0308
IO-mIgG	0	3	-0.4525	0.1772	11.72	-2.55	0.0257
IO-mIgG	0	4	-0.6025	0.2554	10.58	-2.36	0.0387
IO-mIgG	0	5	-0.4675	0.1669	12.45	-2.80	0.0155
IO-mIgG	0	7	-0.5663	0.2249	8.568	-2.52	0.0341
IO-mIgG	0.25	1	-0.3800	0.2217	12.25	-1.71	0.1116
IO-mIgG	0.25	2	-0.5300	0.1864	11.72	-2.84	0.0151
IO-mIgG	0.25	3	-0.5175	0.1782	13.37	-2.90	0.0120
IO-mIgG	0.25	4	-0.6675	0.2532	11.23	-2.64	0.0228
IO-mIgG	0.25	5	-0.5325	0.1686	14.37	-3.16	0.0068
IO-mIgG	0.25	7	-0.6313	0.2247	9.328	-2.81	0.0197
IO-mIgG	1	2	-0.1500	0.2431	20.2	-0.62	0.5441
IO-mIgG	1	3	-0.1375	0.2393	19.77	-0.57	0.5720
IO-mIgG	1	4	-0.2875	0.2817	20.71	-1.02	0.3192
IO-mIgG	1	5	-0.1525	0.2352	18.74	-0.65	0.5247
IO-mIgG	1	7	-0.2513	0.2702	20.04	-0.93	0.3635
IO-mIgG	2	3	0.01250	0.2147	20.37	0.06	0.9541
IO-mIgG	2	4	-0.1375	0.2665	17.97	-0.52	0.6122
IO-mIgG	2	5	-0.00250	0.2091	20.42	-0.01	0.9906
IO-mIgG	2	7	-0.1013	0.2503	17.57	-0.40	0.6906
IO-mIgG	3	4	-0.1500	0.2637	17.08	-0.57	0.5768
IO-mIgG	3	5	-0.01500	0.2035	21.43	-0.07	0.9419
IO-mIgG	3	7	-0.1138	0.2461	16.79	-0.46	0.6499
IO-mIgG	4	5	0.1350	0.2605	16.29	0.52	0.6113
IO-mIgG	4	7	0.03622	0.2908	20.07	0.12	0.9021
IO-mIgG	5	7	-0.09878	0.2418	15.35	-0.41	0.6886

Table F19. *Simple effect comparisons of treatment*day least squares means by treatment for γδ T cell+ CD8+, Study 3* (Cont.)

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
mIgG	0	0.25	-0.2150	0.1015	18.87	-2.12	0.0476
mIgG	0	1	-0.6950	0.2228	11.34	-3.12	0.0094
mIgG	0	2	-0.6475	0.1859	10.3	-3.48	0.0056
mIgG	0	3	-0.4750	0.1772	11.72	-2.68	0.0204
mIgG	0	4	-0.7425	0.2554	10.58	-2.91	0.0148
mIgG	0	5	-0.7550	0.1669	12.45	-4.52	0.0006
mIgG	0	7	-0.3950	0.1985	8.35	-1.99	0.0803
mIgG	0.25	1	-0.4800	0.2217	12.25	-2.17	0.0508
mIgG	0.25	2	-0.4325	0.1864	11.72	-2.32	0.0392
mIgG	0.25	3	-0.2600	0.1782	13.37	-1.46	0.1676
mIgG	0.25	4	-0.5275	0.2532	11.23	-2.08	0.0608
mIgG	0.25	5	-0.5400	0.1686	14.37	-3.20	0.0062
mIgG	0.25	7	-0.1800	0.1984	9.338	-0.91	0.3870
mIgG	1	2	0.04750	0.2431	20.2	0.20	0.8470
mIgG	1	3	0.2200	0.2393	19.77	0.92	0.3689
mIgG	1	4	-0.04750	0.2817	20.71	-0.17	0.8677
mIgG	1	5	-0.06000	0.2352	18.74	-0.26	0.8015
mIgG	1	7	0.3000	0.2488	20.25	1.21	0.2417
mIgG	2	3	0.1725	0.2147	20.37	0.80	0.4309
mIgG	2	4	-0.09500	0.2665	17.97	-0.36	0.7257
mIgG	2	5	-0.1075	0.2091	20.42	-0.51	0.6127
mIgG	2	7	0.2525	0.2269	18.89	1.11	0.2798
mIgG	3	4	-0.2675	0.2637	17.08	-1.01	0.3245
mIgG	3	5	-0.2800	0.2035	21.43	-1.38	0.1830
mIgG	3	7	0.08000	0.2224	18.39	0.36	0.7231
mIgG	4	5	-0.01250	0.2605	16.29	-0.05	0.9623
mIgG	4	7	0.3475	0.2709	18.88	1.28	0.2151
mIgG	5	7	0.3600	0.2176	16.84	1.65	0.1165

Table F20. *Simple effect comparisons of treatment*day least squares means by day for γδ T cell+ CD8+, Study 3*

Simple Effect Level	Treatment	Treatment	Estimate	Std. Error	DF	t Value	Pr > |t|
day 0	Alum-mIGg	IO-mIgG	-0.08000	0.1181	8.37	-0.68	0.5165
day 0	Alum-mIGg	mIgG	0.01500	0.1181	8.37	0.13	0.9020
day 0	IO-mIgG	mIgG	0.09500	0.1181	8.37	0.80	0.4435
day 0.25	Alum-mIGg	IO-mIgG	0.5200	0.1472	9.542	3.53	0.0058
day 0.25	Alum-mIGg	mIgG	0.3350	0.1472	9.542	2.28	0.0473
day 0.25	IO-mIgG	mIgG	-0.1850	0.1472	9.542	-1.26	0.2388
day 1	Alum-mIGg	IO-mIgG	0.7550	0.3477	10.43	2.17	0.0540
day 1	Alum-mIGg	mIgG	0.4700	0.3477	10.43	1.35	0.2051
day 1	IO-mIgG	mIgG	-0.2850	0.3477	10.43	-0.82	0.4308
day 2	Alum-mIGg	IO-mIgG	0.1900	0.2915	8.867	0.65	0.5311
day 2	Alum-mIGg	mIgG	0.1025	0.2915	8.867	0.35	0.7334
day 2	IO-mIgG	mIgG	-0.08750	0.2915	8.867	-0.30	0.7710
day 3	Alum-mIGg	IO-mIgG	0.5175	0.2779	9.924	1.86	0.0924
day 3	Alum-mIGg	mIgG	0.5900	0.2779	9.924	2.12	0.0599
day 3	IO-mIgG	mIgG	0.07250	0.2779	9.924	0.26	0.7995
day 4	Alum-mIGg	IO-mIgG	-0.2350	0.3962	10.04	-0.59	0.5663
day 4	Alum-mIGg	mIgG	-0.2800	0.3962	10.04	-0.71	0.4959
day 4	IO-mIgG	mIgG	-0.04500	0.3962	10.04	-0.11	0.9118
day 5	Alum-mIGg	IO-mIgG	-0.06750	0.2618	10.24	-0.26	0.8016
day 5	Alum-mIGg	mIgG	-0.2600	0.2618	10.24	-0.99	0.3435
day 5	IO-mIgG	mIgG	-0.1925	0.2618	10.24	-0.74	0.4786
day 7	Alum-mIGg	IO-mIgG	-0.4688	0.3284	7.753	-1.43	0.1925
day 7	Alum-mIGg	mIgG	-0.2025	0.3110	7.468	-0.65	0.5345
day 7	IO-mIgG	mIgG	0.2663	0.3284	7.753	0.81	0.4417

Table F21. *Simple effect comparisons of treatment*day least squares means by treatment for γδ T cell+ CD8-, Study 3*

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
Alum-mIGg	0	0.25	-7.0925	1.0085	12.41	-7.03	<.0001
Alum-mIGg	0	1	-14.9575	3.2499	9.289	-4.60	0.0012
Alum-mIGg	0	2	-7.2425	2.2401	9.619	-3.23	0.0094
Alum-mIGg	0	3	-7.7075	1.0114	12.39	-7.62	<.0001
Alum-mIGg	0	4	-2.8450	1.0768	11.95	-2.64	0.0216
Alum-mIGg	0	5	-2.7300	0.6827	16.67	-4.00	0.0010
Alum-mIGg	0	7	-1.3850	0.9973	11.15	-1.39	0.1920
Alum-mIGg	0.25	1	-7.8650	3.3534	10.46	-2.35	0.0399
Alum-mIGg	0.25	2	-0.1500	2.3877	12.06	-0.06	0.9509
Alum-mIGg	0.25	3	-0.6150	1.3061	18	-0.47	0.6434
Alum-mIGg	0.25	4	4.2475	1.3573	17.89	3.13	0.0058
Alum-mIGg	0.25	5	4.3625	1.0719	14.63	4.07	0.0011
Alum-mIGg	0.25	7	5.7075	1.2952	16.96	4.41	0.0004
Alum-mIGg	1	2	7.7150	3.9046	15.9	1.98	0.0658
Alum-mIGg	1	3	7.2500	3.3542	10.47	2.16	0.0548

Table F21. *Simple effect comparisons of treatment*day least squares means by treatment for γδ T cell+ CD8-, Study 3* (Cont.)

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
Alum-mIGg	1	4	12.1125	3.3745	10.7	3.59	0.0044
Alum-mIGg	1	5	12.2275	3.2702	9.517	3.74	0.0042
Alum-mIGg	1	7	13.5725	3.3500	10.42	4.05	0.0021
Alum-mIGg	2	3	-0.4650	2.3889	12.08	-0.19	0.8489
Alum-mIGg	2	4	4.3975	2.4173	12.53	1.82	0.0928
Alum-mIGg	2	5	4.5125	2.2693	10.11	1.99	0.0745
Alum-mIGg	2	7	5.8575	2.3829	11.94	2.46	0.0302
Alum-mIGg	3	4	4.8625	1.3595	17.9	3.58	0.0022
Alum-mIGg	3	5	4.9775	1.0746	14.6	4.63	0.0003
Alum-mIGg	3	7	6.3225	1.2974	16.97	4.87	0.0001
Alum-mIGg	4	5	0.1150	1.1364	13.97	0.10	0.9208
Alum-mIGg	4	7	1.4600	1.3490	16.98	1.08	0.2943
Alum-mIGg	5	7	1.3450	1.0613	13.28	1.27	0.2268
IO-mIgG	0	0.25	-0.2500	1.0085	12.41	-0.25	0.8083
IO-mIgG	0	1	-1.2625	3.2499	9.289	-0.39	0.7064
IO-mIgG	0	2	-3.5350	2.2401	9.619	-1.58	0.1468
IO-mIgG	0	3	-2.9400	1.0114	12.39	-2.91	0.0128
IO-mIgG	0	4	-2.2800	1.0768	11.95	-2.12	0.0559
IO-mIgG	0	5	-1.0975	0.6827	16.67	-1.61	0.1267
IO-mIgG	0	7	-2.3900	1.1271	10.4	-2.12	0.0590
IO-mIgG	0.25	1	-1.0125	3.3534	10.46	-0.30	0.7686
IO-mIgG	0.25	2	-3.2850	2.3877	12.06	-1.38	0.1939
IO-mIgG	0.25	3	-2.6900	1.3061	18	-2.06	0.0542
IO-mIgG	0.25	4	-2.0300	1.3573	17.89	-1.50	0.1522
IO-mIgG	0.25	5	-0.8475	1.0719	14.63	-0.79	0.4418
IO-mIgG	0.25	7	-2.1400	1.3976	16.41	-1.53	0.1448
IO-mIgG	1	2	-2.2725	3.9046	15.9	-0.58	0.5687
IO-mIgG	1	3	-1.6775	3.3542	10.47	-0.50	0.6273
IO-mIgG	1	4	-1.0175	3.3745	10.7	-0.30	0.7688
IO-mIgG	1	5	0.1650	3.2702	9.517	0.05	0.9608
IO-mIgG	1	7	-1.1275	3.3909	10.87	-0.33	0.7458
IO-mIgG	2	3	0.5950	2.3889	12.08	0.25	0.8075
IO-mIgG	2	4	1.2550	2.4173	12.53	0.52	0.6127
IO-mIgG	2	5	2.4375	2.2693	10.11	1.07	0.3078
IO-mIgG	2	7	1.1450	2.4401	12.81	0.47	0.6468
IO-mIgG	3	4	0.6600	1.3595	17.9	0.49	0.6332
IO-mIgG	3	5	1.8425	1.0746	14.6	1.71	0.1076
IO-mIgG	3	7	0.5500	1.3997	16.43	0.39	0.6994
IO-mIgG	4	5	1.1825	1.1364	13.97	1.04	0.3157
IO-mIgG	4	7	-0.1100	1.4476	16.79	-0.08	0.9403
IO-mIgG	5	7	-1.2925	1.1842	12.13	-1.09	0.2963
mIgG	0	0.25	-1.8800	1.0085	12.41	-1.86	0.0861
mIgG	0	1	-2.2950	3.2499	9.289	-0.71	0.4974

Table F21. *Simple effect comparisons of treatment*day least squares means by treatment for γδ T cell+ CD8-, Study 3* (Cont.)

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
mIgG	0	2	-2.4900	2.2401	9.619	-1.11	0.2933
mIgG	0	3	-2.5100	1.0114	12.39	-2.48	0.0283
mIgG	0	4	-2.2275	1.0768	11.95	-2.07	0.0609
mIgG	0	5	-2.1600	0.6827	16.67	-3.16	0.0058
mIgG	0	7	-0.8350	0.9973	11.15	-0.84	0.4200
mIgG	0.25	1	-0.4150	3.3534	10.46	-0.12	0.9039
mIgG	0.25	2	-0.6100	2.3877	12.06	-0.26	0.8027
mIgG	0.25	3	-0.6300	1.3061	18	-0.48	0.6354
mIgG	0.25	4	-0.3475	1.3573	17.89	-0.26	0.8009
mIgG	0.25	5	-0.2800	1.0719	14.63	-0.26	0.7976
mIgG	0.25	7	1.0450	1.2952	16.96	0.81	0.4309
mIgG	1	2	-0.1950	3.9046	15.9	-0.05	0.9608
mIgG	1	3	-0.2150	3.3542	10.47	-0.06	0.9501
mIgG	1	4	0.06750	3.3745	10.7	0.02	0.9844
mIgG	1	5	0.1350	3.2702	9.517	0.04	0.9679
mIgG	1	7	1.4600	3.3500	10.42	0.44	0.6719
mIgG	2	3	-0.02000	2.3889	12.08	-0.01	0.9935
mIgG	2	4	0.2625	2.4173	12.53	0.11	0.9152
mIgG	2	5	0.3300	2.2693	10.11	0.15	0.8872
mIgG	2	7	1.6550	2.3829	11.94	0.69	0.5006
mIgG	3	4	0.2825	1.3595	17.9	0.21	0.8377
mIgG	3	5	0.3500	1.0746	14.6	0.33	0.7493
mIgG	3	7	1.6750	1.2974	16.97	1.29	0.2140
mIgG	4	5	0.06750	1.1364	13.97	0.06	0.9535
mIgG	4	7	1.3925	1.3490	16.98	1.03	0.3164
mIgG	5	7	1.3250	1.0613	13.28	1.25	0.2334

Table F22. *Simple effect comparisons of treatment*day least squares means by day for γδ T cell+ CD8-, Study 3*

Simple Effect Level	Treatment	Treatment	Estimate	Std. Error	DF	t Value	Pr > |t|
day 0	Alum-mIGg	IO-mIgG	-0.7950	0.5781	9	-1.38	0.2024
day 0	Alum-mIGg	mIgG	0.005000	0.5781	9	0.01	0.9933
day 0	IO-mIgG	mIgG	0.8000	0.5781	9	1.38	0.1998
day 0.25	Alum-mIGg	IO-mIgG	6.0475	1.3039	9	4.64	0.0012
day 0.25	Alum-mIGg	mIgG	5.2175	1.3039	9	4.00	0.0031
day 0.25	IO-mIgG	mIgG	-0.8300	1.3039	9	-0.64	0.5403
day 1	Alum-mIGg	IO-mIgG	12.9000	4.5596	9	2.83	0.0197
day 1	Alum-mIGg	mIgG	12.6675	4.5596	9	2.78	0.0215
day 1	IO-mIgG	mIgG	-0.2325	4.5596	9	-0.05	0.9604
day 2	Alum-mIGg	IO-mIgG	2.9125	3.1148	9	0.94	0.3742
day 2	Alum-mIGg	mIgG	4.7575	3.1148	9	1.53	0.1610
day 2	IO-mIgG	mIgG	1.8450	3.1148	9	0.59	0.5682
day 3	Alum-mIGg	IO-mIgG	3.9725	1.3083	9	3.04	0.0141
day 3	Alum-mIGg	mIgG	5.2025	1.3083	9	3.98	0.0032
day 3	IO-mIgG	mIgG	1.2300	1.3083	9	0.94	0.3717
day 4	Alum-mIGg	IO-mIgG	-0.2300	1.4088	9	-0.16	0.8739
day 4	Alum-mIGg	mIgG	0.6225	1.4088	9	0.44	0.6690
day 4	IO-mIgG	mIgG	0.8525	1.4088	9	0.61	0.5600
day 5	Alum-mIGg	IO-mIgG	0.8375	0.7733	9	1.08	0.3069
day 5	Alum-mIGg	mIgG	0.5750	0.7733	9	0.74	0.4761
day 5	IO-mIgG	mIgG	-0.2625	0.7733	9	-0.34	0.7420
day 7	Alum-mIGg	IO-mIgG	-1.8000	1.3895	8	-1.30	0.2313
day 7	Alum-mIGg	mIgG	0.5550	1.2864	8	0.43	0.6776
day 7	IO-mIgG	mIgG	2.3550	1.3895	8	1.69	0.1286

Table F23. *Simple effect comparisons of treatment*day least squares means by treatment for αβ1 T cell+, Study 3*

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
Alum-mIGg	0	0.25	-4.2700	0.9287	17.26	-4.60	0.0002
Alum-mIGg	0	1	-8.1675	1.5796	11.79	-5.17	0.0002
Alum-mIGg	0	2	-10.7375	1.6944	11.39	-6.34	<.0001
Alum-mIGg	0	3	-20.2475	2.7999	9.82	-7.23	<.0001
Alum-mIGg	0	4	-12.9025	4.3145	9.337	-2.99	0.0146
Alum-mIGg	0	5	-12.4750	3.0202	9.701	-4.13	0.0022
Alum-mIGg	0	7	-5.0225	1.7692	9.951	-2.84	0.0177
Alum-mIGg	0.25	1	-3.8975	1.6350	13.11	-2.38	0.0329
Alum-mIGg	0.25	2	-6.4675	1.7461	12.55	-3.70	0.0028
Alum-mIGg	0.25	3	-15.9775	2.8315	10.24	-5.64	0.0002
Alum-mIGg	0.25	4	-8.6325	4.3351	9.512	-1.99	0.0759
Alum-mIGg	0.25	5	-8.2050	3.0496	10.06	-2.69	0.0226
Alum-mIGg	0.25	7	-0.7525	1.8188	10.93	-0.41	0.6871
Alum-mIGg	1	2	-2.5700	2.1637	17.88	-1.19	0.2505
Alum-mIGg	1	3	-12.0800	3.1065	13.78	-3.89	0.0017

Table F23. *Simple effect comparisons of treatment*day least squares means by treatment for αβ1 T cell+, Study 3* (Cont.)

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
Alum-mIGg	1	4	-4.7350	4.5194	11.09	-1.05	0.3171
Alum-mIGg	1	5	-4.3075	3.3064	13.16	-1.30	0.2150
Alum-mIGg	1	7	3.1450	2.2228	16.42	1.41	0.1758
Alum-mIGg	2	3	-9.5100	3.1664	14.45	-3.00	0.0092
Alum-mIGg	2	4	-2.1650	4.5608	11.44	-0.47	0.6439
Alum-mIGg	2	5	-1.7375	3.3628	13.79	-0.52	0.6136
Alum-mIGg	2	7	5.7150	2.3058	16.8	2.48	0.0241
Alum-mIGg	3	4	7.3450	5.0764	15.32	1.45	0.1681
Alum-mIGg	3	5	7.7725	4.0345	17.89	1.93	0.0701
Alum-mIGg	3	7	15.2250	3.2070	14.66	4.75	0.0003
Alum-mIGg	4	5	0.4275	5.2012	16.03	0.08	0.9355
Alum-mIGg	4	7	7.8800	4.5892	11.65	1.72	0.1124
Alum-mIGg	5	7	7.4525	3.4011	14.03	2.19	0.0458
IO-mIgG	0	0.25	0.4925	0.9287	17.26	0.53	0.6027
IO-mIgG	0	1	-1.0350	1.5796	11.79	-0.66	0.5249
IO-mIgG	0	2	-6.5775	1.6944	11.39	-3.88	0.0024
IO-mIgG	0	3	-12.4275	2.7999	9.82	-4.44	0.0013
IO-mIgG	0	4	-7.1725	4.3145	9.337	-1.66	0.1296
IO-mIgG	0	5	-11.1175	3.0202	9.701	-3.68	0.0045
IO-mIgG	0	7	-6.8500	2.0148	9.47	-3.40	0.0073
IO-mIgG	0.25	1	-1.5275	1.6350	13.11	-0.93	0.3671
IO-mIgG	0.25	2	-7.0700	1.7461	12.55	-4.05	0.0015
IO-mIgG	0.25	3	-12.9200	2.8315	10.24	-4.56	0.0010
IO-mIgG	0.25	4	-7.6650	4.3351	9.512	-1.77	0.1090
IO-mIgG	0.25	5	-11.6100	3.0496	10.06	-3.81	0.0034
IO-mIgG	0.25	7	-7.3425	2.0585	10.22	-3.57	0.0050
IO-mIgG	1	2	-5.5425	2.1637	17.88	-2.56	0.0197
IO-mIgG	1	3	-11.3925	3.1065	13.78	-3.67	0.0026
IO-mIgG	1	4	-6.1375	4.5194	11.09	-1.36	0.2014
IO-mIgG	1	5	-10.0825	3.3064	13.16	-3.05	0.0092
IO-mIgG	1	7	-5.8150	2.4228	15.37	-2.40	0.0295
IO-mIgG	2	3	-5.8500	3.1664	14.45	-1.85	0.0852
IO-mIgG	2	4	-0.5950	4.5608	11.44	-0.13	0.8985
IO-mIgG	2	5	-4.5400	3.3628	13.79	-1.35	0.1987
IO-mIgG	2	7	-0.2725	2.4992	16	-0.11	0.9145
IO-mIgG	3	4	5.2550	5.0764	15.32	1.04	0.3166
IO-mIgG	3	5	1.3100	4.0345	17.89	0.32	0.7492
IO-mIgG	3	7	5.5775	3.3488	15.77	1.67	0.1155
IO-mIgG	4	5	-3.9450	5.2012	16.03	-0.76	0.4592
IO-mIgG	4	7	0.3225	4.6893	12.45	0.07	0.9463
IO-mIgG	5	7	4.2675	3.5351	15.17	1.21	0.2458
mIgG	0	0.25	-1.0150	0.9287	17.26	-1.09	0.2894
mIgG	0	1	-6.2375	1.5796	11.79	-3.95	0.0020

Table F23. *Simple effect comparisons of treatment*day least squares means by treatment for αβ1 T cell+, Study 3* (Cont.)

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
mIgG	0	2	-5.3925	1.6944	11.39	-3.18	0.0084
mIgG	0	3	-8.2550	2.7999	9.82	-2.95	0.0149
mIgG	0	4	-7.6975	4.3145	9.337	-1.78	0.1069
mIgG	0	5	-6.5900	3.0202	9.701	-2.18	0.0549
mIgG	0	7	-2.2050	1.7692	9.951	-1.25	0.2412
mIgG	0.25	1	-5.2225	1.6350	13.11	-3.19	0.0070
mIgG	0.25	2	-4.3775	1.7461	12.55	-2.51	0.0268
mIgG	0.25	3	-7.2400	2.8315	10.24	-2.56	0.0280
mIgG	0.25	4	-6.6825	4.3351	9.512	-1.54	0.1558
mIgG	0.25	5	-5.5750	3.0496	10.06	-1.83	0.0973
mIgG	0.25	7	-1.1900	1.8188	10.93	-0.65	0.5265
mIgG	1	2	0.8450	2.1637	17.88	0.39	0.7008
mIgG	1	3	-2.0175	3.1065	13.78	-0.65	0.5267
mIgG	1	4	-1.4600	4.5194	11.09	-0.32	0.7527
mIgG	1	5	-0.3525	3.3064	13.16	-0.11	0.9167
mIgG	1	7	4.0325	2.2228	16.42	1.81	0.0880
mIgG	2	3	-2.8625	3.1664	14.45	-0.90	0.3808
mIgG	2	4	-2.3050	4.5608	11.44	-0.51	0.6229
mIgG	2	5	-1.1975	3.3628	13.79	-0.36	0.7272
mIgG	2	7	3.1875	2.3058	16.8	1.38	0.1849
mIgG	3	4	0.5575	5.0764	15.32	0.11	0.9140
mIgG	3	5	1.6650	4.0345	17.89	0.41	0.6847
mIgG	3	7	6.0500	3.2070	14.66	1.89	0.0792
mIgG	4	5	1.1075	5.2012	16.03	0.21	0.8341
mIgG	4	7	5.4925	4.5892	11.65	1.20	0.2552
mIgG	5	7	4.3850	3.4011	14.03	1.29	0.2181

Table F24. *Simple effect comparisons of treatment*day least squares means by day for αβ1 T cell+, Study 3*

Simple Effect Level	Treatment	Treatment	Estimate	Std. Error	DF	t Value	Pr > |t|
day 0	Alum-mIGg	IO-mIgG	-0.8400	0.8273	9	-1.02	0.3365
day 0	Alum-mIGg	mIgG	0.5150	0.8273	9	0.62	0.5491
day 0	IO-mIgG	mIgG	1.3550	0.8273	9	1.64	0.1359
day 0.25	Alum-mIGg	IO-mIgG	3.9225	1.0201	9	3.85	0.0039
day 0.25	Alum-mIGg	mIgG	3.7700	1.0201	9	3.70	0.0050
day 0.25	IO-mIgG	mIgG	-0.1525	1.0201	9	-0.15	0.8845
day 1	Alum-mIGg	IO-mIgG	6.2925	2.0750	9	3.03	0.0142
day 1	Alum-mIGg	mIgG	2.4450	2.0750	9	1.18	0.2689
day 1	IO-mIgG	mIgG	-3.8475	2.0750	9	-1.85	0.0967
day 2	Alum-mIGg	IO-mIgG	3.3200	2.2488	9	1.48	0.1740
day 2	Alum-mIGg	mIgG	5.8600	2.2488	9	2.61	0.0285
day 2	IO-mIgG	mIgG	2.5400	2.2488	9	1.13	0.2879
day 3	Alum-mIGg	IO-mIgG	6.9800	3.8723	9	1.80	0.1050
day 3	Alum-mIGg	mIgG	12.5075	3.8723	9	3.23	0.0103
day 3	IO-mIgG	mIgG	5.5275	3.8723	9	1.43	0.1872
day 4	Alum-mIGg	IO-mIgG	4.8900	6.0452	9	0.81	0.4394
day 4	Alum-mIGg	mIgG	5.7200	6.0452	9	0.95	0.3688
day 4	IO-mIgG	mIgG	0.8300	6.0452	9	0.14	0.8938
day 5	Alum-mIGg	IO-mIgG	0.5175	4.1904	9	0.12	0.9044
day 5	Alum-mIGg	mIgG	6.4000	4.1904	9	1.53	0.1610
day 5	IO-mIgG	mIgG	5.8825	4.1904	9	1.40	0.1939
day 7	Alum-mIGg	IO-mIgG	-2.6675	2.5505	8	-1.05	0.3262
day 7	Alum-mIGg	mIgG	3.3325	2.3613	8	1.41	0.1958
day 7	IO-mIgG	mIgG	6.0000	2.5505	8	2.35	0.0465

Table F25. *Simple effect comparisons of treatment*day least squares means by treatment for αβ1 T cell+ CD4- CD8+, Study 3*

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
Alum-mIGg	0	0.25	-1.0925	0.2100	12.62	-5.20	0.0002
Alum-mIGg	0	1	-1.6100	0.3258	10.33	-4.94	0.0005
Alum-mIGg	0	2	-3.1575	0.4136	9.764	-7.63	<.0001
Alum-mIGg	0	3	-6.9500	0.8147	9.236	-8.53	<.0001
Alum-mIGg	0	4	-4.7950	1.0189	9.153	-4.71	0.0011
Alum-mIGg	0	5	-3.5200	0.6724	9.098	-5.24	0.0005
Alum-mIGg	0	7	-2.5550	0.4338	8.667	-5.89	0.0003
Alum-mIGg	0.25	1	-0.5175	0.3631	14.28	-1.43	0.1755
Alum-mIGg	0.25	2	-2.0650	0.4428	12.54	-4.66	0.0005
Alum-mIGg	0.25	3	-5.8575	0.8277	9.879	-7.08	<.0001
Alum-mIGg	0.25	4	-3.7025	1.0285	9.559	-3.60	0.0052
Alum-mIGg	0.25	5	-2.4275	0.6892	10.16	-3.52	0.0054
Alum-mIGg	0.25	7	-1.4625	0.4613	10.81	-3.17	0.0091
Alum-mIGg	1	2	-1.5475	0.5044	16.31	-3.07	0.0072
Alum-mIGg	1	3	-5.3400	0.8592	11.28	-6.21	<.0001

Table F25. *Simple effect comparisons of treatment*day least squares means by treatment for αβ1 T cell+ CD4- CD8+, Study 3* (Cont.)

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
Alum-mIGg	1	4	-3.1850	1.0529	10.48	-3.02	0.0122
Alum-mIGg	1	5	-1.9100	0.7283	12.5	-2.62	0.0216
Alum-mIGg	1	7	-0.9450	0.5203	14.19	-1.82	0.0905
Alum-mIGg	2	3	-3.7925	0.8934	12.8	-4.24	0.0010
Alum-mIGg	2	4	-1.6375	1.0804	11.52	-1.52	0.1566
Alum-mIGg	2	5	-0.3625	0.7692	14.48	-0.47	0.6445
Alum-mIGg	2	7	0.6025	0.5772	16.12	1.04	0.3120
Alum-mIGg	3	4	2.1550	1.2748	16.22	1.69	0.1101
Alum-mIGg	3	5	3.4300	1.0313	17.03	3.33	0.0040
Alum-mIGg	3	7	4.3950	0.9015	12.92	4.88	0.0003
Alum-mIGg	4	5	1.2750	1.1952	15.22	1.07	0.3027
Alum-mIGg	4	7	2.2400	1.0868	11.65	2.06	0.0623
Alum-mIGg	5	7	0.9650	0.7789	14.58	1.24	0.2350
IO-mIgG	0	0.25	-0.1000	0.2100	12.62	-0.48	0.6420
IO-mIgG	0	1	-0.3500	0.3258	10.33	-1.07	0.3072
IO-mIgG	0	2	-2.1400	0.4136	9.764	-5.17	0.0004
IO-mIgG	0	3	-3.2825	0.8147	9.236	-4.03	0.0028
IO-mIgG	0	4	-2.7675	1.0189	9.153	-2.72	0.0234
IO-mIgG	0	5	-2.4775	0.6724	9.098	-3.68	0.0049
IO-mIgG	0	7	-1.1268	0.5013	8.513	-2.25	0.0528
IO-mIgG	0.25	1	-0.2500	0.3631	14.28	-0.69	0.5021
IO-mIgG	0.25	2	-2.0400	0.4428	12.54	-4.61	0.0005
IO-mIgG	0.25	3	-3.1825	0.8277	9.879	-3.85	0.0033
IO-mIgG	0.25	4	-2.6675	1.0285	9.559	-2.59	0.0277
IO-mIgG	0.25	5	-2.3775	0.6892	10.16	-3.45	0.0061
IO-mIgG	0.25	7	-1.0268	0.5253	10.15	-1.95	0.0787
IO-mIgG	1	2	-1.7900	0.5044	16.31	-3.55	0.0026
IO-mIgG	1	3	-2.9325	0.8592	11.28	-3.41	0.0056
IO-mIgG	1	4	-2.4175	1.0529	10.48	-2.30	0.0434
IO-mIgG	1	5	-2.1275	0.7283	12.5	-2.92	0.0123
IO-mIgG	1	7	-0.7768	0.5778	13.16	-1.34	0.2015
IO-mIgG	2	3	-1.1425	0.8934	12.8	-1.28	0.2237
IO-mIgG	2	4	-0.6275	1.0804	11.52	-0.58	0.5726
IO-mIgG	2	5	-0.3375	0.7692	14.48	-0.44	0.6673
IO-mIgG	2	7	1.0132	0.6295	15.46	1.61	0.1277
IO-mIgG	3	4	0.5150	1.2748	16.22	0.40	0.6915
IO-mIgG	3	5	0.8050	1.0313	17.03	0.78	0.4458
IO-mIgG	3	7	2.1557	0.9359	14.03	2.30	0.0371
IO-mIgG	4	5	0.2900	1.1952	15.22	0.24	0.8115
IO-mIgG	4	7	1.6407	1.1154	12.53	1.47	0.1660
IO-mIgG	5	7	1.3507	0.8184	15.7	1.65	0.1187
mIgG	0	0.25	-0.9500	0.2100	12.62	-4.52	0.0006
mIgG	0	1	-1.0625	0.3258	10.33	-3.26	0.0082

Table F25. *Simple effect comparisons of treatment*day least squares means by treatment for αβ1 T cell+ CD4- CD8+, Study 3* (Cont.)

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
mIgG	0	2	-1.1400	0.4136	9.764	-2.76	0.0207
mIgG	0	3	-2.2700	0.8147	9.236	-2.79	0.0207
mIgG	0	4	-2.1075	1.0189	9.153	-2.07	0.0680
mIgG	0	5	-1.8050	0.6724	9.098	-2.68	0.0248
mIgG	0	7	-0.9450	0.4338	8.667	-2.18	0.0584
mIgG	0.25	1	-0.1125	0.3631	14.28	-0.31	0.7611
mIgG	0.25	2	-0.1900	0.4428	12.54	-0.43	0.6751
mIgG	0.25	3	-1.3200	0.8277	9.879	-1.59	0.1422
mIgG	0.25	4	-1.1575	1.0285	9.559	-1.13	0.2879
mIgG	0.25	5	-0.8550	0.6892	10.16	-1.24	0.2427
mIgG	0.25	7	0.005000	0.4613	10.81	0.01	0.9915
mIgG	1	2	-0.07750	0.5044	16.31	-0.15	0.8798
mIgG	1	3	-1.2075	0.8592	11.28	-1.41	0.1869
mIgG	1	4	-1.0450	1.0529	10.48	-0.99	0.3433
mIgG	1	5	-0.7425	0.7283	12.5	-1.02	0.3273
mIgG	1	7	0.1175	0.5203	14.19	0.23	0.8245
mIgG	2	3	-1.1300	0.8934	12.8	-1.26	0.2285
mIgG	2	4	-0.9675	1.0804	11.52	-0.90	0.3888
mIgG	2	5	-0.6650	0.7692	14.48	-0.86	0.4014
mIgG	2	7	0.1950	0.5772	16.12	0.34	0.7399
mIgG	3	4	0.1625	1.2748	16.22	0.13	0.9001
mIgG	3	5	0.4650	1.0313	17.03	0.45	0.6578
mIgG	3	7	1.3250	0.9015	12.92	1.47	0.1656
mIgG	4	5	0.3025	1.1952	15.22	0.25	0.8036
mIgG	4	7	1.1625	1.0868	11.65	1.07	0.3064
mIgG	5	7	0.8600	0.7789	14.58	1.10	0.2874

Table F26. *Simple effect comparisons of treatment*day least squares means by day for αβ1 T cell+ CD4- CD8+, Study 3*

Simple Effect Level	Treatment	Treatment	Estimate	Std. Error	DF	t Value	Pr > |t|
day 0	Alum-mIGg	IO-mIgG	-0.02500	0.1308	8.849	-0.19	0.8527
day 0	Alum-mIGg	mIgG	0.1500	0.1308	8.849	1.15	0.2814
day 0	IO-mIgG	mIgG	0.1750	0.1308	8.849	1.34	0.2142
day 0.25	Alum-mIGg	IO-mIgG	0.9675	0.2720	8.911	3.56	0.0063
day 0.25	Alum-mIGg	mIgG	0.2925	0.2720	8.911	1.08	0.3105
day 0.25	IO-mIgG	mIgG	-0.6750	0.2720	8.911	-2.48	0.0352
day 1	Alum-mIGg	IO-mIgG	1.2350	0.4476	9.137	2.76	0.0218
day 1	Alum-mIGg	mIgG	0.6975	0.4476	9.137	1.56	0.1531
day 1	IO-mIgG	mIgG	-0.5375	0.4476	9.137	-1.20	0.2600
day 2	Alum-mIGg	IO-mIgG	0.9925	0.5756	8.976	1.72	0.1188
day 2	Alum-mIGg	mIgG	2.1675	0.5756	8.976	3.77	0.0045
day 2	IO-mIgG	mIgG	1.1750	0.5756	8.976	2.04	0.0717
day 3	Alum-mIGg	IO-mIgG	3.6425	1.1504	9.107	3.17	0.0113
day 3	Alum-mIGg	mIgG	4.8300	1.1504	9.107	4.20	0.0023
day 3	IO-mIgG	mIgG	1.1875	1.1504	9.107	1.03	0.3286
day 4	Alum-mIGg	IO-mIgG	2.0025	1.4406	9.074	1.39	0.1977
day 4	Alum-mIGg	mIgG	2.8375	1.4406	9.074	1.97	0.0801
day 4	IO-mIgG	mIgG	0.8350	1.4406	9.074	0.58	0.5763
day 5	Alum-mIGg	IO-mIgG	1.0175	0.9472	8.782	1.07	0.3114
day 5	Alum-mIGg	mIgG	1.8650	0.9472	8.782	1.97	0.0813
day 5	IO-mIgG	mIgG	0.8475	0.9472	8.782	0.89	0.3948
day 7	Alum-mIGg	IO-mIgG	1.4032	0.6552	8.126	2.14	0.0641
day 7	Alum-mIGg	mIgG	1.7600	0.6052	8.133	2.91	0.0193
day 7	IO-mIgG	mIgG	0.3568	0.6552	8.126	0.54	0.6007

Table F27. *Simple effect comparisons of treatment*day least squares means by treatment for B cell+ IgM-, Study 3*

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
Alum-mIGg	0	0.25	-0.3775	0.06440	15.04	-5.86	<.0001
Alum-mIGg	0	1	-0.9900	0.3233	9.903	-3.06	0.0121
Alum-mIGg	0	2	-3.2800	0.6095	10.06	-5.38	0.0003
Alum-mIGg	0	3	-2.6050	0.5721	10.24	-4.55	0.0010
Alum-mIGg	0	4	-1.0675	0.5603	8.325	-1.91	0.0918
Alum-mIGg	0	5	-2.7425	0.6818	8.871	-4.02	0.0031
Alum-mIGg	0	7	-1.6975	0.6946	8.462	-2.44	0.0387
Alum-mIGg	0.25	1	-0.6125	0.3210	10.06	-1.91	0.0853
Alum-mIGg	0.25	2	-2.9025	0.6057	10.07	-4.79	0.0007
Alum-mIGg	0.25	3	-2.2275	0.5683	10.24	-3.92	0.0027
Alum-mIGg	0.25	4	-0.6900	0.5566	8.363	-1.24	0.2488
Alum-mIGg	0.25	5	-2.3650	0.6778	8.89	-3.49	0.0070
Alum-mIGg	0.25	7	-1.3200	0.6905	8.454	-1.91	0.0903

Table F27. *Simple effect comparisons of treatment*day least squares means by treatment for B cell+ IgM-, Study 3* (Cont.)

