PARP14 IN ALLERGIC INFLAMMATION

Purna Krishnamurthy

Submitted to the faculty of the University Graduate School
in partial fulfillment of the requirements
for the degree
Doctor of philosophy
in the Department of Microbiology and Immunology,
Indiana University

April 2017

Accepted by the Graduate Faculty, of Indiana University, in partial fulfillment of the requirements for the degree of Doctor of Philosophy

Mark H. Kaplan, Ph.D. – Chair

Janice S. Blum, Ph.D.

Doctoral Committee

Robert S. Tepper, M.D.

January 13, 2017

Matthew J. Turner, M.D., Ph.D.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my graduate school mentor and my thesis advisor Dr. Mark Kaplan, for his guidance, motivation and patience through out my time in his laboratory. I have had the great opportunity to train under his guidance and grow as a scientist. He helped me to develop and improve my scientific writing and presentation skills. Though he is not physically among us today, I would like to thank Dr. Shreevrat Goenka, my first mentor in graduate school. I learnt a lot about science in the short time I spent as his first graduate student.

I would also like to thank my committee members, Drs. Janice Blum, Robert Tepper, Matthew Turner and Jie Sun who have helped me build my critical thinking abilities and for their suggestions, insightful comments and encouragement.

I would like to thank members of the Kaplan lab for all the discussions and fun. I would like to sincerely thank Purvi Mehrotra, my initial go to person after my transition to the Kaplan lab and Ana Serezani, for all their guidance and mentoring. I would like to thank past and present members of the Kaplan lab for their inspiration and friendship – Sarita Sehra, Rukhsana Jabeen, Matt Hufford, Gunseli Bozdogan, Felipe Fortino, Nicky Glosson, Kemi Awe, Matt Olson,

Byunghee Koh, Ben Ulrich, Yongyao Fu, Pegah Mehrpouya-Bahrami and Nahid Akhtar.

Lastly, I would like to thank my parents, my support system and my sister for being there at every step, for their constant love and moral support. I am thankful to my fiancé, Siddharth for always being there, for his encouragement and support through graduate school.

Purna Krishnamurthy

PARP14 IN ALLERGIC INFLAMMATION

Allergic inflammation is a result of damaging immune responses stimulated by innocuous proteins and is mediated by a complex interplay between inflammatory cells including eosinophils, basophils, neutrophils, mast cells, dendritic cells and lymphocytes. The anatomic location of allergic inflammation determines the structural cells that become targets, such as airway smooth muscle cells, mucous secreting cells, keratinocytes, and other epithelial cells. The cytokine IL-4 is critical for this process and promotes activation of the transcription factor STAT6 and transcriptional co-factors that regulate pro-allergic gene expression. STAT6 is required for IL-4 mediated responses, Th2 cell differentiation and class switching to IgE and has been implicated in the progression of asthma, atopic dermatitis (AD), and eosinophilic esophagitis (EoE). STAT6 interacts with the co-factor Poly-ADP ribose polymerse-14 (PARP-14 or ARTD8), one of 17 PARPs with ADP-ribosyl transferase activity. PARP14 was initially identified as a transcriptional co-activator for STAT6, and PARP14 and the enzymatic activity associated with it promote Th2 cell differentiation. Allergic airway disease is attenuated in *Parp14*$^{-/-}$ mice or in mice treated with PARP inhibitor, PJ34. To study the role of PARP14 in EoE, we examined esophageal biopsies from children and demonstrated a correlation between the expression of eosinophilic chemo-attractant, *CCL26* and *PARP14.* To study

allergic skin inflammation, we used a mouse model of spontaneous inflammation (Stat6VT mice). We generated Stat6VTx*Parp14*$^{-/-}$ mice and observed that Stat6VTx*Parp14*$^{-/-}$ mice develop more severe AD-like lesions with increased morbidity compared to Stat6VT mice. However, PARP14 is not required in keratinocytes to mediate the expression of IL4 and Stat6 responsive genes important for skin barrier function, suggesting that PARP14 contributes to a hematopoietic cell-intrinsic function. Thus, the data suggests that PARP14 serves specific roles in allergic disease that vary with the target organ.

Mark H Kaplan, Ph.D. - Chair

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AD	Atopic dermatitis
AAD	Allergic airway disease
ADP	Adenosine diphosphate
AHR	Airway hyperresponsiveness
Alum	Aluminium hydroxide
APC	Antigen presenting cell
ART	ADP-ribosyl transferase
ARTD	ADP-ribosyl transferase diphtheria toxin-like
BAL	Bronchoalveolar lavage
BCR	B cell receptor
CBP	CREB-binding protein
CCL	C-C chemokine ligand
CCR	C-C chemokine receptor
CD	Cluster of differentiation
CE	Cornified envelope
ChIP-Seq	Chromatin immunoprecipitation-sequencing
CTL	Cytotoxic T cell
DC	Dendritic cell
DETC	Dendritic epidermal T cell
DNA	Deoxyribonucleic acid
DTT	Dithiothreitol
EAE	Experimental autoimmune encephalomyelitis

EASI	Eczema area severity index
ELISA	Enzyme-linked immunosorbent assay
EoE	Eosinophilic esophagitis
EDC	Epidermal differentiation complex
FBS	Fetal bovine serum
GATA3	GATA binding protein 3
GC	Germinal center
GFP	Green fluorescent protein
GM-CSF	Granulocyte macrophage colony stimulating factor
HAT	Histone acetyl transferase
HDAC	Histone deacetylase
HPF	High power field
ICS	Intracellular staining
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
iNOS	Inducible nitric oxide synthase
IP	intraperitoneal
JAK	Janus activated kinase
K1	Keratin 1
K5	Keratin 5
K10	Keratin 10
K14	Keratin 14

LPS	Lipopolysaccharide
LES	Lower esophageal sphincter
MEASI	Mouse eczema area severity index
MHC	Major histocompatibility complex
mRNA	messenger ribonucleic acid
NAD	Nicotinamide adenine dinucleotide
NCoA	Nuclear receptor co-activators
NFAT	Nuclear factor of activated T cells
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NK	Natural killer
NKT	Natural killer T
Ova	Ovalbumin
PAMPs	Pattern associated molecular patterns
PARP	Poly ADP-ribose polymerase
PBS	Phosphate buffered saline
PMA	Phorbol 12-myristate 13-acetate
PRR	Pathogen recognition receptor
qRT-PCR	quantitative real-time polymerase chain reaction
RNA	Ribonucleic acid
STAT	Signal transducer and activator of transcription
SH2	Src - homology 2
SPRR	Small proline-rich proteins

SRBC	Sheep red blood cell
TCF-1	T cell factor 1
TCR	T cell receptor
Tfh	T follicular helper
TGF	Transforming growth factor
Th	T helper
TNF	Tumor necrosis factor
Treg	T regulatory
TEWL	Transepidermal water loss
TSLP	Thymic stromal lymphopoietin
UES	Upper esophageal sphincter

INTRODUCTION

Immune System

The function of the immune system is to defend the body against infectious microbes. Components of microbes as well as macromolecules, such as proteins, polysaccharides and small chemicals that are recognized as foreign can elicit an immune response. Innate and the adaptive immunity form important aspects of the immune system that work together to generate effective protective responses against infectious pathogens.

Innate Immunity

Innate immunity provides the early line of defense; the defense mechanisms are in place before an infection and respond rapidly to an infection. Skin provides a physical barrier and forms the first line of defense. In addition to forming a physical barrier, the enzymes (anti-microbial peptides) produced by the skin can kill microbes and the cytokines produced can promote and regulate immune responses (1). Epithelial cells held together by tight junctions line the gastrointestinal, respiratory and urogenital tracts. Internal epithelia is coated with mucous that prevents the adhesion of microorganisms, and cilia on epithelial cell layers in the respiratory, urogenital and gastrointestinal tracts help to continuously expel mucous and microorganisms. When this barrier is broken, microorganisms can cross the epithelial barrier where various immune cells then recognize and try to eliminate the pathogen. Cell mediated immunity of the innate

immune system is mediated by phagocytes – macrophages, neutrophils, dendritic cells (DCs), and cells that release inflammatory mediators – mast cells, basophils, eosinophils, natural killer cells (NK cells) (2).

Cells of the innate immune system recognize certain highly conserved structures on the surface of microorganisms referred to as pathogen associated molecular patterns (PAMPs) (eg. LPS, double stranded RNA, mannans). PAMPs are recognized by pathogen recognition receptors (PRRs) on the surface of macrophages, dendritic cells and neutrophils. Recognition of a PAMP by PRR on macrophages leads to phagocytosis and killing of the pathogen. Neutrophils migrate to site of infection within a few hours of pathogen entry and like macrophages, sequester and destroy microbes. Eosinophils and basophils carry cytoplasmic granules containing cytokines and cytotoxic proteins that are released into the extracellular space for defense against parasites. Mast cells provide defense against helminths by degranulating thereby releasing proinflammatory substances including cytokines, acidic proteoglycans and histamine. They also release inflammatory mediators like cytokines and chemotactic factors that attract other immune cells to the site of inflammation and enhance the immune response. Unlike other cells that recognize pathogen via PAMPs, NK cells lack antigen specific receptors. NK cells monitor the expression of major histocompatibility complex class I (MHC I) on all nucleated cells and kill virus-infected cells and tumor cells that downregulate MHC I. Immature dendritic

cells (DCs) are also capable of phagocytosis of pathogens upon recognition of PAMPs. Mature DCs then present antigens to T-cells in the lymph nodes (3).

Adaptive immunity

Unlike innate immune pathways that are activated relatively rapidly, the adaptive immune responses become effective only after several days and show great specificity to target antigens. T cells express αβ T cell receptor (TCR), the majority express αβ TCR, while others γδ TCR. T - and B-cells develop antigen specificity via somatic rearrangement within T cell receptor (TCR) and B cell receptor (BCR) genes. This results in a pool of lymphocytes expressing a diverse repertoire of antigen specific receptors.

Professional antigen presenting cells (APCs) such as dendritic cells, macrophages and B-cells break down foreign protein into peptides that are then loaded on MHC molecules and presented on cell surface. Upon interaction of naïve T cells with APC expressing MHC-peptide complex along with co-stimulatory signals- CD80 and CD86, T cells become activated. CD4+ T cells recognize peptides presented on MHC class II molecules whereas CD8+ T cells recognize peptides presented on MHC class I molecules.

Upon activation, naïve CD4+ T cells undergo clonal expansion and differentiation into effector T cells. The specific cytokine milieu encountered by naïve T cels determines which subset of T helper cells develops (e.g. Th1, Th2, Th9, Th17,

Treg or Tfh cells). These subsets are often characterized by the cytokines they secrete and the immune response they elicit. Th1 cells secrete IFNγ and TNF-α. These cytokines are important in mediating host defense against intracellular pathogens. However Th1 cells can also cause autoimmune diseases. Th2 cells secrete IL-4, IL-5 and IL-13 and are important for immunity to extracellular pathogens and allergic disease. Th9 cells are characterized by IL-9, IL-10 and IL-21 production and are important to clear extracellular pathogens and mediate allergic diseases. Th17 cells secrete IL-17A, IL-17F, IL-21 and IL-22 and are important for host defense against bacterial and fungal pathogens; they also mediate autoimmunity and allergic diseases. Treg cells secrete IL-10, TGF-β and IL-2 and are responsible for establishing immune tolerance. Treg cells can be sub-classified into those that differentiate in the thymus (tTreg cells), the periphery (pTreg cells) and in vitro (iTreg cells). Tfh cells secrete IL-21 and IL-4 and are important in humoral immunity by activating follicular B cells in lymphoid tissues (3, 4).

Peptide antigens presented on MHC class I molecules activate CD8 T cells, also referred to as cytotoxic T cells (CTL). CTLs contain cytoplasmic granules filled with toxic proteins like granzymes and perforins. When CTLs recognize cells bearing non-self antigens such as virus infected cells or tumor cells, toxins from the granules are released only in the direction of the target cells by the formation of an immune synapse, thus avoiding damage to healthy neighboring cells (2).

Similar to T cells, naïve B cells, upon antigen binding to BCR, get activated. Both protein and non-protein antigens stimulate antibody responses. Naïve mature B cells express surface IgM and IgD. B cell responses to protein antigens require involvement of a CD4+ helper T cell specific for that antigen. Activated T cells express CD40L that engages with CD40 expressed on B cells. Cytokines secreted by these T cells bind to cytokine receptors on B cells. This stimulates B cell proliferation and differentiation. Some of the B cells undergo isotype class switching leading to the generation of various immunoglobulin (Ig) isotypes. Isotype class switching is regulated by cytokines produced by T helper cells that are activated in response to the microbes. The immunoglobulin isotypes are IgD, IgM, IgG, IgE and IgA, with IgG and IgA further classified into subclasses each with distinct effector functions. Affinity maturation leads to the generation of antibodies with increased affinity for a particular antigen, and results in antibodies, which more efficiently bind to, neutralize and eliminate microbes. B cell activation can lead to the selective survival of B cells producing antibodies with the highest affinity (3,5).

Th2 cells

Th2 (T helper type 2) cells secrete IL-4, IL-5, IL-9, IL-10 and IL-13. Th2 cells mediate host defense against extracellular pathogens, such as helminths. Th2 cells also mediate allergic disease like asthma and atopic dermatitis and play an important role in disease pathology. T cells can be polarized *in vitro* by IL-4 into Th2 cells. IL-4 signaling through the IL-4R, leads to the phosphorylation and

homodimerization of STAT6. IL-4 via STAT6 upregulates the expression of GATA3, the master transcription regulator of Th2 cell differentiation. STAT6-independent pathways, such as Notch signaling pathway, Wnt signaling pathway through β-catenin and T-cell factor 1 (TCF-1) can also promote GATA3 expression. IL-2 activation of STAT5 is also crucial for Th2 cell differentiation in vitro. In addition to STAT6, STAT5 and STAT3 are also important for Th2 cell differentiation. STAT5 binds to regions on the *Il4* locus and in combination with GATA3, STAT5 can induce IL-4 expression. STAT3 also becomes activated during Th2 differentiation and STAT3 is required for STAT6 ability to bind to target genes (6). Epithelial cells are a source of cytokines such as Thymic stromal lymphopoietin (TSLP), IL-25 and IL-33. These cytokines also contribute to type 2 immune responses (7).

IL-4 induces B cell class switching to IgG1 and IgE. IL-4 induces class switching to IgE and IgE can bind to high affinity Fc receptors (FcεRI) on mast cells, basophils and eosinophils. Binding of antigen to IgE, cross-links IgE bound to its receptor resulting in activation and degranulation of these cells. Upon activation, mast cells and basophils secrete inflammatory mediators such as histamine, chemokines, cytokines and lipid mediators like prostaglandins and leukotrienes. IgE-dependent mast cell activation in the gastrointestinal tract promotes expulsion of parasites by increasing peristalsis and mucus secretion. In mucosal tissues, IL-5 can promote recruitment of eosinophils, and IL-9 produced can recruit mast cells, resulting in tissue eosinophilia and mast cell hyperplasia,

respectively. IL-4, IL-9 and IL-13 can act directly on epithelial cells and smooth muscles leading to goblet cell hyperplasia, mucous production. Th2 immune responses can cause airway hyperresponsiveness (AHR) in the lung and can cause barrier dysfunction in the skin and esophagus. Thus, though Th2 mediated responses are important for host immunity, they can also be harmful to the host (7).

STAT6

STATs (Signal Transducer and Activator of Transcription) are latent cytoplasmic transcription factors that activate gene transcription in response to cytokines. In response to extracellular cytokine signals and growth factors, STAT proteins undergo tyrosine phosphorylation and are involved in many different regulatory events such as hematopoiesis, immunomodulation and development (8). Phosphorylated STAT6 is transported to the nucleus, where along with co-factors, STAT6 regulates the expression of genes that are involved in allergic inflammatory responses. Upon exposure to an environmental allergen, epithelial cells in the airways, esophagus and the keratinocytes in the skin secrete cytokines that initiate the development of an immune response (9). Chronic challenge with allergen results in organ-specific diseases such as asthma or food allergy that are characterized by pulmonary and gastrointestinal inflammation respectively.

Signal transducer and activator of transcription 6 (STAT6) is a member of the STAT family of proteins, which consists of STAT1, 2, 3, 4, 5A, 5B and 6. Human STAT6 is located on chromosome 12q13.3. The cytokines IL-4 and IL-13 activate STAT6 (10). The type I IL-4 receptor is composed of the common gamma chain (γc) and the IL4Rα chain and binds only IL-4. The type-II IL-4 receptor is composed of IL4Rα chain and IL13Rα1 chain and binds to and transmits signals of both IL-4 and IL-13. Three central tyrosine residues in IL4Rα chain (Y575, Y603 and Y631) become phosphorylated by stimulation of the receptor and are important for the activation of STAT6. These phosphotyrosines on the receptor chain become docking sites for SH2 domains of STAT6 monomers. The SH2 domains then get tyrosine phosphorylated themselves by receptor associated Jak kinases (Jak1 & 3). This allows for dimerization of STAT proteins via their SH2 domains. The resulting STAT6 dimer can now migrate to the nucleus where it can bind DNA directly (11–13)

Early studies in Stat6$^{-/-}$ mice showed that STAT6 is involved in IL-4 mediated functions including Th2 cell differentiation, class switching to IgE and expression of cell surface markers such as MHC-II and CD23 (14–16). STAT6 regulates the expression of Gata-3, and Gata-3 reconstitutes Th2 development in STAT6-deficient T cells, making it a critical factor for Th2 cell differentiation and function (17).

Mechanisms of STAT6-dependent gene regulation

The protein structure and domain organization of STAT6 includes the N-terminal domain that is involved in dimerization, coiled-coil domain required for interaction with other proteins, DNA binding domain, SH2 domain (src homology domain) that interacts with phosphorylated tyrosine residues in the receptor and the transactivation domain at the C-terminal. STAT6 preferentially binds to $TTCN_4GAA$ sequence and activates gene transcription. STAT6 recruits specific sets of co-activators to provide specificity for promoter activation (12). STAT6 recruits co-activators such as CBP/p300 and NCoAs to activate gene expression by providing histone acetyltransferase (HAT) activity. PARP14 has been identified to be one such co-factor that associates with STAT6 and regulates gene expression.

PARPs

Poly (ADP–ribose) polymerases (PARPs) family of proteins contains 17 homologues, of which PARP1 is the founding member. PARPs are enzymes that catalyze the transfer of ADP ribose moieties from donor NAD+ molecules to target proteins although members of the family vary in their enzymatic abilities. Mono- and poly- ADP ribosylation is a protein modification that impacts the regulation of transcription, cell proliferation, apoptosis, signaling cascades and cell differentiation. Since the catalytic product of many of the members is unclear and some don't have a catalytic activity, a new nomenclature was proposed. Based on the type of enzymatic reaction and on structural features, PARP family

members are now referred to as ADP-ribosyl transferase Diphtheria-toxin like (ARTD) family of proteins (18).

PARPs are well studied in DNA damage and cancer. As shown by Catalogue of somatic mutations in cancer (COSMIC), there are four known mutations in PARP14 that have been implicated in cancer. The mutations are – c.4754A>G (Pancreas, carcinoma), c.2780T>G (Endometrium, carcinoma), c.1308G>A (Large intestine, carcinoma), c.1698C>G (Urinary tract, carcinoma). Though no mutations or SNPs have been related to allergic inflammation.