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
Alum-mIGg	1	2	-2.2900	0.6230	13.76	-3.68	0.0026
Alum-mIGg	1	3	-1.6150	0.5912	14.3	-2.73	0.0160
Alum-mIGg	1	4	-0.07750	0.5822	12.99	-0.13	0.8961
Alum-mIGg	1	5	-1.7525	0.6867	11.94	-2.55	0.0254
Alum-mIGg	1	7	-0.7075	0.6976	10.94	-1.01	0.3324
Alum-mIGg	2	3	0.6750	0.7331	18.7	0.92	0.3689
Alum-mIGg	2	4	2.2125	0.7276	18.83	3.04	0.0068
Alum-mIGg	2	5	0.5375	0.8028	18.12	0.67	0.5116
Alum-mIGg	2	7	1.5825	0.8104	17.04	1.95	0.0675
Alum-mIGg	3	4	1.5375	0.7036	18.6	2.19	0.0419
Alum-mIGg	3	5	-0.1375	0.7824	17.89	-0.18	0.8625
Alum-mIGg	3	7	0.9075	0.7906	16.24	1.15	0.2677
Alum-mIGg	4	5	-1.6750	0.7777	17.93	-2.15	0.0451
Alum-mIGg	4	7	-0.6300	0.7861	16.15	-0.80	0.4345
Alum-mIGg	5	7	1.0450	0.8530	17.53	1.23	0.2367
IO-mIgG	0	0.25	0.07750	0.06440	15.04	1.20	0.2475
IO-mIgG	0	1	-0.2550	0.3233	9.903	-0.79	0.4488
IO-mIgG	0	2	-1.1975	0.6095	10.06	-1.96	0.0777
IO-mIgG	0	3	-1.2725	0.5721	10.24	-2.22	0.0497
IO-mIgG	0	4	-0.8325	0.5603	8.325	-1.49	0.1742
IO-mIgG	0	5	-1.0750	0.6818	8.871	-1.58	0.1498
IO-mIgG	0	7	-1.2509	0.7906	8.673	-1.58	0.1493
IO-mIgG	0.25	1	-0.3325	0.3210	10.06	-1.04	0.3245
IO-mIgG	0.25	2	-1.2750	0.6057	10.07	-2.11	0.0614
IO-mIgG	0.25	3	-1.3500	0.5683	10.24	-2.38	0.0384
IO-mIgG	0.25	4	-0.9100	0.5566	8.363	-1.63	0.1391
IO-mIgG	0.25	5	-1.1525	0.6778	8.89	-1.70	0.1237
IO-mIgG	0.25	7	-1.3284	0.7870	8.654	-1.69	0.1270
IO-mIgG	1	2	-0.9425	0.6230	13.76	-1.51	0.1530
IO-mIgG	1	3	-1.0175	0.5912	14.3	-1.72	0.1068
IO-mIgG	1	4	-0.5775	0.5822	12.99	-0.99	0.3393
IO-mIgG	1	5	-0.8200	0.6867	11.94	-1.19	0.2556
IO-mIgG	1	7	-0.9959	0.7932	10.45	-1.26	0.2366
IO-mIgG	2	3	-0.07500	0.7331	18.7	-0.10	0.9196
IO-mIgG	2	4	0.3650	0.7276	18.83	0.50	0.6217
IO-mIgG	2	5	0.1225	0.8028	18.12	0.15	0.8804
IO-mIgG	2	7	-0.05342	0.8940	15.86	-0.06	0.9531
IO-mIgG	3	4	0.4400	0.7036	18.6	0.63	0.5393
IO-mIgG	3	5	0.1975	0.7824	17.89	0.25	0.8036
IO-mIgG	3	7	0.02158	0.8761	15	0.02	0.9807
IO-mIgG	4	5	-0.2425	0.7777	17.93	-0.31	0.7588
IO-mIgG	4	7	-0.4184	0.8721	14.93	-0.48	0.6383
IO-mIgG	5	7	-0.1759	0.9328	16.9	-0.19	0.8527

Table F27. *Simple effect comparisons of treatment*day least squares means by treatment for B cell+ IgM-, Study 3* (Cont.)

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
mIgG	0	0.25	-0.07750	0.06440	15.04	-1.20	0.2475
mIgG	0	1	-0.6900	0.3233	9.903	-2.13	0.0589
mIgG	0	2	-1.4800	0.6095	10.06	-2.43	0.0354
mIgG	0	3	-1.4000	0.5721	10.24	-2.45	0.0339
mIgG	0	4	-1.6475	0.5603	8.325	-2.94	0.0179
mIgG	0	5	-1.3900	0.6818	8.871	-2.04	0.0724
mIgG	0	7	-0.7025	0.6946	8.462	-1.01	0.3399
mIgG	0.25	1	-0.6125	0.3210	10.06	-1.91	0.0853
mIgG	0.25	2	-1.4025	0.6057	10.07	-2.32	0.0429
mIgG	0.25	3	-1.3225	0.5683	10.24	-2.33	0.0417
mIgG	0.25	4	-1.5700	0.5566	8.363	-2.82	0.0215
mIgG	0.25	5	-1.3125	0.6778	8.89	-1.94	0.0852
mIgG	0.25	7	-0.6250	0.6905	8.454	-0.91	0.3905
mIgG	1	2	-0.7900	0.6230	13.76	-1.27	0.2258
mIgG	1	3	-0.7100	0.5912	14.3	-1.20	0.2493
mIgG	1	4	-0.9575	0.5822	12.99	-1.64	0.1240
mIgG	1	5	-0.7000	0.6867	11.94	-1.02	0.3282
mIgG	1	7	-0.01250	0.6976	10.94	-0.02	0.9860
mIgG	2	3	0.08000	0.7331	18.7	0.11	0.9143
mIgG	2	4	-0.1675	0.7276	18.83	-0.23	0.8204
mIgG	2	5	0.09000	0.8028	18.12	0.11	0.9120
mIgG	2	7	0.7775	0.8104	17.04	0.96	0.3508
mIgG	3	4	-0.2475	0.7036	18.6	-0.35	0.7290
mIgG	3	5	0.01000	0.7824	17.89	0.01	0.9899
mIgG	3	7	0.6975	0.7906	16.24	0.88	0.3905
mIgG	4	5	0.2575	0.7777	17.93	0.33	0.7444
mIgG	4	7	0.9450	0.7861	16.15	1.20	0.2467
mIgG	5	7	0.6875	0.8530	17.53	0.81	0.4310

Table F28. *Simple effect comparisons of treatment*day least squares means by day for B cell+ IgM-, Study 3*

Simple Effect Level	Treatment	Treatment	Estimate	Std. Error	DF	t Value	Pr > |t|
day 0	Alum-mIGg	IO-mIgG	-0.05500	0.05598	8.241	-0.98	0.3539
day 0	Alum-mIGg	mIgG	-0.02000	0.05598	8.241	-0.36	0.7299
day 0	IO-mIgG	mIgG	0.03500	0.05598	8.241	0.63	0.5488
day 0.25	Alum-mIGg	IO-mIgG	0.4000	0.08713	9.363	4.59	0.0012
day 0.25	Alum-mIGg	mIgG	0.2800	0.08713	9.363	3.21	0.0101
day 0.25	IO-mIgG	mIgG	-0.1200	0.08713	9.363	-1.38	0.2005
day 1	Alum-mIGg	IO-mIgG	0.6800	0.4680	9.963	1.45	0.1770
day 1	Alum-mIGg	mIgG	0.2800	0.4680	9.963	0.60	0.5630
day 1	IO-mIgG	mIgG	-0.4000	0.4680	9.963	-0.85	0.4128
day 2	Alum-mIGg	IO-mIgG	2.0275	0.8743	10.16	2.32	0.0425
day 2	Alum-mIGg	mIgG	1.7800	0.8743	10.16	2.04	0.0687
day 2	IO-mIgG	mIgG	-0.2475	0.8743	10.16	-0.28	0.7828
day 3	Alum-mIGg	IO-mIgG	1.2775	0.8213	10.35	1.56	0.1499
day 3	Alum-mIGg	mIgG	1.1850	0.8213	10.35	1.44	0.1786
day 3	IO-mIgG	mIgG	-0.09250	0.8213	10.35	-0.11	0.9125
day 4	Alum-mIGg	IO-mIgG	0.1800	0.8044	8.35	0.22	0.8283
day 4	Alum-mIGg	mIgG	-0.6000	0.8044	8.35	-0.75	0.4762
day 4	IO-mIgG	mIgG	-0.7800	0.8044	8.35	-0.97	0.3595
day 5	Alum-mIGg	IO-mIgG	1.6125	0.9767	8.916	1.65	0.1334
day 5	Alum-mIGg	mIgG	1.3325	0.9767	8.916	1.36	0.2059
day 5	IO-mIgG	mIgG	-0.2800	0.9767	8.916	-0.29	0.7809
day 7	Alum-mIGg	IO-mIgG	0.3916	1.0641	8.673	0.37	0.7217
day 7	Alum-mIGg	mIgG	0.9750	0.9949	8.538	0.98	0.3540
day 7	IO-mIgG	mIgG	0.5834	1.0641	8.673	0.55	0.5973

Table F29. *Simple effect comparisons of treatment*day least squares means by treatment for CD3+, Study 3*

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
Alum-mIGg	0	0.25	-13.4550	1.5051	17.76	-8.94	<.0001
Alum-mIGg	0	1	-24.4000	5.2199	9.685	-4.67	0.0010
Alum-mIGg	0	2	-28.0525	3.2938	10.81	-8.52	<.0001
Alum-mIGg	0	3	-35.0775	4.3593	9.997	-8.05	<.0001
Alum-mIGg	0	4	-19.8425	6.6738	9.413	-2.97	0.0149
Alum-mIGg	0	5	-21.6850	4.7388	8.747	-4.58	0.0014
Alum-mIGg	0	7	-5.2950	3.5584	9.37	-1.49	0.1696
Alum-mIGg	0.25	1	-10.9450	5.2451	9.865	-2.09	0.0639
Alum-mIGg	0.25	2	-14.5975	3.3336	11.27	-4.38	0.0010
Alum-mIGg	0.25	3	-21.6225	4.3895	10.26	-4.93	0.0006
Alum-mIGg	0.25	4	-6.3875	6.6936	9.522	-0.95	0.3635
Alum-mIGg	0.25	5	-8.2300	4.7666	8.943	-1.73	0.1185
Alum-mIGg	0.25	7	8.1600	3.5953	9.728	2.27	0.0473

Table F29. *Simple effect comparisons of treatment*day least squares means by treatment for CD3+, Study 3* (Cont.)

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
Alum-mIGg	1	2	-3.6525	6.0079	14.92	-0.61	0.5524
Alum-mIGg	1	3	-10.6775	6.6520	17.4	-1.61	0.1265
Alum-mIGg	1	4	4.5575	8.3538	16.96	0.55	0.5925
Alum-mIGg	1	5	2.7150	6.9066	16.97	0.39	0.6991
Alum-mIGg	1	7	19.1050	6.1570	15.36	3.10	0.0071
Alum-mIGg	2	3	-7.0250	5.2775	16.58	-1.33	0.2012
Alum-mIGg	2	4	8.2100	7.3067	12.87	1.12	0.2817
Alum-mIGg	2	5	6.3675	5.5950	14.34	1.14	0.2737
Alum-mIGg	2	7	22.7575	4.6379	16.66	4.91	0.0001
Alum-mIGg	3	4	15.2350	7.8449	15.36	1.94	0.0707
Alum-mIGg	3	5	13.3925	6.2816	16.64	2.13	0.0482
Alum-mIGg	3	7	29.7825	5.4465	16.6	5.47	<.0001
Alum-mIGg	4	5	-1.8425	8.0619	15.75	-0.23	0.8222
Alum-mIGg	4	7	14.5475	7.4297	13.39	1.96	0.0714
Alum-mIGg	5	7	16.3900	5.7547	14.71	2.85	0.0124
IO-mIgG	0	0.25	-1.4750	1.5051	17.76	-0.98	0.3402
IO-mIgG	0	1	-3.1150	5.2199	9.685	-0.60	0.5644
IO-mIgG	0	2	-11.7075	3.2938	10.81	-3.55	0.0046
IO-mIgG	0	3	-19.1175	4.3593	9.997	-4.39	0.0014
IO-mIgG	0	4	-12.6600	6.6738	9.413	-1.90	0.0889
IO-mIgG	0	5	-10.0800	4.7388	8.747	-2.13	0.0632
IO-mIgG	0	7	-11.8400	4.0681	9.03	-2.91	0.0172
IO-mIgG	0.25	1	-1.6400	5.2451	9.865	-0.31	0.7610
IO-mIgG	0.25	2	-10.2325	3.3336	11.27	-3.07	0.0104
IO-mIgG	0.25	3	-17.6425	4.3895	10.26	-4.02	0.0023
IO-mIgG	0.25	4	-11.1850	6.6936	9.522	-1.67	0.1272
IO-mIgG	0.25	5	-8.6050	4.7666	8.943	-1.81	0.1047
IO-mIgG	0.25	7	-10.3650	4.1004	9.3	-2.53	0.0316
IO-mIgG	1	2	-8.5925	6.0079	14.92	-1.43	0.1733
IO-mIgG	1	3	-16.0025	6.6520	17.4	-2.41	0.0275
IO-mIgG	1	4	-9.5450	8.3538	16.96	-1.14	0.2691
IO-mIgG	1	5	-6.9650	6.9066	16.97	-1.01	0.3274
IO-mIgG	1	7	-8.7250	6.4649	16.36	-1.35	0.1955
IO-mIgG	2	3	-7.4100	5.2775	16.58	-1.40	0.1787
IO-mIgG	2	4	-0.9525	7.3067	12.87	-0.13	0.8983
IO-mIgG	2	5	1.6275	5.5950	14.34	0.29	0.7753
IO-mIgG	2	7	-0.1325	5.0396	15.73	-0.03	0.9794
IO-mIgG	3	4	6.4575	7.8449	15.36	0.82	0.4230
IO-mIgG	3	5	9.0375	6.2816	16.64	1.44	0.1688
IO-mIgG	3	7	7.2775	5.7924	17	1.26	0.2260
IO-mIgG	4	5	2.5800	8.0619	15.75	0.32	0.7532
IO-mIgG	4	7	0.8200	7.6869	14.5	0.11	0.9165
IO-mIgG	5	7	-1.7600	6.0831	15.6	-0.29	0.7761

Table F29. *Simple effect comparisons of treatment*day least squares means by treatment for CD3+, Study 3* (Cont.)

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
mIgG	0	0.25	-2.9850	1.5051	17.76	-1.98	0.0630
mIgG	0	1	-9.8325	5.2199	9.685	-1.88	0.0899
mIgG	0	2	-9.9700	3.2938	10.81	-3.03	0.0117
mIgG	0	3	-13.1925	4.3593	9.997	-3.03	0.0128
mIgG	0	4	-12.5425	6.6738	9.413	-1.88	0.0915
mIgG	0	5	-13.2583	5.4413	8.56	-2.44	0.0389
mIgG	0	7	-4.0625	3.5584	9.37	-1.14	0.2819
mIgG	0.25	1	-6.8475	5.2451	9.865	-1.31	0.2213
mIgG	0.25	2	-6.9850	3.3336	11.27	-2.10	0.0595
mIgG	0.25	3	-10.2075	4.3895	10.26	-2.33	0.0418
mIgG	0.25	4	-9.5575	6.6936	9.522	-1.43	0.1853
mIgG	0.25	5	-10.2733	5.4655	8.708	-1.88	0.0940
mIgG	0.25	7	-1.0775	3.5953	9.728	-0.30	0.7707
mIgG	1	2	-0.1375	6.0079	14.92	-0.02	0.9820
mIgG	1	3	-3.3600	6.6520	17.4	-0.51	0.6198
mIgG	1	4	-2.7100	8.3538	16.96	-0.32	0.7496
mIgG	1	5	-3.4258	7.4063	16.82	-0.46	0.6496
mIgG	1	7	5.7700	6.1570	15.36	0.94	0.3632
mIgG	2	3	-3.2225	5.2775	16.58	-0.61	0.5497
mIgG	2	4	-2.5725	7.3067	12.87	-0.35	0.7305
mIgG	2	5	-3.2883	6.2012	13.08	-0.53	0.6048
mIgG	2	7	5.9075	4.6379	16.66	1.27	0.2202
mIgG	3	4	0.6500	7.8449	15.36	0.08	0.9350
mIgG	3	5	-0.06583	6.8272	15.71	-0.01	0.9924
mIgG	3	7	9.1300	5.4465	16.6	1.68	0.1124
mIgG	4	5	-0.7158	8.4939	16.63	-0.08	0.9338
mIgG	4	7	8.4800	7.4297	13.39	1.14	0.2737
mIgG	5	7	9.1958	6.3458	13.59	1.45	0.1700

Table F30. *Simple effect comparisons of treatment*day least squares means by day for CD3+, Study 3*

Simple Effect Level	Treatment	Treatment	Estimate	Std. Error	DF	t Value	Pr > |t|
day 0	Alum-mIGg	IO-mIgG	-2.0000	1.4146	9	-1.41	0.1911
day 0	Alum-mIGg	mIgG	0.1350	1.4146	9	0.10	0.9261
day 0	IO-mIgG	mIgG	2.1350	1.4146	9	1.51	0.1655
day 0.25	Alum-mIGg	IO-mIgG	9.9800	1.5904	9	6.28	0.0001
day 0.25	Alum-mIGg	mIgG	10.6050	1.5904	9	6.67	<.0001
day 0.25	IO-mIgG	mIgG	0.6250	1.5904	9	0.39	0.7035
day 1	Alum-mIGg	IO-mIgG	19.2850	7.2452	9	2.66	0.0260
day 1	Alum-mIGg	mIgG	14.7025	7.2452	9	2.03	0.0730
day 1	IO-mIgG	mIgG	-4.5825	7.2452	9	-0.63	0.5428
day 2	Alum-mIGg	IO-mIgG	14.3450	4.4381	9	3.23	0.0103
day 2	Alum-mIGg	mIgG	18.2175	4.4381	9	4.10	0.0027
day 2	IO-mIgG	mIgG	3.8725	4.4381	9	0.87	0.4056
day 3	Alum-mIGg	IO-mIgG	13.9600	6.0005	9	2.33	0.0450
day 3	Alum-mIGg	mIgG	22.0200	6.0005	9	3.67	0.0052
day 3	IO-mIgG	mIgG	8.0600	6.0005	9	1.34	0.2121
day 4	Alum-mIGg	IO-mIgG	5.1825	9.3316	9	0.56	0.5922
day 4	Alum-mIGg	mIgG	7.4350	9.3316	9	0.80	0.4461
day 4	IO-mIgG	mIgG	2.2525	9.3316	9	0.24	0.8147
day 5	Alum-mIGg	IO-mIgG	9.6050	6.5506	8	1.47	0.1807
day 5	Alum-mIGg	mIgG	8.5617	7.0755	8	1.21	0.2608
day 5	IO-mIgG	mIgG	-1.0433	7.0755	8	-0.15	0.8864
day 7	Alum-mIGg	IO-mIgG	-8.5450	5.2164	8	-1.64	0.1400
day 7	Alum-mIGg	mIgG	1.3675	4.8294	8	0.28	0.7842
day 7	IO-mIgG	mIgG	9.9125	5.2164	8	1.90	0.0939

Table F31. *Simple effect comparisons of treatment*day least squares means by treatment for CD4/CD8 ratio, Study 3*

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
Alum-mIGg	0.25	1	1.3732	0.4648	9.053	2.95	0.0160
Alum-mIGg	0.25	2	0.8164	0.8385	9.121	0.97	0.3553
Alum-mIGg	0.25	3	1.0537	0.6638	9.044	1.59	0.1467
Alum-mIGg	0.25	4	1.4693	0.6991	9.091	2.10	0.0646
Alum-mIGg	0.25	5	1.3021	0.7086	9.088	1.84	0.0990
Alum-mIGg	0.25	7	1.3747	0.4179	8.932	3.29	0.0095
Alum-mIGg	1	2	-0.5568	0.7283	9.081	-0.76	0.4640
Alum-mIGg	1	3	-0.3195	0.5443	9.026	-0.59	0.5716
Alum-mIGg	1	4	0.09612	0.4468	9.067	0.22	0.8344
Alum-mIGg	1	5	-0.07115	0.5628	9.056	-0.13	0.9022
Alum-mIGg	1	7	0.001506	0.6003	8.981	0.00	0.9981
Alum-mIGg	2	3	0.2373	0.6698	9.083	0.35	0.7312
Alum-mIGg	2	4	0.6529	0.6248	9.003	1.04	0.3233

Table F31. *Simple effect comparisons of treatment*day least squares means by treatment for CD4/CD8 ratio, Study 3* (Cont.)

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
Alum-mIGg	2	5	0.4856	0.5013	9.012	0.97	0.3579
Alum-mIGg	2	7	0.5583	0.9948	9.076	0.56	0.5883
Alum-mIGg	3	4	0.4156	0.5490	9.038	0.76	0.4683
Alum-mIGg	3	5	0.2483	0.4437	9.05	0.56	0.5893
Alum-mIGg	3	7	0.3210	0.6527	8.998	0.49	0.6346
Alum-mIGg	4	5	-0.1673	0.3523	9.015	-0.47	0.6462
Alum-mIGg	4	7	-0.09462	0.6861	9.086	-0.14	0.8933
Alum-mIGg	5	7	0.07265	0.6479	9.101	0.11	0.9131
IO-mIgG	0.25	1	2.3322	0.4648	9.053	5.02	0.0007
IO-mIgG	0.25	2	1.3725	0.8384	9.121	1.64	0.1356
IO-mIgG	0.25	3	1.6422	0.6638	9.044	2.47	0.0352
IO-mIgG	0.25	4	2.5461	0.6991	9.091	3.64	0.0053
IO-mIgG	0.25	5	0.8879	0.7086	9.088	1.25	0.2415
IO-mIgG	0.25	7	0.7626	0.4200	9.001	1.82	0.1028
IO-mIgG	1	2	-0.9597	0.7283	9.081	-1.32	0.2199
IO-mIgG	1	3	-0.6900	0.5443	9.026	-1.27	0.2366
IO-mIgG	1	4	0.2139	0.4468	9.067	0.48	0.6434
IO-mIgG	1	5	-1.4443	0.5628	9.056	-2.57	0.0302
IO-mIgG	1	7	-1.5696	0.6017	9.015	-2.61	0.0283
IO-mIgG	2	3	0.2697	0.6698	9.083	0.40	0.6966
IO-mIgG	2	4	1.1736	0.6248	9.003	1.88	0.0931
IO-mIgG	2	5	-0.4846	0.5013	9.012	-0.97	0.3589
IO-mIgG	2	7	-0.6099	0.9957	9.089	-0.61	0.5552
IO-mIgG	3	4	0.9039	0.5490	9.038	1.65	0.1339
IO-mIgG	3	5	-0.7543	0.4437	9.05	-1.70	0.1232
IO-mIgG	3	7	-0.8796	0.6540	9.027	-1.34	0.2114
IO-mIgG	4	5	-1.6582	0.3523	9.015	-4.71	0.0011
IO-mIgG	4	7	-1.7835	0.6873	9.112	-2.59	0.0287
IO-mIgG	5	7	-0.1253	0.6492	9.131	-0.19	0.8511
mIgG	0.25	1	-0.1474	0.4648	9.053	-0.32	0.7584
mIgG	0.25	2	-0.7977	0.8384	9.121	-0.95	0.3659
mIgG	0.25	3	-1.1368	0.6638	9.044	-1.71	0.1208
mIgG	0.25	4	-0.8564	0.6991	9.091	-1.23	0.2513
mIgG	0.25	5	-0.6846	0.7086	9.088	-0.97	0.3590
mIgG	0.25	7	-0.2611	0.4179	8.932	-0.62	0.5477
mIgG	1	2	-0.6503	0.7282	9.081	-0.89	0.3949
mIgG	1	3	-0.9894	0.5443	9.026	-1.82	0.1024
mIgG	1	4	-0.7091	0.4468	9.067	-1.59	0.1467
mIgG	1	5	-0.5372	0.5628	9.056	-0.95	0.3646
mIgG	1	7	-0.1137	0.6003	8.981	-0.19	0.8539
mIgG	2	3	-0.3391	0.6698	9.083	-0.51	0.6248
mIgG	2	4	-0.05874	0.6248	9.003	-0.09	0.9272
mIgG	2	5	0.1131	0.5013	9.012	0.23	0.8266

Table F31. *Simple effect comparisons of treatment*day least squares means by treatment for CD4/CD8 ratio, Study 3* (Cont.)

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
mIgG	2	7	0.5366	0.9948	9.076	0.54	0.6026
mIgG	3	4	0.2804	0.5490	9.038	0.51	0.6218
mIgG	3	5	0.4522	0.4437	9.05	1.02	0.3346
mIgG	3	7	0.8757	0.6527	8.998	1.34	0.2126
mIgG	4	5	0.1718	0.3523	9.015	0.49	0.6374
mIgG	4	7	0.5953	0.6861	9.086	0.87	0.4079
mIgG	5	7	0.4235	0.6479	9.101	0.65	0.5295

Table F32. *Simple effect comparisons of treatment*day least squares means by day for CD4/CD8 ratio, Study 3*

Simple Effect Level	Treatment	Treatment	Estimate	Std. Error	DF	t Value	Pr > |t|
day 0.25	Alum-mIGg	IO-mIgG	-1.1001	0.7664	9.122	-1.44	0.1846
day 0.25	Alum-mIGg	mIgG	1.2784	0.7664	9.122	1.67	0.1292
day 0.25	IO-mIgG	mIgG	2.3785	0.7664	9.122	3.10	0.0124
day 1	Alum-mIGg	IO-mIgG	-0.1411	0.6029	9.061	-0.23	0.8201
day 1	Alum-mIGg	mIgG	-0.2422	0.6029	9.061	-0.40	0.6972
day 1	IO-mIgG	mIgG	-0.1011	0.6029	9.061	-0.17	0.8706
day 2	Alum-mIGg	IO-mIgG	-0.5440	0.7414	9.025	-0.73	0.4817
day 2	Alum-mIGg	mIgG	-0.3357	0.7414	9.025	-0.45	0.6614
day 2	IO-mIgG	mIgG	0.2083	0.7414	9.025	0.28	0.7851
day 3	Alum-mIGg	IO-mIgG	-0.5116	0.6636	9.042	-0.77	0.4604
day 3	Alum-mIGg	mIgG	-0.9121	0.6636	9.042	-1.37	0.2024
day 3	IO-mIgG	mIgG	-0.4005	0.6636	9.042	-0.60	0.5610
day 4	Alum-mIGg	IO-mIgG	-0.02333	0.4604	9.002	-0.05	0.9607
day 4	Alum-mIGg	mIgG	-1.0473	0.4604	9.002	-2.27	0.0490
day 4	IO-mIgG	mIgG	-1.0240	0.4604	9.002	-2.22	0.0532
day 5	Alum-mIGg	IO-mIgG	-1.5142	0.4136	9.013	-3.66	0.0052
day 5	Alum-mIGg	mIgG	-0.7083	0.4136	9.013	-1.71	0.1209
day 5	IO-mIgG	mIgG	0.8060	0.4136	9.013	1.95	0.0831
day 7	Alum-mIGg	IO-mIgG	-1.7122	0.8448	9.111	-2.03	0.0729
day 7	Alum-mIGg	mIgG	-0.3574	0.8438	9.094	-0.42	0.6817
day 7	IO-mIgG	mIgG	1.3548	0.8448	9.111	1.60	0.1428

APPENDIX G

Type III Test of Fixed Effects Results for

Primary Response in Intramuscular Injection Studies

Table G1. *Type III test of fixed effects results for primary response in intramuscular injection studies*

		Study A		Study B	
Response Factor	**Fixed Effect**	**F Value**	**P Value**	**F Value**	**P Value**
CD45+	Day	59.59	< 0.0001	-	-
	Treatment	0.81	NS	-	-
	Treatment*Day	1.11	NS	-	-
Macrophages	Day	7.49	0.0037	-	-
	Treatment	2.37	NS	-	-
	Treatment*Day	1.89	NS	-	-
MHCII+	Day	35.30	< 0.0001	38.57	0.0062
Macrophages+	Treatment	0.33	NS	6.23	0.0200
	Treatment*Day	1.24	NS	10.31	0.0238
MHCII+	Day	11.38	0.0009	23.71	0.0119
B cells+	Treatment	0.54	NS	2.30	NS
	Treatment*Day	0.45	NS	6.53	NS
CD4- CD8+	Day	16.24	0.0003	9.44	0.0460
	Treatment	1.72	NS	0.56	NS
	Treatment*Day	1.43	NS	2.31	NS
CD4+ CD8+	Day	8.44	0.0024	7.10	NS
	Treatment	1.80	NS	0.60	NS
	Treatment*Day	1.06	NS	0.19	NS
CD4+ CD8-	Day	48.05	< 0.0001	112.43	0.0013
	Treatment	1.57	NS	0.83	NS
	Treatment*Day	2.94	0.0365	2.48	NS
CD25+ CD4+	Day	3.63	0.0398	-	-
	Treatment	0.29	NS	-	-
	Treatment*Day	0.89	NS	-	-
CD25+ CD4-	Day	10.63	0.0013	-	-
	Treatment	3.52	NS	-	-
	Treatment*Day	0.65	NS	-	-
γδ T cell+ CD8+	Day	14.33	0.0004	3.83	NS
	Treatment	3.72	0.0488	1.53	NS
	Treatment*Day	0.74	NS	1.57	NS
γδ T cell+ CD8-	Day	15.64	0.0003	11.15	0.0366
	Treatment	1.56	NS	0.35	NS
	Treatment*Day	4.90	0.0050	0.73	NS
αβ1 Tcell+	Day	41.49	< 0.0001	149.72	0.0008
	Treatment	2.50	NS	1.78	NS
	Treatment*Day	1.21	NS	4.78	NS

Table G1. *Type III test of fixed effects results for primary response in intramuscular injection studies* (Cont.)