Of the 17 PARP family members, PARP1 is the most extensively studied. Reports using PARP inhibitors and knockout mice have shown that, in addition to its role in surveillance and maintaining genome integrity, PARP1 also functions in inflammatory diseases by triggering cell death in injured tissue (19). PARP family members including PARP1 and PARP2 are involved in T cell development in the thymus and in the periphery (20, 21). PARP1 regulates T cell activation and absence of PARP1 alters expression of genes and chemokines that are involved in maintaining Th1-Th2 balance (22). PARP1 and PARP14 have been studied to have a role in allergic inflammation.

PARP1 (ARTD1) and allergic inflammation

PARP1 was originally characterized for its role as a DNA damage sensor and its function in DNA repair. More recently, PARP1 has been identified for its role in

transcription, chromatin structure modification, cell death pathways and in the mitotic apparatus among many others, thus having outcomes in carcinogenesis, genome maintenance, inflammation, neuronal function and aging (23). PARP1 participates in inflammation by directly or indirectly regulating the expression of inflammatory factors including cytokines, adhesion molecules and iNOS (24). Inflammation in the lung or any other organ causes an increase in PARP expression. PARP1 has emerged as a critical player in the development and the progression of allergic airway disease. Animal models of allergen (Ovalbumin; Ova) induced airway inflammation elucidates the positive involvement of PARP1 in airway inflammation and airway hyper responsiveness (AHR) (25, 26).

In an Ova triggered model of asthma, *Parp1-/-* mice and mice treated using pharmacological PARP inhibitors to target PARP1 had diminished inflammation with diminished infiltration of inflammatory cells into the lung, reducing AHR and mucus secretion (25–28). This protective effect in the absence of PARP1 is attributed to inhibition of NF-κB activation and the regulated expression of molecules like TNF-α, iNOS and NFATc (25, 29). Decreases in the amount of IL-5 were linked to reductions in the number of eosinophils infiltrating the lung of *Parp1-/-* mice, suggesting that PARP1 regulates IL-5 production (24). The absence of PARP1 or its enzymatic activity severely decreases STAT6 protein without having an effect on STAT6 mRNA levels. STAT6 protein degradation was allergen stimulated and mediated by calpain as observed in an asthma model in *Parp1-/-* mice. This in turn results in reduced IL-5 levels, decreased eosinophilia

and lung inflammation (30). Together, studies of PARP1 in models of asthma suggest that PARP1 directly regulates STAT6 and its absence can mitigate all signs of inflammation.

PARP14 (ARTD8) and allergic inflammation

PARP family members are classified into sub-families based on their domain architecture. One such sub-family is macro-PARPs that include PARP9, PARP14 and PARP15. Members of this family contain repeats of a domain found in the non-histone-like region of variant histone macroH2A (31, 32). PARP14 is enzymatically active and catalyzes mono-ADP ribosylation. Studies from many labs have shown that PARP14 functions in transcriptional regulation, signal transduction pathways and in actin cytoskeleton regulation (33). PARP14 lacks the conventional transcriptional co-regulator domains such as HAT, Bromo, Chromo, SET or ATPase domains.

PARP14 associates with STAT6 in the presence or the absence of IL-4, but not with STAT1, and brings about transcriptional enhancement to IL-4 dependent and not IFNγ dependent gene activation (Figure 1) (34). Although the macro domains of histone macro H2A are known to participate in gene silencing, the macro domains of PARP14 interact with STAT6 and increase IL-4 induced gene expression (34). PARP14 and its associated PARP catalytic activity can mono-ADP-ribosylate itself and a STAT6 co-activator, p100 (Figure 4). The enzymatic activity of PARP14 is required to enhance gene transcription. Most PARP inhibitors function as competitive inhibitors, as they occupy the NAD binding site

within the catalytic domain of the enzyme. Blocking PARP activity using pharmacological inhibitors blocked IL-4 dependent gene transcription *in vivo* (35). Studies show that PARP14 regulates STAT6-dependent gene transcription by functioning as a transcriptional switch. Under non-stimulating conditions, PARP14 binds to STAT6-responsive promoters and recruits HDAC2 and 3 and represses gene transcription. Upon IL-4 stimulation and STAT6 activation, the catalytic activity of PARP14 is increased resulting in ADP-ribosylation of itself and HDACs. The HDAC repressor complex leaves the promoter relieving gene repression and making the promoter more accessible to histone acetyl transferases (CBP/p300, NCoA-1 and NCoA-3) and increase gene transcription (Figure 1) (36). As PARP14 functions as a transcriptional switch for STAT6 dependent gene transcription, naïve T cells from *Parp14-/-* mice cultured under Th2 conditions secrete reduced amounts of Th2 cytokines IL-4, IL-5 and IL-13 (37). Inhibiting PARP enzymatic activity using a pharmacological inhibitor confirmed a dose dependent reduction in Th2 cytokines, with similar amounts of IFNγ produced from Th1 skewed cells (37). The mechanism for promoting Th2 differentiation is based on the ability of PARP14 or its enzymatic activity to regulate the binding of STAT6 to the *Gata3* promoter. In a model of allergic airway disease (AAD), *Parp14-/-* mice have reduced numbers of inflammatory cells in BAL and reduced AHR.

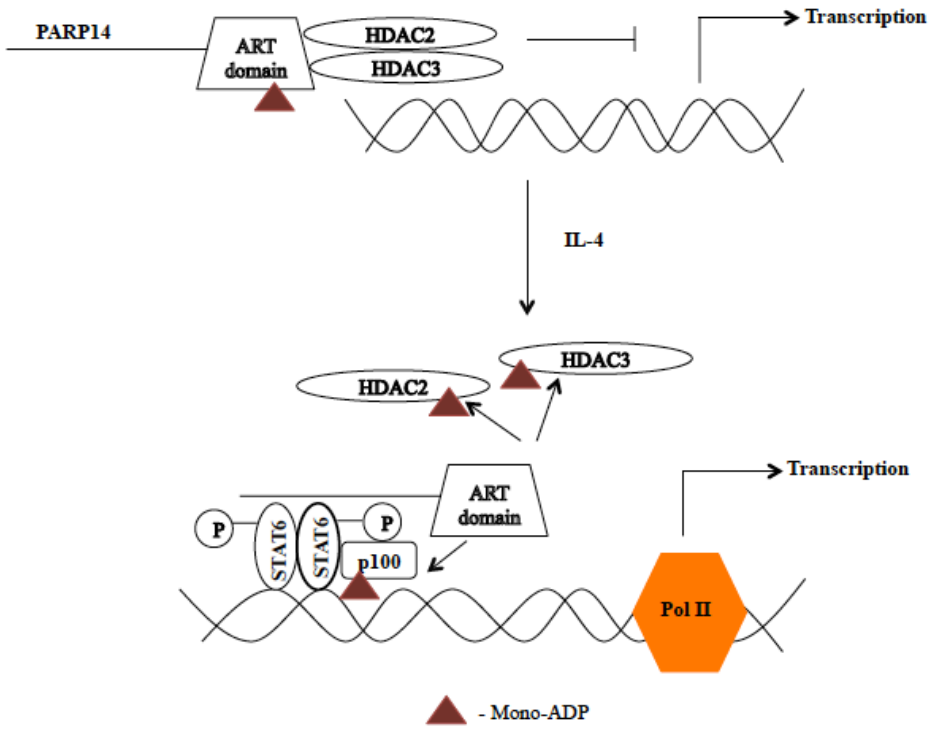

Figure 1. PARP14 (ARTD8) and its enzymatic activity functions as an activator of STAT6. In the presence of IL-4, PARP14 mono-ADP ribosylates histone deacetylase-2 and -3 (HDAC-2 and HDAC-3), leading to their dissociation from the IL-4 responsive promoter. This allows STAT6 and transcription co-factors such as p100 to bind to the promoter, resulting in active gene transcription.

Until recently, the role of PARP14 in other T helper cell subset differentiation and development was not known. Th17 cells are defined by their ability to produce IL-17A, IL-17F and IL-22 and to a lesser degree TNF and IL-6. They play a pivotal role in host defense and in induction and propagation of autoimmunity (38). Increases in IL-17A are correlated with increases in neutrophil recruitment

and airway resistance in patients with asthma (39). T follicular helper (Tfh) cells are responsible for providing cognate help to B cells during formation of germinal centers. CD4 Tfh cells secrete IL-4, IL-10 and IL-21. IL-21 is required for germinal center formation for T-cell dependent antigens (40). Tfh cells are essential for IgE production and play an important role in allergic responses (41). ChIP-Seq analysis of wild-type and *Parp14*$^{-/-}$ Th2 cells identified that PARP14 along with its enzymatic activity regulated the expression of *Il21*, and IL-21 promotes Th17 and Tfh development and function (42). It was also identified from the ChIP-Seq data that the expression of a number genes were dependent on the enzymatic activity of PARP14 but were not regulated by STAT6, suggesting that PARP14 has STAT6-independent functions (42). Upon further studies, *in vitro Parp14-/-* Th17 cells had reduced frequency of IL-17A and IL-17F positive cells. Decreased frequencies of IL-17A and IL-17F were also observed when PARP catalytic activity was inhibited using PARP inhibitor, PJ34. *In vivo* in a model of AAD, *Parp14-/-* mice had decreased numbers of IL-17A+ CD4 cells and reduced IL-17A concentrations from antigen-stimulated splenocytes. PARP14 regulates *Maf* and *Irf4* that are important transcription factors for Tfh development. Diminished Tfh and GC B cell frequency and numbers were observed in *Parp14-/-* mice in response to sheep red blood cell (SRBC) and influenza virus immunizations with decreased titres of SRBC-specific IgG. This has been attributed to decreased STAT3 phosphorylation potentially due to reduced IL-6 receptor alpha (*Il6ra)* expression in both Th17 and Tfh cells (43).