		Study A		Study B	
Response Factor	**Fixed Effect**	**F Value**	**P Value**	**F Value**	**P Value**
αβ1 T cell+	Day	-	-	20.51	0.0154
CD4-CD8+	Treatment	-	-	2.48	NS
	Treatment*Day	-	-	6.01	NS
αβ1 T cell+	Day	-	-	7.85	NS
CD4+CD8+	Treatment	-	-	1.24	NS
	Treatment*Day	-	-	0.72	NS
αβ1 T cell+	Day	-	-	33.57	0.0075
CD4-CD8-	Treatment	-	-	1.97	NS
	Treatment*Day	-	-	0.79	NS
αβ1 T cell+	Day	-	-	79.81	0.0021
CD4+CD8-	Treatment	-	-	1.69	NS
	Treatment*Day	-	-	1.57	NS
αβ2 T cell+	Day	39.89	< 0.0001	24.32	0.0121
	Treatment	2.56	NS	0.29	NS
	Treatment*Day	1.85	NS	0.66	NS
αβ2 T cell+	Day	-	-	24.29	0.0121
CD4-CD8+	Treatment	-	-	0.39	NS
	Treatment*Day	-	-	1.80	NS
αβ2 T cell+	Day	-	-	42.76	0.0053
CD4+CD8+	Treatment	-	-	0.15	NS
	Treatment*Day	-	-	8.35	0.0339
αβ2 T cell+	Day	-	-	6.46	NS
CD4-CD8-	Treatment	-	-	1.01	NS
	Treatment*Day	-	-	0.26	NS
αβ2 T cell+	Day	-	-	257.06	0.0458
CD4+CD8-	Treatment	-	-	0.04	NS
	Treatment*Day	-	-	0.04	NS
B cell	Day	48.19	< 0.0001	-	-
	Treatment	16.04	0.0002	-	-
	Treatment*Day	5.02	0.0045	-	-
B cell+ IgM+	Day	-	-	21.65	0.0143
	Treatment	-	-	1.88	NS
	Treatment*Day	-	-	6.31	NS
B cell+ IgM-	Day	-	-	29.00	0.0093
	Treatment	-	-	4.47	0.0449
	Treatment*Day	-	-	2.96	NS
B cell+ IgG+	Day	-	-	5.87	NS
	Treatment	-	-	0.86	NS
	Treatment*Day	-	-	0.79	NS
B cell+ IgG-	Day	-	-	106.05	0.0013
	Treatment	-	-	2.57	NS
	Treatment*Day	-	-	14.54	0.0132

APPENDIX H

Difference of Least Squares Means Results for Primary Response in Intramuscular Injection Studies

Table H1. *Difference of least squares means for pulp cell responses by day*

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
Study A							
CD45+	0	0.25	-15.4800	1.2634	14.99	-12.25	<.0001
	0	1	-16.7111	1.2270	15	-13.62	<.0001
	0	2	-14.6950	1.6447	14.98	-8.93	<.0001
	0	3	-8.4694	1.3065	15	-6.48	<.0001
	0	4	-6.3896	1.2527	15.08	-5.10	0.0001
	0	5	-3.4341	1.0765	12.29	-3.19	0.0076
	0	7	2.7261	1.3168	15.01	2.07	0.0561
	0.25	1	-1.2311	1.6911	14.99	-0.73	0.4778
	0.25	2	0.7850	2.2212	14.98	0.35	0.7287
	0.25	3	7.0106	1.5596	15	4.49	0.0004
	0.25	4	9.0904	1.9196	15.19	4.74	0.0003
	0.25	5	12.0459	1.8222	12.96	6.61	<.0001
	0.25	7	18.2061	1.5822	15	11.51	<.0001
	1	2	2.0161	1.7803	14.99	1.13	0.2752
	1	3	8.2417	1.5033	15	5.48	<.0001
	1	4	10.3216	1.6808	14.87	6.14	<.0001
	1	5	13.2770	1.4757	15.17	9.00	<.0001
	1	7	19.4372	1.7096	15.01	11.37	<.0001
	2	3	6.2256	1.7385	14.97	3.58	0.0027
	2	4	8.3054	2.1101	15.16	3.94	0.0013
	2	5	11.2609	1.7025	14.21	6.61	<.0001
	2	7	17.4211	1.5384	14.97	11.32	<.0001
	3	4	2.0799	1.5135	11.01	1.37	0.1967
	3	5	5.0354	1.2842	14.07	3.92	0.0015
	3	7	11.1956	1.2049	15	9.29	<.0001
	4	5	2.9555	0.8615	14.34	3.43	0.0039
	4	7	9.1157	1.4928	15.23	6.11	<.0001
	5	7	6.1602	1.2882	14.77	4.78	0.0003
Macrophages	0	0.25	-3.9006	0.5027	15	-7.76	<.0001
	0	1	0.1061	0.3279	15	0.32	0.7507
	0	2	-0.01722	0.2626	15.01	-0.07	0.9486
	0	3	0.1194	0.2949	15	0.41	0.6912
	0	4	0.2461	0.2006	15.22	1.23	0.2384
	0	5	0.07227	0.3819	14.58	0.19	0.8525
	0	7	0.8789	0.3650	15	2.41	0.0294
	0.25	1	4.0067	0.6499	15.02	6.17	<.0001

Table H1. *Difference of least squares means for pulp cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
	0.25	2	3.8833	0.6121	15.01	6.34	<.0001
	0.25	3	4.0200	0.5789	15.01	6.94	<.0001
	0.25	4	4.1467	0.6107	15.04	6.79	<.0001
	0.25	5	3.9728	0.6749	15.09	5.89	<.0001
	0.25	7	4.7794	0.7133	15	6.70	<.0001
	1	2	-0.1233	0.3406	14.99	-0.36	0.7223
	1	3	0.01333	0.2231	14.99	0.06	0.9531
	1	4	0.1400	0.2485	15.16	0.56	0.5814
	1	5	-0.03384	0.3012	14.62	-0.11	0.9121
	1	7	0.7728	0.3413	14.97	2.26	0.0389
	2	3	0.1367	0.2743	15	0.50	0.6256
	2	4	0.2633	0.2510	15.19	1.05	0.3105
	2	5	0.08950	0.2992	14.86	0.30	0.7690
	2	7	0.8961	0.3647	15	2.46	0.0267
	3	4	0.1267	0.2163	14.93	0.59	0.5669
	3	5	-0.04717	0.2899	13.26	-0.16	0.8732
	3	7	0.7594	0.3125	14.97	2.43	0.0281
	4	5	-0.1738	0.2866	14.92	-0.61	0.5532
	4	7	0.6328	0.2528	15.15	2.50	0.0242
	5	7	0.8066	0.3653	15.09	2.21	0.0431
MHCII+	0	0.25	-4.4467	0.4785	15	-9.29	<.0001
Macrophages+	0	1	-1.2117	0.1685	15	-7.19	<.0001
	0	2	-1.5467	0.1768	14.98	-8.75	<.0001
	0	3	-0.8494	0.1479	14.99	-5.74	<.0001
	0	4	-0.9311	0.2054	15.01	-4.53	0.0004
	0	5	-0.5238	0.1353	12.34	-3.87	0.0021
	0	7	0.4744	0.1621	14.98	2.93	0.0104
	0.25	1	3.2350	0.5204	15.02	6.22	<.0001
	0.25	2	2.9000	0.5682	14.98	5.10	0.0001
	0.25	3	3.5972	0.5261	15	6.84	<.0001
	0.25	4	3.5156	0.5395	15.02	6.52	<.0001
	0.25	5	3.9229	0.5307	14.96	7.39	<.0001
	0.25	7	4.9211	0.5419	14.98	9.08	<.0001
	1	2	-0.3350	0.2280	14.97	-1.47	0.1624
	1	3	0.3622	0.1723	14.98	2.10	0.0529
	1	4	0.2806	0.2182	15.01	1.29	0.2179
	1	5	0.6879	0.1981	14.24	3.47	0.0036
	1	7	1.6861	0.2326	14.98	7.25	<.0001
	2	3	0.6972	0.1662	14.99	4.19	0.0008
	2	4	0.6156	0.2787	14.99	2.21	0.0432
	2	5	1.0229	0.1816	14.2	5.63	<.0001
	2	7	2.0211	0.1529	15	13.22	<.0001
	3	4	-0.08167	0.1683	15.01	-0.49	0.6345
	3	5	0.3257	0.1557	13.96	2.09	0.0553

Table H1. *Difference of least squares means for pulp cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
	3	7	1.3239	0.1690	14.98	7.84	<.0001
	4	5	0.4073	0.1683	14.81	2.42	0.0288
	4	7	1.4056	0.2756	15	5.10	0.0001
	5	7	0.9982	0.2010	14.95	4.97	0.0002
Lymphocytes	0	0.25	-3.6533	0.5767	15.02	-6.34	<.0001
	0	1	-12.4206	1.0393	15.12	-11.95	<.0001
	0	2	-11.9700	1.0501	14.94	-11.40	<.0001
	0	3	-5.7156	0.8677	14.98	-6.59	<.0001
	0	4	-3.6022	0.7507	14.94	-4.80	0.0002
	0	5	-1.0857	0.3890	14.25	-2.79	0.0142
	0	7	-0.9456	0.2653	15.05	-3.56	0.0028
	0.25	1	-8.7672	1.0269	15.04	-8.54	<.0001
	0.25	2	-8.3167	0.9878	14.97	-8.42	<.0001
	0.25	3	-2.0622	1.0421	15.02	-1.98	0.0665
	0.25	4	0.05111	1.0701	14.92	0.05	0.9625
	0.25	5	2.5676	0.5539	15.11	4.64	0.0003
	0.25	7	2.7078	0.6050	15.01	4.48	0.0004
	1	2	0.4506	1.2964	14.93	0.35	0.7330
	1	3	6.7050	1.2206	15.01	5.49	<.0001
	1	4	8.8183	1.1067	15.06	7.97	<.0001
	1	5	11.3348	0.8942	14.86	12.68	<.0001
	1	7	11.4750	0.8861	15.12	12.95	<.0001
	2	3	6.2544	1.2204	14.94	5.12	0.0001
	2	4	8.3678	1.4941	14.88	5.60	<.0001
	2	5	10.8843	1.1253	14.59	9.67	<.0001
	2	7	11.0244	1.0402	14.93	10.60	<.0001
	3	4	2.1133	0.9958	14.86	2.12	0.0510
	3	5	4.6298	0.9345	15	4.95	0.0002
	3	7	4.7700	0.8543	14.97	5.58	<.0001
	4	5	2.5165	0.6585	15.05	3.82	0.0017
	4	7	2.6567	0.8436	14.95	3.15	0.0066
	5	7	0.1402	0.4603	14.82	0.30	0.7649
CD4+ CD8+	0	0.25	-0.1422	0.1495	15	-0.95	0.3566
	0	1	-0.2400	0.04141	15.03	-5.80	<.0001
	0	2	-0.5789	0.1663	15.02	-3.48	0.0033
	0	3	-0.1911	0.08126	15	-2.35	0.0328
	0	4	-0.1389	0.04034	15.01	-3.44	0.0036
	0	5	-0.05325	0.02840	15.03	-1.87	0.0804
	0	7	-0.00167	0.02009	15	-0.08	0.9350
	0.25	1	-0.09778	0.1345	14.98	-0.73	0.4784
	0.25	2	-0.4367	0.1138	15.01	-3.84	0.0016
	0.25	3	-0.04889	0.08191	15	-0.60	0.5595
	0.25	4	0.003333	0.1640	15	0.02	0.9841
	0.25	5	0.08897	0.1499	14.98	0.59	0.5618
	0.25	7	0.1406	0.1398	15	1.01	0.3308
	1	2	-0.3389	0.1650	15	-2.05	0.0579

Table H1. *Difference of least squares means for pulp cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
	1	3	0.04889	0.07695	14.95	0.64	0.5348
	1	4	0.1011	0.06219	15.01	1.63	0.1248
	1	5	0.1867	0.03932	15.12	4.75	0.0003
	1	7	0.2383	0.03609	15.02	6.60	<.0001
	2	3	0.3878	0.09886	15.04	3.92	0.0014
	2	4	0.4400	0.1629	15.02	2.70	0.0164
	2	5	0.5256	0.1647	14.99	3.19	0.0061
	2	7	0.5772	0.1663	15.01	3.47	0.0034
	3	4	0.05222	0.09104	15	0.57	0.5747
	3	5	0.1379	0.08355	14.96	1.65	0.1198
	3	7	0.1894	0.07675	14.98	2.47	0.0261
	4	5	0.08564	0.04520	15.04	1.89	0.0775
	4	7	0.1372	0.05004	15	2.74	0.0151
	5	7	0.05159	0.03323	15.12	1.55	0.1412
CD4- CD8+	0	0.25	-2.2533	0.4541	14.96	-4.96	0.0002
	0	1	-2.9100	0.8957	14.99	-3.25	0.0054
	0	2	-2.0911	0.3107	15.02	-6.73	<.0001
	0	3	-0.6772	0.1731	15.15	-3.91	0.0014
	0	4	-0.3706	0.08908	14.98	-4.16	0.0008
	0	5	-0.02488	0.1084	7.644	-0.23	0.8245
	0	7	0.02556	0.03694	14.94	0.69	0.4997
	0.25	1	-0.6567	0.9172	15.13	-0.72	0.4849
	0.25	2	0.1622	0.2975	14.61	0.55	0.5938
	0.25	3	1.5761	0.3440	14.85	4.58	0.0004
	0.25	4	1.8828	0.4974	14.95	3.79	0.0018
	0.25	5	2.2285	0.4826	14.81	4.62	0.0003
	0.25	7	2.2789	0.4381	14.96	5.20	0.0001
	1	2	0.8189	0.9478	14.84	0.86	0.4014
	1	3	2.2328	0.8845	14.92	2.52	0.0234
	1	4	2.5394	0.9153	14.99	2.77	0.0142
	1	5	2.8851	0.8826	14.89	3.27	0.0052
	1	7	2.9356	0.8820	15	3.33	0.0046
	2	3	1.4139	0.1880	14.89	7.52	<.0001
	2	4	1.7206	0.3419	15.04	5.03	0.0001
	2	5	2.0662	0.3106	14.97	6.65	<.0001
	2	7	2.1167	0.3056	14.98	6.93	<.0001
	3	4	0.3067	0.1977	15.14	1.55	0.1416
	3	5	0.6523	0.1984	14.47	3.29	0.0052
	3	7	0.7028	0.1586	15.11	4.43	0.0005
	4	5	0.3457	0.1305	12.4	2.65	0.0207
	4	7	0.3961	0.09865	14.97	4.02	0.0011
	5	7	0.05043	0.1135	7.619	0.44	0.6692
CD25+ CD4-	0	0.25	-0.04333	0.01647	15	-2.63	0.0189
	0	1	-0.06833	0.01752	14.99	-3.90	0.0014
	0	2	-0.02278	0.02027	15	-1.12	0.2789
	0	3	0.02944	0.009654	14.98	3.05	0.0081

Table H1. *Difference of least squares means for pulp cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
	0	4	0.006667	0.01306	14.99	0.51	0.6170
	0	5	-0.00361	0.01345	14.56	-0.27	0.7920
	0	7	-0.1878	0.02924	14.99	-6.42	<.0001
	0.25	1	-0.02500	0.01660	15	-1.51	0.1529
	0.25	2	0.02056	0.02386	15	0.86	0.4026
	0.25	3	0.07278	0.01702	15	4.28	0.0007
	0.25	4	0.05000	0.01956	15	2.56	0.0219
	0.25	5	0.03972	0.01923	13.94	2.07	0.0580
	0.25	7	-0.1444	0.03700	15	-3.90	0.0014
	1	2	0.04556	0.02405	14.99	1.89	0.0776
	1	3	0.09778	0.01705	14.99	5.73	<.0001
	1	4	0.07500	0.01443	14.99	5.20	0.0001
	1	5	0.06472	0.01978	15.2	3.27	0.0051
	1	7	-0.1194	0.03254	14.99	-3.67	0.0023
	2	3	0.05222	0.01716	15	3.04	0.0082
	2	4	0.02944	0.01965	15	1.50	0.1547
	2	5	0.01917	0.01980	14.88	0.97	0.3486
	2	7	-0.1650	0.03668	15	-4.50	0.0004
	3	4	-0.02278	0.009166	15	-2.48	0.0252
	3	5	-0.03306	0.008386	13.75	-3.94	0.0015
	3	7	-0.2172	0.02930	15	-7.41	<.0001
	4	5	-0.01028	0.01186	13.9	-0.87	0.4008
	4	7	-0.1944	0.02857	15	-6.81	<.0001
	5	7	-0.1842	0.02869	15.11	-6.42	<.0001
CD25+ CD4+	0	0.25	-0.00111	0.01302	15	-0.09	0.9331
	0	1	-0.04444	0.01820	15.01	-2.44	0.0275
	0	2	0.02111	0.01233	15	1.71	0.1075
	0	3	0.01167	0.01246	15	0.94	0.3641
	0	4	-0.05000	0.01886	15	-2.65	0.0182
	0	5	-0.04230	0.01817	13.09	-2.33	0.0366
	0	7	-0.04667	0.01459	15	-3.20	0.0060
	0.25	1	-0.04333	0.01427	15.01	-3.04	0.0083
	0.25	2	0.02222	0.008876	14.99	2.50	0.0243
	0.25	3	0.01278	0.01005	14.99	1.27	0.2231
	0.25	4	-0.04889	0.01486	15	-3.29	0.0050
	0.25	5	-0.04119	0.01157	14.68	-3.56	0.0029
	0.25	7	-0.04556	0.01289	15.01	-3.53	0.0030
	1	2	0.06556	0.01615	15	4.06	0.0010
	1	3	0.05611	0.01703	15	3.29	0.0049
	1	4	-0.00556	0.02195	15	-0.25	0.8036
	1	5	0.002143	0.01330	15.28	0.16	0.8741
	1	7	-0.00222	0.01902	15.01	-0.12	0.9085
	2	3	-0.00944	0.006464	15	-1.46	0.1646
	2	4	-0.07111	0.01659	15	-4.29	0.0007
	2	5	-0.06341	0.01291	14.6	-4.91	0.0002
	2	7	-0.06778	0.01294	15.01	-5.24	<.0001

Table H1. *Difference of least squares means for pulp cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
	3	4	-0.06167	0.01557	14.99	-3.96	0.0013
	3	5	-0.05397	0.01281	14.4	-4.21	0.0008
	3	7	-0.05833	0.01274	15	-4.58	0.0004
	4	5	0.007698	0.01538	14.94	0.50	0.6240
	4	7	0.003333	0.01373	14.99	0.24	0.8114
	5	7	-0.00436	0.01323	13.73	-0.33	0.7465
γδ T cell+ CD8+	0	0.25	-0.03000	0.01652	15	-1.82	0.0894
	0	1	-0.3611	0.04807	15	-7.51	<.0001
	0	2	-0.2644	0.04139	14.99	-6.39	<.0001
	0	3	-0.04444	0.02453	15	-1.81	0.0901
	0	4	-0.03889	0.02071	15.01	-1.88	0.0799
	0	5	0.01617	0.02236	14.9	0.72	0.4807
	0	7	-0.00389	0.02687	14.99	-0.14	0.8868
	0.25	1	-0.3311	0.05262	15	-6.29	<.0001
	0.25	2	-0.2344	0.04315	14.99	-5.43	<.0001
	0.25	3	-0.01444	0.02879	14.99	-0.50	0.6231
	0.25	4	-0.00889	0.02271	15.01	-0.39	0.7010
	0.25	5	0.04617	0.02571	14.98	1.80	0.0927
	0.25	7	0.02611	0.02989	14.99	0.87	0.3961
	1	2	0.09667	0.04329	15	2.23	0.0412
	1	3	0.3167	0.03585	15	8.83	<.0001
	1	4	0.3222	0.04826	15	6.68	<.0001
	1	5	0.3773	0.03869	14.64	9.75	<.0001
	1	7	0.3572	0.04694	15.01	7.61	<.0001
	2	3	0.2200	0.03424	15	6.42	<.0001
	2	4	0.2256	0.04295	15	5.25	<.0001
	2	5	0.2806	0.04057	14.58	6.92	<.0001
	2	7	0.2606	0.03328	15	7.83	<.0001
	3	4	0.005556	0.02668	15	0.21	0.8379
	3	5	0.06062	0.02474	13.96	2.45	0.0281
	3	7	0.04056	0.02474	15.01	1.64	0.1219
	4	5	0.05506	0.02606	15.27	2.11	0.0515
	4	7	0.03500	0.03290	15	1.06	0.3042
	5	7	-0.02006	0.02576	15.22	-0.78	0.4481
αβ1 T cell+	0	0.25	-0.8367	0.1532	15.01	-5.46	<.0001
	0	1	-4.5689	0.3644	15.02	-12.54	<.0001
	0	2	-4.4339	0.3673	14.99	-12.07	<.0001
	0	3	-2.1406	0.3239	15.06	-6.61	<.0001
	0	4	-1.8533	0.3201	15	-5.79	<.0001
	0	5	-0.9435	0.3376	14.9	-2.79	0.0137
	0	7	0.1183	0.07208	14.99	1.64	0.1215
	0.25	1	-3.7322	0.3499	15.03	-10.67	<.0001
	0.25	2	-3.5972	0.4076	15	-8.82	<.0001
	0.25	3	-1.3039	0.3669	15.05	-3.55	0.0029
	0.25	4	-1.0167	0.3931	14.99	-2.59	0.0207
	0.25	5	-0.1068	0.3426	14.1	-0.31	0.7597

Table H1. *Difference of least squares means for pulp cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
	0.25	7	0.9550	0.1380	15.01	6.92	<.0001
	1	2	0.1350	0.4158	14.96	0.32	0.7499
	1	3	2.4283	0.3846	14.96	6.31	<.0001
	1	4	2.7156	0.4121	14.91	6.59	<.0001
	1	5	3.6254	0.4393	14.11	8.25	<.0001
	1	7	4.6872	0.3188	15.03	14.70	<.0001
	2	3	2.2933	0.3916	14.97	5.86	<.0001
	2	4	2.5806	0.4314	14.86	5.98	<.0001
	2	5	3.4904	0.4971	12.35	7.02	<.0001
	2	7	4.5522	0.3725	14.99	12.22	<.0001
	3	4	0.2872	0.3209	14.95	0.90	0.3849
	3	5	1.1971	0.3880	14.92	3.09	0.0076
	3	7	2.2589	0.3068	15.06	7.36	<.0001
	4	5	0.9098	0.3806	14.09	2.39	0.0313
	4	7	1.9717	0.3341	14.99	5.90	<.0001
	5	7	1.0618	0.3424	14.79	3.10	0.0074
αβ2 T cell+	0	0.25	-0.2433	0.05426	15.01	-4.48	0.0004
	0	1	-1.4228	0.1082	14.99	-13.14	<.0001
	0	2	-1.3194	0.1143	15.02	-11.54	<.0001
	0	3	-0.6083	0.09285	14.98	-6.55	<.0001
	0	4	-0.4444	0.07202	15.01	-6.17	<.0001
	0	5	-0.2215	0.05426	14.29	-4.08	0.0011
	0	7	0.03722	0.02593	15	1.44	0.1716
	0.25	1	-1.1794	0.09478	14.99	-12.44	<.0001
	0.25	2	-1.0761	0.1232	15.02	-8.73	<.0001
	0.25	3	-0.3650	0.09588	14.97	-3.81	0.0017
	0.25	4	-0.2011	0.1012	15.01	-1.99	0.0655
	0.25	5	0.02185	0.05811	14.9	0.38	0.7122
	0.25	7	0.2806	0.04392	15	6.39	<.0001
	1	2	0.1033	0.1399	15.01	0.74	0.4715
	1	3	0.8144	0.09800	15	8.31	<.0001
	1	4	0.9783	0.1399	15.01	6.99	<.0001
	1	5	1.2013	0.1161	14.77	10.35	<.0001
	1	7	1.4600	0.09836	14.99	14.84	<.0001
	2	3	0.7111	0.1322	15.01	5.38	<.0001
	2	4	0.8750	0.1343	15.01	6.52	<.0001
	2	5	1.0980	0.1301	13.56	8.44	<.0001
	2	7	1.3567	0.1139	15.02	11.92	<.0001
	3	4	0.1639	0.1056	14.99	1.55	0.1415
	3	5	0.3868	0.08625	15.13	4.49	0.0004
	3	7	0.6456	0.08056	14.97	8.01	<.0001
	4	5	0.2230	0.07949	13.71	2.81	0.0143
	4	7	0.4817	0.07988	15.01	6.03	<.0001
	5	7	0.2587	0.04575	14.64	5.66	<.0001
MHCII+ B cell+	0	0.25	-0.1367	0.03147	14.99	-4.34	0.0006
	0	1	-0.5672	0.05878	14.99	-9.65	<.0001

Table H1. *Difference of least squares means for pulp cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
	0	2	-0.8300	0.1225	15.12	-6.77	<.0001
	0	3	-0.2700	0.05053	14.99	-5.34	<.0001
	0	4	-0.3767	0.08272	15.02	-4.55	0.0004
	0	5	-0.3181	0.08252	14.93	-3.85	0.0016
	0	7	-0.2283	0.05846	15	-3.91	0.0014
	0.25	1	-0.4306	0.04945	15	-8.71	<.0001
	0.25	2	-0.6933	0.1289	15.12	-5.38	<.0001
	0.25	3	-0.1333	0.04449	15	-3.00	0.0090
	0.25	4	-0.2400	0.07859	15.04	-3.05	0.0080
	0.25	5	-0.1814	0.07934	15.09	-2.29	0.0371
	0.25	7	-0.09167	0.04968	15	-1.85	0.0849
	1	2	-0.2628	0.1269	15.1	-2.07	0.0560
	1	3	0.2972	0.04608	15	6.45	<.0001
	1	4	0.1906	0.07586	15.03	2.51	0.0239
	1	5	0.2492	0.07274	15.23	3.43	0.0037
	1	7	0.3389	0.06818	15	4.97	0.0002
	2	3	0.5600	0.1304	15.11	4.29	0.0006
	2	4	0.4533	0.1089	15.06	4.16	0.0008
	2	5	0.5119	0.1234	14.63	4.15	0.0009
	2	7	0.6017	0.1423	15.11	4.23	0.0007
	3	4	-0.1067	0.07514	15.04	-1.42	0.1762
	3	5	-0.04806	0.06395	14	-0.75	0.4648
	3	7	0.04167	0.06501	15	0.64	0.5312
	4	5	0.05861	0.06956	14.7	0.84	0.4130
	4	7	0.1483	0.09311	15.04	1.59	0.1319
	5	7	0.08973	0.1111	15.12	0.81	0.4319
Study B							
MHCII+	0	0.25	-0.2992	0.1907	9.001	-1.57	0.1512
B cells+	0	1	-4.4825	0.5134	9	-8.73	<.0001
	0	2	-5.7983	1.6145	9	-3.59	0.0058
	0	3	-11.4775	1.9290	9	-5.95	0.0002
	0	4	-9.1417	1.9239	9	-4.75	0.0010
	0	5	-4.9858	0.6365	9	-7.83	<.0001
	0	7	-2.0308	0.2865	9	-7.09	<.0001
	0.25	1	-4.1833	0.5433	9	-7.70	<.0001
	0.25	2	-5.4992	1.4904	9	-3.69	0.0050
	0.25	3	-11.1783	1.8197	9	-6.14	0.0002
	0.25	4	-8.8425	1.8534	9	-4.77	0.0010
	0.25	5	-4.6867	0.6475	9	-7.24	<.0001
	0.25	7	-1.7317	0.3717	9	-4.66	0.0012
	1	2	-1.3158	1.3321	9	-0.99	0.3491
	1	3	-6.9950	2.0636	9	-3.39	0.0080
	1	4	-4.6592	2.0468	9	-2.28	0.0489
	1	5	-0.5033	0.9610	9	-0.52	0.6131
	1	7	2.4517	0.6297	9	3.89	0.0037

Table H1. *Difference of least squares means for pulp cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
	2	3	-5.6792	1.8986	9	-2.99	0.0152
	2	4	-3.3433	2.4562	9	-1.36	0.2066
	2	5	0.8125	1.9403	9	0.42	0.6852
	2	7	3.7675	1.6685	9	2.26	0.0503
	3	4	2.3358	2.7256	9	0.86	0.4137
	3	5	6.4917	2.1369	9	3.04	0.0141
	3	7	9.4467	1.8021	9	5.24	0.0005
	4	5	4.1558	1.5915	9	2.61	0.0282
	4	7	7.1108	1.9509	9	3.64	0.0054
	5	7	2.9550	0.6884	9	4.29	0.0020
CD4- CD8+	0	0.25	-0.6708	0.1766	9	-3.80	0.0042
	0	1	-2.9425	0.5710	9	-5.15	0.0006
	0	2	-3.1525	0.5201	9	-6.06	0.0002
	0	3	-2.9667	0.7971	9	-3.72	0.0048
	0	4	-1.3600	0.2489	9	-5.46	0.0004
	0	5	-0.8683	0.1293	9	-6.71	<.0001
	0	7	-1.0392	0.2511	9	-4.14	0.0025
	0.25	1	-2.2717	0.5673	9	-4.00	0.0031
	0.25	2	-2.4817	0.3811	9	-6.51	0.0001
	0.25	3	-2.2958	0.7639	9	-3.01	0.0148
	0.25	4	-0.6892	0.3239	9	-2.13	0.0622
	0.25	5	-0.1975	0.1888	9	-1.05	0.3229
	0.25	7	-0.3683	0.1957	9	-1.88	0.0925
	1	2	-0.2100	0.8466	9	-0.25	0.8097
	1	3	-0.02417	0.9281	9	-0.03	0.9798
	1	4	1.5825	0.5369	9	2.95	0.0163
	1	5	2.0742	0.4898	9	4.23	0.0022
	1	7	1.9033	0.5816	9	3.27	0.0096
	2	3	0.1858	0.8324	9	0.22	0.8283
	2	4	1.7925	0.6128	9	2.93	0.0169
	2	5	2.2842	0.5396	9	4.23	0.0022
	2	7	2.1133	0.4111	9	5.14	0.0006
	3	4	1.6067	0.8221	9	1.95	0.0824
	3	5	2.0983	0.7402	9	2.84	0.0196
	3	7	1.9275	0.7207	9	2.67	0.0254
	4	5	0.4917	0.2356	9	2.09	0.0665
	4	7	0.3208	0.3168	9	1.01	0.3376
	5	7	-0.1708	0.2762	9	-0.62	0.5516
CD4+ CD8-	0	0.25	-1.7933	0.6322	9	-2.84	0.0195
	0	1	-7.5408	0.6470	9	-11.66	<.0001
	0	2	-7.6783	0.7985	9	-9.62	<.0001
	0	3	-6.3408	0.7174	9	-8.84	<.0001
	0	4	-4.6775	0.7850	9	-5.96	0.0002
	0	5	-2.7983	0.6666	9	-4.20	0.0023
	0	7	-2.4342	0.6039	9	-4.03	0.0030
	0.25	1	-5.7475	0.8723	9	-6.59	0.0001

Table H1. *Difference of least squares means for pulp cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
	0.25	2	-5.8850	0.5992	9	-9.82	<.0001
	0.25	3	-4.5475	0.6260	9	-7.26	<.0001
	0.25	4	-2.8842	0.9452	9	-3.05	0.0138
	0.25	5	-1.0050	0.8681	9	-1.16	0.2768
	0.25	7	-0.6408	0.4035	9	-1.59	0.1467
	1	2	-0.1375	1.2408	9	-0.11	0.9142
	1	3	1.2000	0.9022	9	1.33	0.2162
	1	4	2.8633	1.1274	9	2.54	0.0317
	1	5	4.7425	0.7891	9	6.01	0.0002
	1	7	5.1067	0.8430	9	6.06	0.0002
	2	3	1.3375	0.9324	9	1.43	0.1852
	2	4	3.0008	0.9975	9	3.01	0.0148
	2	5	4.8800	1.0444	9	4.67	0.0012
	2	7	5.2442	0.7709	9	6.80	<.0001
	3	4	1.6633	1.0952	9	1.52	0.1631
	3	5	3.5425	0.9797	9	3.62	0.0056
	3	7	3.9067	0.5859	9	6.67	<.0001
	4	5	1.8792	0.5768	9	3.26	0.0099
	4	7	2.2433	0.8475	9	2.65	0.0266
	5	7	0.3642	0.9088	9	0.40	0.6980
γδ T cell+ CD8-	0	0.25	-3.9350	1.4936	9	-2.63	0.0272
	0	1	-5.1767	0.8841	9	-5.86	0.0002
	0	2	-1.3850	0.3048	9	-4.54	0.0014
	0	3	-1.5017	0.4940	9	-3.04	0.0140
	0	4	-0.9675	0.2206	9	-4.39	0.0018
	0	5	-0.6208	0.1737	9	-3.57	0.0060
	0	7	-0.6175	0.2466	9	-2.50	0.0336
	0.25	1	-1.2417	1.8891	9	-0.66	0.5275
	0.25	2	2.5500	1.4446	9	1.77	0.1113
	0.25	3	2.4333	1.3440	9	1.81	0.1037
	0.25	4	2.9675	1.3977	9	2.12	0.0627
	0.25	5	3.3142	1.3907	9	2.38	0.0410
	0.25	7	3.3175	1.4686	9	2.26	0.0503
	1	2	3.7917	1.0520	9	3.60	0.0057
	1	3	3.6750	1.2476	9	2.95	0.0163
	1	4	4.2092	0.8817	9	4.77	0.0010
	1	5	4.5558	0.8295	9	5.49	0.0004
	1	7	4.5592	0.9854	9	4.63	0.0012
	2	3	-0.1167	0.2917	9	-0.40	0.6986
	2	4	0.4175	0.2623	9	1.59	0.1459
	2	5	0.7642	0.3064	9	2.49	0.0342
	2	7	0.7675	0.3205	9	2.39	0.0402
	3	4	0.5342	0.4394	9	1.22	0.2550
	3	5	0.8808	0.4835	9	1.82	0.1018
	3	7	0.8842	0.4347	9	2.03	0.0725
	4	5	0.3467	0.1324	9	2.62	0.0279

Table H1. *Difference of least squares means for pulp cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
	4	7	0.3500	0.3045	9	1.15	0.2800
	5	7	0.003333	0.3063	9	0.01	0.9916
αβ1 T cell+	0	0.25	-1.7450	0.6045	9	-2.89	0.0180
	0	1	-8.4300	1.1364	9	-7.42	<.0001
	0	2	-7.9742	0.7253	9	-10.99	<.0001
	0	3	-5.1258	0.7627	9	-6.72	<.0001
	0	4	-4.4108	0.9482	9	-4.65	0.0012
	0	5	-2.9692	0.9514	9	-3.12	0.0123
	0	7	-2.4008	0.7093	9	-3.38	0.0081
	0.25	1	-6.6850	1.1314	9	-5.91	0.0002
	0.25	2	-6.2292	0.5551	9	-11.22	<.0001
	0.25	3	-3.3808	0.4340	9	-7.79	<.0001
	0.25	4	-2.6658	1.0733	9	-2.48	0.0348
	0.25	5	-1.2242	0.9808	9	-1.25	0.2435
	0.25	7	-0.6558	0.5527	9	-1.19	0.2658
	1	2	0.4558	1.4897	9	0.31	0.7666
	1	3	3.3042	1.1156	9	2.96	0.0159
	1	4	4.0192	1.2477	9	3.22	0.0105
	1	5	5.4608	0.9096	9	6.00	0.0002
	1	7	6.0292	1.1808	9	5.11	0.0006
	2	3	2.8483	0.7667	9	3.72	0.0048
	2	4	3.5633	1.1779	9	3.03	0.0144
	2	5	5.0050	1.1431	9	4.38	0.0018
	2	7	5.5733	0.7542	9	7.39	<.0001
	3	4	0.7150	1.1953	9	0.60	0.5645
	3	5	2.1567	1.1242	9	1.92	0.0873
	3	7	2.7250	0.5099	9	5.34	0.0005
	4	5	1.4417	0.7233	9	1.99	0.0774
	4	7	2.0100	1.0752	9	1.87	0.0944
	5	7	0.5683	1.2203	9	0.47	0.6525
αβ1 T cell+	0	0.25	-0.7025	0.1351	9	-5.20	0.0006
CD4- CD8+	0	1	-2.1100	0.4115	9	-5.13	0.0006
	0	2	-2.1800	0.3079	9	-7.08	<.0001
	0	3	-1.3058	0.2409	9	-5.42	0.0004
	0	4	-0.7467	0.2038	9	-3.66	0.0052
	0	5	-0.9133	0.1952	9	-4.68	0.0012
	0	7	-0.9708	0.2317	9	-4.19	0.0023
	0.25	1	-1.4075	0.3472	9	-4.05	0.0029
	0.25	2	-1.4775	0.2799	9	-5.28	0.0005
	0.25	3	-0.6033	0.1861	9	-3.24	0.0101
	0.25	4	-0.04417	0.2150	9	-0.21	0.8418
	0.25	5	-0.2108	0.1573	9	-1.34	0.2131
	0.25	7	-0.2683	0.1746	9	-1.54	0.1586
	1	2	-0.07000	0.5098	9	-0.14	0.8938
	1	3	0.8042	0.2601	9	3.09	0.0129
	1	4	1.3633	0.3122	9	4.37	0.0018

Table H1. *Difference of least squares means for pulp cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
	1	5	1.1967	0.2657	9	4.50	0.0015
	1	7	1.1392	0.3899	9	2.92	0.0170
	2	3	0.8742	0.3556	9	2.46	0.0363
	2	4	1.4333	0.3590	9	3.99	0.0031
	2	5	1.2667	0.3116	9	4.06	0.0028
	2	7	1.2092	0.2449	9	4.94	0.0008
	3	4	0.5592	0.1863	9	3.00	0.0149
	3	5	0.3925	0.2080	9	1.89	0.0917
	3	7	0.3350	0.2044	9	1.64	0.1356
	4	5	-0.1667	0.1938	9	-0.86	0.4121
	4	7	-0.2242	0.2534	9	-0.88	0.3994
	5	7	-0.05750	0.2679	9	-0.21	0.8348
αβ1 T cell+	0	0.25	-0.2025	0.05549	9	-3.65	0.0053
CD4- CD8-	0	1	-0.2350	0.03540	9	-6.64	<.0001
	0	2	-0.1958	0.02725	9	-7.19	<.0001
	0	3	-0.7692	0.1093	9	-7.04	<.0001
	0	4	-0.2167	0.04111	9	-5.27	0.0005
	0	5	-0.2417	0.03201	9	-7.55	<.0001
	0	7	-0.2617	0.04010	9	-6.53	0.0001
	0.25	1	-0.03250	0.05655	9	-0.57	0.5796
	0.25	2	0.006667	0.06381	9	0.10	0.9191
	0.25	3	-0.5667	0.1009	9	-5.61	0.0003
	0.25	4	-0.01417	0.06130	9	-0.23	0.8224
	0.25	5	-0.03917	0.07465	9	-0.52	0.6125
	0.25	7	-0.05917	0.05704	9	-1.04	0.3267
	1	2	0.03917	0.03194	9	1.23	0.2512
	1	3	-0.5342	0.1012	9	-5.28	0.0005
	1	4	0.01833	0.05172	9	0.35	0.7311
	1	5	-0.00667	0.04743	9	-0.14	0.8913
	1	7	-0.02667	0.05743	9	-0.46	0.6534
	2	3	-0.5733	0.1050	9	-5.46	0.0004
	2	4	-0.02083	0.04972	9	-0.42	0.6851
	2	5	-0.04583	0.04254	9	-1.08	0.3093
	2	7	-0.06583	0.03978	9	-1.66	0.1323
	3	4	0.5525	0.1296	9	4.26	0.0021
	3	5	0.5275	0.1185	9	4.45	0.0016
	3	7	0.5075	0.1195	9	4.25	0.0022
	4	5	-0.02500	0.04377	9	-0.57	0.5819
	4	7	-0.04500	0.04927	9	-0.91	0.3849
	5	7	-0.02000	0.06447	9	-0.31	0.7634
αβ1 T cell+	0	0.25	-1.4717	0.4902	9	-3.00	0.0149
CD4+ CD8-	0	1	-6.1867	0.5954	9	-10.39	<.0001
	0	2	-6.0600	0.5668	9	-10.69	<.0001
	0	3	-3.6233	0.5056	9	-7.17	<.0001
	0	4	-3.9642	0.6801	9	-5.83	0.0003
	0	5	-2.3542	0.6434	9	-3.66	0.0052