PARP14 and its enzymatic activity also have functions in other cell types. In B-cells, PARP14 is required to maintain the balance of B-cell subsets in the spleen and in generating IgA responses to antigen. PARP14 induces the expression of B-cell survival factors such as Pim-1 and Mcl-1 and protects IL-4 treated cells from apoptosis (44). PARP14 deficient mice have significantly reduced numbers and frequency of Tfh cells. Tfh cells promote GC B cell development and a 45% reduction in the numbers and frequency of GC B cells was observed along with reductions in serum IgG1 and IgG2a/c titres in PARP14 deficient mice in response to SRBC immunizations. These could either be B-cell intrinsic effects or extrinsic mechanisms due to reduced Tfh numbers (43). Epithelial cells express PARP14 and this pathway has a significant role in airway epithelial cells where PARP14 deficient mice subjected to Ova induced AAD had diminished AHR (37). In esophageal epithelial cells, STAT6 regulates the expression of eotaxin-3 and it is found to be dramatically increased in patients with EoE (45, 46). PARP14 expression is increased in EoE biopsy samples with a positive correlation to eotaxin-3 expression, which was entirely dependent on STAT6 binding to eotaxin-3 promoter (47). Inhibiting the enzymatic activity of PARP14 in human esophageal epithelial cells cultured with IL4 and/or IL13, decreased the expression of eosinophil chemoattractant – eotaxin-3 (*CCL26)* (47). Keratinocytes, which are highly specialized epithelial cells also express PARP14.

Contrasting the roles of PARP1 and PARP14 in allergic airway inflammation

In T cells, PARP1 has also been shown to modulate transcription factors such as

STAT6, NFAT and NF-κB (25, 29), while PARP14 primarily regulates STAT6 gene expression (36) and the activation of STAT3 (43). Though PARP1 and PARP14 seem to play similar roles in STAT6-mediated allergic inflammation, they regulate GATA3 gene expression and hence Th2 differentiation in very different ways. In the absence of PARP1, calpain mediates the degradation of STAT6 to reduce protein concentration within the cell (30), whereas in the absence of PARP14, HDACs remain bound to the *Gata3* promoter, inhibiting gene expression (87). The absence of PARP14 and its enzymatic activity resulted in a reduced expression of IL17A, IL17F and IL21 (36), whereas the absence of PARP1 has no effect on IL17 production (48). Lung cells of Ova challenged *Parp1*$^{-/-}$ mice fail to synthesize GM-CSF and IL-5 resulting in impaired eosinophil recruitment (24). In comparison, the significant reduction in lung eosinophilia in *Parp14*$^{-/-}$ mice is attributed to the decrease in expression of CCL24 (37). The production of IL-4 was inhibited to a much lower extent compared to IL-5 and IL-13 by PARP-1 inhibition (24). While there was a positive correlation between PARP14 and CCL26 expression in EoE patient biopsies, the same was not observed between PARP1 and CCL26 (47). Together, these studies suggest that PARP1 and PARP14 interact with different partners and regulate inflammation through independent functions.

STAT6 plays varied functions in allergic inflammation both in immune cells and in resident tissue cells such as airway, esophageal, and intestinal epithelial cells, and keratinocytes (Figure 2). Current therapies to treat allergic disease are

targeted towards disease symptoms rather than the causative factor. Since STAT6 plays a pivotal role in allergic diseases, targeting the function of STAT6 has been an appealing therapeutic strategy. Along with STAT6, inhibiting the transcriptional co-activator PARP14 would be helpful. PARP inhibition might have beneficial effects in inflammatory conditions such as asthma (24, 28) and contact hypersensitivity (49, 50). In a mouse model of AAD, *Parp14*$^{-/-}$ mice have decreased lung inflammation, airway hyper responsiveness and serum IgE (37). Administering a pharmacological PARP inhibitor – PJ34 (that inhibits the enzymatic activity of PARPs) to wild-type mice that were subjected to AAD, reduced the severity of airway hyperesponsiveness and lung inflammation along with a reduction in the Th2 responses. Administering PJ34 either during challenge or sensitization and challenge or multiple times during the sensitization phase were effective (37). Recent studies show that PARP14 is required for STAT3 activation (43), suggesting that it could be a potential therapeutic target not only for allergic inflammation but also in other immune responses where this signaling pathway is important. The studies in this dissertation are focused on gaining a better understanding of the function of PARP14 in allergic inflammation.

As allergic airway disease is attenuated in *Parp14*$^{-/-}$ mice or in mice treated with PARP inhibitor, PJ34, in this dissertation we study the role of PARP14 in allergic inflammation of the esophagus (eosinophilic esophagitis, EoE) and of the skin (atopic dermatitis, AD).

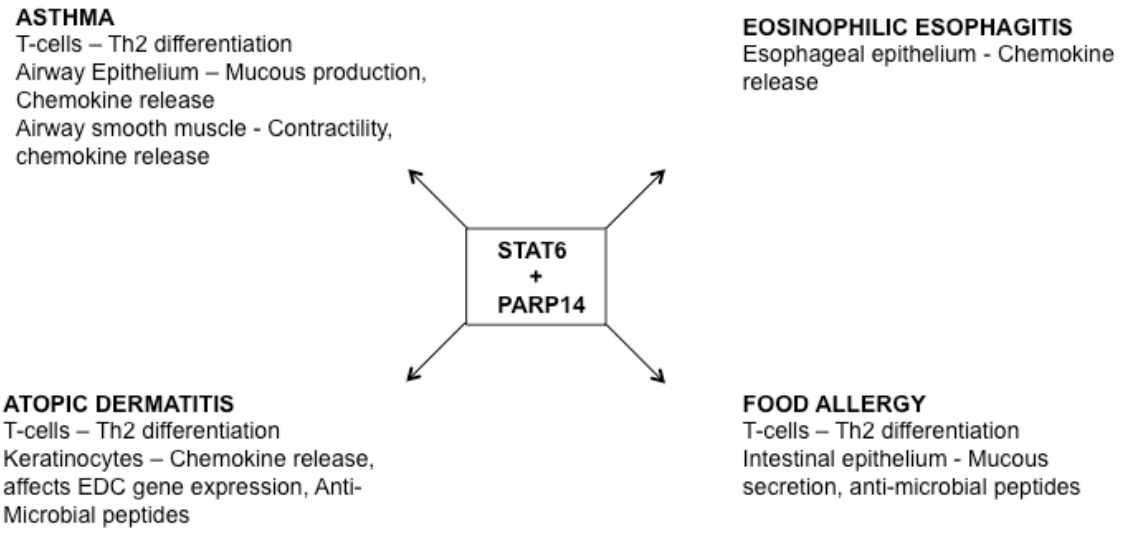

Figure 2. Function of PARP14 and STAT6 in allergic inflammation. Although the studies on PARP14 are still emerging, this figure illustrates the function of PARP14 and STAT6 in asthma, atopic dermatitis, eosinophilic esophagitis and food allergy.

Esophagus physiology

The esophagus is a tubular muscular structure connecting the throat with the stomach and is approximately 8-10 cm at birth and 18-26 cm in adults. The primary function of the esophagus is to propel the swallowed food, fluids and saliva from the throat into the stomach. A bundle of muscles at the upper end of the esophagus constitutes the upper esophageal sphincter (UES), is under conscious control and used during breathing, eating, vomiting and belching. Lower esophageal sphincter (LES) is a bundle of muscles located at the lower end of the esophagus, where the esophagus meets the stomach. LES muscles

are not under voluntary control and prevent the backward flow of stomach acids and contents (51).

The esophageal wall is composed of mucosa, submucosa and muscularis propria. The mucosa is made up of stratified squamous epithelial cells, the submucosa contains the esophageal glands and the muscularis propria is composed of the striated and the smooth muscle. The secretions of the esophageal submucosal glands are involved in mucosal clearance, peristalsis and salivary secretion.

Defense provided by the esophagus to injury due to acid refluxate include luminal acid clearance and tissue resistance. Tissue resistance is a term that is used for all structural and functional components of the esophagus that withstand contact with the luminal acid. Mucous layer, unstirred water and bicarbonate ions comprise the pre-epithelial defense of tissue resistance. The esophageal mucosa consists of 30 cell layers of keratinized stratified squamous epithelium with three functional regions: stratum corneum, stratum spinosum and stratum germinativum. These epithelial layers form a part of the epithelial defense of tissue resistance. The uppermost seven to eight layers of cells comprises the stratum corneum that is designed to protect against luminal contents. Apical cell membranes and adjacent intercellular cell junctions such as tight junctions and zonula adherens provide barrier function.

Acid from the lumen can destroy the epithelium by crossing the cell membrane to lower the cytosolic pH and sustain the acidic pH long enough to destroy cell respiration. Acid from the lumen can also enter the cell cytosol from across the junctional complex into the intercellular space and then across the basolateral membrane. This increase in junctional permeability leads to the development of dilated intercellular spaces within the epithelium. Post-epithelial defense is provided by the blood supply to the esophageal epithelium. Along with nutrients and oxygen provided to the epithelium for cellular function and repair, the blood also brings in bicarbonates that neutralize the acid in the intercellular spaces. Dilated intercellular spaces are observed in acid exposed esophageal epithelium. Prolonged acidification of the cytosol can lead to cell injury as reflected by cell swelling.

Cells of the lower layers, that is, stratum germinativum, are capable of replication and repair the injured epithelia. Replication is shown to begin 30 minutes after acid exposure. Repair of the epithelia is stimulated by epidermal growth factor, hepatocyte growth factor and insulin-like growth factor. Cell turn over in the esophageal epithelium is 5 to 8 days (52).

In susceptible individuals, eosinophilic esophagitis (EoE) is triggered by an immune response to antigens acquired via food or inhalation of environmental antigens. Recognition of these antigens leads to a series of cytokine-mediated immune responses resulting in the infiltration of eosinophils, basal zone

hyperplasia and subepithelial fibrosis with increased collagen deposition. Microscopic structural changes to the esophagus leads to some of the symptoms of EoE – food impaction, dysphagia and chest or abdominal pain.

Eosinophilic Esophagitis

Eosinophilic esophagitis (EoE) is a chronic inflammation of the esophagus and an emerging disease worldwide with a high rate of associated atopic disease (53). It is currently defined as a chronic, immune-mediated or antigen-mediated esophageal disease that is characterized by esophageal dysfunction and eosinophil-predominant inflammation. The dominant antigens that mediate disease are food based. Children present with food impaction, dysphagia and have esophageal mucosal eosinophilia of >15 eosinophils per high power field (scale – 0.04mm). Other histological features include aggregates of eosinophils and eosinophil layering along the luminal surface. Numbers of inflammatory cells, including lymphocytes, mast cells and basophils are also increased in the affected epithelial space (54). EoE is characterized by a Th2 response with the increase in the expression of IL-4, IL-5 and IL-13 (45). STAT6 mediates the expression of eotaxin-3/CCL26 (55, 53, 56) and hence eosinophil accumulation in EoE (57–59). Esophageal biopsies from children with EoE have increased expression of CCL26 (47). Genome wide association studies have reported three genes – TSLP, CCL26 and Calpain-14 as being altered in EoE (54). Th2 cytokines IL-5 and IL-13 downregulate the expression of desmoglein-1 (DSG1) and genes of the epidermal differentiation complex (EDC) like filaggrin (FLG) (60,

61) and impair barrier function. Altered esophageal barrier function could thus lead to enhanced antigen presentation and greater eosinophil recruitment.

Skin Physiology

The skin is the largest organ of the body with varied and important functions. It is composed of two main layers, the epidermis (uppermost layer) and the dermis, with the subcutaneous layer beneath the dermis (Figure 3A). Thickness of the skin varies depending on the area of the body and function and can range from 0.5mm in the eyelids to 3-4mm in the soles of the feet with an average thickness of 1-2mm (62).

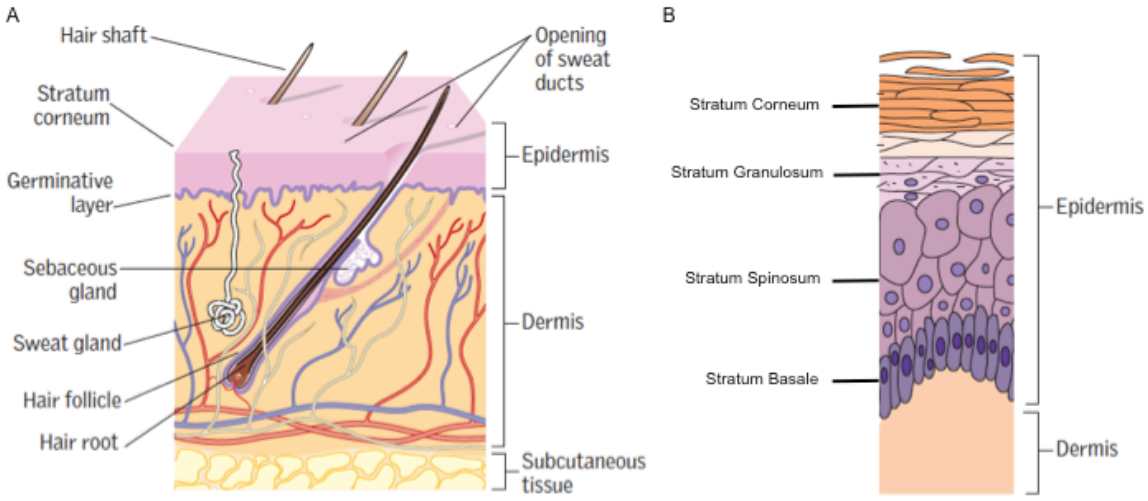

Figure 3. Skin Physiology. (A) Cross section of skin tissue showing structure of skin (B) Cross section of the epidermis showing different layers of keratinocytes. *Modified from McLafferty et al. Nurs. Stand. 2012.*

The epidermis is composed of many layers of stratified keratinized squamous epithelium. The function of the epidermis is mainly to guard against infection, to prevent dehydration, and for re-epithelialization after wounding (63). Epidermis mainly consists of keratinocytes (90%), Langerhans cells, melanocytes and merkel cells (sensory cells). The epidermis has 4 distinct layers based on the stage of differentiation stratum basale, stratum spinousum, startum granulosum and startum corneum (Figure 3B).

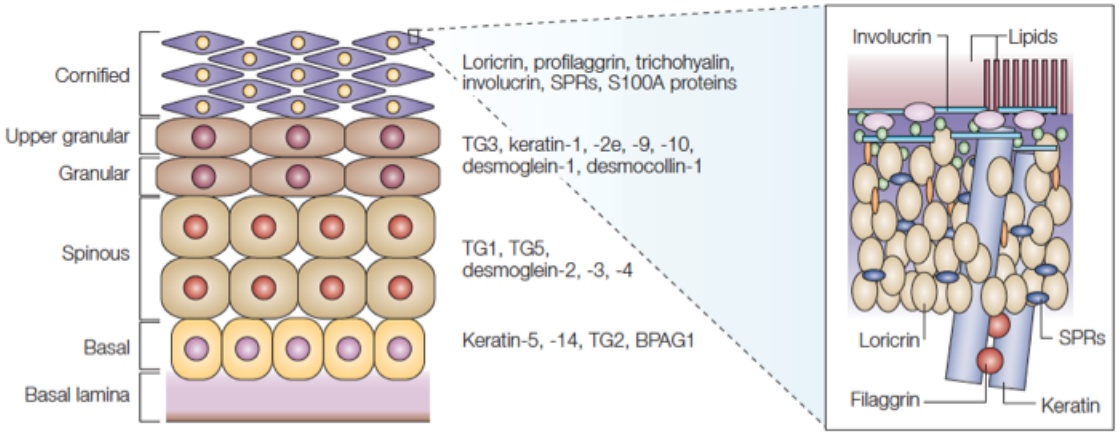

Figure 4. Terminal differentiation of keratinocytes. *Modified from Candi E et al. Nat Rev Mol Cell Biol. 2005.*

Basal layer (Stratum basale or Stratum germinativum) is one cell layer thick, and consists of proliferating cells that constantly divide to renew the basal layer. Basal keratinocytes express keratins 5 and 14 (K5 and K14), commit to differentiation by exiting the cell cycle and migrate upwards. Upon cell division, the daughter cell migrates up through the many layers to the surface of the

epidermis where they get sloughed from the skin surface. As the skin is of varied thickness based on its location in the body, this process takes approximately 28 days (62). The cells of the stratum basale migrate upwards and form the cells of the Stratum spinosum which is 5 - 12 cells thick (62). As the cells exit the basal layer and form the spinous layer, they switch the expression of keratin from K14 and K5 to K1 and K10 (64, 65), a sign that the cell has committed to terminal differentiation. The cells join together via desmosomes and cytoskeleton made of K1/K10 filament bundles that connect to the desmosomes forming a scaffold. Proteins like involucrin (IVL) and loricrin (LOR) are deposited on this scaffold to increase mechanical strength and resistance needed for the cornified envelope (CE) (66). As the cells move towards the surface, they become longer and form horizontally flattened cells of the stratum granulosum. The differentiation into the granular layer is associated with a decrease in the expression of K1 and K10 (66), increase of calcium and synthesis of keratohyalin granules (63, 66). Keratohyalin granules are made up of profilaggrin (along with loricrin in murine epidermis), which when cleaved forms filaggrin (FLG) peptides that bind to keratin with small proline-rich proteins (SPRRs) and LOR on the inside of the CE forming tight bundles that are cross-linked by transglutaminase1 (TG1) (Figure 4) (64, 66). Lamellar granules called Odland's bodies are seen in the granular cells. They contain lipids like ceramides that are secreted in to the intercellular space and play an important role in barrier function (62). By this time, the cells have lost their nuclei and cytoplasmic organelles, FLG is further processed into amino acids, forming the mature cornified envelope. Cornified envelope (CE) is an

insoluble structure of 5-20 nm thick, the proteins are cross-linked by transglutaminases that hold the keratin macrofibrils (63, 66). This layer of dead cells forms a protective barrier and is continuously sloughed from the surface and replenished by cells from the inner layers that move outwards.

Apart from keratinocytes, the epidermis also contains immune cells like the Langerhans cells and dendritic epidermal T cells (DETCs). CD8+ T cells are usually found in the startum basale and stratum spinosum. In contrast, the dermis contains many specialized immune cells like dendritic cells, CD4+ T cells, γδ T cells, NKT cells, macrophages and mast cells. Keratinocytes are also a main source of antimicrobial peptides like β-defensins and cathelicidins (67, 68). Genes that encode for major proteins of the late epidermal differentiation are located in a region on human chromosome 1q21; known as the Epidermal Differentiation Complex (EDC) (66). EDC contains three families of genes – (a) the late cornified envelope proteins that include IVL, LOR (b) calcium binding proteins (S100 proteins) and (c) fused gene proteins that include FLG, FLG-2 and HRNR (66).