Table H1. *Difference of least squares means for pulp cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
	0	7	-1.7758	0.4450	9	-3.99	0.0032
	0.25	1	-4.7150	0.7969	9	-5.92	0.0002
	0.25	2	-4.5883	0.4150	9	-11.06	<.0001
	0.25	3	-2.1517	0.3514	9	-6.12	0.0002
	0.25	4	-2.4925	0.7667	9	-3.25	0.0100
	0.25	5	-0.8825	0.7196	9	-1.23	0.2512
	0.25	7	-0.3042	0.3485	9	-0.87	0.4055
	1	2	0.1267	0.9778	9	0.13	0.8998
	1	3	2.5633	0.7877	9	3.25	0.0099
	1	4	2.2225	0.9775	9	2.27	0.0491
	1	5	3.8325	0.6581	9	5.82	0.0003
	1	7	4.4108	0.7515	9	5.87	0.0002
	2	3	2.4367	0.5348	9	4.56	0.0014
	2	4	2.0958	0.8046	9	2.60	0.0285
	2	5	3.7058	0.8148	9	4.55	0.0014
	2	7	4.2842	0.5434	9	7.88	<.0001
	3	4	-0.3408	0.9042	9	-0.38	0.7150
	3	5	1.2692	0.8626	9	1.47	0.1753
	3	7	1.8475	0.3200	9	5.77	0.0003
	4	5	1.6100	0.5340	9	3.02	0.0146
	4	7	2.1883	0.7857	9	2.79	0.0212
	5	7	0.5783	0.8115	9	0.71	0.4941
αβ2 T cell+	0	0.25	-1.0900	0.2399	9	-4.54	0.0014
	0	1	-3.6292	0.4540	9	-7.99	<.0001
	0	2	-2.6858	0.2904	9	-9.25	<.0001
	0	3	-1.9542	0.2872	9	-6.81	<.0001
	0	4	-2.3275	0.4881	9	-4.77	0.0010
	0	5	-0.7283	0.2756	9	-2.64	0.0268
	0	7	-0.8342	0.2642	9	-3.16	0.0116
	0.25	1	-2.5392	0.3695	9	-6.87	<.0001
	0.25	2	-1.5958	0.3264	9	-4.89	0.0009
	0.25	3	-0.8642	0.1935	9	-4.47	0.0016
	0.25	4	-1.2375	0.5346	9	-2.31	0.0459
	0.25	5	0.3617	0.3571	9	1.01	0.3376
	0.25	7	0.2558	0.2374	9	1.08	0.3093
	1	2	0.9433	0.5954	9	1.58	0.1476
	1	3	1.6750	0.4114	9	4.07	0.0028
	1	4	1.3017	0.6500	9	2.00	0.0763
	1	5	2.9008	0.4525	9	6.41	0.0001
	1	7	2.7950	0.4581	9	6.10	0.0002
	2	3	0.7317	0.2555	9	2.86	0.0187
	2	4	0.3583	0.5225	9	0.69	0.5101
	2	5	1.9575	0.4099	9	4.78	0.0010
	2	7	1.8517	0.3106	9	5.96	0.0002
	3	4	-0.3733	0.5875	9	-0.64	0.5409
	3	5	1.2258	0.4302	9	2.85	0.0191

Table H1. *Difference of least squares means for pulp cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
	3	7	1.1200	0.2535	9	4.42	0.0017
	4	5	1.5992	0.4240	9	3.77	0.0044
	4	7	1.4933	0.4618	9	3.23	0.0103
	5	7	-0.1058	0.4235	9	-0.25	0.8083
αβ2 T cell+	0	0.25	-0.5583	0.09746	9	-5.73	0.0003
CD4- CD8+	0	1	-1.2775	0.2046	9	-6.24	0.0002
	0	2	-0.8025	0.1276	9	-6.29	0.0001
	0	3	-0.4583	0.1021	9	-4.49	0.0015
	0	4	-0.8058	0.1503	9	-5.36	0.0005
	0	5	-0.2042	0.06874	9	-2.97	0.0157
	0	7	-0.2633	0.07356	9	-3.58	0.0059
	0.25	1	-0.7192	0.1637	9	-4.39	0.0017
	0.25	2	-0.2442	0.1499	9	-1.63	0.1378
	0.25	3	0.1000	0.05504	9	1.82	0.1026
	0.25	4	-0.2475	0.1753	9	-1.41	0.1916
	0.25	5	0.3542	0.1055	9	3.36	0.0084
	0.25	7	0.2950	0.09658	9	3.05	0.0137
	1	2	0.4750	0.2354	9	2.02	0.0744
	1	3	0.8192	0.1467	9	5.58	0.0003
	1	4	0.4717	0.2067	9	2.28	0.0484
	1	5	1.0733	0.1759	9	6.10	0.0002
	1	7	1.0142	0.1945	9	5.21	0.0006
	2	3	0.3442	0.1206	9	2.85	0.0190
	2	4	-0.00333	0.1966	9	-0.02	0.9868
	2	5	0.5983	0.1393	9	4.29	0.0020
	2	7	0.5392	0.1169	9	4.61	0.0013
	3	4	-0.3475	0.1823	9	-1.91	0.0890
	3	5	0.2542	0.09974	9	2.55	0.0313
	3	7	0.1950	0.09615	9	2.03	0.0731
	4	5	0.6017	0.1381	9	4.36	0.0018
	4	7	0.5425	0.1686	9	3.22	0.0105
	5	7	-0.05917	0.1203	9	-0.49	0.6347
αβ2 T cell+	0	0.25	-0.5742	0.1686	9	-3.41	0.0078
CD4+ CD8-	0	1	-2.2208	0.1819	9	-12.21	<.0001
	0	2	-1.9942	0.2216	9	-9.00	<.0001
	0	3	-1.5850	0.1964	9	-8.07	<.0001
	0	4	-1.6117	0.3718	9	-4.34	0.0019
	0	5	-0.6550	0.1762	9	-3.72	0.0048
	0	7	-0.6442	0.1752	9	-3.68	0.0051
	0.25	1	-1.6467	0.2888	9	-5.70	0.0003
	0.25	2	-1.4200	0.1899	9	-7.48	<.0001
	0.25	3	-1.0108	0.1535	9	-6.59	0.0001
	0.25	4	-1.0375	0.3546	9	-2.93	0.0169
	0.25	5	-0.08083	0.1981	9	-0.41	0.6927
	0.25	7	-0.07000	0.1050	9	-0.67	0.5217
	1	2	0.2267	0.3697	9	0.61	0.5550

Table H1. *Difference of least squares means for pulp cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
	1	3	0.6358	0.2713	9	2.34	0.0438
	1	4	0.6092	0.4731	9	1.29	0.2300
	1	5	1.5658	0.2502	9	6.26	0.0001
	1	7	1.5767	0.2756	9	5.72	0.0003
	2	3	0.4092	0.1851	9	2.21	0.0544
	2	4	0.3825	0.3328	9	1.15	0.2801
	2	5	1.3392	0.2568	9	5.22	0.0006
	2	7	1.3500	0.2096	9	6.44	0.0001
	3	4	-0.02667	0.3986	9	-0.07	0.9481
	3	5	0.9300	0.2651	9	3.51	0.0066
	3	7	0.9408	0.1555	9	6.05	0.0002
	4	5	0.9567	0.2908	9	3.29	0.0094
	4	7	0.9675	0.3157	9	3.06	0.0135
	5	7	0.01083	0.2182	9	0.05	0.9615
B cell+ IgM+	0	0.25	-0.2642	0.1690	9	-1.56	0.1524
	0	1	-3.4100	0.4113	9	-8.29	<.0001
	0	2	-7.9050	1.2724	9	-6.21	0.0002
	0	3	-10.8850	1.6984	9	-6.41	0.0001
	0	4	-9.2183	1.5669	9	-5.88	0.0002
	0	5	-4.4900	0.6490	9	-6.92	<.0001
	0	7	-1.4058	0.2225	9	-6.32	0.0001
	0.25	1	-3.1458	0.4436	9	-7.09	<.0001
	0.25	2	-7.6408	1.1640	9	-6.56	0.0001
	0.25	3	-10.6208	1.6066	9	-6.61	<.0001
	0.25	4	-8.9542	1.5273	9	-5.86	0.0002
	0.25	5	-4.2258	0.6674	9	-6.33	0.0001
	0.25	7	-1.1417	0.3105	9	-3.68	0.0051
	1	2	-4.4950	1.3293	9	-3.38	0.0081
	1	3	-7.4750	1.8021	9	-4.15	0.0025
	1	4	-5.8083	1.6705	9	-3.48	0.0070
	1	5	-1.0800	0.8398	9	-1.29	0.2305
	1	7	2.0042	0.4996	9	4.01	0.0031
	2	3	-2.9800	1.0670	9	-2.79	0.0210
	2	4	-1.3133	1.5889	9	-0.83	0.4298
	2	5	3.4150	1.4429	9	2.37	0.0421
	2	7	6.4992	1.2677	9	5.13	0.0006
	3	4	1.6667	2.0763	9	0.80	0.4428
	3	5	6.3950	1.9231	9	3.33	0.0089
	3	7	9.4792	1.6121	9	5.88	0.0002
	4	5	4.7283	1.1183	9	4.23	0.0022
	4	7	7.8125	1.5429	9	5.06	0.0007
	5	7	3.0842	0.6328	9	4.87	0.0009
B cell+ IgM-	0	0.25	-0.1142	0.04209	9.001	-2.71	0.0239
	0	1	-0.9008	0.1175	9	-7.67	<.0001
	0	2	-1.9092	0.3959	9	-4.82	0.0009
	0	3	-1.5283	0.2654	9	-5.76	0.0003

Table H1. *Difference of least squares means for pulp cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
	0	4	-1.6067	0.2578	9	-6.23	0.0002
	0	5	-1.2367	0.1722	9	-7.18	<.0001
	0	7	-0.4733	0.09084	9	-5.21	0.0006
	0.25	1	-0.7867	0.1246	9	-6.31	0.0001
	0.25	2	-1.7950	0.3721	9	-4.82	0.0009
	0.25	3	-1.4142	0.2340	9	-6.04	0.0002
	0.25	4	-1.4925	0.2521	9	-5.92	0.0002
	0.25	5	-1.1225	0.1843	9	-6.09	0.0002
	0.25	7	-0.3592	0.1111	9	-3.23	0.0103
	1	2	-1.0083	0.3756	9	-2.68	0.0250
	1	3	-0.6275	0.2803	9	-2.24	0.0520
	1	4	-0.7058	0.2672	9	-2.64	0.0268
	1	5	-0.3358	0.1905	9	-1.76	0.1118
	1	7	0.4275	0.1623	9	2.63	0.0272
	2	3	0.3808	0.2105	9	1.81	0.1038
	2	4	0.3025	0.2976	9	1.02	0.3359
	2	5	0.6725	0.4692	9	1.43	0.1856
	2	7	1.4358	0.4354	9	3.30	0.0093
	3	4	-0.07833	0.3080	9	-0.25	0.8049
	3	5	0.2917	0.3632	9	0.80	0.4426
	3	7	1.0550	0.2998	9	3.52	0.0065
	4	5	0.3700	0.3116	9	1.19	0.2654
	4	7	1.1333	0.2875	9	3.94	0.0034
	5	7	0.7633	0.2026	9	3.77	0.0044
CD3+	0	0.25	-5.2133	1.6677	9	-3.13	0.0122
	0	1	-15.7692	2.0218	9	-7.80	<.0001
	0	2	-12.3567	1.2383	9	-9.98	<.0001
	0	3	-11.5425	1.4058	9	-8.21	<.0001
	0	4	-7.7417	1.2744	9	-6.07	0.0002
	0	5	-4.6483	1.1255	9	-4.13	0.0026
	0	7	-4.1300	1.0407	9	-3.97	0.0033
	0.25	1	-10.5558	2.1289	9	-4.96	0.0008
	0.25	2	-7.1433	1.9560	9	-3.65	0.0053
	0.25	3	-6.3292	1.8547	9	-3.41	0.0077
	0.25	4	-2.5283	1.6907	9	-1.50	0.1690
	0.25	5	0.5650	1.3036	9	0.43	0.6749
	0.25	7	1.0833	1.4435	9	0.75	0.4721
	1	2	3.4125	2.9749	9	1.15	0.2809
	1	3	4.2267	2.7260	9	1.55	0.1554
	1	4	8.0275	2.1216	9	3.78	0.0043
	1	5	11.1208	1.6000	9	6.95	<.0001
	1	7	11.6392	2.4501	9	4.75	0.0010
	2	3	0.8142	1.3886	9	0.59	0.5721
	2	4	4.6150	1.7868	9	2.58	0.0296
	2	5	7.7083	1.6692	9	4.62	0.0013
	2	7	8.2267	1.2047	9	6.83	<.0001

Table H1. *Difference of least squares means for pulp cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
	3	4	3.8008	2.0504	9	1.85	0.0968
	3	5	6.8942	1.8338	9	3.76	0.0045
	3	7	7.4125	1.2176	9	6.09	0.0002
	4	5	3.0933	1.0253	9	3.02	0.0146
	4	7	3.6117	1.5700	9	2.30	0.0470
	5	7	0.5183	1.5981	9	0.32	0.7531

Table H2. *Difference of least squares means for pulp cell responses by treatment*

Study/Factor	Treatment	Treatment	Estimate	Std. Error	DF	t Value	Pr > |t|
Study A							
γδ T cell+ CD8+	ALUmIgG	IOmIgG	-0.08752	0.03281	14.95	-2.67	0.0176
	ALUmIgG	mIgG	-0.02773	0.03281	14.95	-0.85	0.4113
	IOmIgG	mIgG	0.05979	0.03276	14.9	1.83	0.0881
Study B							
B cell+ IgM-	AlummIgG	IOmIgG	-0.2609	0.2518	9	-1.04	0.3271
	AlummIgG	mIgG	-0.7419	0.2518	9	-2.95	0.0163
	IOmIgG	mIgG	-0.4809	0.2518	9	-1.91	0.0885

Table H3. *Simple effect comparisons of treatment*day least squares means by treatment for B cells, Study A*

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
Alum-mIgG	0	0.25	-0.2567	0.09742	15.04	-2.63	0.0187
Alum-mIgG	0	1	-2.5717	0.4586	14.96	-5.61	<.0001
Alum-mIgG	0	2	-2.1750	0.9113	15.03	-2.39	0.0306
Alum-mIgG	0	3	-1.6200	0.8019	15	-2.02	0.0616
Alum-mIgG	0	4	-0.9267	0.5831	14.89	-1.59	0.1330
Alum-mIgG	0	5	-0.4919	0.2118	15.19	-2.32	0.0345
Alum-mIgG	0	7	-0.09000	0.2619	14.98	-0.34	0.7359
Alum-mIgG	0.25	1	-2.3150	0.3937	14.95	-5.88	<.0001
Alum-mIgG	0.25	2	-1.9183	0.8754	15.01	-2.19	0.0446
Alum-mIgG	0.25	3	-1.3633	0.8007	15.01	-1.70	0.1092
Alum-mIgG	0.25	4	-0.6700	0.6215	14.91	-1.08	0.2981
Alum-mIgG	0.25	5	-0.2352	0.1984	14.41	-1.19	0.2549
Alum-mIgG	0.25	7	0.1667	0.2758	15.01	0.60	0.5546
Alum-mIgG	1	2	0.3967	0.9900	14.9	0.40	0.6944
Alum-mIgG	1	3	0.9517	1.0122	15	0.94	0.3620
Alum-mIgG	1	4	1.6450	0.7257	14.98	2.27	0.0387
Alum-mIgG	1	5	2.0798	0.3830	15.28	5.43	<.0001
Alum-mIgG	1	7	2.4817	0.5307	14.96	4.68	0.0003
Alum-mIgG	2	3	0.5550	1.0431	15.06	0.53	0.6024
Alum-mIgG	2	4	1.2483	1.3803	14.94	0.90	0.3801
Alum-mIgG	2	5	1.6831	0.9335	15	1.80	0.0915
Alum-mIgG	2	7	2.0850	0.8393	15.1	2.48	0.0252
Alum-mIgG	3	4	0.6933	1.0183	14.96	0.68	0.5064
Alum-mIgG	3	5	1.1281	0.8774	15.07	1.29	0.2179
Alum-mIgG	3	7	1.5300	0.6869	15.01	2.23	0.0416
Alum-mIgG	4	5	0.4348	0.5297	15.12	0.82	0.4245
Alum-mIgG	4	7	0.8367	0.7249	14.89	1.15	0.2666
Alum-mIgG	5	7	0.4019	0.3780	15.35	1.06	0.3042
IO-mIgG	0	0.25	-0.2800	0.09742	15.04	-2.87	0.0116
IO-mIgG	0	1	-3.1783	0.4586	14.96	-6.93	<.0001
IO-mIgG	0	2	-7.3800	0.9113	15.03	-8.10	<.0001
IO-mIgG	0	3	-3.6748	0.8019	15	-4.58	0.0004
IO-mIgG	0	4	-2.4533	0.5831	14.89	-4.21	0.0008
IO-mIgG	0	5	-0.7700	0.2025	14.21	-3.80	0.0019
IO-mIgG	0	7	-0.3533	0.2619	14.98	-1.35	0.1974
IO-mIgG	0.25	1	-2.8983	0.3937	14.95	-7.36	<.0001
IO-mIgG	0.25	2	-7.1000	0.8754	15.01	-8.11	<.0001
IO-mIgG	0.25	3	-3.3948	0.8007	15.01	-4.24	0.0007
IO-mIgG	0.25	4	-2.1733	0.6215	14.91	-3.50	0.0033
IO-mIgG	0.25	5	-0.4900	0.1884	13.37	-2.60	0.0216
IO-mIgG	0.25	7	-0.07333	0.2758	15.01	-0.27	0.7939
IO-mIgG	1	2	-4.2017	0.9900	14.9	-4.24	0.0007
IO-mIgG	1	3	-0.4965	1.0122	15	-0.49	0.6309
IO-mIgG	1	4	0.7250	0.7257	14.98	1.00	0.3337
IO-mIgG	1	5	2.4083	0.3779	14.92	6.37	<.0001

Table H3. *Simple effect comparisons of treatment*day least squares means by treatment for B cells, Study A* (Cont.)

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
IO-mIgG	1	7	2.8250	0.5307	14.96	5.32	<.0001
IO-mIgG	2	3	3.7052	1.0431	15.06	3.55	0.0029
IO-mIgG	2	4	4.9267	1.3803	14.94	3.57	0.0028
IO-mIgG	2	5	6.6100	0.9315	14.94	7.10	<.0001
IO-mIgG	2	7	7.0267	0.8393	15.1	8.37	<.0001
IO-mIgG	3	4	1.2215	1.0183	14.96	1.20	0.2490
IO-mIgG	3	5	2.9048	0.8752	15	3.32	0.0047
IO-mIgG	3	7	3.3215	0.6869	15.01	4.84	0.0002
IO-mIgG	4	5	1.6833	0.5261	14.92	3.20	0.0060
IO-mIgG	4	7	2.1000	0.7249	14.89	2.90	0.0111
IO-mIgG	5	7	0.4167	0.3729	14.97	1.12	0.2814
mIgG	0	0.25	0.006667	0.09742	15.04	0.07	0.9463
mIgG	0	1	-2.1017	0.4586	14.96	-4.58	0.0004
mIgG	0	2	-2.8317	0.9113	15.03	-3.11	0.0072
mIgG	0	3	-1.3650	0.8019	15	-1.70	0.1093
mIgG	0	4	-1.1567	0.5831	14.89	-1.98	0.0660
mIgG	0	5	-0.6433	0.2025	14.21	-3.18	0.0066
mIgG	0	7	0.005000	0.2619	14.98	0.02	0.9850
mIgG	0.25	1	-2.1083	0.3937	14.95	-5.36	<.0001
mIgG	0.25	2	-2.8383	0.8754	15.01	-3.24	0.0055
mIgG	0.25	3	-1.3717	0.8007	15.01	-1.71	0.1073
mIgG	0.25	4	-1.1633	0.6215	14.91	-1.87	0.0810
mIgG	0.25	5	-0.6500	0.1884	13.37	-3.45	0.0041
mIgG	0.25	7	-0.00167	0.2758	15.01	-0.01	0.9953
mIgG	1	2	-0.7300	0.9900	14.9	-0.74	0.4724
mIgG	1	3	0.7367	1.0122	15	0.73	0.4780
mIgG	1	4	0.9450	0.7257	14.98	1.30	0.2125
mIgG	1	5	1.4583	0.3779	14.92	3.86	0.0016
mIgG	1	7	2.1067	0.5307	14.96	3.97	0.0012
mIgG	2	3	1.4667	1.0431	15.06	1.41	0.1800
mIgG	2	4	1.6750	1.3803	14.94	1.21	0.2438
mIgG	2	5	2.1883	0.9315	14.94	2.35	0.0330
mIgG	2	7	2.8367	0.8393	15.1	3.38	0.0041
mIgG	3	4	0.2083	1.0183	14.96	0.20	0.8407
mIgG	3	5	0.7217	0.8752	15	0.82	0.4225
mIgG	3	7	1.3700	0.6869	15.01	1.99	0.0646
mIgG	4	5	0.5133	0.5261	14.92	0.98	0.3447
mIgG	4	7	1.1617	0.7249	14.89	1.60	0.1300
mIgG	5	7	0.6483	0.3729	14.97	1.74	0.1026

Table H4. *Simple effect comparisons of treatment*day least squares means by day for, B cells, Study A*

Simple Effect Level	Treatment	Treatment	Estimate	Std. Error	DF	t Value	Pr > |t|
day 0	ALUmIgG	IOmIgG	-0.04333	0.06154	15.01	-0.70	0.4921
day 0	ALUmIgG	mIgG	-0.08667	0.06154	15.01	-1.41	0.1794
day 0	IOmIgG	mIgG	-0.04333	0.06154	15.01	-0.70	0.4921
day 0.25	ALUmIgG	IOmIgG	-0.06667	0.1450	15.07	-0.46	0.6523
day 0.25	ALUmIgG	mIgG	0.1767	0.1450	15.07	1.22	0.2419
day 0.25	IOmIgG	mIgG	0.2433	0.1450	15.07	1.68	0.1140
day 1	ALUmIgG	IOmIgG	-0.6500	0.6419	14.97	-1.01	0.3273
day 1	ALUmIgG	mIgG	0.3833	0.6419	14.97	0.60	0.5593
day 1	IOmIgG	mIgG	1.0333	0.6419	14.97	1.61	0.1283
day 2	ALUmIgG	IOmIgG	-5.2483	1.2909	15.04	-4.07	0.0010
day 2	ALUmIgG	mIgG	-0.7433	1.2909	15.04	-0.58	0.5732
day 2	IOmIgG	mIgG	4.5050	1.2909	15.04	3.49	0.0033
day 3	ALUmIgG	IOmIgG	-2.0982	1.1271	15	-1.86	0.0824
day 3	ALUmIgG	mIgG	0.1683	1.1271	15	0.15	0.8833
day 3	IOmIgG	mIgG	2.2665	1.1271	15	2.01	0.0627
day 4	ALUmIgG	IOmIgG	-1.5700	0.8185	14.89	-1.92	0.0745
day 4	ALUmIgG	mIgG	-0.3167	0.8185	14.89	-0.39	0.7043
day 4	IOmIgG	mIgG	1.2533	0.8185	14.89	1.53	0.1467
day 5	ALUmIgG	IOmIgG	-0.3215	0.2999	14.91	-1.07	0.3009
day 5	ALUmIgG	mIgG	-0.2381	0.2999	14.91	-0.79	0.4397
day 5	IOmIgG	mIgG	0.08333	0.2934	14.43	0.28	0.7805
day 7	ALUmIgG	IOmIgG	-0.3067	0.3531	14.97	-0.87	0.3988
day 7	ALUmIgG	mIgG	0.008333	0.3531	14.97	0.02	0.9815
day 7	IOmIgG	mIgG	0.3150	0.3531	14.97	0.89	0.3864

Table H5. *Simple effect comparisons of treatment*day least squares means by treatment for CD4+ CD8-, Study A*

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
Alum-mIgG	0	0.25	-0.3167	0.2957	14.98	-1.07	0.3012
Alum-mIgG	0	1	-5.1200	0.7280	15.19	-7.03	<.0001
Alum-mIgG	0	2	-4.3800	0.8176	15.06	-5.36	<.0001
Alum-mIgG	0	3	-2.5767	0.6334	15	-4.07	0.0010
Alum-mIgG	0	4	-2.4083	0.6886	15.07	-3.50	0.0032
Alum-mIgG	0	5	-0.7241	0.3722	15.45	-1.95	0.0701
Alum-mIgG	0	7	-0.2700	0.2124	14.99	-1.27	0.2231
Alum-mIgG	0.25	1	-4.8033	0.6483	15.19	-7.41	<.0001
Alum-mIgG	0.25	2	-4.0633	0.7624	15.05	-5.33	<.0001
Alum-mIgG	0.25	3	-2.2600	0.5914	14.98	-3.82	0.0017
Alum-mIgG	0.25	4	-2.0917	0.8409	15.01	-2.49	0.0251
Alum-mIgG	0.25	5	-0.4074	0.4682	15.59	-0.87	0.3974
Alum-mIgG	0.25	7	0.04667	0.1984	14.91	0.24	0.8173

Table H5. *Simple effect comparisons of treatment*day least squares means by treatment for CD4+ CD8-, Study A* (Cont.)

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
Alum-mIgG	1	2	0.7400	0.8240	14.83	0.90	0.3835
Alum-mIgG	1	3	2.5433	0.7240	14.98	3.51	0.0031
Alum-mIgG	1	4	2.7117	0.8501	14.94	3.19	0.0061
Alum-mIgG	1	5	4.3959	0.5693	15.37	7.72	<.0001
Alum-mIgG	1	7	4.8500	0.6457	15.2	7.51	<.0001
Alum-mIgG	2	3	1.8033	0.8289	14.95	2.18	0.0460
Alum-mIgG	2	4	1.9717	0.8994	14.9	2.19	0.0447
Alum-mIgG	2	5	3.6559	0.7858	14.34	4.65	0.0004
Alum-mIgG	2	7	4.1100	0.8128	15.02	5.06	0.0001
Alum-mIgG	3	4	0.1683	0.7366	14.95	0.23	0.8223
Alum-mIgG	3	5	1.8526	0.6128	15.39	3.02	0.0084
Alum-mIgG	3	7	2.3067	0.6257	14.96	3.69	0.0022
Alum-mIgG	4	5	1.6843	0.6777	15.21	2.49	0.0250
Alum-mIgG	4	7	2.1383	0.7201	15.04	2.97	0.0095
Alum-mIgG	5	7	0.4541	0.3966	15.56	1.15	0.2695
IO-mIgG	0	0.25	-0.6100	0.2957	14.98	-2.06	0.0569
IO-mIgG	0	1	-6.4450	0.7280	15.19	-8.85	<.0001
IO-mIgG	0	2	-6.1050	0.8176	15.06	-7.47	<.0001
IO-mIgG	0	3	-3.1467	0.6334	15	-4.97	0.0002
IO-mIgG	0	4	-2.6733	0.6886	15.07	-3.88	0.0015
IO-mIgG	0	5	-1.0533	0.3546	14.42	-2.97	0.0099
IO-mIgG	0	7	-0.01000	0.2124	14.99	-0.05	0.9631
IO-mIgG	0.25	1	-5.8350	0.6483	15.19	-9.00	<.0001
IO-mIgG	0.25	2	-5.4950	0.7624	15.05	-7.21	<.0001
IO-mIgG	0.25	3	-2.5367	0.5914	14.98	-4.29	0.0006
IO-mIgG	0.25	4	-2.0633	0.8409	15.01	-2.45	0.0268
IO-mIgG	0.25	5	-0.4433	0.4543	14.84	-0.98	0.3448
IO-mIgG	0.25	7	0.6000	0.1984	14.91	3.02	0.0086
IO-mIgG	1	2	0.3400	0.8240	14.83	0.41	0.6858
IO-mIgG	1	3	3.2983	0.7240	14.98	4.56	0.0004
IO-mIgG	1	4	3.7717	0.8501	14.94	4.44	0.0005
IO-mIgG	1	5	5.3917	0.5579	14.84	9.66	<.0001
IO-mIgG	1	7	6.4350	0.6457	15.2	9.97	<.0001
IO-mIgG	2	3	2.9583	0.8289	14.95	3.57	0.0028
IO-mIgG	2	4	3.4317	0.8994	14.9	3.82	0.0017
IO-mIgG	2	5	5.0517	0.7776	14.07	6.50	<.0001
IO-mIgG	2	7	6.0950	0.8128	15.02	7.50	<.0001
IO-mIgG	3	4	0.4733	0.7366	14.95	0.64	0.5302
IO-mIgG	3	5	2.0933	0.6022	14.93	3.48	0.0034
IO-mIgG	3	7	3.1367	0.6257	14.96	5.01	0.0002
IO-mIgG	4	5	1.6200	0.6682	14.83	2.42	0.0286
IO-mIgG	4	7	2.6633	0.7201	15.04	3.70	0.0021
IO-mIgG	5	7	1.0433	0.3801	14.61	2.74	0.0153

Table H5. *Simple effect comparisons of treatment*day least squares means by treatment for CD4+ CD8-, Study A* (Cont.)

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
mIgG	0	0.25	0.3567	0.2957	14.98	1.21	0.2465
mIgG	0	1	-5.4467	0.7280	15.19	-7.48	<.0001
mIgG	0	2	-4.7867	0.8176	15.06	-5.85	<.0001
mIgG	0	3	-1.8917	0.6334	15	-2.99	0.0092
mIgG	0	4	-1.6233	0.6886	15.07	-2.36	0.0323
mIgG	0	5	-0.5633	0.3546	14.42	-1.59	0.1338
mIgG	0	7	0.1800	0.2124	14.99	0.85	0.4101
mIgG	0.25	1	-5.8033	0.6483	15.19	-8.95	<.0001
mIgG	0.25	2	-5.1433	0.7624	15.05	-6.75	<.0001
mIgG	0.25	3	-2.2483	0.5914	14.98	-3.80	0.0017
mIgG	0.25	4	-1.9800	0.8409	15.01	-2.35	0.0326
mIgG	0.25	5	-0.9200	0.4543	14.84	-2.03	0.0612
mIgG	0.25	7	-0.1767	0.1984	14.91	-0.89	0.3874
mIgG	1	2	0.6600	0.8240	14.83	0.80	0.4358
mIgG	1	3	3.5550	0.7240	14.98	4.91	0.0002
mIgG	1	4	3.8233	0.8501	14.94	4.50	0.0004
mIgG	1	5	4.8833	0.5579	14.84	8.75	<.0001
mIgG	1	7	5.6267	0.6457	15.2	8.71	<.0001
mIgG	2	3	2.8950	0.8289	14.95	3.49	0.0033
mIgG	2	4	3.1633	0.8994	14.9	3.52	0.0031
mIgG	2	5	4.2233	0.7776	14.07	5.43	<.0001
mIgG	2	7	4.9667	0.8128	15.02	6.11	<.0001
mIgG	3	4	0.2683	0.7366	14.95	0.36	0.7208
mIgG	3	5	1.3283	0.6022	14.93	2.21	0.0435
mIgG	3	7	2.0717	0.6257	14.96	3.31	0.0048
mIgG	4	5	1.0600	0.6682	14.83	1.59	0.1338
mIgG	4	7	1.8033	0.7201	15.04	2.50	0.0243
mIgG	5	7	0.7433	0.3801	14.61	1.96	0.0699

Table H6. *Simple effect comparisons of treatment*day least squares means by day for CD4+ CD8-, Study A*

Simple Effect Level	Treatment	Treatment	Estimate	Std. Error	DF	t Value	Pr > |t|
day 0	ALUmIgG	IOmIgG	-0.1467	0.1830	14.96	-0.80	0.4355
day 0	ALUmIgG	mIgG	-0.4433	0.1830	14.96	-2.42	0.0286
day 0	IOmIgG	mIgG	-0.2967	0.1830	14.96	-1.62	0.1259
day 0.25	ALUmIgG	IOmIgG	-0.4400	0.3841	14.95	-1.15	0.2700
day 0.25	ALUmIgG	mIgG	0.2300	0.3841	14.95	0.60	0.5583
day 0.25	IOmIgG	mIgG	0.6700	0.3841	14.95	1.74	0.1016
day 1	ALUmIgG	IOmIgG	-1.4717	0.9422	15.22	-1.56	0.1389
day 1	ALUmIgG	mIgG	-0.7700	0.9422	15.22	-0.82	0.4264
day 1	IOmIgG	mIgG	0.7017	0.9422	15.22	0.74	0.4678
day 2	ALUmIgG	IOmIgG	-1.8717	1.1601	15.03	-1.61	0.1275
day 2	ALUmIgG	mIgG	-0.8500	1.1601	15.03	-0.73	0.4750
day 2	IOmIgG	mIgG	1.0217	1.1601	15.03	0.88	0.3924
day 3	ALUmIgG	IOmIgG	-0.7167	0.8680	14.98	-0.83	0.4219
day 3	ALUmIgG	mIgG	0.2417	0.8680	14.98	0.28	0.7845
day 3	IOmIgG	mIgG	0.9583	0.8680	14.98	1.10	0.2870
day 4	ALUmIgG	IOmIgG	-0.4117	0.9774	15.06	-0.42	0.6796
day 4	ALUmIgG	mIgG	0.3417	0.9774	15.06	0.35	0.7315
day 4	IOmIgG	mIgG	0.7533	0.9774	15.06	0.77	0.4528
day 5	ALUmIgG	IOmIgG	-0.4759	0.4417	14.15	-1.08	0.2993
day 5	ALUmIgG	mIgG	-0.2826	0.4417	14.15	-0.64	0.5325
day 5	IOmIgG	mIgG	0.1933	0.4270	13.54	0.45	0.6579
day 7	ALUmIgG	IOmIgG	0.1133	0.2214	14.98	0.51	0.6162
day 7	ALUmIgG	mIgG	0.006667	0.2214	14.98	0.03	0.9764
day 7	IOmIgG	mIgG	-0.1067	0.2214	14.98	-0.48	0.6370

Table H5. *Simple effect comparisons of treatment*day least squares means by treatment for γδ T cell+ CD8-, Study A*

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
Alum-mIgG	0	0.25	-2.6250	0.6789	15	-3.87	0.0015
Alum-mIgG	0	1	-2.0817	0.6004	15	-3.47	0.0034
Alum-mIgG	0	2	-0.4200	0.2492	15.01	-1.69	0.1126
Alum-mIgG	0	3	0.02833	0.2070	14.99	0.14	0.8930
Alum-mIgG	0	4	0.3067	0.1892	14.97	1.62	0.1258
Alum-mIgG	0	5	0.3935	0.1952	15.06	2.02	0.0620
Alum-mIgG	0	7	0.2800	0.1979	15	1.41	0.1776
Alum-mIgG	0.25	1	0.5433	0.7195	14.99	0.76	0.4619
Alum-mIgG	0.25	2	2.2050	0.5520	14.99	3.99	0.0012
Alum-mIgG	0.25	3	2.6533	0.6047	15	4.39	0.0005
Alum-mIgG	0.25	4	2.9317	0.6208	15.01	4.72	0.0003
Alum-mIgG	0.25	5	3.0185	0.5767	15.02	5.23	0.0001
Alum-mIgG	0.25	7	2.9050	0.5823	15	4.99	0.0002

Table H5. *Simple effect comparisons of treatment*day least squares means by treatment for γδ T cell+ CD8-, Study A* (Cont.)