The skin barrier maintenance genes that we look at in this dissertation that are altered during allergic inflammation include the following: FLG is synthesized as profilaggrin consisting of 10-12 tandem repeats of filaggrin peptide. FLG peptides are further broken down into hydrophilic amino acids or amino acid by-products that maintain the pH levels of the skin. In the absence of FLG, the integrity and

cohesion of the corneocytes is weakened, making the barrier leaky, allowing for the entry of irritants, microorganisms and increasing trans-epidermal water loss (TEWL) (63, 66, 69). Involucrin is a minor component of the human cornified envelope and is important during the early stages of CE formation by forming a protein layer for the attachment of ceramides (64, 66). Loricrin is one of the most abundant proteins in the CE, glycine-rich allowing for flexibility and expression is induced by calcium in the late granular layers (64,66).

Other proteins that are important for maintaining skin barrier integrity and regulated by IL-4 are CA II, CISH, CCL26, HAS3, SERPINB 3/4. Carbonic anhydrase II (CA II) is expressed in varied tissues and this enzyme is involved in the maintenance of cellular pH, water transport and in ion homeostasis. Studies show that CA II expression in regulated by Th2 cytokines (70).

Cytokine inducible SH2 containing protein (CISH) belongs to the SOCS family of proteins and is an inhibitor of the JAK/STAT pathway. CISH expression in keratinocytes is induced by both IFNγ and IL-4. In allergic inflammation like atopic dermatitis, the expression of CISH is increased (71, 72).

Eosinophils are one of the important inflammatory cells that migrate to a tissue during allergic inflammation. The chemokine CCL26 or eotaxin-3 is a chemoattractant for eosinophils as their receptor CCR3 is preferentially

expressed on eosinophils. Th2 cytokines IL-4 and IL-13 induce the expression of CCL26 in keratinocytes (55).

Hyaluronan synthase 3 (HAS3) is responsible for the synthesis of hyaluronic acid that is abundant in the dermis and epidermis (73). Hyaluronic acid is expressed both in the spinous layer and in the stratum corneum. Hyaluronic acid and HAS3 are involved in proliferation, differentiation and migration of keratinocytes and in maintaining the epidermal barrier. HAS3 is upregulated by proinflammatory cytokines IL-4, IL-13 and IFNγ and an increase in HAS3 is observed during inflammation along (74) with an increase in hyaluronic acid causing spongiosis (75).

SERPINB3/4 are serine protease inhibitors that were first identified to inhibit proteases and caspases. SERPINB3/4 have been implicated in allergic disorders like asthma and in atopic dermatitis and IL-4 and IL-13 via STAT6 induce their expression in bronchial epithelial cells. Serine proteases are critical for epidermal barrier homeostasis and elevated levels of SERPINB3/4 are implicated in atopic dermatitis and psoriasis (76, 77).

Atopic Dermatitis

Atopic dermatitis (AD) is a chronic, relapsing inflammatory condition of the skin. AD usually presents during early infancy and childhood but can persist into adulthood or start during adulthood. The key characteristics of AD include

pruritus, scratching and chronic relapsing lesions. AD is characterized by increases in total IgE levels, infiltration of mast cells and eosinophils, decreases in anti-microbial peptides and increased expression of Th2 cytokines (78). Many researchers favor to differentiate AD into extrinsic and intrinsic types, where extrinsic AD patients show high total serum IgE levels that are specific to environmental or food allergens and intrinsic AD patients show normal IgE levels (79). The cause of AD is still unknown though genetic factors play an important role. Mutations in the epidermal differentiation complex (EDC, important during terminal differentiation of keratinocytes) gene, filaggrin (*FLG*) can cause skin barrier impairment (80, 81). Loss-of-function mutation in *FLG* along with copy number variation is a strong predisposing factor for atopic dermatitis and this mutation is carried by approximately 9% of the European population (80, 82, 83). Flg mutant mice (flaky tail mice - Ft) carry mutations similar to mutations seen in AD patients and these mice have increased propensity to develop spontaneous AD-like skin lesions (84–86).

STAT6 and Th2 cytokines, IL-4 and IL-13 decrease the expression of EDC genes filaggrin (*FLG*), loricrin (*LOR*) and involucrin (*IVL*) in human skin biopsies and in differentiated primary human keratinocytes (87, 88). IL-4 and IL-13 are elevated in the skin of patients with AD and either cytokine can lead to decreased expression of antimicrobial genes like human β defensins (HBD)-2 and 3 (78, 89). Polymorphisms in genes encoding the cytokines IL-4 and IL-13 have shown association with AD. Polymorphisms in the promoter of IL-4 in an Egyptian

population and Czech population (90, 91) and polymorphisms in IL-13 gene in Caucasian population (92) and Japanese populations (93) show association with AD. Several polymorphisms in IL-4Rα have been found to be associated with AD (94–96). SNPs in Stat6 gene also show association with allergic disease including AD (90, 97).

Atopy is generally associated with increased activation of STAT6, hence a transgenic mouse model with constitutively phosphorylated STAT6 in T cells was generated to define a direct role for STAT6 in allergic inflammation (98). STAT6 transgenic mice, termed STAT6VT express a constitutively active form of STAT6 in T cells generated by mutating V-547 and T-548 to alanine in the SH2 domain resulting in constitutive phosphorylation of tyrosine residue, Y-641, independent of IL-4 stimulation (98). STAT6VT mice have increased Th2 cell differentiation and increased serum IgE levels (98). STAT6VT mice develop spontaneous allergic inflammation in the tissue around the eye, in the lung and in the skin (99-101). Slower recovery of skin injury caused by treatment with retinoic acid, detergent and vitamin D analogs were observed in STAT6VT mice (101-103). STAT6VT mice are also more susceptible to infections (104). STAT6VT mice skin has diminished expression of epidermal differentiation complex (EDC) genes and decreased barrier function (100). The absence of endogenous IL-4 protects the STAT6VT transgenic mice from allergic inflammation (99, 100). Many murine models of AD exist that require either gene overexpression in the skin, like transgenic mice expressing IL-4 or IL-13 in the epidermis of the skin (105, 106)

or experimental skin exposure to irritants. However, the STAT6VT model of AD primes a Th2 immune response that results in the spontaneous development of allergic skin inflammation under specific-pathogen free (SPF) conditions, thus making it an ideal model to study AD.

Atopic diseases start early in life and have a typical progression. This progression of atopic disorders from atopic dermatitis in infants to allergic rhinitis and asthma in children is referred to as atopic march. Atopy is considered to be critical in linking AD, allergic rhinitis and asthma. Genetic and environmental factors strongly influence disease development. Once diagnosed, children may develop a sequence of atopic diseases at certain ages. Studies have identified PARP14 to promote AAD in a murine model. The role of PARP14 in other atopic diseases has not yet been determined. Thus, the studies in this dissertation are focused on gaining a better understanding of the function of PARP14 in other allergic diseases such as atopic dermatitis and eosinophilic esophagitis.

Research goals

Poly ADP-ribose polymerase 14 is one of a family of 17 PARPs with mono-ADP ribosyl transferase activity. PARP14 was initially identified as a transcriptional co-activator, where in the presence of IL-4 the catalytic activity associated with PARP14 facilitates the binding of STAT6 to the promoter and aids in transcription. PARP14 and its catalytic activity promote the differentiation of T cells into Th2 cells by facilitating in the binding of STAT6 to Gata3 promoter. Studies on PARP14 deficient mice show that, allergen induced airway inflammation is attenuated and administration of a PARP inhibitor alleviated disease. The goal of this research is to investigate the role and function of PARP14 in allergic inflammation of the esophagus and the skin. We aim to determine the function of PARP14 in regulating the expression of eotaxin-3 and hence in the pathogenesis of eosinophilic esophagitis. Next, we studied the role of PARP14 in allergic skin inflammation using a model of spontaneous inflammation (Stat6VT mice). Together, our studies would further our understanding of the function of PARP14 in the pathogenesis of allergic inflammation.

MATERIALS AND METHODS

Patient Population

EoE biopsies (n=16) were obtained from children who were diagnosed with EoE (>15 eosinophils per high power field (hpf)). Biopsies from control subjects were obtained from children undergoing endoscopy but with no disease on pathological examination with 0 eosinophils per hpf (n=17). This study was approved by the Institutional Review Board of Indiana University, and informed consent for the participation in this study was obtained from parents of children enrolled in the study. We would like to thank Dr. Sandeep Gupta, Indiana University School of Medicine for the patient samples.

RNA isolation from Esophageal Biopsy and Gene Expression

RNA was isolated from esophageal biopsies using RNeasy fibrous tissue mini kit (Qiagen) according to manufacturer's protocol. RNA was reverse transcribed to make cDNA according to the manufacturer's instruction. Quantitative PCR reactions were setup by adding cDNA, Taqman primers (ThermoFisher Scientific), Taqman Fast Universal Master Mix (Applied Biosystems) and DEPC to a final volume of 10µl in MicroAmp Fast Optical 96-well plates (Applied Biosystems). Quantitative PCR was performed on duplicate samples using 7500 Fast Real-Time PCR system. Samples were normalized and gene expression of the indicated genes was assessed using quantitative PCR. Samples were

normalized to β_2- microglobulin mRNA and relative expression was calculated using the change-in-threshold method.

Esophageal epithelial cell culture

TE-7 and TE-1 esophageal epithelial cells, derived from human esophageal squamous cell carcinoma, obtained from Drs. Pierre Hainaut and Jean-Yves Scoazec, were cultured in RPMI-1640 medium (supplemented with 10% (vol/vol) FBS (Atlanta Biologicals), 1 mM glutamine (Lonza-BioWhittaker), 100 U/ml penicillin/streptomycin (Lonza-BioWhittaker), 10 mM HEPES, pH 7.3 (Lonza-BioWhittaker), 1 mM sodium pyruvate (Lonza-BioWhittaker) and 50 μM 2-mercaptoehtanol). Cells were incubated in the presence or in the absence of 2.5 ng/ml IL-4 or 20 ng/ml IL-13. The cells were then treated with 25 μM PARP inhibitor, PJ34.

Luciferase assay

TE-7 esophageal epithelial cells were transfected using Transfectin (BioRad) with a CCL26 luciferase reporter or a reporter with a mutated STAT6 binding site and control, STAT6 or PARP14 expressing plasmids. Twenty-four hours later cells were cultured in fresh RPMI media and incubated in the presence of 25 ng/ml of IL-4 or 25 ng/ml of IL-13 for 24 hours. The cells were then trypsinized and washed in PBS containing 10% FBS. GFP expression from control samples was assessed using flow cytometry. Luciferase substrate (Promega) was added to test samples, before luciferase activity was assessed.

Mice

C57BL/6 (Wild-type) mice were purchased from Harlan Biosciences. *Parp14-/-* mice on C57BL/6 background were generated by an insertion into 5' end of the first exon of PARP14 locus (44). Stat6VT transgenic mice were previously described (98). Transgene positive cofounders were (CD2:Stat6VT (78) line) carrying human Stat6 with V547 and T548 mutated to alanine under the control of CD2 locus control region (restricting expression to lymphoid populations) and backcrossed to C57BL/6 mice. Stat6VT is constitutively phosphorylated on the critical tyrosine, Y-641. This phosphorylation is important for the dimerization of Stat6VT and its ability to activate transcription. *Parp14-/-* mice were mated to Stat6VT mice to generate *Parp14-/-* deficient transgene positive mice. Mice were kept in specific pathogen-free condition and all studies were approved by Indiana University Institutional Animal Care and Use Committee.

CD4 T cell differentiation

Naïve CD4+ CD62L+ T cells were purified from spleens using magnetic isolation (Miltenyi Biotec). Naïve cells were cultured in complete RPMI medium (supplemented with 10% (vol/vol) FBS (Atlanta Biologicals), 1 mM glutamine (Lonza-BioWhittaker), 100 U/ml Penicillin (Lonza-BioWhittaker), 100 µg/ml Streptomycin (Lonza-BioWhittaker), 10 mM HEPES, pH7.3 (Lonza-BioWhittaker), 1 mM Sodium pyruvate (Lonza-BioWhittaker), 50 µM 2-mercaptoehtanol). Naïve cells were activated with plate bound anti-CD3 (2 µg/ml – 2C11) and soluble anti-CD28 (0.5 µg/ml). Cells were polarized to generate Th0 (anti-IFNγ/XMG -10

μg/ml, anti-IL-4/11B11 – 10 μg/ml, hIL-2 -50 U/ml) and Th2 (IL4 -10 ng/ml, anti-IFNγ/XMG – 10 μg/ml) cells. Cells were expanded after 3 days with media alone. After five days, cells in culture were harvested for analysis.

Surface and Intracellular staining

For splenocytes, cells were stimulated with PMA and Ionomycin or anti-CD3 (2 μg/ml) for 5 hours at 37°C, with the addition of 3 μM monensin during the last 4 hours of stimulation. After 5 hours, the cells were collected and stained with a fixable viability dye dissolved in PBS (eBioscience) along with surface markers for CD4 for 20 min at 4°C. The cells were then fixed with 4% formaldehyde for 10 min at RT, permeabilized (BD Biosciences) and with fluorochrome conjugated antibodies for IL-4, IL-13, IFNγ and IL-17A.

For phospho-STAT6 staining, differentiated Th0 and Th2 cells were fixed with the viability dye (eBioscience) and fixed with 4% formaldehyde at RT for 10 min. Cells are permeabilized in 100% methanol overnight at 4°C and stained the next day using fluorochrome conjugated antibody for phospho-STAT6 for 1 hour at RT. Cells were analyzed by flow cytometry using Attune Flow Cytometer (ThermoFisher Scientific) and results were analyzed using FlowJo.

ELISA

To obtain sera, blood obtained by cardiac puncture from mice was centrifuged at 12,000 rpm for 15 min at 4°C and sera collected was used for ELISA. To

generate cell-free supernatants, splenocytes were stimulated with plate-bound anti-CD3 (4 µg/ml) for 72 hours at 37°C and cell free supernatants were collected. 96-well Nunc MaxiSorp plates were coated with capture antibodies (2 µg/ml; BD Biosciences) dissolved in PBS or 0.1 M NaHCo3 buffer (pH 9) overnight at 4°C. Plates were then washed three times with ELISA wash buffer (0.1% Tween-20 in PBS) and blocked with ELISA blocking buffer (2% BSA, 0.01% NaN_3 in PBS) for 1 hour at room temperature. After washing the plates, diluted standards and samples (sera or cell free supernatants) were added and incubated at 4°C overnight. The wells were washed four times with ELISA wash buffer and biotinylated antibody (1 µg/ml; BD Biosciences) dissolved in ELISA blocking buffer were added (2 µg/ml, BD Biosciences) and the plates were incubated at 4°C overnight. Wells were washed four times with the wash buffer and incubated with avidin-alkaline phosphatase (1:2000; Sigma) dissolved in ELISA blocking buffer and incubated for 1 hour at RT. Wells were washed four times with wash buffer and phosphatase substrate (4 mg/ml; Sigma) dissolved in ELISA substrate buffer (10% diethanolamine, 0.05mM $MgCl_2$, 0.02% NaN_3; pH 9.8) was added. Absorbance was read at 405 nm (Bio-Rad microplate reader model 680).

Adoptive transfer

Splenic CD4+ cells were magnetically sorted (Miltenyi Biotec) from wild-type, Stat6VT and Stat6VTx*Parp14-/-* mice. Cells were re-suspended in PBS at a concentration of $1.5x10^6$ cells/ml – $3x10^6$ cell/ml and cells were transferred by

retro-orbital injections into 8-10 week-old *Rag1-/-* mice. Mice were monitored for 10-20 weeks for the development of skin inflammation and spleens and skin were harvested.

MEASI Scoring

Stat6VT and Stat6VTx*Parp14-/-* mice were scored using Mouse Eczema Area Severity Index (MEASI) scoring system. EASI (Eczema area severity index) method of scoring skin lesions in humans has been modified and adapted to mice and referred to as MEASI. Here the skin lesions in different mouse body areas (Head, trunk, tail) are given a score from 0 – 3 (nil, mild, moderate, severe respectively) for erythema, excoriation, lichenification and infiltration. Stat6VT and Stat6VTx*Parp14-/-* mice with blepharitis and a MEASI score <1 were characterized as having mild disease. Mice with skin lesions and a MEASI score >1 were characterized as having severe disease.

Histological examination of skin sections

Skin tissues were fixed in neutral buffered formalin. Paraffin embedded tissue sections were stained with hematoxylin and eosin to evaluate the infiltration of inflammatory cells by light microscopy. Slides were scored using a semi-quantitative scoring scale: 0= no inflammation, 1= 5-25%, 2= 26-50%, 3= 51-70% 4 = 71-90% 5= 91-100% infiltration of cells.

Keratinocyte cell culture

Primary human keratinocytes (HK) were isolated from excised foreskin tissue as previously described (107) and washed with antibiotics. The tissue was minced and the individual cells were released from the tissue using trypsin digestion. Keratinocytes and fibroblasts were separated by differential resistance to treatment with EDTA. Isolated HK were grown in EpiLife Complete media (Cascade Biologics) with human keratinocyte growth supplement (containing 5 μg/ml insulin; Cascade Biologics) and 1000 U penicillin–streptomycin (Roche, Indianapolis, IN, USA). To stimulate keratinocyte differentiation, HK were treated with 2 mM of $CaCl_2$ every other day and stimulated with recombinant human IL-4 (R&D Systems) as indicated.

Gene expression analysis (quantitative RT-PCR)

RNA was isolated using TRIzol (Ambion Life Technologies) and reverse transcribed to make cDNA using First-strand cloned AMV kit (Invitrogen). Quantitative PCR reactions were setup by adding cDNA, primers (Applied Biosystems), Taqman Fast Universal Master Mix (Applied Biosystems) and DEPC to a final volume of 10 μl in MicroAmp Fast Optical 96-well plates (Applied Biosystems). Quantitative PCR was performed on duplicate samples using 7500 Fast Real-Time PCR system. Samples were normalized and gene expression of the indicated genes was assessed using quantitative PCR. Samples were normalized to β_2 microglobulin mRNA and relative expression was calculated using the change-in-threshold method.

Cell isolation from the skin

Ear skin from mice were split and placed in a 6-well dish containing 1.5 ml RPMI with Liberase (2 mg/ml; Roche) with the dermis facing down. Skin samples were incubated at 37°C, 5% CO2 for 1.5 hours. At the end of 1.5 hours, liberase was inactivated with RPMI containing FBS and the samples were disrupted to dissociate the cells using gentleMACS Dissociator (Miltenyi Biotec). Cells were stained for specific surface markers based on the cell type. Basophils – $CD19^{-}$ $CD117^{-}NK1.1^{-}CD3^{-}CD11c^{-}CD49b^{+}Fc\varepsilon RIa^{+}$, Eosniophils – $CD11c^{-}CD11b^{-}Gr1^{-}$ $SiglecF^{+}$, Neutrophils – $CD11b^{+}Gr1^{+}$, Mast cells – $CD3^{-}CD117^{+}Fc\varepsilon RIa^{+}$, T-cells - $CD3^{+}CD4^{+}$ or $CD3^{+}CD8^{+}$

Retroviral transduction

Retroviral expression vector expressing eGFP (MIEG), or vectors that express STAT6VT along with eGFP have been previously described (102). Two days after naïve T cells were cultured under Th0 or Th2 conditions, the cells were transduced with retroviral supernatant for, MIEG control vector or STAT6VT-MIEG in the presence of polybrene (8 μg/ml) and IL-2. Cells were expanded on day 3 of culture and harvested on day 5. After 5 days, cells were stained with fluorochrome conjugated IL-4, IL-13, phospho-STAT6 analysis was carried out by flow cytometry for cells that were positive for GFP.