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
Alum-mIgG	1	2	1.6617	0.5280	15	3.15	0.0066
Alum-mIgG	1	3	2.1100	0.5323	14.99	3.96	0.0012
Alum-mIgG	1	4	2.3883	0.5108	15	4.68	0.0003
Alum-mIgG	1	5	2.4751	0.5448	15.03	4.54	0.0004
Alum-mIgG	1	7	2.3617	0.5582	15	4.23	0.0007
Alum-mIgG	2	3	0.4483	0.1404	15.02	3.19	0.0060
Alum-mIgG	2	4	0.7267	0.2212	15.02	3.29	0.0050
Alum-mIgG	2	5	0.8135	0.1987	14.95	4.09	0.0010
Alum-mIgG	2	7	0.7000	0.1822	15.01	3.84	0.0016
Alum-mIgG	3	4	0.2783	0.1284	14.98	2.17	0.0467
Alum-mIgG	3	5	0.3651	0.1285	15.46	2.84	0.0121
Alum-mIgG	3	7	0.2517	0.1056	14.99	2.38	0.0308
Alum-mIgG	4	5	0.08681	0.09519	15.88	0.91	0.3754
Alum-mIgG	4	7	-0.02667	0.1008	15.05	-0.26	0.7950
Alum-mIgG	5	7	-0.1135	0.06078	14.78	-1.87	0.0819
IO-mIgG	0	0.25	-1.8867	0.6789	15	-2.78	0.0141
IO-mIgG	0	1	-3.0250	0.6004	15	-5.04	0.0001
IO-mIgG	0	2	-1.9383	0.2492	15.01	-7.78	<.0001
IO-mIgG	0	3	-0.4350	0.2070	14.99	-2.10	0.0529
IO-mIgG	0	4	-0.1500	0.1892	14.97	-0.79	0.4402
IO-mIgG	0	5	0.02167	0.1933	14.79	0.11	0.9123
IO-mIgG	0	7	0.08333	0.1979	15	0.42	0.6797
IO-mIgG	0.25	1	-1.1383	0.7195	14.99	-1.58	0.1345
IO-mIgG	0.25	2	-0.05167	0.5520	14.99	-0.09	0.9267
IO-mIgG	0.25	3	1.4517	0.6047	15	2.40	0.0298
IO-mIgG	0.25	4	1.7367	0.6208	15.01	2.80	0.0135
IO-mIgG	0.25	5	1.9083	0.5760	14.99	3.31	0.0047
IO-mIgG	0.25	7	1.9700	0.5823	15	3.38	0.0041
IO-mIgG	1	2	1.0867	0.5280	15	2.06	0.0574
IO-mIgG	1	3	2.5900	0.5323	14.99	4.87	0.0002
IO-mIgG	1	4	2.8750	0.5108	15	5.63	<.0001
IO-mIgG	1	5	3.0467	0.5442	14.99	5.60	<.0001
IO-mIgG	1	7	3.1083	0.5582	15	5.57	<.0001
IO-mIgG	2	3	1.5033	0.1404	15.02	10.71	<.0001
IO-mIgG	2	4	1.7883	0.2212	15.02	8.09	<.0001
IO-mIgG	2	5	1.9600	0.1969	14.69	9.95	<.0001
IO-mIgG	2	7	2.0217	0.1822	15.01	11.10	<.0001
IO-mIgG	3	4	0.2850	0.1284	14.98	2.22	0.0423
IO-mIgG	3	5	0.4567	0.1256	14.87	3.64	0.0025
IO-mIgG	3	7	0.5183	0.1056	14.99	4.91	0.0002
IO-mIgG	4	5	0.1717	0.09128	14.91	1.88	0.0797
IO-mIgG	4	7	0.2333	0.1008	15.05	2.31	0.0352
IO-mIgG	5	7	0.06167	0.05445	13.6	1.13	0.2770

Table H5. *Simple effect comparisons of treatment*day least squares means by treatment for γδ T cell+ CD8-, Study A* (Cont.)

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
mIgG	0	0.25	-1.5033	0.6789	15	-2.21	0.0427
mIgG	0	1	-2.1550	0.6004	15	-3.59	0.0027
mIgG	0	2	-0.7250	0.2492	15.01	-2.91	0.0108
mIgG	0	3	0.02167	0.2070	14.99	0.10	0.9180
mIgG	0	4	0.1267	0.1892	14.97	0.67	0.5133
mIgG	0	5	0.1700	0.1933	14.79	0.88	0.3933
mIgG	0	7	0.2117	0.1979	15	1.07	0.3018
mIgG	0.25	1	-0.6517	0.7195	14.99	-0.91	0.3794
mIgG	0.25	2	0.7783	0.5520	14.99	1.41	0.1789
mIgG	0.25	3	1.5250	0.6047	15	2.52	0.0235
mIgG	0.25	4	1.6300	0.6208	15.01	2.63	0.0191
mIgG	0.25	5	1.6733	0.5760	14.99	2.90	0.0109
mIgG	0.25	7	1.7150	0.5823	15	2.95	0.0100
mIgG	1	2	1.4300	0.5280	15	2.71	0.0162
mIgG	1	3	2.1767	0.5323	14.99	4.09	0.0010
mIgG	1	4	2.2817	0.5108	15	4.47	0.0005
mIgG	1	5	2.3250	0.5442	14.99	4.27	0.0007
mIgG	1	7	2.3667	0.5582	15	4.24	0.0007
mIgG	2	3	0.7467	0.1404	15.02	5.32	<.0001
mIgG	2	4	0.8517	0.2212	15.02	3.85	0.0016
mIgG	2	5	0.8950	0.1969	14.69	4.55	0.0004
mIgG	2	7	0.9367	0.1822	15.01	5.14	0.0001
mIgG	3	4	0.1050	0.1284	14.98	0.82	0.4264
mIgG	3	5	0.1483	0.1256	14.87	1.18	0.2563
mIgG	3	7	0.1900	0.1056	14.99	1.80	0.0921
mIgG	4	5	0.04333	0.09128	14.91	0.47	0.6418
mIgG	4	7	0.08500	0.1008	15.05	0.84	0.4123
mIgG	5	7	0.04167	0.05445	13.6	0.77	0.4572

Table H6. *Simple effect comparisons of treatment*day least squares means by day for γδ T cell+ CD8-, Study A*

Simple Effect Level	Treatment	Treatment	Estimate	Std. Error	DF	t Value	Pr > |t|
day 0	ALUmIgG	IOmIgG	0.2500	0.2499	15	1.00	0.3329
day 0	ALUmIgG	mIgG	0.1533	0.2499	15	0.61	0.5486
day 0	IOmIgG	mIgG	-0.09667	0.2499	15	-0.39	0.7043
day 0.25	ALUmIgG	IOmIgG	0.9883	0.8557	15	1.15	0.2662
day 0.25	ALUmIgG	mIgG	1.2750	0.8557	15	1.49	0.1570
day 0.25	IOmIgG	mIgG	0.2867	0.8557	15	0.33	0.7423
day 1	ALUmIgG	IOmIgG	-0.6933	0.7757	15	-0.89	0.3855
day 1	ALUmIgG	mIgG	0.08000	0.7757	15	0.10	0.9192
day 1	IOmIgG	mIgG	0.7733	0.7757	15	1.00	0.3346
day 2	ALUmIgG	IOmIgG	-1.2683	0.2765	15.01	-4.59	0.0004
day 2	ALUmIgG	mIgG	-0.1517	0.2765	15.01	-0.55	0.5914
day 2	IOmIgG	mIgG	1.1167	0.2765	15.01	4.04	0.0011
day 3	ALUmIgG	IOmIgG	-0.2133	0.1628	14.97	-1.31	0.2099
day 3	ALUmIgG	mIgG	0.1467	0.1628	14.97	0.90	0.3820
day 3	IOmIgG	mIgG	0.3600	0.1628	14.97	2.21	0.0430
day 4	ALUmIgG	IOmIgG	-0.2067	0.1148	15.03	-1.80	0.0919
day 4	ALUmIgG	mIgG	-0.02667	0.1148	15.03	-0.23	0.8194
day 4	IOmIgG	mIgG	0.1800	0.1148	15.03	1.57	0.1376
day 5	ALUmIgG	IOmIgG	-0.1218	0.08906	15.26	-1.37	0.1912
day 5	ALUmIgG	mIgG	-0.07015	0.08906	15.26	-0.79	0.4430
day 5	IOmIgG	mIgG	0.05167	0.08487	14.6	0.61	0.5520
day 7	ALUmIgG	IOmIgG	0.05333	0.06039	14.97	0.88	0.3911
day 7	ALUmIgG	mIgG	0.08500	0.06039	14.97	1.41	0.1797
day 7	IOmIgG	mIgG	0.03167	0.06039	14.97	0.52	0.6077

Table H5. *Simple effect comparisons of treatment*day least squares means by treatment for MHCII+ Macrophages+, Study B*

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
Alum-mIgG	0	0.25	-6.2525	1.8208	9	-3.43	0.0075
Alum-mIgG	0	1	-0.4800	0.3573	9	-1.34	0.2120
Alum-mIgG	0	2	-0.4125	0.3801	9	-1.09	0.3060
Alum-mIgG	0	3	-1.1900	1.0421	9	-1.14	0.2829
Alum-mIgG	0	4	0.7425	0.6134	9	1.21	0.2569
Alum-mIgG	0	5	0.8225	0.4984	9	1.65	0.1333
Alum-mIgG	0	7	0.5550	0.5341	9	1.04	0.3258
Alum-mIgG	0.25	1	5.7725	1.8465	9	3.13	0.0122
Alum-mIgG	0.25	2	5.8400	1.8526	9	3.15	0.0117
Alum-mIgG	0.25	3	5.0625	2.4376	9	2.08	0.0676
Alum-mIgG	0.25	4	6.9950	1.8722	9	3.74	0.0047
Alum-mIgG	0.25	5	7.0750	1.8602	9	3.80	0.0042
Alum-mIgG	0.25	7	6.8075	2.0383	9	3.34	0.0087

Table H5. *Simple effect comparisons of treatment*day least squares means by treatment for MHCII+ Macrophages+, Study B* (Cont.)

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
Alum-mIgG	1	2	0.06750	0.07688	9	0.88	0.4028
Alum-mIgG	1	3	-0.7100	1.0354	9	-0.69	0.5101
Alum-mIgG	1	4	1.2225	0.6071	9	2.01	0.0749
Alum-mIgG	1	5	1.3025	0.5555	9	2.34	0.0437
Alum-mIgG	1	7	1.0350	0.5543	9	1.87	0.0947
Alum-mIgG	2	3	-0.7775	1.0382	9	-0.75	0.4730
Alum-mIgG	2	4	1.1550	0.5899	9	1.96	0.0819
Alum-mIgG	2	5	1.2350	0.5335	9	2.32	0.0459
Alum-mIgG	2	7	0.9675	0.5498	9	1.76	0.1123
Alum-mIgG	3	4	1.9325	0.6741	9	2.87	0.0186
Alum-mIgG	3	5	2.0125	0.9032	9	2.23	0.0529
Alum-mIgG	3	7	1.7450	0.5967	9	2.92	0.0169
Alum-mIgG	4	5	0.08000	0.3335	9	0.24	0.8158
Alum-mIgG	4	7	-0.1875	0.3270	9	-0.57	0.5804
Alum-mIgG	5	7	-0.2675	0.4242	9	-0.63	0.5440
IO-mIgG	0	0.25	0.1050	1.8208	9	0.06	0.9553
IO-mIgG	0	1	0.09500	0.3572	9	0.27	0.7963
IO-mIgG	0	2	0.2850	0.3801	9	0.75	0.4725
IO-mIgG	0	3	0.8450	1.0420	9	0.81	0.4383
IO-mIgG	0	4	1.3200	0.6134	9	2.15	0.0598
IO-mIgG	0	5	1.2200	0.4984	9	2.45	0.0369
IO-mIgG	0	7	0.4400	0.5341	9	0.82	0.4313
IO-mIgG	0.25	1	-0.01000	1.8465	9	-0.01	0.9958
IO-mIgG	0.25	2	0.1800	1.8526	9	0.10	0.9247
IO-mIgG	0.25	3	0.7400	2.4376	9	0.30	0.7684
IO-mIgG	0.25	4	1.2150	1.8722	9	0.65	0.5326
IO-mIgG	0.25	5	1.1150	1.8602	9	0.60	0.5637
IO-mIgG	0.25	7	0.3350	2.0383	9	0.16	0.8731
IO-mIgG	1	2	0.1900	0.07688	9	2.47	0.0355
IO-mIgG	1	3	0.7500	1.0353	9	0.72	0.4872
IO-mIgG	1	4	1.2250	0.6071	9	2.02	0.0744
IO-mIgG	1	5	1.1250	0.5555	9	2.03	0.0735
IO-mIgG	1	7	0.3450	0.5542	9	0.62	0.5491
IO-mIgG	2	3	0.5600	1.0382	9	0.54	0.6027
IO-mIgG	2	4	1.0350	0.5899	9	1.75	0.1132
IO-mIgG	2	5	0.9350	0.5335	9	1.75	0.1136
IO-mIgG	2	7	0.1550	0.5497	9	0.28	0.7844
IO-mIgG	3	4	0.4750	0.6741	9	0.70	0.4988
IO-mIgG	3	5	0.3750	0.9032	9	0.42	0.6877
IO-mIgG	3	7	-0.4050	0.5967	9	-0.68	0.5144
IO-mIgG	4	5	-0.1000	0.3335	9	-0.30	0.7711
IO-mIgG	4	7	-0.8800	0.3270	9	-2.69	0.0247
IO-mIgG	5	7	-0.7800	0.4242	9	-1.84	0.0991

Table H5. *Simple effect comparisons of treatment*day least squares means by treatment for MHCII+ Macrophages+, Study B* (Cont.)

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
mIgG	0	0.25	-2.4175	1.8208	9	-1.33	0.2170
mIgG	0	1	-0.4250	0.3573	9	-1.19	0.2646
mIgG	0	2	-0.2600	0.3801	9	-0.68	0.5111
mIgG	0	3	0.5075	1.0421	9	0.49	0.6379
mIgG	0	4	0.9425	0.6134	9	1.54	0.1588
mIgG	0	5	0.5600	0.4984	9	1.12	0.2902
mIgG	0	7	0.4725	0.5341	9	0.88	0.3993
mIgG	0.25	1	1.9925	1.8465	9	1.08	0.3086
mIgG	0.25	2	2.1575	1.8526	9	1.16	0.2741
mIgG	0.25	3	2.9250	2.4376	9	1.20	0.2608
mIgG	0.25	4	3.3600	1.8722	9	1.79	0.1063
mIgG	0.25	5	2.9775	1.8603	9	1.60	0.1439
mIgG	0.25	7	2.8900	2.0383	9	1.42	0.1899
mIgG	1	2	0.1650	0.07688	9	2.15	0.0604
mIgG	1	3	0.9325	1.0354	9	0.90	0.3912
mIgG	1	4	1.3675	0.6071	9	2.25	0.0508
mIgG	1	5	0.9850	0.5555	9	1.77	0.1099
mIgG	1	7	0.8975	0.5543	9	1.62	0.1398
mIgG	2	3	0.7675	1.0382	9	0.74	0.4786
mIgG	2	4	1.2025	0.5899	9	2.04	0.0719
mIgG	2	5	0.8200	0.5335	9	1.54	0.1586
mIgG	2	7	0.7325	0.5498	9	1.33	0.2155
mIgG	3	4	0.4350	0.6741	9	0.65	0.5348
mIgG	3	5	0.05250	0.9032	9	0.06	0.9549
mIgG	3	7	-0.03500	0.5967	9	-0.06	0.9545
mIgG	4	5	-0.3825	0.3335	9	-1.15	0.2809
mIgG	4	7	-0.4700	0.3270	9	-1.44	0.1844
mIgG	5	7	-0.08750	0.4242	9	-0.21	0.8412

Table H6. *Simple effect comparisons of treatment*day least squares means by day for MHCII+ Macrophages+, Study B*

Simple Effect Level	Treatment	Treatment	Estimate	Std. Error	DF	t Value	Pr > |t|
day 0	AlummIgG	IOmIgG	0.09500	0.5808	9	0.16	0.8737
day 0	AlummIgG	mIgG	0.3525	0.5808	9	0.61	0.5589
day 0	IOmIgG	mIgG	0.2575	0.5808	9	0.44	0.6680
day 0.25	AlummIgG	IOmIgG	6.4525	2.5884	9	2.49	0.0343
day 0.25	AlummIgG	mIgG	4.1875	2.5884	9	1.62	0.1402
day 0.25	IOmIgG	mIgG	-2.2650	2.5884	9	-0.88	0.4043
day 1	AlummIgG	IOmIgG	0.6700	0.7372	9	0.91	0.3871
day 1	AlummIgG	mIgG	0.4075	0.7372	9	0.55	0.5939
day 1	IOmIgG	mIgG	-0.2625	0.7372	9	-0.36	0.7300
day 2	AlummIgG	IOmIgG	0.7925	0.7069	9	1.12	0.2913
day 2	AlummIgG	mIgG	0.5050	0.7069	9	0.71	0.4931
day 2	IOmIgG	mIgG	-0.2875	0.7069	9	-0.41	0.6937
day 3	AlummIgG	IOmIgG	2.1300	1.2584	9	1.69	0.1248
day 3	AlummIgG	mIgG	2.0500	1.2584	9	1.63	0.1377
day 3	IOmIgG	mIgG	-0.08000	1.2584	9	-0.06	0.9507
day 4	AlummIgG	IOmIgG	0.6725	0.4916	9	1.37	0.2045
day 4	AlummIgG	mIgG	0.5525	0.4916	9	1.12	0.2901
day 4	IOmIgG	mIgG	-0.1200	0.4916	9	-0.24	0.8126
day 5	AlummIgG	IOmIgG	0.4925	0.3153	9	1.56	0.1527
day 5	AlummIgG	mIgG	0.09000	0.3153	9	0.29	0.7818
day 5	IOmIgG	mIgG	-0.4025	0.3153	9	-1.28	0.2337
day 7	AlummIgG	IOmIgG	-0.02000	0.5215	9	-0.04	0.9702
day 7	AlummIgG	mIgG	0.2700	0.5215	9	0.52	0.6171
day 7	IOmIgG	mIgG	0.2900	0.5215	9	0.56	0.5917

Table H7. *Simple effect comparisons of treatment*day least squares means by treatment for αβ2 T cell+ CD4+ CD8+, Study B*

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
Alum-mIgG	0	0.25	0.6350	0.1988	9	3.19	0.0109
Alum-mIgG	0	1	0.1925	0.2816	9	0.68	0.5114
Alum-mIgG	0	2	0.3225	0.2338	9	1.38	0.2010
Alum-mIgG	0	3	0.4425	0.2107	9	2.10	0.0651
Alum-mIgG	0	4	0.3850	0.2019	9	1.91	0.0889
Alum-mIgG	0	5	0.4325	0.1972	9	2.19	0.0560
Alum-mIgG	0	7	0.4550	0.2316	9	1.96	0.0810
Alum-mIgG	0.25	1	-0.4425	0.1207	9	-3.67	0.0052
Alum-mIgG	0.25	2	-0.3125	0.1030	9	-3.03	0.0142
Alum-mIgG	0.25	3	-0.1925	0.1165	9	-1.65	0.1329
Alum-mIgG	0.25	4	-0.2500	0.1285	9	-1.95	0.0836
Alum-mIgG	0.25	5	-0.2025	0.1938	9	-1.05	0.3232
Alum-mIgG	0.25	7	-0.1800	0.1528	9	-1.18	0.2691
Alum-mIgG	1	2	0.1300	0.1647	9	0.79	0.4502
Alum-mIgG	1	3	0.2500	0.1713	9	1.46	0.1784

Table H7. *Simple effect comparisons of treatment*day least squares means by treatment for αβ2 T cell+ CD4+ CD8+, Study B* (Cont.)

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
Alum-mIgG	1	4	0.1925	0.2042	9	0.94	0.3705
Alum-mIgG	1	5	0.2400	0.2567	9	0.93	0.3743
Alum-mIgG	1	7	0.2625	0.2213	9	1.19	0.2659
Alum-mIgG	2	3	0.1200	0.06227	9	1.93	0.0861
Alum-mIgG	2	4	0.06250	0.07389	9	0.85	0.4196
Alum-mIgG	2	5	0.1100	0.1551	9	0.71	0.4960
Alum-mIgG	2	7	0.1325	0.09269	9	1.43	0.1866
Alum-mIgG	3	4	-0.05750	0.1013	9	-0.57	0.5842
Alum-mIgG	3	5	-0.01000	0.1615	9	-0.06	0.9520
Alum-mIgG	3	7	0.01250	0.1032	9	0.12	0.9063
Alum-mIgG	4	5	0.04750	0.1150	9	0.41	0.6893
Alum-mIgG	4	7	0.07000	0.1111	9	0.63	0.5442
Alum-mIgG	5	7	0.02250	0.1850	9	0.12	0.9059
IO-mIgG	0	0.25	0.4850	0.1988	9	2.44	0.0374
IO-mIgG	0	1	0.1175	0.2816	9	0.42	0.6862
IO-mIgG	0	2	0.4250	0.2338	9	1.82	0.1024
IO-mIgG	0	3	0.4500	0.2107	9	2.14	0.0614
IO-mIgG	0	4	0.4750	0.2019	9	2.35	0.0431
IO-mIgG	0	5	0.5750	0.1972	9	2.92	0.0172
IO-mIgG	0	7	0.3700	0.2316	9	1.60	0.1446
IO-mIgG	0.25	1	-0.3675	0.1207	9	-3.04	0.0139
IO-mIgG	0.25	2	-0.06000	0.1030	9	-0.58	0.5745
IO-mIgG	0.25	3	-0.03500	0.1165	9	-0.30	0.7707
IO-mIgG	0.25	4	-0.01000	0.1285	9	-0.08	0.9397
IO-mIgG	0.25	5	0.09000	0.1938	9	0.46	0.6533
IO-mIgG	0.25	7	-0.1150	0.1528	9	-0.75	0.4710
IO-mIgG	1	2	0.3075	0.1647	9	1.87	0.0947
IO-mIgG	1	3	0.3325	0.1713	9	1.94	0.0841
IO-mIgG	1	4	0.3575	0.2042	9	1.75	0.1140
IO-mIgG	1	5	0.4575	0.2567	9	1.78	0.1084
IO-mIgG	1	7	0.2525	0.2213	9	1.14	0.2833
IO-mIgG	2	3	0.02500	0.06227	9	0.40	0.6974
IO-mIgG	2	4	0.05000	0.07389	9	0.68	0.5156
IO-mIgG	2	5	0.1500	0.1551	9	0.97	0.3586
IO-mIgG	2	7	-0.05500	0.09269	9	-0.59	0.5675
IO-mIgG	3	4	0.02500	0.1013	9	0.25	0.8106
IO-mIgG	3	5	0.1250	0.1615	9	0.77	0.4588
IO-mIgG	3	7	-0.08000	0.1032	9	-0.78	0.4581
IO-mIgG	4	5	0.1000	0.1150	9	0.87	0.4072
IO-mIgG	4	7	-0.1050	0.1111	9	-0.95	0.3692
IO-mIgG	5	7	-0.2050	0.1850	9	-1.11	0.2965
mIgG	0	0.25	0.1500	0.1988	9	0.75	0.4697
mIgG	0	1	0.1325	0.2816	9	0.47	0.6491

Table H7. *Simple effect comparisons of treatment*day least squares means by treatment for αβ2 T cell+ CD4+ CD8+, Study B* (Cont.)

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
mIgG	0	2	0.4000	0.2338	9	1.71	0.1212
mIgG	0	3	0.4250	0.2107	9	2.02	0.0745
mIgG	0	4	0.4125	0.2019	9	2.04	0.0714
mIgG	0	5	0.3175	0.1972	9	1.61	0.1419
mIgG	0	7	0.3650	0.2316	9	1.58	0.1494
mIgG	0.25	1	-0.01750	0.1207	9	-0.14	0.8879
mIgG	0.25	2	0.2500	0.1030	9	2.43	0.0381
mIgG	0.25	3	0.2750	0.1165	9	2.36	0.0426
mIgG	0.25	4	0.2625	0.1285	9	2.04	0.0714
mIgG	0.25	5	0.1675	0.1938	9	0.86	0.4098
mIgG	0.25	7	0.2150	0.1528	9	1.41	0.1931
mIgG	1	2	0.2675	0.1647	9	1.62	0.1388
mIgG	1	3	0.2925	0.1713	9	1.71	0.1218
mIgG	1	4	0.2800	0.2042	9	1.37	0.2036
mIgG	1	5	0.1850	0.2567	9	0.72	0.4895
mIgG	1	7	0.2325	0.2213	9	1.05	0.3208
mIgG	2	3	0.02500	0.06227	9	0.40	0.6974
mIgG	2	4	0.01250	0.07389	9	0.17	0.8694
mIgG	2	5	-0.08250	0.1551	9	-0.53	0.6076
mIgG	2	7	-0.03500	0.09269	9	-0.38	0.7145
mIgG	3	4	-0.01250	0.1013	9	-0.12	0.9045
mIgG	3	5	-0.1075	0.1615	9	-0.67	0.5223
mIgG	3	7	-0.06000	0.1032	9	-0.58	0.5753
mIgG	4	5	-0.09500	0.1150	9	-0.83	0.4301
mIgG	4	7	-0.04750	0.1111	9	-0.43	0.6790
mIgG	5	7	0.04750	0.1850	9	0.26	0.8031

Table H8. *Simple effect comparisons of treatment*day least squares means by day for αβ2 T cell+ CD4+ CD8+, Study B*

Simple Effect Level	Treatment	Treatment	Estimate	Std. Error	DF	t Value	Pr > |t|
day 0	AlummIgG	IOmIgG	0.03750	0.2145	9	0.17	0.8651
day 0	AlummIgG	mIgG	0.1625	0.2145	9	0.76	0.4680
day 0	IOmIgG	mIgG	0.1250	0.2145	9	0.58	0.5743
day 0.25	AlummIgG	IOmIgG	-0.1125	0.2084	9	-0.54	0.6025
day 0.25	AlummIgG	mIgG	-0.3225	0.2084	9	-1.55	0.1562
day 0.25	IOmIgG	mIgG	-0.2100	0.2084	9	-1.01	0.3400
day 1	AlummIgG	IOmIgG	-0.03750	0.3494	9	-0.11	0.9169
day 1	AlummIgG	mIgG	0.1025	0.3494	9	0.29	0.7759
day 1	IOmIgG	mIgG	0.1400	0.3494	9	0.40	0.6980
day 2	AlummIgG	IOmIgG	0.1400	0.1896	9	0.74	0.4791
day 2	AlummIgG	mIgG	0.2400	0.1896	9	1.27	0.2373
day 2	IOmIgG	mIgG	0.1000	0.1896	9	0.53	0.6106
day 3	AlummIgG	IOmIgG	0.04500	0.1861	9	0.24	0.8144
day 3	AlummIgG	mIgG	0.1450	0.1861	9	0.78	0.4560
day 3	IOmIgG	mIgG	0.1000	0.1861	9	0.54	0.6041
day 4	AlummIgG	IOmIgG	0.1275	0.1618	9	0.79	0.4510
day 4	AlummIgG	mIgG	0.1900	0.1618	9	1.17	0.2704
day 4	IOmIgG	mIgG	0.06250	0.1618	9	0.39	0.7083
day 5	AlummIgG	IOmIgG	0.1800	0.1705	9	1.06	0.3185
day 5	AlummIgG	mIgG	0.04750	0.1705	9	0.28	0.7868
day 5	IOmIgG	mIgG	-0.1325	0.1705	9	-0.78	0.4569
day 7	AlummIgG	IOmIgG	-0.04750	0.1916	9	-0.25	0.8098
day 7	AlummIgG	mIgG	0.07250	0.1916	9	0.38	0.7139
day 7	IOmIgG	mIgG	0.1200	0.1916	9	0.63	0.5467

Table H9. *Simple effect comparisons of treatment*day least squares means by treatment for B cell+ IgG-, Study B*

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
Alum-mIgG	0	0.25	-0.2825	0.3275	9.003	-0.86	0.4108
Alum-mIgG	0	1	-4.2125	0.9337	9	-4.51	0.0015
Alum-mIgG	0	2	-7.8200	2.7561	9	-2.84	0.0195
Alum-mIgG	0	3	-9.7425	3.6333	9	-2.68	0.0251
Alum-mIgG	0	4	-6.1000	2.9839	9	-2.04	0.0713
Alum-mIgG	0	5	-1.7000	1.1836	9	-1.44	0.1847
Alum-mIgG	0	7	-1.9100	0.4480	9	-4.26	0.0021
Alum-mIgG	0.25	1	-3.9300	0.9823	9	-4.00	0.0031
Alum-mIgG	0.25	2	-7.5375	2.5035	9	-3.01	0.0147
Alum-mIgG	0.25	3	-9.4600	3.4281	9	-2.76	0.0221
Alum-mIgG	0.25	4	-5.8175	2.9216	9	-1.99	0.0776
Alum-mIgG	0.25	5	-1.4175	1.2325	9	-1.15	0.2798
Alum-mIgG	0.25	7	-1.6275	0.6182	9	-2.63	0.0272
Alum-mIgG	1	2	-3.6075	2.9867	9	-1.21	0.2579
Alum-mIgG	1	3	-5.5300	4.0177	9	-1.38	0.2020

Table H9. *Simple effect comparisons of treatment*day least squares means by treatment for B cell+ IgG-, Study B* (Cont.)

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
Alum-mIgG	1	4	-1.8875	3.3280	9	-0.57	0.5845
Alum-mIgG	1	5	2.5125	1.7489	9	1.44	0.1847
Alum-mIgG	1	7	2.3025	1.2163	9	1.89	0.0909
Alum-mIgG	2	3	-1.9225	2.3777	9	-0.81	0.4396
Alum-mIgG	2	4	1.7200	3.1825	9	0.54	0.6020
Alum-mIgG	2	5	6.1200	3.1412	9	1.95	0.0832
Alum-mIgG	2	7	5.9100	2.8213	9	2.09	0.0657
Alum-mIgG	3	4	3.6425	4.3859	9	0.83	0.4277
Alum-mIgG	3	5	8.0425	4.1664	9	1.93	0.0856
Alum-mIgG	3	7	7.8325	3.4995	9	2.24	0.0520
Alum-mIgG	4	5	4.4000	2.3152	9	1.90	0.0898
Alum-mIgG	4	7	4.1900	2.8997	9	1.44	0.1824
Alum-mIgG	5	7	-0.2100	1.1801	9	-0.18	0.8627
IO-mIgG	0	0.25	-0.5550	0.3275	9.003	-1.69	0.1244
IO-mIgG	0	1	-3.2125	0.9337	9	-3.44	0.0074
IO-mIgG	0	2	-8.9875	2.7561	9	-3.26	0.0098
IO-mIgG	0	3	-10.4425	3.6333	9	-2.87	0.0184
IO-mIgG	0	4	-16.2775	2.9839	9	-5.46	0.0004
IO-mIgG	0	5	-7.7000	1.1836	9	-6.51	0.0001
IO-mIgG	0	7	-2.5525	0.4480	9	-5.70	0.0003
IO-mIgG	0.25	1	-2.6575	0.9823	9	-2.71	0.0242
IO-mIgG	0.25	2	-8.4325	2.5035	9	-3.37	0.0083
IO-mIgG	0.25	3	-9.8875	3.4281	9	-2.88	0.0181
IO-mIgG	0.25	4	-15.7225	2.9216	9	-5.38	0.0004
IO-mIgG	0.25	5	-7.1450	1.2325	9	-5.80	0.0003
IO-mIgG	0.25	7	-1.9975	0.6182	9	-3.23	0.0103
IO-mIgG	1	2	-5.7750	2.9867	9	-1.93	0.0852
IO-mIgG	1	3	-7.2300	4.0177	9	-1.80	0.1055
IO-mIgG	1	4	-13.0650	3.3280	9	-3.93	0.0035
IO-mIgG	1	5	-4.4875	1.7489	9	-2.57	0.0304
IO-mIgG	1	7	0.6600	1.2163	9	0.54	0.6005
IO-mIgG	2	3	-1.4550	2.3777	9	-0.61	0.5557
IO-mIgG	2	4	-7.2900	3.1825	9	-2.29	0.0477
IO-mIgG	2	5	1.2875	3.1412	9	0.41	0.6915
IO-mIgG	2	7	6.4350	2.8213	9	2.28	0.0485
IO-mIgG	3	4	-5.8350	4.3859	9	-1.33	0.2161
IO-mIgG	3	5	2.7425	4.1664	9	0.66	0.5269
IO-mIgG	3	7	7.8900	3.4995	9	2.25	0.0506
IO-mIgG	4	5	8.5775	2.3152	9	3.70	0.0049
IO-mIgG	4	7	13.7250	2.8997	9	4.73	0.0011
IO-mIgG	5	7	5.1475	1.1801	9	4.36	0.0018
mIgG	0	0.25	0.04250	0.3275	9.003	0.13	0.8996
mIgG	0	1	-5.8275	0.9337	9	-6.24	0.0002

Table H9. *Simple effect comparisons of treatment*day least squares means by treatment for B cell+ IgG-, Study B* (Cont.)

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
mIgG	0	2	-12.3850	2.7561	9	-4.49	0.0015
mIgG	0	3	-21.5650	3.6333	9	-5.94	0.0002
mIgG	0	4	-8.1025	2.9839	9	-2.72	0.0238
mIgG	0	5	-5.2250	1.1836	9	-4.41	0.0017
mIgG	0	7	-1.0650	0.4480	9	-2.38	0.0414
mIgG	0.25	1	-5.8700	0.9823	9	-5.98	0.0002
mIgG	0.25	2	-12.4275	2.5035	9	-4.96	0.0008
mIgG	0.25	3	-21.6075	3.4281	9	-6.30	0.0001
mIgG	0.25	4	-8.1450	2.9216	9	-2.79	0.0211
mIgG	0.25	5	-5.2675	1.2325	9	-4.27	0.0021
mIgG	0.25	7	-1.1075	0.6182	9	-1.79	0.1068
mIgG	1	2	-6.5575	2.9867	9	-2.20	0.0557
mIgG	1	3	-15.7375	4.0177	9	-3.92	0.0035
mIgG	1	4	-2.2750	3.3280	9	-0.68	0.5114
mIgG	1	5	0.6025	1.7489	9	0.34	0.7384
mIgG	1	7	4.7625	1.2163	9	3.92	0.0035
mIgG	2	3	-9.1800	2.3777	9	-3.86	0.0038
mIgG	2	4	4.2825	3.1825	9	1.35	0.2113
mIgG	2	5	7.1600	3.1411	9	2.28	0.0486
mIgG	2	7	11.3200	2.8213	9	4.01	0.0031
mIgG	3	4	13.4625	4.3859	9	3.07	0.0134
mIgG	3	5	16.3400	4.1664	9	3.92	0.0035
mIgG	3	7	20.5000	3.4995	9	5.86	0.0002
mIgG	4	5	2.8775	2.3152	9	1.24	0.2453
mIgG	4	7	7.0375	2.8997	9	2.43	0.0382
mIgG	5	7	4.1600	1.1801	9	3.53	0.0065

Table H10. *Simple effect comparisons of treatment*day least squares means by day for B cell+ IgG-, Study B*

Simple Effect Level	Treatment	Treatment	Estimate	Std. Error	DF	t Value	Pr > |t|
day 0	AlummIgG	IOmIgG	-0.00500	0.2608	9	-0.02	0.9851
day 0	AlummIgG	mIgG	-0.06750	0.2608	9	-0.26	0.8016
day 0	IOmIgG	mIgG	-0.06250	0.2608	9	-0.24	0.8160
day 0.25	AlummIgG	IOmIgG	-0.2775	0.3895	9	-0.71	0.4942
day 0.25	AlummIgG	mIgG	0.2575	0.3895	9	0.66	0.5251
day 0.25	IOmIgG	mIgG	0.5350	0.3895	9	1.37	0.2028
day 1	AlummIgG	IOmIgG	0.9950	1.3991	9	0.71	0.4950
day 1	AlummIgG	mIgG	-1.6825	1.3991	9	-1.20	0.2598
day 1	IOmIgG	mIgG	-2.6775	1.3991	9	-1.91	0.0879
day 2	AlummIgG	IOmIgG	-1.1725	3.8872	9	-0.30	0.7698
day 2	AlummIgG	mIgG	-4.6325	3.8872	9	-1.19	0.2638
day 2	IOmIgG	mIgG	-3.4600	3.8872	9	-0.89	0.3966
day 3	AlummIgG	IOmIgG	-0.7050	5.1406	9	-0.14	0.8939
day 3	AlummIgG	mIgG	-11.8900	5.1406	9	-2.31	0.0460
day 3	IOmIgG	mIgG	-11.1850	5.1406	9	-2.18	0.0576
day 4	AlummIgG	IOmIgG	-10.1825	4.1669	9	-2.44	0.0371
day 4	AlummIgG	mIgG	-2.0700	4.1669	9	-0.50	0.6313
day 4	IOmIgG	mIgG	8.1125	4.1669	9	1.95	0.0834
day 5	AlummIgG	IOmIgG	-6.0050	1.5779	9	-3.81	0.0042
day 5	AlummIgG	mIgG	-3.5925	1.5779	9	-2.28	0.0488
day 5	IOmIgG	mIgG	2.4125	1.5779	9	1.53	0.1606
day 7	AlummIgG	IOmIgG	-0.6475	0.7520	9	-0.86	0.4116
day 7	AlummIgG	mIgG	0.7775	0.7520	9	1.03	0.3281
day 7	IOmIgG	mIgG	1.4250	0.7520	9	1.90	0.0906

APPENDIX I

Type III Test of Fixed Effects Results for

Memory Response in Intramuscular Injection Studies

Table I1. *Type III test of fixed effects results for memory response in intramuscular injection studies*

		Study A		Study B	
Response Factor	**Fixed Effect**	**F Value**	**P Value**	**F Value**	**P Value**
Live cells	Day	-	-	63.36	0.0006
	Treatment	-	-	0.22	NS
	Treatment*Day	-	-	2.52	NS
CD45+	Day	20.51	< 0.0001	-	-
	Treatment	8.19	0.0044	-	-
	Treatment*Day	2.05	NS	-	-
MHCII+	Day	20.98	< 0.0001	4.70	NS
Macrophages+	Treatment	1.05	NS	1.71	NS
	Treatment*Day	1.53	NS	6.55	0.0326
MHCII+	Day	2.06	NS	15.44	0.0097
B cells+	Treatment	2.47	NS	1.49	NS
	Treatment*Day	1.44	NS	5.13	NS
CD3+	Day	18.55	< 0.0001	86.56	< 0.0001
	Treatment	3.03	NS	0.20	NS
	Treatment*Day	1.00	NS	0.92	NS
CD4- CD8+	Day	22.45	< 0.0001	38.89	0.0017
	Treatment	0.63	NS	0.07	NS
	Treatment*Day	1.00	NS	2.88	NS
CD4+ CD8+	Day	5.68	0.0107	8.93	0.0263
	Treatment	3.15	NS	0.80	NS
	Treatment*Day	1.34	NS	10.04	0.0140
CD4+ CD8-	Day	8.93	0.0023	11.63	0.0164
	Treatment	4.78	0.0262	0.14	NS
	Treatment*Day	1.03	NS	1.32	NS
CD25+ CD4+	Day	5.97	0.0091	-	-
	Treatment	2.39	NS	-	-
	Treatment*Day	0.67	NS	-	-
CD25+ CD4-	Day	5.81	0.0099	-	-
	Treatment	1.91	NS	-	-
	Treatment*Day	0.93	NS	-	-
γδ T cell+ CD8+	Day	25.98	< 0.0001	289.44	< 0.0001
	Treatment	0.57	NS	2.09	NS
	Treatment*Day	2.06	NS	7.52	0.0249
γδ T cell+ CD8-	Day	24.85	< 0.0001	23.70	0.0043
	Treatment	2.09	NS	5.23	0.0312
	Treatment*Day	1.01	NS	0.71	NS

Table I1. *Type III test of fixed effects results for memory response in intramuscular injection studies* (Cont.)

		Study A		Study B	
Response Factor	**Fixed Effect**	**F Value**	**P Value**	**F Value**	**P Value**
αβ1 Tcell+	Day	21.42	< 0.0001	18.90	0.0067
	Treatment	5.69	0.0155	1.44	NS
	Treatment*Day	1.25	NS	4.00	NS
αβ1 T cell+	Day	-	-	8.77	0.0272
CD4-CD8+	Treatment	-	-	2.37	NS
	Treatment*Day	-	-	2.33	NS
αβ1 T cell+	Day	-	-	19.11	0.0065
CD4+CD8+	Treatment	-	-	0.76	NS
	Treatment*Day	-	-	2.92	NS
αβ1 T cell+	Day	-	-	12.19	0.0150
CD4-CD8-	Treatment	-	-	1.35	NS
	Treatment*Day	-	-	8.29	0.0206
αβ1 T cell+	Day	-	-	18.84	0.0067
CD4+CD8-	Treatment	-	-	0.06	NS
	Treatment*Day	-	-	2.04	NS
αβ2 T cell+	Day	25.58	< 0.0001	216.43	< 0.0001
	Treatment	2.72	NS	0.09	NS
	Treatment*Day	0.66	NS	4.51	NS
αβ2 T cell+	Day	-	-	20.87	0.0055
CD4-CD8+	Treatment	-	-	1.09	NS
	Treatment*Day	-	-	3.11	NS
αβ2 T cell+	Day	-	-	6.28	0.0484
CD4+CD8+	Treatment	-	-	0.92	NS
	Treatment*Day	-	-	3.14	NS
αβ2 T cell+	Day	-	-	30.46	0.0027
CD4-CD8-	Treatment	-	-	0.96	NS
	Treatment*Day	-	-	0.73	NS
αβ2 T cell+	Day	-	-	7.28	0.0376
CD4+CD8-	Treatment	-	-	0.63	NS
	Treatment*Day	-	-	1.80	NS
B cells	Day	36.38	< 0.0001	-	-
	Treatment	4.08	0.0402	-	-
	Treatment*Day	1.43	NS	-	-
B cell+ IgM+	Day	-	-	44.39	0.0013
	Treatment	-	-	1.38	NS
	Treatment*Day	-	-	5.38	0.0475
B cell+ IgM-	Day	-	-	10.48	0.0198
	Treatment	-	-	0.18	NS
	Treatment*Day	-	-	0.68	NS
B cell+ IgG+	Day	-	-	15.45	0.0097
	Treatment	-	-	1.67	NS
	Treatment*Day	-	-	0.74	NS
B cell+ IgG-	Day	-	-	11.25	0.0174
	Treatment	-	-	1.52	NS
	Treatment*Day	-	-	4.23	NS

APPENDIX J

Difference of Least Squares Means Results for Memory Response in Intramuscular Injection Studies

Table J1. *Difference of least squares means for pulp cell responses by day*

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
Study A							
CD45+	0	0.25	-21.7877	2.9063	14	-7.50	<.0001
	0	1	-18.9327	2.7888	14	-6.79	<.0001
	0	2	-21.3263	2.9461	14	-7.24	<.0001
	0	3	-10.7033	2.4912	14	-4.30	0.0007
	0	4	-9.2061	2.4231	14	-3.80	0.0020
	0	5	-5.8034	1.1620	14	-4.99	0.0002
	0.25	1	2.8550	4.3062	14	0.66	0.5181
	0.25	2	0.4613	3.4048	14	0.14	0.8942
	0.25	3	11.0843	4.4154	14	2.51	0.0250
	0.25	4	12.5816	3.6513	14	3.45	0.0039
	0.25	5	15.9842	2.8999	14	5.51	<.0001
	1	2	-2.3937	4.4810	14	-0.53	0.6016
	1	3	8.2293	3.6433	14	2.26	0.0404
	1	4	9.7266	4.1312	14	2.35	0.0337
	1	5	13.1292	2.5416	14	5.17	0.0001
	2	3	10.6230	3.1694	14	3.35	0.0047
	2	4	12.1202	3.0460	14	3.98	0.0014
	2	5	15.5229	3.1316	14	4.96	0.0002
	3	4	1.4972	2.9716	14	0.50	0.6222
	3	5	4.8999	2.5444	14	1.93	0.0747
	4	5	3.4027	3.0134	14	1.13	0.2778
MHCII+	0	0.25	-1.1486	0.3993	14	-2.88	0.0122
Macrophages+	0	1	0.1271	0.05785	14	2.20	0.0453
	0	2	0.3261	0.06054	14	5.39	<.0001
	0	3	0.1359	0.1006	14	1.35	0.1983
	0	4	-0.06456	0.1059	14	-0.61	0.5519
	0	5	0.1692	0.06728	14	2.52	0.0247
	0.25	1	1.2757	0.4106	14	3.11	0.0077
	0.25	2	1.4747	0.3998	14	3.69	0.0024
	0.25	3	1.2844	0.4363	14	2.94	0.0107
	0.25	4	1.0840	0.4324	14	2.51	0.0251
	0.25	5	1.3178	0.4155	14	3.17	0.0068
	1	2	0.1990	0.04347	14	4.58	0.0004
	1	3	0.008778	0.08728	14	0.10	0.9213
	1	4	-0.1917	0.07846	14	-2.44	0.0284

Table J1. *Difference of least squares means for pulp cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
	1	5	0.04211	0.06045	14	0.70	0.4974
	2	3	-0.1902	0.08913	14	-2.13	0.0510
	2	4	-0.3907	0.07344	14	-5.32	0.0001
	2	5	-0.1569	0.03437	14	-4.56	0.0004
	3	4	-0.2004	0.09345	14	-2.15	0.0500
	3	5	0.03333	0.08093	14	0.41	0.6867
	4	5	0.2338	0.07807	14	2.99	0.0097
CD4+ CD8+	0	0.25	-0.04422	0.02983	14	-1.48	0.1604
	0	1	-0.06500	0.03184	14	-2.04	0.0605
	0	2	-0.01711	0.02421	14	-0.71	0.4913
	0	3	-0.4812	0.09480	14	-5.08	0.0002
	0	4	-0.2661	0.07623	14	-3.49	0.0036
	0	5	-0.3301	0.05495	14	-6.01	<.0001
	0.25	1	-0.02078	0.03208	14	-0.65	0.5277
	0.25	2	0.02711	0.03172	14	0.85	0.4071
	0.25	3	-0.4370	0.09220	14	-4.74	0.0003
	0.25	4	-0.2219	0.08429	14	-2.63	0.0197
	0.25	5	-0.2859	0.05696	14	-5.02	0.0002
	1	2	0.04789	0.03519	14	1.36	0.1950
	1	3	-0.4162	0.09911	14	-4.20	0.0009
	1	4	-0.2011	0.09171	14	-2.19	0.0457
	1	5	-0.2651	0.06550	14	-4.05	0.0012
	2	3	-0.4641	0.09130	14	-5.08	0.0002
	2	4	-0.2490	0.06885	14	-3.62	0.0028
	2	5	-0.3130	0.06642	14	-4.71	0.0003
	3	4	0.2151	0.07543	14	2.85	0.0128
	3	5	0.1511	0.08329	14	1.81	0.0911
	4	5	-0.06400	0.08068	14	-0.79	0.4409
CD4+ CD8-	0	0.25	-3.0803	0.8306	14	-3.71	0.0023
	0	1	-5.8193	1.1818	14	-4.92	0.0002
	0	2	-5.4109	0.8328	14	-6.50	<.0001
	0	3	-2.8750	0.8153	14	-3.53	0.0034
	0	4	-1.5489	0.6026	14	-2.57	0.0222
	0	5	-1.0569	0.4365	14	-2.42	0.0296
	0.25	1	-2.7390	1.4675	14	-1.87	0.0831
	0.25	2	-2.3306	1.0702	14	-2.18	0.0470
	0.25	3	0.2053	1.2687	14	0.16	0.8737
	0.25	4	1.5314	1.0157	14	1.51	0.1539
	0.25	5	2.0234	0.7178	14	2.82	0.0137
	1	2	0.4084	1.3583	14	0.30	0.7681
	1	3	2.9443	1.2260	14	2.40	0.0308
	1	4	4.2704	1.1934	14	3.58	0.0030
	1	5	4.7624	1.0801	14	4.41	0.0006
	2	3	2.5359	1.0535	14	2.41	0.0304

Table J1. *Difference of least squares means for pulp cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
	2	4	3.8620	0.8730	14	4.42	0.0006
	2	5	4.3540	0.9605	14	4.53	0.0005
	3	4	1.3261	0.8035	14	1.65	0.1211
	3	5	1.8181	0.7257	14	2.51	0.0252
	4	5	0.4920	0.7116	14	0.69	0.5006
CD4- CD8+	0	0.25	-1.9666	0.3817	14	-5.15	0.0001
	0	1	-2.5330	0.4617	14	-5.49	<.0001
	0	2	-2.4003	0.3122	14	-7.69	<.0001
	0	3	-2.7570	0.5166	14	-5.34	0.0001
	0	4	-1.1937	0.2322	14	-5.14	0.0001
	0	5	-1.1458	0.1741	14	-6.58	<.0001
	0.25	1	-0.5664	0.6129	14	-0.92	0.3710
	0.25	2	-0.4338	0.5093	14	-0.85	0.4087
	0.25	3	-0.7904	0.7284	14	-1.09	0.2962
	0.25	4	0.7729	0.5089	14	1.52	0.1511
	0.25	5	0.8208	0.3651	14	2.25	0.0412
	1	2	0.1327	0.5458	14	0.24	0.8115
	1	3	-0.2240	0.6944	14	-0.32	0.7518
	1	4	1.3393	0.5461	14	2.45	0.0279
	1	5	1.3872	0.4674	14	2.97	0.0102
	2	3	-0.3567	0.5937	14	-0.60	0.5576
	2	4	1.2067	0.3610	14	3.34	0.0048
	2	5	1.2546	0.3790	14	3.31	0.0052
	3	4	1.5633	0.4986	14	3.14	0.0073
	3	5	1.6112	0.4860	14	3.32	0.0051
	4	5	0.04789	0.2386	14	0.20	0.8438
CD25+ CD4+	0	0.25	0.05956	0.03374	14	1.76	0.0994
	0	1	-0.2738	0.08314	14	-3.29	0.0053
	0	2	-0.06089	0.04237	14	-1.44	0.1726
	0	3	-0.1439	0.04340	14	-3.32	0.0051
	0	4	-0.08167	0.03631	14	-2.25	0.0411
	0	5	-0.08944	0.03684	14	-2.43	0.0293
	0.25	1	-0.3333	0.08304	14	-4.01	0.0013
	0.25	2	-0.1204	0.04138	14	-2.91	0.0114
	0.25	3	-0.2034	0.04070	14	-5.00	0.0002
	0.25	4	-0.1412	0.04184	14	-3.38	0.0045
	0.25	5	-0.1490	0.03027	14	-4.92	0.0002
	1	2	0.2129	0.07889	14	2.70	0.0173
	1	3	0.1299	0.07693	14	1.69	0.1135
	1	4	0.1921	0.09935	14	1.93	0.0736
	1	5	0.1843	0.08810	14	2.09	0.0551
	2	3	-0.08300	0.05497	14	-1.51	0.1533
	2	4	-0.02078	0.05684	14	-0.37	0.7202
	2	5	-0.02856	0.05533	14	-0.52	0.6138
	3	4	0.06222	0.04571	14	1.36	0.1950
	3	5	0.05444	0.03639	14	1.50	0.1568

Table J1. *Difference of least squares means for pulp cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
	4	5	-0.00778	0.03474	14	-0.22	0.8261
CD25+ CD4-	0	0.25	-0.07533	0.06612	14	-1.14	0.2737
	0	1	-0.4262	0.1029	14	-4.14	0.0010
	0	2	0.1004	0.08237	14	1.22	0.2428
	0	3	0.1856	0.08705	14	2.13	0.0512
	0	4	0.1129	0.06602	14	1.71	0.1093
	0	5	0.1520	0.06374	14	2.38	0.0318
	0.25	1	-0.3509	0.1085	14	-3.23	0.0060
	0.25	2	0.1758	0.09042	14	1.94	0.0723
	0.25	3	0.2609	0.08154	14	3.20	0.0064
	0.25	4	0.1882	0.06433	14	2.93	0.0111
	0.25	5	0.2273	0.08845	14	2.57	0.0222
	1	2	0.5267	0.1172	14	4.49	0.0005
	1	3	0.6118	0.1111	14	5.51	<.0001
	1	4	0.5391	0.09268	14	5.82	<.0001
	1	5	0.5782	0.1011	14	5.72	<.0001
	2	3	0.08511	0.04860	14	1.75	0.1018
	2	4	0.01244	0.05570	14	0.22	0.8264
	2	5	0.05156	0.06101	14	0.84	0.4123
	3	4	-0.07267	0.05369	14	-1.35	0.1974
	3	5	-0.03356	0.05905	14	-0.57	0.5789
	4	5	0.03911	0.05845	14	0.67	0.5143
γδ T cell+ CD8+	0	0.25	-0.4692	0.05912	14	-7.94	<.0001
	0	1	-0.3897	0.07367	14	-5.29	0.0001
	0	2	-0.2857	0.03830	14	-7.46	<.0001
	0	3	-0.5936	0.06892	14	-8.61	<.0001
	0	4	-0.1984	0.04007	14	-4.95	0.0002
	0	5	-0.3780	0.03541	14	-10.67	<.0001
	0.25	1	0.07956	0.09932	14	0.80	0.4365
	0.25	2	0.1836	0.07739	14	2.37	0.0326
	0.25	3	-0.1243	0.09613	14	-1.29	0.2168
	0.25	4	0.2708	0.08100	14	3.34	0.0048
	0.25	5	0.09122	0.06092	14	1.50	0.1565
	1	2	0.1040	0.07202	14	1.44	0.1707
	1	3	-0.2039	0.1001	14	-2.04	0.0609
	1	4	0.1912	0.08406	14	2.27	0.0392
	1	5	0.01167	0.08631	14	0.14	0.8944
	2	3	-0.3079	0.06060	14	-5.08	0.0002
	2	4	0.08722	0.02364	14	3.69	0.0024
	2	5	-0.09233	0.04872	14	-1.90	0.0789
	3	4	0.3951	0.05392	14	7.33	<.0001
	3	5	0.2156	0.05949	14	3.62	0.0028
	4	5	-0.1796	0.04541	14	-3.95	0.0014
γδ T cell+ CD8-	0	0.25	-4.6739	0.9623	14	-4.86	0.0003
	0	1	-3.0680	0.3891	14	-7.88	<.0001
	0	2	-1.1196	0.1669	14	-6.71	<.0001

Table J1. *Difference of least squares means for pulp cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
	0	3	-0.8113	0.1345	14	-6.03	<.0001
	0	4	-0.4184	0.1730	14	-2.42	0.0298
	0	5	-0.4967	0.1306	14	-3.80	0.0019
	0.25	1	1.6059	1.1013	14	1.46	0.1668
	0.25	2	3.5543	0.9347	14	3.80	0.0019
	0.25	3	3.8626	0.9849	14	3.92	0.0015
	0.25	4	4.2554	0.9227	14	4.61	0.0004
	0.25	5	4.1772	0.9873	14	4.23	0.0008
	1	2	1.9484	0.4727	14	4.12	0.0010
	1	3	2.2567	0.4399	14	5.13	0.0002
	1	4	2.6496	0.4348	14	6.09	<.0001
	1	5	2.5713	0.3806	14	6.76	<.0001
	2	3	0.3082	0.1442	14	2.14	0.0507
	2	4	0.7011	0.1546	14	4.54	0.0005
	2	5	0.6229	0.1899	14	3.28	0.0055
	3	4	0.3929	0.1902	14	2.07	0.0579
	3	5	0.3147	0.1714	14	1.84	0.0877
	4	5	-0.07822	0.1742	14	-0.45	0.6603
αβ1 T cell+	0	0.25	-3.4014	0.6301	14	-5.40	<.0001
	0	1	-5.7460	0.9658	14	-5.95	<.0001
	0	2	-5.3638	0.6428	14	-8.34	<.0001
	0	3	-3.9572	0.7030	14	-5.63	<.0001
	0	4	-1.6892	0.3405	14	-4.96	0.0002
	0	5	-1.8833	0.3233	14	-5.83	<.0001
	0.25	1	-2.3446	1.3052	14	-1.80	0.0941
	0.25	2	-1.9623	0.8816	14	-2.23	0.0430
	0.25	3	-0.5558	1.1125	14	-0.50	0.6251
	0.25	4	1.7122	0.7645	14	2.24	0.0419
	0.25	5	1.5181	0.6299	14	2.41	0.0303
	1	2	0.3822	1.2005	14	0.32	0.7549
	1	3	1.7888	1.0295	14	1.74	0.1042
	1	4	4.0568	0.9637	14	4.21	0.0009
	1	5	3.8627	0.8929	14	4.33	0.0007
	2	3	1.4066	0.8497	14	1.66	0.1201
	2	4	3.6746	0.6291	14	5.84	<.0001
	2	5	3.4804	0.7662	14	4.54	0.0005
	3	4	2.2680	0.6534	14	3.47	0.0037
	3	5	2.0739	0.6180	14	3.36	0.0047
	4	5	-0.1941	0.4673	14	-0.42	0.6842
αβ2 T cell+	0	0.25	-1.7231	0.3737	14	-4.61	0.0004
	0	1	-2.1064	0.3528	14	-5.97	<.0001
	0	2	-1.7837	0.2511	14	-7.10	<.0001
	0	3	-1.4296	0.2532	14	-5.65	<.0001
	0	4	-0.5901	0.1236	14	-4.77	0.0003
	0	5	-0.6206	0.1504	14	-4.13	0.0010

Table J1. *Difference of least squares means for pulp cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
	0.25	1	-0.3833	0.5621	14	-0.68	0.5064
	0.25	2	-0.06056	0.4472	14	-0.14	0.8942
	0.25	3	0.2936	0.5117	14	0.57	0.5753
	0.25	4	1.1330	0.4048	14	2.80	0.0142
	0.25	5	1.1026	0.3558	14	3.10	0.0079
	1	2	0.3228	0.4250	14	0.76	0.4602
	1	3	0.6769	0.4128	14	1.64	0.1234
	1	4	1.5163	0.3508	14	4.32	0.0007
	1	5	1.4859	0.3286	14	4.52	0.0005
	2	3	0.3541	0.3719	14	0.95	0.3572
	2	4	1.1936	0.2452	14	4.87	0.0002
	2	5	1.1631	0.3115	14	3.73	0.0022
	3	4	0.8394	0.2575	14	3.26	0.0057
	3	5	0.8090	0.2146	14	3.77	0.0021
	4	5	-0.03044	0.1897	14	-0.16	0.8748
CD3+	0	0.25	-9.2509	1.6671	14	-5.55	<.0001
	0	1	-9.8456	1.6019	14	-6.15	<.0001
	0	2	-9.5484	1.2316	14	-7.75	<.0001
	0	3	-6.2850	0.9423	14	-6.67	<.0001
	0	4	-3.3744	0.7157	14	-4.71	0.0003
	0	5	-3.1467	0.5182	14	-6.07	<.0001
	0.25	1	-0.5947	2.4631	14	-0.24	0.8127
	0.25	2	-0.2976	2.0104	14	-0.15	0.8844
	0.25	3	2.9659	2.1160	14	1.40	0.1828
	0.25	4	5.8764	1.6728	14	3.51	0.0034
	0.25	5	6.1042	1.7224	14	3.54	0.0032
	1	2	0.2971	2.1401	14	0.14	0.8916
	1	3	3.5606	1.7968	14	1.98	0.0675
	1	4	6.4711	1.6966	14	3.81	0.0019
	1	5	6.6989	1.4336	14	4.67	0.0004
	2	3	3.2634	1.0813	14	3.02	0.0092
	2	4	6.1740	1.0929	14	5.65	<.0001
	2	5	6.4018	1.3715	14	4.67	0.0004
	3	4	2.9106	0.9459	14	3.08	0.0082
	3	5	3.1383	0.9675	14	3.24	0.0059
	4	5	0.2278	0.8611	14	0.26	0.7952
B cells	0	0.25	-0.8827	0.1437	14	-6.14	<.0001
	0	1	-4.3372	0.6659	14	-6.51	<.0001
	0	2	-10.0049	1.2916	14	-7.75	<.0001
	0	3	-7.6129	1.3606	14	-5.60	<.0001
	0	4	-5.0324	0.9314	14	-5.40	<.0001
	0	5	-3.1478	0.3652	14	-8.62	<.0001
	0.25	1	-3.4546	0.6999	14	-4.94	0.0002
	0.25	2	-9.1222	1.2831	14	-7.11	<.0001
	0.25	3	-6.7302	1.4131	14	-4.76	0.0003
	0.25	4	-4.1498	0.9375	14	-4.43	0.0006

Table J1. *Difference of least squares means for pulp cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
	0.25	5	-2.2651	0.4484	14	-5.05	0.0002
	1	2	-5.6677	1.6244	14	-3.49	0.0036
	1	3	-3.2757	1.5804	14	-2.07	0.0571
	1	4	-0.6952	1.2346	14	-0.56	0.5823
	1	5	1.1894	0.7127	14	1.67	0.1173
	2	3	2.3920	1.5239	14	1.57	0.1388
	2	4	4.9724	1.3927	14	3.57	0.0031
	2	5	6.8571	1.3179	14	5.20	0.0001
	3	4	2.5804	1.2711	14	2.03	0.0618
	3	5	4.4651	1.2967	14	3.44	0.0040
	4	5	1.8847	1.0353	14	1.82	0.0901
Study B							
Live cells	0	0.25	-9.6550	1.7301	9	-5.58	0.0003
	0	1	-13.5367	1.6694	9	-8.11	<.0001
	0	2	-12.1867	1.7722	9	-6.88	<.0001
	0	3	-11.5925	1.2064	9	-9.61	<.0001
	0	4	-0.1900	1.2066	9	-0.16	0.8784
	0	5	-2.9425	1.4683	9	-2.00	0.0761
	0.25	1	-3.8817	3.0501	9	-1.27	0.2350
	0.25	2	-2.5317	2.9000	9	-0.87	0.4054
	0.25	3	-1.9375	2.1577	9	-0.90	0.3926
	0.25	4	9.4650	2.0933	9	4.52	0.0014
	0.25	5	6.7125	2.1182	9	3.17	0.0114
	1	2	1.3500	1.2434	9	1.09	0.3058
	1	3	1.9442	2.0167	9	0.96	0.3602
	1	4	13.3467	1.8016	9	7.41	<.0001
	1	5	10.5942	2.3511	9	4.51	0.0015
	2	3	0.5942	1.4174	9	0.42	0.6849
	2	4	11.9967	2.2002	9	5.45	0.0004
	2	5	9.2442	2.3339	9	3.96	0.0033
	3	4	11.4025	1.8690	9	6.10	0.0002
	3	5	8.6500	1.6635	9	5.20	0.0006
	4	5	-2.7525	1.2970	9	-2.12	0.0628
MHCII+ B cells+	0	0.25	-1.2667	0.1253	9	-10.11	<.0001
	0	1	-12.1825	2.9036	9	-4.20	0.0023
	0	2	-12.0450	1.8015	9	-6.69	<.0001
	0	3	-12.6717	1.6376	9	-7.74	<.0001
	0	4	-2.5392	0.7223	9	-3.52	0.0066
	0	5	-1.7633	0.4898	9	-3.60	0.0057
	0.25	1	-10.9158	2.8871	9	-3.78	0.0043
	0.25	2	-10.7783	1.7187	9	-6.27	0.0001
	0.25	3	-11.4050	1.6067	9	-7.10	<.0001
	0.25	4	-1.2725	0.6945	9	-1.83	0.1002
	0.25	5	-0.4967	0.4827	9	-1.03	0.3303
	1	2	0.1375	2.6393	9	0.05	0.9596

Table J1. *Difference of least squares means for pulp cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
	1	3	-0.4892	2.8525	9	-0.17	0.8676
	1	4	9.6433	3.3100	9	2.91	0.0172
	1	5	10.4192	3.0033	9	3.47	0.0071
	2	3	-0.6267	1.5080	9	-0.42	0.6875
	2	4	9.5058	2.0114	9	4.73	0.0011
	2	5	10.2817	1.8816	9	5.46	0.0004
	3	4	10.1325	1.6181	9	6.26	0.0001
	3	5	10.9083	1.5070	9	7.24	<.0001
	4	5	0.7758	0.7442	9	1.04	0.3244
CD4- CD8+	0	0.25	-1.9000	0.3498	9	-5.43	0.0004
	0	1	-3.6533	0.4453	9	-8.20	<.0001
	0	2	-2.4850	0.4484	9	-5.54	0.0004
	0	3	-2.0900	0.3416	9	-6.12	0.0002
	0	4	-0.5458	0.09998	9	-5.46	0.0004
	0	5	-0.4142	0.1609	9	-2.57	0.0300
	0.25	1	-1.7533	0.6649	9	-2.64	0.0270
	0.25	2	-0.5850	0.7063	9	-0.83	0.4290
	0.25	3	-0.1900	0.4845	9	-0.39	0.7041
	0.25	4	1.3542	0.3905	9	3.47	0.0071
	0.25	5	1.4858	0.3806	9	3.90	0.0036
	1	2	1.1683	0.4093	9	2.85	0.0190
	1	3	1.5633	0.5074	9	3.08	0.0131
	1	4	3.1075	0.4507	9	6.89	<.0001
	1	5	3.2392	0.4553	9	7.11	<.0001
	2	3	0.3950	0.3672	9	1.08	0.3100
	2	4	1.9392	0.4899	9	3.96	0.0033
	2	5	2.0708	0.4341	9	4.77	0.0010
	3	4	1.5442	0.4113	9	3.75	0.0045
	3	5	1.6758	0.3678	9	4.56	0.0014
	4	5	0.1317	0.1820	9	0.72	0.4878
CD4+ CD8-	0	0.25	-3.4767	0.4284	9	-8.12	<.0001
	0	1	-8.3225	1.0370	9	-8.03	<.0001
	0	2	-6.0192	0.9518	9	-6.32	0.0001
	0	3	-5.3975	0.7370	9	-7.32	<.0001
	0	4	-1.4058	0.4048	9	-3.47	0.0070
	0	5	-1.4033	0.4901	9	-2.86	0.0187
	0.25	1	-4.8458	1.0217	9	-4.74	0.0011
	0.25	2	-2.5425	1.1173	9	-2.28	0.0489
	0.25	3	-1.9208	0.7909	9	-2.43	0.0381
	0.25	4	2.0708	0.4359	9	4.75	0.0010
	0.25	5	2.0733	0.3150	9	6.58	0.0001
	1	2	2.3033	0.9657	9	2.39	0.0409
	1	3	2.9250	0.9820	9	2.98	0.0155
	1	4	6.9167	1.1051	9	6.26	0.0001
	1	5	6.9192	1.0525	9	6.57	0.0001
	2	3	0.6217	0.6214	9	1.00	0.3432

Table J1. *Difference of least squares means for pulp cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
	2	4	4.6133	1.0560	9	4.37	0.0018
	2	5	4.6158	1.1737	9	3.93	0.0034
	3	4	3.9917	0.7742	9	5.16	0.0006
	3	5	3.9942	0.8594	9	4.65	0.0012
	4	5	0.002500	0.4264	9	0.01	0.9954
γδ T cell+ CD8-	0	0.25	-7.1725	1.4801	9	-4.85	0.0009
	0	1	-5.2842	0.6724	9	-7.86	<.0001
	0	2	-1.8517	0.3129	9	-5.92	0.0002
	0	3	-1.6792	0.3783	9	-4.44	0.0016
	0	4	-0.4275	0.1672	9	-2.56	0.0309
	0	5	-0.4692	0.2361	9	-1.99	0.0782
	0.25	1	1.8883	1.8649	9	1.01	0.3377
	0.25	2	5.3208	1.5416	9	3.45	0.0073
	0.25	3	5.4933	1.7005	9	3.23	0.0103
	0.25	4	6.7450	1.4759	9	4.57	0.0013
	0.25	5	6.7033	1.4355	9	4.67	0.0012
	1	2	3.4325	0.7360	9	4.66	0.0012
	1	3	3.6050	0.5954	9	6.05	0.0002
	1	4	4.8567	0.6571	9	7.39	<.0001
	1	5	4.8150	0.7792	9	6.18	0.0002
	2	3	0.1725	0.3455	9	0.50	0.6295
	2	4	1.4242	0.2451	9	5.81	0.0003
	2	5	1.3825	0.4092	9	3.38	0.0081
	3	4	1.2517	0.3414	9	3.67	0.0052
	3	5	1.2100	0.4283	9	2.83	0.0199
	4	5	-0.04167	0.2534	9	-0.16	0.8730
αβ1 T cell+	0	0.25	-3.8400	0.5452	9	-7.04	<.0001
	0	1	-9.3992	0.9718	9	-9.67	<.0001
	0	2	-7.5258	0.9393	9	-8.01	<.0001
	0	3	-6.1992	0.9740	9	-6.36	0.0001
	0	4	-2.3200	0.5778	9	-4.02	0.0030
	0	5	-4.2983	0.4524	9	-9.50	<.0001
	0.25	1	-5.5592	1.0418	9	-5.34	0.0005
	0.25	2	-3.6858	1.2010	9	-3.07	0.0134
	0.25	3	-2.3592	1.0929	9	-2.16	0.0592
	0.25	4	1.5200	0.4753	9	3.20	0.0109
	0.25	5	-0.4583	0.5240	9	-0.87	0.4045
	1	2	1.8733	0.8797	9	2.13	0.0621
	1	3	3.2000	0.9263	9	3.45	0.0072
	1	4	7.0792	1.1294	9	6.27	0.0001
	1	5	5.1008	1.0182	9	5.01	0.0007
	2	3	1.3267	0.7762	9	1.71	0.1216
	2	4	5.2058	1.0260	9	5.07	0.0007
	2	5	3.2275	1.1638	9	2.77	0.0216
	3	4	3.8792	1.0383	9	3.74	0.0047
	3	5	1.9008	1.0975	9	1.73	0.1173

Table J1. *Difference of least squares means for pulp cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
	4	5	-1.9783	0.7520	9	-2.63	0.0273
αβ1 T cell+	0	0.25	-0.8233	0.2108	9	-3.91	0.0036
CD4- CD8+	0	1	-2.0908	0.3376	9	-6.19	0.0002
	0	2	-1.5767	0.2838	9	-5.55	0.0004
	0	3	-1.0575	0.2278	9	-4.64	0.0012
	0	4	-0.2258	0.1397	9	-1.62	0.1405
	0	5	-1.9275	0.2568	9	-7.51	<.0001
	0.25	1	-1.2675	0.2588	9	-4.90	0.0008
	0.25	2	-0.7533	0.2334	9	-3.23	0.0104
	0.25	3	-0.2342	0.2914	9	-0.80	0.4424
	0.25	4	0.5975	0.1347	9	4.44	0.0016
	0.25	5	-1.1042	0.3683	9	-3.00	0.0150
	1	2	0.5142	0.2672	9	1.92	0.0865
	1	3	1.0333	0.2978	9	3.47	0.0070
	1	4	1.8650	0.2573	9	7.25	<.0001
	1	5	0.1633	0.4373	9	0.37	0.7174
	2	3	0.5192	0.2768	9	1.88	0.0935
	2	4	1.3508	0.2531	9	5.34	0.0005
	2	5	-0.3508	0.3436	9	-1.02	0.3339
	3	4	0.8317	0.2113	9	3.94	0.0034
	3	5	-0.8700	0.2605	9	-3.34	0.0087
	4	5	-1.7017	0.3246	9	-5.24	0.0005
αβ1 T cell+	0	0.25	-0.02083	0.05928	9	-0.35	0.7334
CD4+ CD8+	0	1	-0.5383	0.1729	9	-3.11	0.0124
	0	2	-0.5517	0.1371	9	-4.02	0.0030
	0	3	-0.4700	0.1700	9	-2.77	0.0219
	0	4	-0.3067	0.1524	9	-2.01	0.0751
	0	5	-0.8717	0.1110	9	-7.85	<.0001
	0.25	1	-0.5175	0.1383	9	-3.74	0.0046
	0.25	2	-0.5308	0.1145	9	-4.63	0.0012
	0.25	3	-0.4492	0.1476	9	-3.04	0.0140
	0.25	4	-0.2858	0.1319	9	-2.17	0.0583
	0.25	5	-0.8508	0.1480	9	-5.75	0.0003
	1	2	-0.01333	0.1049	9	-0.13	0.9017
	1	3	0.06833	0.08464	9	0.81	0.4403
	1	4	0.2317	0.1377	9	1.68	0.1267
	1	5	-0.3333	0.2425	9	-1.37	0.2025
	2	3	0.08167	0.09909	9	0.82	0.4311
	2	4	0.2450	0.1244	9	1.97	0.0805
	2	5	-0.3200	0.2212	9	-1.45	0.1819
	3	4	0.1633	0.1232	9	1.33	0.2176
	3	5	-0.4017	0.2561	9	-1.57	0.1512
	4	5	-0.5650	0.2234	9	-2.53	0.0323
αβ1 T cell+	0	0.25	-3.0350	0.3482	9	-8.72	<.0001
CD4+ CD8-	0	1	-6.7408	0.8250	9	-8.17	<.0001
	0	2	-5.5250	0.7908	9	-6.99	<.0001

Table J1. *Difference of least squares means for pulp cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
	0	3	-4.2958	0.6360	9	-6.75	<.0001
	0	4	-1.3542	0.3040	9	-4.46	0.0016
	0	5	-1.5867	0.4756	9	-3.34	0.0087
	0.25	1	-3.7058	0.8826	9	-4.20	0.0023
	0.25	2	-2.4900	0.9509	9	-2.62	0.0279
	0.25	3	-1.2608	0.6794	9	-1.86	0.0965
	0.25	4	1.6808	0.2893	9	5.81	0.0003
	0.25	5	1.4483	0.3082	9	4.70	0.0011
	1	2	1.2158	0.7350	9	1.65	0.1325
	1	3	2.4450	0.6672	9	3.66	0.0052
	1	4	5.3867	0.8853	9	6.08	0.0002
	1	5	5.1542	0.9208	9	5.60	0.0003
	2	3	1.2292	0.5617	9	2.19	0.0564
	2	4	4.1708	0.8358	9	4.99	0.0007
	2	5	3.9383	1.0413	9	3.78	0.0043
	3	4	2.9417	0.6929	9	4.25	0.0022
	3	5	2.7092	0.7812	9	3.47	0.0071
	4	5	-0.2325	0.4355	9	-0.53	0.6063
αβ2 T cell+	0	0.25	-2.1192	0.2736	9	-7.75	<.0001
	0	1	-3.8750	0.5214	9	-7.43	<.0001
	0	2	-3.0550	0.2601	9	-11.75	<.0001
	0	3	-2.8775	0.3644	9	-7.90	<.0001
	0	4	-1.2067	0.2830	9	-4.26	0.0021
	0	5	-3.5258	0.1212	9	-29.10	<.0001
	0.25	1	-1.7558	0.4231	9	-4.15	0.0025
	0.25	2	-0.9358	0.4002	9	-2.34	0.0441
	0.25	3	-0.7583	0.4161	9	-1.82	0.1017
	0.25	4	0.9125	0.3111	9	2.93	0.0167
	0.25	5	-1.4067	0.2815	9	-5.00	0.0007
	1	2	0.8200	0.4613	9	1.78	0.1092
	1	3	0.9975	0.5060	9	1.97	0.0802
	1	4	2.6683	0.5569	9	4.79	0.0010
	1	5	0.3492	0.5450	9	0.64	0.5377
	2	3	0.1775	0.3265	9	0.54	0.5999
	2	4	1.8483	0.3187	9	5.80	0.0003
	2	5	-0.4708	0.3462	9	-1.36	0.2069
	3	4	1.6708	0.3695	9	4.52	0.0014
	3	5	-0.6483	0.4351	9	-1.49	0.1704
	4	5	-2.3192	0.3411	9	-6.80	<.0001
αβ2 T cell+	0	0.25	-0.8108	0.1155	9	-7.02	<.0001
CD4- CD8+	0	1	-1.1425	0.1448	9	-7.89	<.0001
	0	2	-0.9833	0.1069	9	-9.20	<.0001
	0	3	-0.7258	0.08936	9	-8.12	<.0001
	0	4	-0.4142	0.08032	9	-5.16	0.0006
	0	5	-1.6608	0.2383	9	-6.97	<.0001
	0.25	1	-0.3317	0.1478	9	-2.24	0.0515

Table J1. *Difference of least squares means for pulp cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
	0.25	2	-0.1725	0.07937	9	-2.17	0.0578
	0.25	3	0.08500	0.1281	9	0.66	0.5238
	0.25	4	0.3967	0.1072	9	3.70	0.0049
	0.25	5	-0.8500	0.2802	9	-3.03	0.0142
	1	2	0.1592	0.1512	9	1.05	0.3200
	1	3	0.4167	0.1380	9	3.02	0.0145
	1	4	0.7283	0.1565	9	4.65	0.0012
	1	5	-0.5183	0.3062	9	-1.69	0.1248
	2	3	0.2575	0.1497	9	1.72	0.1194
	2	4	0.5692	0.1106	9	5.15	0.0006
	2	5	-0.6775	0.2903	9	-2.33	0.0445
	3	4	0.3117	0.09727	9	3.20	0.0108
	3	5	-0.9350	0.2228	9	-4.20	0.0023
	4	5	-1.2467	0.2511	9	-4.97	0.0008
αβ2 T cell+	0	0.25	0.02917	0.02392	9	1.22	0.2536
CD4+ CD8+	0	1	-0.1675	0.07394	9	-2.27	0.0497
	0	2	-0.2100	0.06299	9	-3.33	0.0087
	0	3	-0.2450	0.08964	9	-2.73	0.0231
	0	4	-0.2100	0.1416	9	-1.48	0.1721
	0	5	-1.0042	0.1653	9	-6.08	0.0002
	0.25	1	-0.1967	0.06789	9	-2.90	0.0177
	0.25	2	-0.2392	0.05787	9	-4.13	0.0025
	0.25	3	-0.2742	0.07821	9	-3.51	0.0067
	0.25	4	-0.2392	0.1285	9	-1.86	0.0956
	0.25	5	-1.0333	0.1806	9	-5.72	0.0003
	1	2	-0.04250	0.04950	9	-0.86	0.4129
	1	3	-0.07750	0.04695	9	-1.65	0.1332
	1	4	-0.04250	0.1166	9	-0.36	0.7239
	1	5	-0.8367	0.1829	9	-4.57	0.0013
	2	3	-0.03500	0.07160	9	-0.49	0.6366
	2	4	-11E-14	0.1056	9	-0.00	1.0000
	2	5	-0.7942	0.1735	9	-4.58	0.0013
	3	4	0.03500	0.1233	9	0.28	0.7830
	3	5	-0.7592	0.2117	9	-3.59	0.0059
	4	5	-0.7942	0.2366	9	-3.36	0.0084
αβ2 T cell+	0	0.25	-0.3900	0.07349	9	-5.31	0.0005
CD4- CD8-	0	1	-0.2217	0.09384	9	-2.36	0.0424
	0	2	-0.1158	0.02535	9	-4.57	0.0013
	0	3	-0.2075	0.02453	9	-8.46	<.0001
	0	4	-0.2583	0.04538	9	-5.69	0.0003
	0	5	-0.6875	0.1077	9	-6.38	0.0001
	0.25	1	0.1683	0.1072	9	1.57	0.1507
	0.25	2	0.2742	0.07508	9	3.65	0.0053
	0.25	3	0.1825	0.07999	9	2.28	0.0484
	0.25	4	0.1317	0.08967	9	1.47	0.1761
	0.25	5	-0.2975	0.09502	9	-3.13	0.0121

Table J1. *Difference of least squares means for pulp cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
	1	2	0.1058	0.08347	9	1.27	0.2366
	1	3	0.01417	0.09326	9	0.15	0.8826
	1	4	-0.03667	0.09139	9	-0.40	0.6976
	1	5	-0.4658	0.09556	9	-4.87	0.0009
	2	3	-0.09167	0.02957	9	-3.10	0.0127
	2	4	-0.1425	0.04107	9	-3.47	0.0071
	2	5	-0.5717	0.1133	9	-5.04	0.0007
	3	4	-0.05083	0.05460	9	-0.93	0.3762
	3	5	-0.4800	0.1166	9	-4.12	0.0026
	4	5	-0.4292	0.1213	9	-3.54	0.0063
αβ2 T cell+	0	0.25	-1.0417	0.1770	9	-5.88	0.0002
CD4+ CD8-	0	1	-2.3567	0.4116	9	-5.73	0.0003
	0	2	-1.8967	0.2859	9	-6.63	<.0001
	0	3	-1.5242	0.2263	9	-6.73	<.0001
	0	4	-0.3558	0.1113	9	-3.20	0.0109
	0	5	-0.5100	0.1412	9	-3.61	0.0057
	0.25	1	-1.3150	0.3301	9	-3.98	0.0032
	0.25	2	-0.8550	0.3396	9	-2.52	0.0329
	0.25	3	-0.4825	0.2239	9	-2.15	0.0596
	0.25	4	0.6858	0.1478	9	4.64	0.0012
	0.25	5	0.5317	0.1241	9	4.28	0.0020
	1	2	0.4600	0.3778	9	1.22	0.2543
	1	3	0.8325	0.3249	9	2.56	0.0306
	1	4	2.0008	0.3919	9	5.11	0.0006
	1	5	1.8467	0.3940	9	4.69	0.0011
	2	3	0.3725	0.1651	9	2.26	0.0505
	2	4	1.5408	0.3038	9	5.07	0.0007
	2	5	1.3867	0.3428	9	4.05	0.0029
	3	4	1.1683	0.2189	9	5.34	0.0005
	3	5	1.0142	0.2391	9	4.24	0.0022
	4	5	-0.1542	0.1426	9	-1.08	0.3079
B cell+ IgM-	0	0.25	0.05250	0.05795	9	0.91	0.3886
	0	1	-2.2942	0.6005	9	-3.82	0.0041
	0	2	-1.5167	0.4673	9	-3.25	0.0101
	0	3	-1.9233	0.4147	9	-4.64	0.0012
	0	4	-0.3283	0.1518	9	-2.16	0.0588
	0	5	-0.1333	0.1500	9	-0.89	0.3972
	0.25	1	-2.3467	0.5794	9	-4.05	0.0029
	0.25	2	-1.5692	0.4342	9	-3.61	0.0056
	0.25	3	-1.9758	0.4025	9	-4.91	0.0008
	0.25	4	-0.3808	0.1268	9	-3.00	0.0149
	0.25	5	-0.1858	0.1114	9	-1.67	0.1297
	1	2	0.7775	0.7217	9	1.08	0.3094
	1	3	0.3708	0.7354	9	0.50	0.6262
	1	4	1.9658	0.6293	9	3.12	0.0122
	1	5	2.1608	0.5914	9	3.65	0.0053

Table J1. *Difference of least squares means for pulp cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
	2	3	-0.4067	0.3323	9	-1.22	0.2521
	2	4	1.1883	0.3779	9	3.14	0.0118
	2	5	1.3833	0.4182	9	3.31	0.0091
	3	4	1.5950	0.3959	9	4.03	0.0030
	3	5	1.7900	0.3773	9	4.74	0.0011
	4	5	0.1950	0.1891	9	1.03	0.3293
B cell+ IgG+	0	0.25	-0.09750	0.03542	9	-2.75	0.0224
	0	1	-0.3942	0.1246	9	-3.16	0.0115
	0	2	-0.2100	0.02716	9	-7.73	<.0001
	0	3	-0.4558	0.07417	9	-6.15	0.0002
	0	4	-0.2117	0.05054	9	-4.19	0.0023
	0	5	-0.1425	0.04342	9	-3.28	0.0095
	0.25	1	-0.2967	0.1416	9	-2.09	0.0657
	0.25	2	-0.1125	0.04265	9	-2.64	0.0270
	0.25	3	-0.3583	0.06358	9	-5.64	0.0003
	0.25	4	-0.1142	0.04829	9	-2.36	0.0423
	0.25	5	-0.04500	0.02161	9	-2.08	0.0670
	1	2	0.1842	0.1331	9	1.38	0.1999
	1	3	-0.06167	0.1337	9	-0.46	0.6557
	1	4	0.1825	0.1444	9	1.26	0.2380
	1	5	0.2517	0.1353	9	1.86	0.0958
	2	3	-0.2458	0.06002	9	-4.10	0.0027
	2	4	-0.00167	0.05164	9	-0.03	0.9750
	2	5	0.06750	0.05223	9	1.29	0.2284
	3	4	0.2442	0.08114	9	3.01	0.0147
	3	5	0.3133	0.06591	9	4.75	0.0010
	4	5	0.06917	0.05132	9	1.35	0.2107
B cell+ IgG-	0	0.25	-1.0100	0.1082	9	-9.33	<.0001
	0	1	-11.7142	2.8034	9	-4.18	0.0024
	0	2	-12.4742	1.7649	9	-7.07	<.0001
	0	3	-11.7875	1.5359	9	-7.67	<.0001
	0	4	-2.4192	0.6575	9	-3.68	0.0051
	0	5	-1.8267	0.4548	9	-4.02	0.0030
	0.25	1	-10.7042	2.7626	9	-3.87	0.0038
	0.25	2	-11.4642	1.7484	9	-6.56	0.0001
	0.25	3	-10.7775	1.5146	9	-7.12	<.0001
	0.25	4	-1.4092	0.6257	9	-2.25	0.0508
	0.25	5	-0.8167	0.4318	9	-1.89	0.0912
	1	2	-0.7600	2.3889	9	-0.32	0.7576
	1	3	-0.07333	2.7145	9	-0.03	0.9790
	1	4	9.2950	3.1601	9	2.94	0.0165
	1	5	9.8875	2.8382	9	3.48	0.0069
	2	3	0.6867	1.5557	9	0.44	0.6694
	2	4	10.0550	1.9633	9	5.12	0.0006
	2	5	10.6475	1.9010	9	5.60	0.0003
	3	4	9.3683	1.4303	9	6.55	0.0001

Table J1. *Difference of least squares means for pulp cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
	3	5	9.9608	1.4453	9	6.89	<.0001
	4	5	0.5925	0.6558	9	0.90	0.3898
Study B PBS Control							
Live cells	0	0.25	1.4725	1.6522	5.279	0.89	0.4116
	0	1	-4.4525	3.3081	3.508	-1.35	0.2587
	0	2	-7.8125	1.6390	5.313	-4.77	0.0043
	0	3	-5.2925	3.2474	3.529	-1.63	0.1879
	0	4	-9.1200	1.5164	5.642	-6.01	0.0012
	0	5	-5.9125	6.0654	3.144	-0.97	0.3986
	0.25	1	-5.9250	3.4573	4.075	-1.71	0.1604
	0.25	2	-9.2850	1.9224	5.999	-4.83	0.0029
	0.25	3	-6.7650	3.3992	4.117	-1.99	0.1154
	0.25	4	-10.5925	1.8190	5.9	-5.82	0.0012
	0.25	5	-7.3850	6.1480	3.311	-1.20	0.3085
	1	2	-3.3600	3.4510	4.052	-0.97	0.3847
	1	3	-0.8400	4.4462	5.998	-0.19	0.8564
	1	4	-4.6675	3.3945	3.839	-1.38	0.2439
	1	5	-1.4600	6.7832	4.561	-0.22	0.8389
	2	3	2.5200	3.3928	4.092	0.74	0.4980
	2	4	-1.3075	1.8070	5.917	-0.72	0.4969
	2	5	1.9000	6.1445	3.304	0.31	0.7756
	3	4	-3.8275	3.3353	3.872	-1.15	0.3171
	3	5	-0.6200	6.7538	4.508	-0.09	0.9308
	4	5	3.2075	6.1129	3.24	0.52	0.6336
MHCII+ B cells+	0	0.25	0.04000	0.1775	5.821	0.23	0.8294
	0	1	-4.6425	0.5247	3.43	-8.85	0.0018
	0	2	-3.7675	1.0339	3.106	-3.64	0.0337
	0	3	-5.1525	1.9781	3.029	-2.60	0.0793
	0	4	-7.5275	1.0736	3.098	-7.01	0.0054
	0	5	-9.8175	3.6717	3.008	-2.67	0.0752
	0.25	1	-4.6825	0.5194	3.303	-9.02	0.0019
	0.25	2	-3.8075	1.0312	3.074	-3.69	0.0331
	0.25	3	-5.1925	1.9767	3.02	-2.63	0.0780
	0.25	4	-7.5675	1.0711	3.069	-7.07	0.0054
	0.25	5	-9.8575	3.6710	3.006	-2.69	0.0746
	1	2	0.8750	1.1433	4.384	0.77	0.4832
	1	3	-0.5100	2.0374	3.394	-0.25	0.8167
	1	4	-2.8850	1.1794	4.292	-2.45	0.0664
	1	5	-5.1750	3.7040	3.114	-1.40	0.2536
	2	3	-1.3850	2.2237	4.509	-0.62	0.5635
	2	4	-3.7600	1.4781	5.991	-2.54	0.0439
	2	5	-6.0500	3.8096	3.465	-1.59	0.1983
	3	4	-2.3750	2.2424	4.611	-1.06	0.3418
	3	5	-4.6650	4.1662	4.602	-1.12	0.3178
	4	5	-2.2900	3.8206	3.502	-0.60	0.5855

Table J1. *Difference of least squares means for pulp cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
CD4- CD8+	0	0.25	-0.2750	0.1599	3.5	-1.72	0.1707
	0	1	-3.1825	0.8222	2.916	-3.87	0.0321
	0	2	-1.7625	0.4279	3.009	-4.12	0.0258
	0	3	-2.3325	0.8815	3.007	-2.65	0.0771
	0	4	-2.1250	0.3613	2.804	-5.88	0.0118
	0	5	-1.2850	0.5266	3.001	-2.44	0.0925
	0.25	1	-2.9075	0.8300	3.008	-3.50	0.0392
	0.25	2	-1.4875	0.4450	3.387	-3.34	0.0370
	0.25	3	-2.0575	0.8891	3.113	-2.31	0.1005
	0.25	4	-1.8500	0.3847	3.774	-4.81	0.0099
	0.25	5	-1.0100	0.5421	3.444	-1.86	0.1473
	1	2	1.4200	0.9174	3.913	1.55	0.1981
	1	3	0.8500	1.1968	5.086	0.71	0.5088
	1	4	1.0575	0.9002	3.86	1.17	0.3075
	1	5	1.8975	0.9758	4.713	1.94	0.1129
	2	3	-0.5700	0.9727	4.035	-0.59	0.5891
	2	4	-0.3625	0.5577	5.741	-0.65	0.5408
	2	5	0.4775	0.6749	5.676	0.71	0.5072
	3	4	0.2075	0.9569	3.882	0.22	0.8392
	3	5	1.0475	1.0290	4.718	1.02	0.3580
	4	5	0.8400	0.6434	5.114	1.31	0.2473
CD4+ CD8+	0	0.25	-0.00750	0.02874	5.61	-0.26	0.8035
	0	1	-0.7850	0.09235	2.923	-8.50	0.0038
	0	2	-0.5675	0.1622	3.279	-3.50	0.0343
	0	3	-1.0175	0.4712	3.301	-2.16	0.1114
	0	4	-0.8225	0.1459	3.182	-5.64	0.0094
	0	5	-0.6700	0.3896	2.695	-1.72	0.1942
	0.25	1	-0.7775	0.09317	3.049	-8.34	0.0034
	0.25	2	-0.5600	0.1624	3.298	-3.45	0.0354
	0.25	3	-1.0100	0.4709	3.295	-2.14	0.1132
	0.25	4	-0.8150	0.1461	3.213	-5.58	0.0095
	0.25	5	-0.6625	0.3895	2.704	-1.70	0.1974
	1	2	0.2175	0.1756	4.69	1.24	0.2739
	1	3	-0.2325	0.4683	3.36	-0.50	0.6502
	1	4	-0.03750	0.1621	4.89	-0.23	0.8264
	1	5	0.1150	0.3900	2.947	0.29	0.7877
	2	3	-0.4500	0.4734	3.67	-0.95	0.4002
	2	4	-0.2550	0.2012	5.622	-1.27	0.2550
	2	5	-0.1025	0.4006	3.55	-0.26	0.8122
	3	4	0.1950	0.4714	3.572	0.41	0.7027
	3	5	0.3475	0.5645	5.834	0.62	0.5614
	4	5	0.1525	0.3969	3.4	0.38	0.7236
CD4+ CD8-	0	0.25	0.1825	0.2279	5.538	0.80	0.4563
	0	1	-4.7875	0.6236	3.367	-7.68	0.0030
	0	2	-3.7125	0.7369	3.229	-5.04	0.0126
	0	3	-4.2925	2.0529	3.069	-2.09	0.1256

Table J1. *Difference of least squares means for pulp cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
	0	4	-3.6625	0.4068	3.956	-9.00	0.0009
	0	5	-2.9425	1.2723	2.979	-2.31	0.1044
	0.25	1	-4.9700	0.6202	3.289	-8.01	0.0029
	0.25	2	-3.8950	0.7344	3.189	-5.30	0.0112
	0.25	3	-4.4750	2.0522	3.066	-2.18	0.1154
	0.25	4	-3.8450	0.4011	3.829	-9.59	0.0008
	0.25	5	-3.1250	1.2709	2.961	-2.46	0.0921
	1	2	1.0750	0.9171	5.306	1.17	0.2911
	1	3	0.4950	2.1092	3.442	0.23	0.8277
	1	4	1.1250	0.7019	4.898	1.60	0.1711
	1	5	1.8450	1.3815	4.116	1.34	0.2508
	2	3	-0.5800	2.1270	3.593	-0.27	0.8000
	2	4	0.05000	0.7997	4.225	0.06	0.9530
	2	5	0.7700	1.4211	4.55	0.54	0.6134
	3	4	0.6300	2.0713	3.178	0.30	0.7799
	3	5	1.3500	2.3519	4.833	0.57	0.5916
	4	5	0.7200	1.3097	3.369	0.55	0.6168
γδ T cell+ CD8+	0	0.25	-0.1150	0.06486	4.256	-1.77	0.1466
	0	1	-0.8575	0.1816	3.027	-4.72	0.0176
	0	2	-0.4625	0.09146	3.511	-5.06	0.0101
	0	3	-0.6200	0.2307	3.054	-2.69	0.0731
	0	4	-0.4650	0.08387	3.506	-5.54	0.0076
	0	5	-0.3250	0.1545	3.219	-2.10	0.1200
	0.25	1	-0.7425	0.1858	3.261	-4.00	0.0240
	0.25	2	-0.3475	0.1014	4.317	-3.43	0.0236
	0.25	3	-0.5050	0.2341	3.299	-2.16	0.1117
	0.25	4	-0.3500	0.09563	5.372	-3.66	0.0129
	0.25	5	-0.2100	0.1602	3.742	-1.31	0.2646
	1	2	0.3950	0.1950	3.692	2.03	0.1186
	1	3	0.2375	0.2849	5.263	0.83	0.4407
	1	4	0.3925	0.1946	3.792	2.02	0.1178
	1	5	0.5325	0.2302	5.099	2.31	0.0676
	2	3	-0.1575	0.2416	3.737	-0.65	0.5524
	2	4	-0.00250	0.1153	5.872	-0.02	0.9834
	2	5	0.1375	0.1717	4.561	0.80	0.4629
	3	4	0.1550	0.2420	3.59	0.64	0.5604
	3	5	0.2950	0.2717	5.127	1.09	0.3261
	4	5	0.1400	0.1705	4.34	0.82	0.4543
γδ T cell+ CD8-	0	0.25	-1.3150	0.6237	2.828	-2.11	0.1311
	0	1	-2.1975	0.2877	4.199	-7.64	0.0013
	0	2	-1.1900	0.3477	3.839	-3.42	0.0285
	0	3	-0.9025	0.5096	3.367	-1.77	0.1646
	0	4	-0.6400	0.2722	4.306	-2.35	0.0738
	0	5	-0.8900	0.5322	3.031	-1.67	0.1921
	0.25	1	-0.8825	0.6437	3.634	-1.37	0.2489
	0.25	2	0.1250	0.6636	4.165	0.19	0.8594

Table J1. *Difference of least squares means for pulp cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
	0.25	3	0.4125	0.7425	5.667	0.56	0.5997
	0.25	4	0.6750	0.6400	3.504	1.05	0.3588
	0.25	5	0.4250	0.7605	5.585	0.56	0.5980
	1	2	1.0075	0.4008	5.453	2.51	0.0496
	1	3	1.2950	0.5380	4.404	2.41	0.0680
	1	4	1.5575	0.3459	5.598	4.50	0.0049
	1	5	1.3075	0.5601	4.071	2.33	0.0787
	2	3	0.2875	0.5634	4.915	0.51	0.6319
	2	4	0.5500	0.3923	5.371	1.40	0.2159
	2	5	0.3000	0.5841	4.812	0.51	0.6303
	3	4	0.2625	0.5330	4.258	0.49	0.6467
	3	5	0.01250	0.6757	6.1	0.02	0.9858
	4	5	-0.2500	0.5553	3.903	-0.45	0.6764
αβ1 T cell+	0	0.25	-0.04500	0.3361	4.946	-0.13	0.8988
	0	1	-6.7625	1.2331	3.343	-5.48	0.0089
	0	2	-5.0000	1.0960	3.441	-4.56	0.0146
	0	3	-6.0750	2.9523	3.057	-2.06	0.1301
	0	4	-4.4975	0.7893	3.896	-5.70	0.0051
	0	5	-5.0475	1.0899	3.446	-4.63	0.0139
	0.25	1	-6.7175	1.2118	3.127	-5.54	0.0104
	0.25	2	-4.9550	1.0719	3.163	-4.62	0.0170
	0.25	3	-6.0300	2.9434	3.021	-2.05	0.1323
	0.25	4	-4.4525	0.7555	3.337	-5.89	0.0072
	0.25	5	-5.0025	1.0657	3.165	-4.69	0.0162
	1	2	1.7625	1.5989	5.908	1.10	0.3132
	1	3	0.6875	3.1735	3.972	0.22	0.8392
	1	4	2.2650	1.4065	4.976	1.61	0.1685
	1	5	1.7150	1.5948	5.899	1.08	0.3242
	2	3	-1.0750	3.1228	3.765	-0.34	0.7490
	2	4	0.5025	1.2880	5.35	0.39	0.7115
	2	5	-0.04750	1.4913	6	-0.03	0.9756
	3	4	1.5775	3.0288	3.374	0.52	0.6348
	3	5	1.0275	3.1207	3.756	0.33	0.7595
	4	5	-0.5500	1.2829	5.367	-0.43	0.6848
αβ1 T cell+	0	0.25	-0.05500	0.2074	5.703	-0.27	0.8002
CD4+ CD8-	0	1	-4.2150	0.5800	3.311	-7.27	0.0038
	0	2	-3.4375	0.6549	3.241	-5.25	0.0111
	0	3	-3.4875	1.8068	3.031	-1.93	0.1482
	0	4	-3.3375	0.4397	3.559	-7.59	0.0025
	0	5	-2.7375	1.1453	3.077	-2.39	0.0946
	0.25	1	-4.1600	0.5884	3.492	-7.07	0.0035
	0.25	2	-3.3825	0.6624	3.383	-5.11	0.0108
	0.25	3	-3.4325	1.8095	3.049	-1.90	0.1526
	0.25	4	-3.2825	0.4507	3.878	-7.28	0.0021

Table J1. *Difference of least squares means for pulp cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
	0.25	5	-2.6825	1.1496	3.122	-2.33	0.0984
	1	2	0.7775	0.8557	5.906	0.91	0.3991
	1	3	0.7275	1.8888	3.585	0.39	0.7219
	1	4	0.8775	0.7046	5.54	1.25	0.2631
	1	5	1.4775	1.2708	4.396	1.16	0.3042
	2	3	-0.05000	1.9132	3.75	-0.03	0.9805
	2	4	0.1000	0.7675	5.172	0.13	0.9013
	2	5	0.7000	1.3067	4.735	0.54	0.6164
	3	4	0.1500	1.8506	3.325	0.08	0.9400
	3	5	0.7500	2.1314	5.064	0.35	0.7391
	4	5	0.6000	1.2132	3.804	0.49	0.6481
αβ2 T cell+	0	0.25	-0.7000	0.1322	5.888	-5.29	0.0020
	0	1	-2.2475	0.6957	2.24	-3.23	0.0722
	0	2	-2.0250	0.5253	2.943	-3.86	0.0319
	0	3	-1.8775	0.6322	3.438	-2.97	0.0498
	0	4	-1.4050	0.1073	6.972	-13.10	<.0001
	0	5	-2.7050	0.1641	4.619	-16.48	<.0001
	0.25	1	-1.5475	0.6840	2.271	-2.26	0.1368
	0.25	2	-1.3250	0.5156	3.03	-2.57	0.0817
	0.25	3	-1.1775	0.6209	3.483	-1.90	0.1413
	0.25	4	-0.7050	0.1335	6.101	-5.28	0.0018
	0.25	5	-2.0050	0.1744	6.18	-11.50	<.0001
	1	2	0.2225	0.7088	4.911	0.31	0.7665
	1	3	0.3700	0.7493	6.393	0.49	0.6380
	1	4	0.8425	0.6952	2.255	1.21	0.3372
	1	5	-0.4575	0.6747	2.319	-0.68	0.5591
	2	3	0.1475	0.6616	6.733	0.22	0.8302
	2	4	0.6200	0.5244	2.951	1.18	0.3235
	2	5	-0.6800	0.5095	3.171	-1.33	0.2697
	3	4	0.4725	0.6310	3.433	0.75	0.5020
	3	5	-0.8275	0.6126	3.565	-1.35	0.2561
	4	5	-1.3000	0.1645	4.785	-7.90	0.0006
αβ2 T cell+	0	0.25	0.02250	0.04452	5.102	0.51	0.6343
CD4+ CD8-	0	1	-1.2700	0.2887	3.124	-4.40	0.0200
	0	2	-1.0100	0.2374	3.551	-4.25	0.0169
	0	3	-0.9125	0.4971	2.44	-1.84	0.1845
	0	4	-0.9450	0.08677	3.794	-10.89	0.0005
	0	5	-0.9325	0.3692	2.97	-2.53	0.0866
	0.25	1	-1.2925	0.2851	3.157	-4.53	0.0180
	0.25	2	-1.0325	0.2342	3.585	-4.41	0.0148
	0.25	3	-0.9350	0.4926	2.439	-1.90	0.1747
	0.25	4	-0.9675	0.08791	4.414	-11.01	0.0002
	0.25	5	-0.9550	0.3651	2.983	-2.62	0.0798
	1	2	0.2600	0.3205	5.862	0.81	0.4489

Table J1. *Difference of least squares means for pulp cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
	1	3	0.3575	0.5044	4.035	0.71	0.5173
	1	4	0.3250	0.2813	3.398	1.16	0.3226
	1	5	0.3375	0.4028	5.562	0.84	0.4366
	2	3	0.09750	0.4908	3.425	0.20	0.8538
	2	4	0.06500	0.2325	3.961	0.28	0.7938
	2	5	0.07750	0.3811	4.833	0.20	0.8471
	3	4	-0.03250	0.4848	2.51	-0.07	0.9515
	3	5	-0.02000	0.5341	5.249	-0.04	0.9715
	4	5	0.01250	0.3590	3.113	0.03	0.9743
B cell+ IgM+	0	0.25	-0.02250	0.08215	5.854	-0.27	0.7936
	0	1	-3.6900	0.3604	3.134	-10.24	0.0016
	0	2	-3.5375	0.9120	3.021	-3.88	0.0300
	0	3	-4.2175	1.5801	3.007	-2.67	0.0756
	0	4	-6.8950	1.0553	3.015	-6.53	0.0072
	0	5	-9.3175	3.4314	3.001	-2.72	0.0728
	0.25	1	-3.6675	0.3619	3.184	-10.13	0.0016
	0.25	2	-3.5150	0.9126	3.028	-3.85	0.0304
	0.25	3	-4.1950	1.5804	3.009	-2.65	0.0765
	0.25	4	-6.8725	1.0558	3.021	-6.51	0.0072
	0.25	5	-9.2950	3.4315	3.002	-2.71	0.0732
	1	2	0.1525	0.9778	3.898	0.16	0.8838
	1	3	-0.5275	1.6189	3.305	-0.33	0.7641
	1	4	-3.2050	1.1126	3.677	-2.88	0.0497
	1	5	-5.6275	3.4494	3.065	-1.63	0.1994
	2	3	-0.6800	1.8228	4.796	-0.37	0.7250
	2	4	-3.3575	1.3927	5.876	-2.41	0.0534
	2	5	-5.7800	3.5497	3.42	-1.63	0.1907
	3	4	-2.6775	1.8986	5.23	-1.41	0.2151
	3	5	-5.1000	3.7770	4.217	-1.35	0.2449
	4	5	-2.4225	3.5892	3.561	-0.67	0.5410
B cell+ IgM-	0	0.25	0.07250	0.04151	3.567	1.75	0.1643
	0	1	-0.6200	0.1393	3.525	-4.45	0.0149
	0	2	-0.5775	0.1296	3.614	-4.46	0.0141
	0	3	-0.7950	0.3575	3.075	-2.22	0.1105
	0	4	-0.9050	0.1891	3.276	-4.79	0.0141
	0	5	-1.1650	0.4797	3.041	-2.43	0.0923
	0.25	1	-0.6925	0.1341	3.05	-5.17	0.0135
	0.25	2	-0.6500	0.1240	3.059	-5.24	0.0129
	0.25	3	-0.8675	0.3555	3.007	-2.44	0.0923
	0.25	4	-0.9775	0.1853	3.026	-5.28	0.0130
	0.25	5	-1.2375	0.4782	3.004	-2.59	0.0811
	1	2	0.04250	0.1818	5.963	0.23	0.8229
	1	3	-0.1750	0.3795	3.831	-0.46	0.6697
	1	4	-0.2850	0.2280	5.46	-1.25	0.2623

Table J1. *Difference of least squares means for pulp cell responses by day* (Cont.)

Study/Factor	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
	1	5	-0.5450	0.4964	3.465	-1.10	0.3426
	2	3	-0.2175	0.3761	3.713	-0.58	0.5963
	2	4	-0.3275	0.2222	5.23	-1.47	0.1981
	2	5	-0.5875	0.4937	3.398	-1.19	0.3105
	3	4	-0.1100	0.4005	4.514	-0.27	0.7957
	3	5	-0.3700	0.5956	5.539	-0.62	0.5591
	4	5	-0.2600	0.5126	3.877	-0.51	0.6395
B cell+ IgG-	0	0.25	0.2175	0.1395	4.635	1.56	0.1842
	0	1	-4.3225	0.6853	3.198	-6.31	0.0066
	0	2	-3.8825	1.1470	3.069	-3.38	0.0415
	0	3	-4.7425	1.9007	3.025	-2.50	0.0874
	0	4	-7.6550	0.9856	3.094	-7.77	0.0040
	0	5	-7.6925	2.8197	3.011	-2.73	0.0718
	0.25	1	-4.5400	0.6775	3.059	-6.70	0.0064
	0.25	2	-4.1000	1.1424	3.021	-3.59	0.0366
	0.25	3	-4.9600	1.8979	3.007	-2.61	0.0792
	0.25	4	-7.8725	0.9802	3.028	-8.03	0.0039
	0.25	5	-7.9100	2.8178	3.003	-2.81	0.0674
	1	2	0.4400	1.3248	4.869	0.33	0.7536
	1	3	-0.4200	2.0130	3.746	-0.21	0.8456
	1	4	-3.3325	1.1879	5.326	-2.81	0.0352
	1	5	-3.3700	2.8966	3.343	-1.16	0.3209
	2	3	-0.8600	2.2132	4.918	-0.39	0.7138
	2	4	-3.7725	1.5023	5.864	-2.51	0.0467
	2	5	-3.8100	3.0391	3.958	-1.25	0.2789
	3	4	-2.9125	2.1340	4.49	-1.36	0.2367
	3	5	-2.9500	3.3961	5.256	-0.87	0.4229
	4	5	-0.03750	2.9820	3.713	-0.01	0.9906

Table J2. *Difference of least squares means for pulp cell responses by treatment*

Study/Factor	Treatment	Treatment	Estimate	Std. Error	DF	t Value	Pr > |t|
Study A							
CD45+	AlumIgG	IOmIgG	-7.0014	2.0504	14	-3.41	0.0042
	AlumIgG	mIgG	0.6082	2.1505	14	0.28	0.7814
	IOmIgG	mIgG	7.6097	2.1505	14	3.54	0.0033
CD4+ CD8-	AlumIgG	IOmIgG	-1.7921	0.6739	14	-2.66	0.0187
	AlumIgG	mIgG	0.08333	0.7068	14	0.12	0.9078
	IOmIgG	mIgG	1.8755	0.7068	14	2.65	0.0189
αβ1 T cell+	AlumIgG	IOmIgG	-1.5483	0.5370	14	-2.88	0.0120
	AlumIgG	mIgG	0.09343	0.5633	14	0.17	0.8706
	IOmIgG	mIgG	1.6418	0.5633	14	2.91	0.0113
B cells	AlumIgG	IOmIgG	-2.5900	0.9154	14	-2.83	0.0134
	AlumIgG	mIgG	-0.9621	0.9601	14	-1.00	0.3333
	IOmIgG	mIgG	1.6279	0.9601	14	1.70	0.1121
Study B							
γδ T cell+ CD8-	AlummIgG	IOmIgG	1.0971	0.4098	9	2.68	0.0253
	AlummIgG	mIgG	1.1918	0.4098	9	2.91	0.0174
	IOmIgG	mIgG	0.09464	0.4098	9	0.23	0.8225

Table J3. *Simple effect comparisons of treatment*day least squares means by treatment for MHCII+ Macrophages+, Study B*

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
Alum-mIgG	0	0.25	-5.9175	2.0039	9	-2.95	0.0161
Alum-mIgG	0	1	-3.6900	0.8162	9	-4.52	0.0014
Alum-mIgG	0	2	-1.2225	0.1749	9	-6.99	<.0001
Alum-mIgG	0	3	-1.5275	0.4000	9	-3.82	0.0041
Alum-mIgG	0	4	-1.1425	0.7372	9	-1.55	0.1556
Alum-mIgG	0	5	-2.0350	0.6880	9	-2.96	0.0160
Alum-mIgG	0.25	1	2.2275	2.1347	9	1.04	0.3239
Alum-mIgG	0.25	2	4.6950	2.0032	9	2.34	0.0437
Alum-mIgG	0.25	3	4.3900	2.0467	9	2.14	0.0605
Alum-mIgG	0.25	4	4.7750	1.6066	9	2.97	0.0156
Alum-mIgG	0.25	5	3.8825	1.9913	9	1.95	0.0830
Alum-mIgG	1	2	2.4675	0.6780	9	3.64	0.0054
Alum-mIgG	1	3	2.1625	0.9872	9	2.19	0.0562
Alum-mIgG	1	4	2.5475	1.0412	9	2.45	0.0370
Alum-mIgG	1	5	1.6550	1.2472	9	1.33	0.2172
Alum-mIgG	2	3	-0.3050	0.5129	9	-0.59	0.5667
Alum-mIgG	2	4	0.08000	0.7712	9	0.10	0.9197
Alum-mIgG	2	5	-0.8125	0.7684	9	-1.06	0.3179
Alum-mIgG	3	4	0.3850	0.8077	9	0.48	0.6450
Alum-mIgG	3	5	-0.5075	0.6716	9	-0.76	0.4692
Alum-mIgG	4	5	-0.8925	0.8804	9	-1.01	0.3372
IO-mIgG	0	0.25	-4.1300	2.0039	9	-2.06	0.0694
IO-mIgG	0	1	-1.0450	0.8162	9	-1.28	0.2324
IO-mIgG	0	2	0.4050	0.1749	9	2.32	0.0458
IO-mIgG	0	3	-0.6275	0.4000	9	-1.57	0.1511
IO-mIgG	0	4	-1.5775	0.7372	9	-2.14	0.0610
IO-mIgG	0	5	-1.0025	0.6880	9	-1.46	0.1791
IO-mIgG	0.25	1	3.0850	2.1347	9	1.45	0.1823
IO-mIgG	0.25	2	4.5350	2.0032	9	2.26	0.0499
IO-mIgG	0.25	3	3.5025	2.0467	9	1.71	0.1212
IO-mIgG	0.25	4	2.5525	1.6066	9	1.59	0.1466
IO-mIgG	0.25	5	3.1275	1.9913	9	1.57	0.1507
IO-mIgG	1	2	1.4500	0.6780	9	2.14	0.0612
IO-mIgG	1	3	0.4175	0.9872	9	0.42	0.6823
IO-mIgG	1	4	-0.5325	1.0412	9	-0.51	0.6214
IO-mIgG	1	5	0.04250	1.2472	9	0.03	0.9736
IO-mIgG	2	3	-1.0325	0.5129	9	-2.01	0.0749
IO-mIgG	2	4	-1.9825	0.7712	9	-2.57	0.0301
IO-mIgG	2	5	-1.4075	0.7684	9	-1.83	0.1002
IO-mIgG	3	4	-0.9500	0.8077	9	-1.18	0.2697
IO-mIgG	3	5	-0.3750	0.6716	9	-0.56	0.5902
IO-mIgG	4	5	0.5750	0.8804	9	0.65	0.5300
mIgG	0	0.25	-1.9725	2.0039	9	-0.98	0.3507
mIgG	0	1	-0.6925	0.8162	9	-0.85	0.4182
mIgG	0	2	0.09250	0.1749	9	0.53	0.6096

Table J3. *Simple effect comparisons of treatment*day least squares means by treatment for MHCII+ Macrophages+, Study B* (Cont.)

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
mIgG	0	3	-0.4450	0.4000	9	-1.11	0.2947
mIgG	0	4	-0.5125	0.7372	9	-0.70	0.5045
mIgG	0	5	-1.1250	0.6880	9	-1.64	0.1364
mIgG	0.25	1	1.2800	2.1347	9	0.60	0.5636
mIgG	0.25	2	2.0650	2.0032	9	1.03	0.3295
mIgG	0.25	3	1.5275	2.0467	9	0.75	0.4745
mIgG	0.25	4	1.4600	1.6066	9	0.91	0.3872
mIgG	0.25	5	0.8475	1.9913	9	0.43	0.6804
mIgG	1	2	0.7850	0.6780	9	1.16	0.2768
mIgG	1	3	0.2475	0.9872	9	0.25	0.8077
mIgG	1	4	0.1800	1.0412	9	0.17	0.8666
mIgG	1	5	-0.4325	1.2472	9	-0.35	0.7367
mIgG	2	3	-0.5375	0.5129	9	-1.05	0.3219
mIgG	2	4	-0.6050	0.7712	9	-0.78	0.4529
mIgG	2	5	-1.2175	0.7684	9	-1.58	0.1476
mIgG	3	4	-0.06750	0.8077	9	-0.08	0.9352
mIgG	3	5	-0.6800	0.6716	9	-1.01	0.3377
mIgG	4	5	-0.6125	0.8804	9	-0.70	0.5042

Table J4. *Simple effect comparisons of treatment*day least squares means by day for MHCII+ Macrophages+, Study B*

Simple Effect Level	Treatment	Treatment	Estimate	Std. Error	DF	t Value	Pr > |t|
day 0	AlummIgG	IOmIgG	-0.4500	0.1990	9	-2.26	0.0501
day 0	AlummIgG	mIgG	-0.3825	0.1990	9	-1.92	0.0868
day 0	IOmIgG	mIgG	0.06750	0.1990	9	0.34	0.7423
day 0.25	AlummIgG	IOmIgG	1.3375	2.8703	9	0.47	0.6523
day 0.25	AlummIgG	mIgG	3.5625	2.8703	9	1.24	0.2459
day 0.25	IOmIgG	mIgG	2.2250	2.8703	9	0.78	0.4581
day 1	AlummIgG	IOmIgG	2.1950	1.1972	9	1.83	0.0999
day 1	AlummIgG	mIgG	2.6150	1.1972	9	2.18	0.0568
day 1	IOmIgG	mIgG	0.4200	1.1972	9	0.35	0.7338
day 2	AlummIgG	IOmIgG	1.1775	0.3642	9	3.23	0.0103
day 2	AlummIgG	mIgG	0.9325	0.3642	9	2.56	0.0307
day 2	IOmIgG	mIgG	-0.2450	0.3642	9	-0.67	0.5180
day 3	AlummIgG	IOmIgG	0.4500	0.5148	9	0.87	0.4047
day 3	AlummIgG	mIgG	0.7000	0.5148	9	1.36	0.2070
day 3	IOmIgG	mIgG	0.2500	0.5148	9	0.49	0.6388
day 4	AlummIgG	IOmIgG	-0.8850	1.0431	9	-0.85	0.4182
day 4	AlummIgG	mIgG	0.2475	1.0431	9	0.24	0.8177
day 4	IOmIgG	mIgG	1.1325	1.0431	9	1.09	0.3058
day 5	AlummIgG	IOmIgG	0.5825	0.9621	9	0.61	0.5598
day 5	AlummIgG	mIgG	0.5275	0.9621	9	0.55	0.5968
day 5	IOmIgG	mIgG	-0.05500	0.9621	9	-0.06	0.9557

Table J5. *Simple effect comparisons of treatment*day least squares means by treatment for CD4+ CD8+, Study B*

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
Alum-mIgG	0	0.25	-0.3500	0.1584	9	-2.21	0.0545
Alum-mIgG	0	1	-0.8025	0.3252	9	-2.47	0.0357
Alum-mIgG	0	2	-1.3050	0.3512	9	-3.72	0.0048
Alum-mIgG	0	3	-0.6000	0.2498	9	-2.40	0.0398
Alum-mIgG	0	4	-0.3800	0.1059	9	-3.59	0.0059
Alum-mIgG	0	5	-0.2475	0.09574	9	-2.59	0.0294
Alum-mIgG	0.25	1	-0.4525	0.2711	9	-1.67	0.1294
Alum-mIgG	0.25	2	-0.9550	0.3329	9	-2.87	0.0185
Alum-mIgG	0.25	3	-0.2500	0.1880	9	-1.33	0.2163
Alum-mIgG	0.25	4	-0.03000	0.1591	9	-0.19	0.8546
Alum-mIgG	0.25	5	0.1025	0.1229	9	0.83	0.4259
Alum-mIgG	1	2	-0.5025	0.2370	9	-2.12	0.0630
Alum-mIgG	1	3	0.2025	0.1804	9	1.12	0.2907
Alum-mIgG	1	4	0.4225	0.3255	9	1.30	0.2266
Alum-mIgG	1	5	0.5550	0.3446	9	1.61	0.1417
Alum-mIgG	2	3	0.7050	0.2082	9	3.39	0.0080

Table J5. *Simple effect comparisons of treatment*day least squares means by treatment for CD4+ CD8+, Study B* (Cont.)

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
Alum-mIgG	2	4	0.9250	0.3412	9	2.71	0.0240
Alum-mIgG	2	5	1.0575	0.3977	9	2.66	0.0261
Alum-mIgG	3	4	0.2200	0.2474	9	0.89	0.3971
Alum-mIgG	3	5	0.3525	0.2558	9	1.38	0.2014
Alum-mIgG	4	5	0.1325	0.1147	9	1.15	0.2779
IO-mIgG	0	0.25	-0.1500	0.1584	9	-0.95	0.3685
IO-mIgG	0	1	-0.5975	0.3252	9	-1.84	0.0993
IO-mIgG	0	2	-0.4300	0.3512	9	-1.22	0.2519
IO-mIgG	0	3	-0.5150	0.2498	9	-2.06	0.0693
IO-mIgG	0	4	-0.1850	0.1059	9	-1.75	0.1147
IO-mIgG	0	5	-0.09250	0.09574	9	-0.97	0.3592
IO-mIgG	0.25	1	-0.4475	0.2711	9	-1.65	0.1331
IO-mIgG	0.25	2	-0.2800	0.3329	9	-0.84	0.4221
IO-mIgG	0.25	3	-0.3650	0.1880	9	-1.94	0.0841
IO-mIgG	0.25	4	-0.03500	0.1591	9	-0.22	0.8307
IO-mIgG	0.25	5	0.05750	0.1229	9	0.47	0.6510
IO-mIgG	1	2	0.1675	0.2370	9	0.71	0.4976
IO-mIgG	1	3	0.08250	0.1804	9	0.46	0.6583
IO-mIgG	1	4	0.4125	0.3255	9	1.27	0.2369
IO-mIgG	1	5	0.5050	0.3446	9	1.47	0.1768
IO-mIgG	2	3	-0.08500	0.2082	9	-0.41	0.6926
IO-mIgG	2	4	0.2450	0.3412	9	0.72	0.4909
IO-mIgG	2	5	0.3375	0.3977	9	0.85	0.4180
IO-mIgG	3	4	0.3300	0.2474	9	1.33	0.2150
IO-mIgG	3	5	0.4225	0.2558	9	1.65	0.1330
IO-mIgG	4	5	0.09250	0.1147	9	0.81	0.4409
mIgG	0	0.25	-0.4500	0.1584	9	-2.84	0.0194
mIgG	0	1	-0.6350	0.3252	9	-1.95	0.0826
mIgG	0	2	-0.6075	0.3512	9	-1.73	0.1177
mIgG	0	3	-0.5475	0.2498	9	-2.19	0.0561
mIgG	0	4	-0.06750	0.1059	9	-0.64	0.5399
mIgG	0	5	-0.06000	0.09574	9	-0.63	0.5464
mIgG	0.25	1	-0.1850	0.2711	9	-0.68	0.5121
mIgG	0.25	2	-0.1575	0.3329	9	-0.47	0.6474
mIgG	0.25	3	-0.09750	0.1880	9	-0.52	0.6165
mIgG	0.25	4	0.3825	0.1591	9	2.40	0.0396
mIgG	0.25	5	0.3900	0.1229	9	3.17	0.0113
mIgG	1	2	0.02750	0.2370	9	0.12	0.9102
mIgG	1	3	0.08750	0.1804	9	0.48	0.6393
mIgG	1	4	0.5675	0.3255	9	1.74	0.1152
mIgG	1	5	0.5750	0.3446	9	1.67	0.1295
mIgG	2	3	0.06000	0.2082	9	0.29	0.7797
mIgG	2	4	0.5400	0.3412	9	1.58	0.1480

Table J5. *Simple effect comparisons of treatment*day least squares means by treatment for CD4+ CD8+, Study B* (Cont.)

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
mIgG	2	5	0.5475	0.3977	9	1.38	0.2019
mIgG	3	4	0.4800	0.2474	9	1.94	0.0843
mIgG	3	5	0.4875	0.2558	9	1.91	0.0890
mIgG	4	5	0.007500	0.1147	9	0.07	0.9493

Table J6. *Simple effect comparisons of treatment*day least squares means by day for CD4+ CD8+, Study B*

Simple Effect Level	Treatment	Treatment	Estimate	Std. Error	DF	t Value	Pr > |t|
day 0	AlummIgG	IOmIgG	0.005000	0.01736	9	0.29	0.7799
day 0	AlummIgG	mIgG	0.01500	0.01736	9	0.86	0.4100
day 0	IOmIgG	mIgG	0.01000	0.01736	9	0.58	0.5787
day 0.25	AlummIgG	IOmIgG	0.2050	0.2317	9	0.88	0.3994
day 0.25	AlummIgG	mIgG	-0.08500	0.2317	9	-0.37	0.7222
day 0.25	IOmIgG	mIgG	-0.2900	0.2317	9	-1.25	0.2423
day 1	AlummIgG	IOmIgG	0.2100	0.4711	9	0.45	0.6663
day 1	AlummIgG	mIgG	0.1825	0.4711	9	0.39	0.7075
day 1	IOmIgG	mIgG	-0.02750	0.4711	9	-0.06	0.9547
day 2	AlummIgG	IOmIgG	0.8800	0.5025	9	1.75	0.1138
day 2	AlummIgG	mIgG	0.7125	0.5025	9	1.42	0.1899
day 2	IOmIgG	mIgG	-0.1675	0.5025	9	-0.33	0.7465
day 3	AlummIgG	IOmIgG	0.09000	0.3623	9	0.25	0.8094
day 3	AlummIgG	mIgG	0.06750	0.3623	9	0.19	0.8563
day 3	IOmIgG	mIgG	-0.02250	0.3623	9	-0.06	0.9518
day 4	AlummIgG	IOmIgG	0.2000	0.1517	9	1.32	0.2200
day 4	AlummIgG	mIgG	0.3275	0.1517	9	2.16	0.0592
day 4	IOmIgG	mIgG	0.1275	0.1517	9	0.84	0.4225
day 5	AlummIgG	IOmIgG	0.1600	0.1385	9	1.16	0.2776
day 5	AlummIgG	mIgG	0.2025	0.1385	9	1.46	0.1776
day 5	IOmIgG	mIgG	0.04250	0.1385	9	0.31	0.7659

Table J7. *Simple effect comparisons of treatment*day least squares means by treatment for γδ T cell+ CD8+, Study B*

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
Alum-mIgG	0	0.25	-0.5175	0.1034	9	-5.00	0.0007
Alum-mIgG	0	1	-0.9475	0.2565	9	-3.69	0.0050
Alum-mIgG	0	2	-0.8000	0.1693	9	-4.73	0.0011
Alum-mIgG	0	3	-0.7750	0.2023	9	-3.83	0.0040
Alum-mIgG	0	4	-0.3300	0.07643	9	-4.32	0.0019
Alum-mIgG	0	5	-0.1975	0.1101	9	-1.79	0.1065
Alum-mIgG	0.25	1	-0.4300	0.2931	9	-1.47	0.1764
Alum-mIgG	0.25	2	-0.2825	0.2471	9	-1.14	0.2824
Alum-mIgG	0.25	3	-0.2575	0.2274	9	-1.13	0.2867
Alum-mIgG	0.25	4	0.1875	0.1341	9	1.40	0.1955
Alum-mIgG	0.25	5	0.3200	0.1703	9	1.88	0.0930
Alum-mIgG	1	2	0.1475	0.2155	9	0.68	0.5109
Alum-mIgG	1	3	0.1725	0.1703	9	1.01	0.3375
Alum-mIgG	1	4	0.6175	0.2791	9	2.21	0.0542
Alum-mIgG	1	5	0.7500	0.3015	9	2.49	0.0346
Alum-mIgG	2	3	0.02500	0.1894	9	0.13	0.8979
Alum-mIgG	2	4	0.4700	0.2205	9	2.13	0.0618
Alum-mIgG	2	5	0.6025	0.2009	9	3.00	0.0150
Alum-mIgG	3	4	0.4450	0.2391	9	1.86	0.0957
Alum-mIgG	3	5	0.5775	0.2383	9	2.42	0.0384
Alum-mIgG	4	5	0.1325	0.1482	9	0.89	0.3947
IO-mIgG	0	0.25	-0.1700	0.1034	9	-1.64	0.1347
IO-mIgG	0	1	-1.5600	0.2565	9	-6.08	0.0002
IO-mIgG	0	2	-0.7275	0.1693	9	-4.30	0.0020
IO-mIgG	0	3	-1.0475	0.2023	9	-5.18	0.0006
IO-mIgG	0	4	-0.2150	0.07643	9	-2.81	0.0203
IO-mIgG	0	5	-0.1800	0.1101	9	-1.63	0.1366
IO-mIgG	0.25	1	-1.3900	0.2931	9	-4.74	0.0011
IO-mIgG	0.25	2	-0.5575	0.2471	9	-2.26	0.0505
IO-mIgG	0.25	3	-0.8775	0.2274	9	-3.86	0.0039
IO-mIgG	0.25	4	-0.04500	0.1341	9	-0.34	0.7449
IO-mIgG	0.25	5	-0.01000	0.1703	9	-0.06	0.9545
IO-mIgG	1	2	0.8325	0.2155	9	3.86	0.0038
IO-mIgG	1	3	0.5125	0.1703	9	3.01	0.0147
IO-mIgG	1	4	1.3450	0.2791	9	4.82	0.0009
IO-mIgG	1	5	1.3800	0.3015	9	4.58	0.0013
IO-mIgG	2	3	-0.3200	0.1894	9	-1.69	0.1254
IO-mIgG	2	4	0.5125	0.2205	9	2.32	0.0452
IO-mIgG	2	5	0.5475	0.2009	9	2.73	0.0234
IO-mIgG	3	4	0.8325	0.2391	9	3.48	0.0069
IO-mIgG	3	5	0.8675	0.2383	9	3.64	0.0054
IO-mIgG	4	5	0.03500	0.1482	9	0.24	0.8186
mIgG	0	0.25	-0.7550	0.1034	9	-7.30	<.0001
mIgG	0	1	-1.0950	0.2565	9	-4.27	0.0021
mIgG	0	2	-0.6150	0.1693	9	-3.63	0.0055

Table J7. *Simple effect comparisons of treatment*day least squares means by treatment for γδ T cell+ CD8+, Study B* (Cont.)

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
mIgG	0	3	-0.9500	0.2023	9	-4.70	0.0011
mIgG	0	4	-0.3200	0.07643	9	-4.19	0.0024
mIgG	0	5	-0.2275	0.1101	9	-2.07	0.0689
mIgG	0.25	1	-0.3400	0.2931	9	-1.16	0.2759
mIgG	0.25	2	0.1400	0.2471	9	0.57	0.5848
mIgG	0.25	3	-0.1950	0.2274	9	-0.86	0.4134
mIgG	0.25	4	0.4350	0.1341	9	3.24	0.0101
mIgG	0.25	5	0.5275	0.1703	9	3.10	0.0128
mIgG	1	2	0.4800	0.2155	9	2.23	0.0529
mIgG	1	3	0.1450	0.1703	9	0.85	0.4165
mIgG	1	4	0.7750	0.2791	9	2.78	0.0215
mIgG	1	5	0.8675	0.3015	9	2.88	0.0183
mIgG	2	3	-0.3350	0.1894	9	-1.77	0.1108
mIgG	2	4	0.2950	0.2205	9	1.34	0.2137
mIgG	2	5	0.3875	0.2009	9	1.93	0.0858
mIgG	3	4	0.6300	0.2391	9	2.63	0.0272
mIgG	3	5	0.7225	0.2383	9	3.03	0.0142
mIgG	4	5	0.09250	0.1482	9	0.62	0.5481

Table J8. *Simple effect comparisons of treatment*day least squares means by day for γδ T cell+ CD8+, Study B*

Simple Effect Level	Treatment	Treatment	Estimate	Std. Error	DF	t Value	Pr > |t|
day 0	AlummIgG	IOmIgG	-0.1675	0.08977	9	-1.87	0.0949
day 0	AlummIgG	mIgG	0.08250	0.08977	9	0.92	0.3820
day 0	IOmIgG	mIgG	0.2500	0.08977	9	2.78	0.0212
day 0.25	AlummIgG	IOmIgG	0.1800	0.1927	9	0.93	0.3746
day 0.25	AlummIgG	mIgG	-0.1550	0.1927	9	-0.80	0.4419
day 0.25	IOmIgG	mIgG	-0.3350	0.1927	9	-1.74	0.1161
day 1	AlummIgG	IOmIgG	-0.7800	0.3857	9	-2.02	0.0739
day 1	AlummIgG	mIgG	-0.06500	0.3857	9	-0.17	0.8699
day 1	IOmIgG	mIgG	0.7150	0.3857	9	1.85	0.0968
day 2	AlummIgG	IOmIgG	-0.09500	0.2083	9	-0.46	0.6591
day 2	AlummIgG	mIgG	0.2675	0.2083	9	1.28	0.2311
day 2	IOmIgG	mIgG	0.3625	0.2083	9	1.74	0.1158
day 3	AlummIgG	IOmIgG	-0.4400	0.3060	9	-1.44	0.1843
day 3	AlummIgG	mIgG	-0.09250	0.3060	9	-0.30	0.7693
day 3	IOmIgG	mIgG	0.3475	0.3060	9	1.14	0.2855
day 4	AlummIgG	IOmIgG	-0.05250	0.1819	9	-0.29	0.7795
day 4	AlummIgG	mIgG	0.09250	0.1819	9	0.51	0.6234
day 4	IOmIgG	mIgG	0.1450	0.1819	9	0.80	0.4460
day 5	AlummIgG	IOmIgG	-0.1500	0.1193	9	-1.26	0.2404
day 5	AlummIgG	mIgG	0.05250	0.1193	9	0.44	0.6704
day 5	IOmIgG	mIgG	0.2025	0.1193	9	1.70	0.1240

Table J9. *Simple effect comparisons of treatment*day least squares means by treatment for αβ1 T cell+ CD4- CD8-, Study B*

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
Alum-mIgG	0	0.25	-0.01500	0.06622	9	-0.23	0.8259
Alum-mIgG	0	1	-0.2900	0.1265	9	-2.29	0.0476
Alum-mIgG	0	2	-0.2900	0.09354	9	-3.10	0.0127
Alum-mIgG	0	3	-0.6800	0.1387	9	-4.90	0.0008
Alum-mIgG	0	4	-0.4000	0.2202	9	-1.82	0.1026
Alum-mIgG	0	5	-0.6750	0.1761	9	-3.83	0.0040
Alum-mIgG	0.25	1	-0.2750	0.1609	9	-1.71	0.1216
Alum-mIgG	0.25	2	-0.2750	0.1104	9	-2.49	0.0344
Alum-mIgG	0.25	3	-0.6650	0.1589	9	-4.18	0.0024
Alum-mIgG	0.25	4	-0.3850	0.2454	9	-1.57	0.1511
Alum-mIgG	0.25	5	-0.6600	0.1682	9	-3.93	0.0035
Alum-mIgG	1	2	-333E-18	0.1435	9	-0.00	1.0000
Alum-mIgG	1	3	-0.3900	0.2342	9	-1.67	0.1302
Alum-mIgG	1	4	-0.1100	0.2130	9	-0.52	0.6180
Alum-mIgG	1	5	-0.3850	0.2294	9	-1.68	0.1275
Alum-mIgG	2	3	-0.3900	0.1722	9	-2.27	0.0498

Table J9. *Simple effect comparisons of treatment*day least squares means by treatment for αβ1 T cell+ CD4- CD8-, Study B* (Cont.)

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
Alum-mIgG	2	4	-0.1100	0.1634	9	-0.67	0.5177
Alum-mIgG	2	5	-0.3850	0.2340	9	-1.64	0.1344
Alum-mIgG	3	4	0.2800	0.2271	9	1.23	0.2488
Alum-mIgG	3	5	0.005000	0.2533	9	0.02	0.9847
Alum-mIgG	4	5	-0.2750	0.3729	9	-0.74	0.4796
IO-mIgG	0	0.25	0.2825	0.06622	9	4.27	0.0021
IO-mIgG	0	1	0.04750	0.1265	9	0.38	0.7161
IO-mIgG	0	2	0.06500	0.09354	9	0.69	0.5047
IO-mIgG	0	3	-0.1750	0.1387	9	-1.26	0.2388
IO-mIgG	0	4	-0.4400	0.2202	9	-2.00	0.0768
IO-mIgG	0	5	-0.3225	0.1761	9	-1.83	0.1003
IO-mIgG	0.25	1	-0.2350	0.1609	9	-1.46	0.1781
IO-mIgG	0.25	2	-0.2175	0.1104	9	-1.97	0.0804
IO-mIgG	0.25	3	-0.4575	0.1589	9	-2.88	0.0182
IO-mIgG	0.25	4	-0.7225	0.2454	9	-2.94	0.0164
IO-mIgG	0.25	5	-0.6050	0.1682	9	-3.60	0.0058
IO-mIgG	1	2	0.01750	0.1435	9	0.12	0.9056
IO-mIgG	1	3	-0.2225	0.2342	9	-0.95	0.3669
IO-mIgG	1	4	-0.4875	0.2130	9	-2.29	0.0479
IO-mIgG	1	5	-0.3700	0.2294	9	-1.61	0.1412
IO-mIgG	2	3	-0.2400	0.1722	9	-1.39	0.1968
IO-mIgG	2	4	-0.5050	0.1634	9	-3.09	0.0129
IO-mIgG	2	5	-0.3875	0.2340	9	-1.66	0.1322
IO-mIgG	3	4	-0.2650	0.2271	9	-1.17	0.2732
IO-mIgG	3	5	-0.1475	0.2533	9	-0.58	0.5747
IO-mIgG	4	5	0.1175	0.3729	9	0.32	0.7599
mIgG	0	0.25	0.02500	0.06622	9	0.38	0.7145
mIgG	0	1	-0.06250	0.1265	9	-0.49	0.6332
mIgG	0	2	-0.06250	0.09354	9	-0.67	0.5208
mIgG	0	3	-0.2325	0.1387	9	-1.68	0.1280
mIgG	0	4	-0.2925	0.2202	9	-1.33	0.2167
mIgG	0	5	-0.3750	0.1761	9	-2.13	0.0621
mIgG	0.25	1	-0.08750	0.1609	9	-0.54	0.5998
mIgG	0.25	2	-0.08750	0.1104	9	-0.79	0.4485
mIgG	0.25	3	-0.2575	0.1589	9	-1.62	0.1397
mIgG	0.25	4	-0.3175	0.2454	9	-1.29	0.2280
mIgG	0.25	5	-0.4000	0.1682	9	-2.38	0.0413
mIgG	1	2	-477E-17	0.1435	9	-0.00	1.0000
mIgG	1	3	-0.1700	0.2342	9	-0.73	0.4863
mIgG	1	4	-0.2300	0.2130	9	-1.08	0.3083
mIgG	1	5	-0.3125	0.2294	9	-1.36	0.2062
mIgG	2	3	-0.1700	0.1722	9	-0.99	0.3493
mIgG	2	4	-0.2300	0.1634	9	-1.41	0.1928

Table J9. *Simple effect comparisons of treatment*day least squares means by treatment for αβ1 T cell+ CD4- CD8-, Study B* (Cont.)

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
mIgG	2	5	-0.3125	0.2340	9	-1.34	0.2146
mIgG	3	4	-0.06000	0.2271	9	-0.26	0.7975
mIgG	3	5	-0.1425	0.2533	9	-0.56	0.5875
mIgG	4	5	-0.08250	0.3729	9	-0.22	0.8298

Table J10. *Simple effect comparisons of treatment*day least squares means by day for αβ1 T cell+ CD4- CD8-, Study B*

Simple Effect Level	Treatment	Treatment	Estimate	Std. Error	DF	t Value	Pr > |t|
day 0	AlummIgG	IOmIgG	-0.1825	0.06590	9	-2.77	0.0218
day 0	AlummIgG	mIgG	-0.06500	0.06590	9	-0.99	0.3498
day 0	IOmIgG	mIgG	0.1175	0.06590	9	1.78	0.1083
day 0.25	AlummIgG	IOmIgG	0.1150	0.06523	9	1.76	0.1118
day 0.25	AlummIgG	mIgG	-0.02500	0.06523	9	-0.38	0.7104
day 0.25	IOmIgG	mIgG	-0.1400	0.06523	9	-2.15	0.0604
day 1	AlummIgG	IOmIgG	0.1550	0.2099	9	0.74	0.4791
day 1	AlummIgG	mIgG	0.1625	0.2099	9	0.77	0.4587
day 1	IOmIgG	mIgG	0.007500	0.2099	9	0.04	0.9723
day 2	AlummIgG	IOmIgG	0.1725	0.1562	9	1.10	0.2980
day 2	AlummIgG	mIgG	0.1625	0.1562	9	1.04	0.3253
day 2	IOmIgG	mIgG	-0.01000	0.1562	9	-0.06	0.9503
day 3	AlummIgG	IOmIgG	0.3225	0.1820	9	1.77	0.1101
day 3	AlummIgG	mIgG	0.3825	0.1820	9	2.10	0.0649
day 3	IOmIgG	mIgG	0.06000	0.1820	9	0.33	0.7492
day 4	AlummIgG	IOmIgG	-0.2225	0.3219	9	-0.69	0.5069
day 4	AlummIgG	mIgG	0.04250	0.3219	9	0.13	0.8979
day 4	IOmIgG	mIgG	0.2650	0.3219	9	0.82	0.4317
day 5	AlummIgG	IOmIgG	0.1700	0.2511	9	0.68	0.5154
day 5	AlummIgG	mIgG	0.2350	0.2511	9	0.94	0.3738
day 5	IOmIgG	mIgG	0.06500	0.2511	9	0.26	0.8016

Table J11. *Simple effect comparisons of treatment*day least squares means by treatment for B cell+ IgM+, Study B*

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
Alum-mIgG	0	0.25	-0.7700	0.1879	9	-4.10	0.0027
Alum-mIgG	0	1	-11.8350	4.9403	9	-2.40	0.0402
Alum-mIgG	0	2	-9.5775	2.5620	9	-3.74	0.0046
Alum-mIgG	0	3	-6.3625	2.0573	9	-3.09	0.0129
Alum-mIgG	0	4	-2.6475	0.9962	9	-2.66	0.0261
Alum-mIgG	0	5	-1.2525	0.7824	9	-1.60	0.1439
Alum-mIgG	0.25	1	-11.0650	4.8665	9	-2.27	0.0491
Alum-mIgG	0.25	2	-8.8075	2.5914	9	-3.40	0.0079
Alum-mIgG	0.25	3	-5.5925	2.0790	9	-2.69	0.0248
Alum-mIgG	0.25	4	-1.8775	0.9620	9	-1.95	0.0827
Alum-mIgG	0.25	5	-0.4825	0.7042	9	-0.69	0.5105
Alum-mIgG	1	2	2.2575	3.7367	9	0.60	0.5607
Alum-mIgG	1	3	5.4725	4.3647	9	1.25	0.2415
Alum-mIgG	1	4	9.1875	5.3753	9	1.71	0.1216
Alum-mIgG	1	5	10.5825	4.9993	9	2.12	0.0634
Alum-mIgG	2	3	3.2150	1.9120	9	1.68	0.1270
Alum-mIgG	2	4	6.9300	2.9425	9	2.36	0.0429
Alum-mIgG	2	5	8.3250	2.9172	9	2.85	0.0190
Alum-mIgG	3	4	3.7150	2.0114	9	1.85	0.0978
Alum-mIgG	3	5	5.1100	2.1563	9	2.37	0.0419
Alum-mIgG	4	5	1.3950	0.9855	9	1.42	0.1906
IO-mIgG	0	0.25	-0.8275	0.1879	9	-4.41	0.0017
IO-mIgG	0	1	-12.7725	4.9403	9	-2.59	0.0294
IO-mIgG	0	2	-12.8425	2.5620	9	-5.01	0.0007
IO-mIgG	0	3	-15.4575	2.0573	9	-7.51	<.0001
IO-mIgG	0	4	-2.9625	0.9962	9	-2.97	0.0156
IO-mIgG	0	5	-3.0075	0.7824	9	-3.84	0.0039
IO-mIgG	0.25	1	-11.9450	4.8665	9	-2.45	0.0365
IO-mIgG	0.25	2	-12.0150	2.5914	9	-4.64	0.0012
IO-mIgG	0.25	3	-14.6300	2.0790	9	-7.04	<.0001
IO-mIgG	0.25	4	-2.1350	0.9620	9	-2.22	0.0536
IO-mIgG	0.25	5	-2.1800	0.7042	9	-3.10	0.0128
IO-mIgG	1	2	-0.07000	3.7367	9	-0.02	0.9855
IO-mIgG	1	3	-2.6850	4.3647	9	-0.62	0.5537
IO-mIgG	1	4	9.8100	5.3753	9	1.83	0.1013
IO-mIgG	1	5	9.7650	4.9993	9	1.95	0.0825
IO-mIgG	2	3	-2.6150	1.9120	9	-1.37	0.2046
IO-mIgG	2	4	9.8800	2.9425	9	3.36	0.0084
IO-mIgG	2	5	9.8350	2.9172	9	3.37	0.0082
IO-mIgG	3	4	12.4950	2.0114	9	6.21	0.0002
IO-mIgG	3	5	12.4500	2.1563	9	5.77	0.0003
IO-mIgG	4	5	-0.04500	0.9855	9	-0.05	0.9646
mIgG	0	0.25	-1.2450	0.1879	9	-6.63	<.0001
mIgG	0	1	-8.0925	4.9403	9	-1.64	0.1358
mIgG	0	2	-10.1550	2.5620	9	-3.96	0.0033

Table J11. *Simple effect comparisons of treatment*day least squares means by treatment for B cell+ IgM+, Study B* (Cont.)

Simple Effect Level	Day	Day	Estimate	Std. Error	DF	t Value	Pr > |t|
mIgG	0	3	-10.9950	2.0573	9	-5.34	0.0005
mIgG	0	4	-0.9200	0.9962	9	-0.92	0.3798
mIgG	0	5	-1.8200	0.7824	9	-2.33	0.0450
mIgG	0.25	1	-6.8475	4.8665	9	-1.41	0.1930
mIgG	0.25	2	-8.9100	2.5914	9	-3.44	0.0074
mIgG	0.25	3	-9.7500	2.0790	9	-4.69	0.0011
mIgG	0.25	4	0.3250	0.9620	9	0.34	0.7432
mIgG	0.25	5	-0.5750	0.7042	9	-0.82	0.4353
mIgG	1	2	-2.0625	3.7367	9	-0.55	0.5944
mIgG	1	3	-2.9025	4.3647	9	-0.66	0.5227
mIgG	1	4	7.1725	5.3753	9	1.33	0.2149
mIgG	1	5	6.2725	4.9993	9	1.25	0.2412
mIgG	2	3	-0.8400	1.9120	9	-0.44	0.6708
mIgG	2	4	9.2350	2.9425	9	3.14	0.0120
mIgG	2	5	8.3350	2.9172	9	2.86	0.0189
mIgG	3	4	10.0750	2.0114	9	5.01	0.0007
mIgG	3	5	9.1750	2.1563	9	4.26	0.0021
mIgG	4	5	-0.9000	0.9855	9	-0.91	0.3849

Table J12. *Simple effect comparisons of treatment*day least squares means by day for B cell+ IgM+, Study B*

Simple Effect Level	Treatment	Treatment	Estimate	Std. Error	DF	t Value	Pr > |t|
day 0	AlummIgG	IOmIgG	-0.1825	0.1428	9	-1.28	0.2334
day 0	AlummIgG	mIgG	0.1000	0.1428	9	0.70	0.5016
day 0	IOmIgG	mIgG	0.2825	0.1428	9	1.98	0.0794
day 0.25	AlummIgG	IOmIgG	-0.2400	0.3599	9	-0.67	0.5216
day 0.25	AlummIgG	mIgG	-0.3750	0.3599	9	-1.04	0.3246
day 0.25	IOmIgG	mIgG	-0.1350	0.3599	9	-0.38	0.7163
day 1	AlummIgG	IOmIgG	-1.1200	7.0164	9	-0.16	0.8767
day 1	AlummIgG	mIgG	3.8425	7.0164	9	0.55	0.5973
day 1	IOmIgG	mIgG	4.9625	7.0164	9	0.71	0.4973
day 2	AlummIgG	IOmIgG	-3.4475	3.5682	9	-0.97	0.3592
day 2	AlummIgG	mIgG	-0.4775	3.5682	9	-0.13	0.8965
day 2	IOmIgG	mIgG	2.9700	3.5682	9	0.83	0.4267
day 3	AlummIgG	IOmIgG	-9.2775	2.8602	9	-3.24	0.0101
day 3	AlummIgG	mIgG	-4.5325	2.8602	9	-1.58	0.1475
day 3	IOmIgG	mIgG	4.7450	2.8602	9	1.66	0.1315
day 4	AlummIgG	IOmIgG	-0.4975	1.3960	9	-0.36	0.7298
day 4	AlummIgG	mIgG	1.8275	1.3960	9	1.31	0.2229
day 4	IOmIgG	mIgG	2.3250	1.3960	9	1.67	0.1302
day 5	AlummIgG	IOmIgG	-1.9375	1.1490	9	-1.69	0.1260
day 5	AlummIgG	mIgG	-0.4675	1.1490	9	-0.41	0.6936
day 5	IOmIgG	mIgG	1.4700	1.1490	9	1.28	0.2328