Epicutaneous sensitization

Induction of skin inflammation via epicutaneous sensitization has been described before (108). 8-10 week-old mice were sensitized by i.p. injection of Ova (20 µg; Sigma) adsorbed in alum (2 mg; Sigma) on day 0 and 7. On day 14, backs of anesthetized mice were then shaven and tape-stripped 6 times with type IV transparent dressing (Blenderm; 3M). 100 µg of Ova in 100 µl PBS or 100 µl PBS was applied on a patch of sterile gauze (1x1 cm), which was secured to the skin with a transparent bio-occlusive dressing (Bioclusive Mini; Systagenix) and dressing tape (Mediapore tape; 3M). Mice were challenged for 2 days with an interval of 2 days for a total five 2-day exposures. Hence, mice were challenged for 2 days, from days 14-30. Mice were sacrificed 4 days after the last challenge (on day 35) and spleens, lymph nodes and serum were harvested. Skin was harvested for histology.

RESULTS

Part I – PARP14 and eosinophilic esophagitis

Macro-PARP gene expression in children with eosinophilic esophagitis (EoE)

PARP14 is known to function as a transcriptional co-factor for STAT6 and in a model of allergic airway inflammation, mice deficient in PARP14 had increased lung function, diminished cellular infiltration and decreased production of Th2 cytokines compared to control mice (37). However, the requirement and function of PARP14 in STAT6 dependent gene expression in non-lymphoid cells and in human disease have not yet been assessed. To study this, we obtained esophageal biopsy samples from children with EoE and control samples from children who did not have EoE but had biopsies for diagnostic purposes (Table 1). To analyze the gene expression of macro-PARPs, we isolated RNA from biopsies, and cDNA was assessed by qPCR. We observed a 5.95 fold average increase in *PARP14* expression and a 3.1 fold average increase in *PARP1* expression, and a decrease in *PARP15* expression in EoE biopsies compared to controls (Figure 5). In contrast, we did not observe a significant difference in the expression of *PARP9*. A similar increase (4.5 fold) in *PARP14* expression was observed in EoE biopsies from Cincinnati Children's Hospital Medical Center through high-throughput RNA sequencing, and this population had more severe inflammation compared to the IU population.

Table 1 – Patient Demographics

	IU population	
	Control	EoE
Number	17	16
Age - Mean (Range)	8.5 (1.5 - 17.3)	10.7 (2.2 - 17.2)
Eos/HPF	0	46.4 ± 7.6
% Male	50	53
% on PPI	23.5	6.3
% on H_2 Blocker	11.8	0
% on Corticosteroid	0	25
% Dicyclomine	5.9	6.3
% Metoclopromide	5.9	0
% PPI & H_2 Blocker	5.9	0
% PPI & Corticosteroid	0	0
% PPI & Dicyclomine	5.9	0
% PPI & Metoclopromide	5.9	0
% H_2 Blocker & Metoclopromide	5.9	0

PPI – proton pump inhibitor, are potent inhibitors of H+, K+ ATPase and used to treat acid reflux

H2 blockers – prevent histamine from binding to its H2 receptors, thus resulting in less acid production

Corticosteroid – widely used to reduce inflammation

Dicyclomine – relaxes smooth muscle of esophagus

Metoclopromide – to treat heartburn

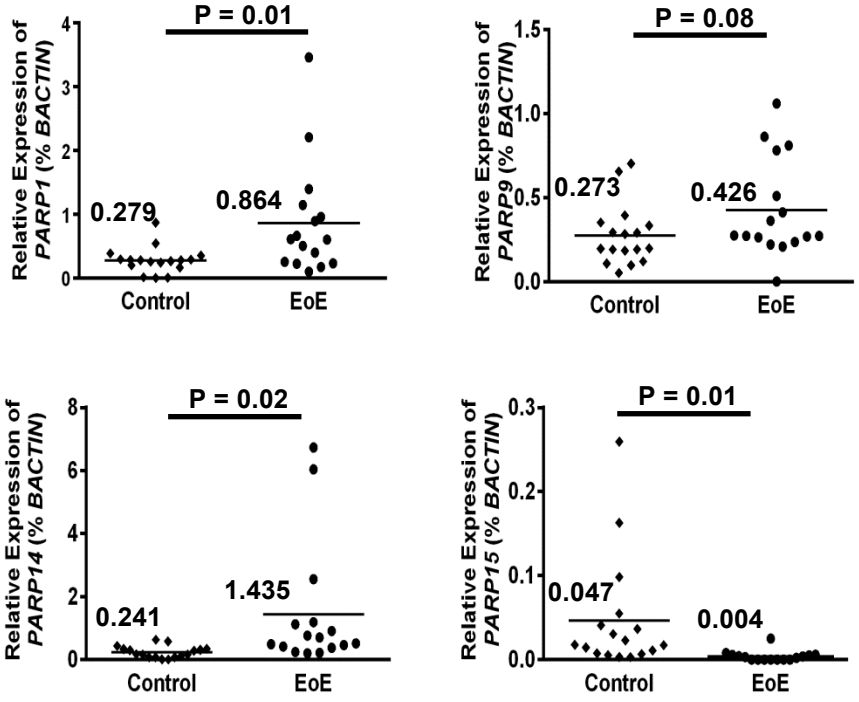

Figure 5. Macro-PARP expression in EoE biopsies. RNA was isolated from control and EoE biopsies, cDNA was synthesized and expression of the indicated macroPARPs was measured by RT-PCR. Data represented as percent β-actin control. Statistical significance was determined using ANOVA.

Correlation of PARP14 and CCL26 expression

The chemokine CCL26 is an important component of the pathology of EoE. Gene expression of *CCL26* is increased in EoE patient biopsies with a strong positive correlation with eosinophil counts in patient biopsies (45, 46). Single nucleotide polymorphisms in *CCL26* gene are also associated with increased disease incidence (45). Additionally, STAT6 regulates *CCL26* expression in esophageal epithelial cells (109). Hence, to determine if *PARP14* expression correlated with *CCL26* expression, we tested the association of these 2 genes in EoE patient

biopsy samples and observed a strong correlation with a correlation coefficient of 0.81 (Figure 6A). In contrast, we did not observe a strong correlation between *PARP1* and *CCL26* (R = 0.30 and P = 0.27) (Figure 6B). We also observe significant heterogeneity in the expression of PARP14 in biopsy samples, with some overlap with control biopsies. Thus, we observed a positive correlation between *PARP14* and *CCL26* expression.

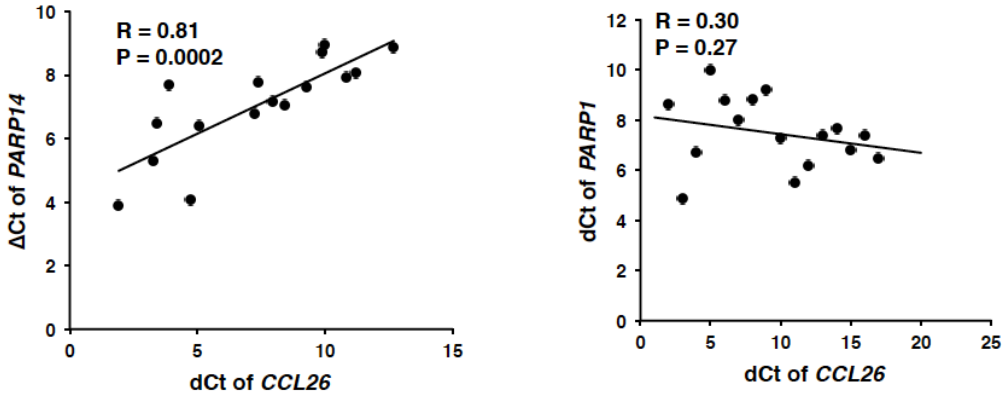

Figure 6. **Correlation of *PARP14* and *CCL26* expression**. The mRNA expression of *CCL26* was graphed against *PARP14* (A) and *PARP1* (B) expression. Significance was determined using ANOVA.

Role of PARP14 in CCL26 expression

As our results suggested a relationship between *PARP14* and *CCL26*, we tested the ability of *PARP14* to regulate *CCL26* directly. To test this, the esophageal epithelial cell line TE-7 was transfected with a CCL26 luciferase reporter vector along with plasmids encoding STAT6 and/or PARP14 and incubated with STAT6 activating cytokines IL-4 or IL-13. Consistent with our previous results,

transfection of STAT6 expressing plasmids increased cytokine induced CCL26 reporter activity (Figure 7A). Moreover, transfection of PARP14 expressing plasmid alone increased both basal and cytokine induced CCL26 reporter activity (Figure 7A). Furthermore, we observed that co-transfection of STAT6 and PARP14 expressing plasmids significantly increased CCL26 reporter activity compared to cells transfected with STAT6 alone (Figure 7A). This effect was not observed when cells were transfected with STAT6 expressing plasmid along with a CCL26 luciferase reporter that had a mutation in the STAT6 binding site (Figure 7A). Suggesting that the effects of PARP14 were completely dependent on STAT6 binding. These results suggest that PARP14 could potentially modulate CCL26 expression.

To study this further, mRNA expression of CCL26 was assessed in TE-7 cells that were incubated with cytokines IL-4 or IL-13 in the presence or the absence of a global PARP inhibitor (PJ34) that binds to the PARP catalytic domain and inhibits PARP activity. We observed that IL-4 and IL-13 increased CCL26 mRNA expression. Incubation with PARP inhibitor PJ34 attenuated the induction that was observed with either cytokine (Figure 7B). Similar results were observed when TE-1 esophageal epithelial cells were used (Figure 7C). Thus, PARP14 along with its enzymatic activity contributes to the regulation of CCL26 in esophageal cells.

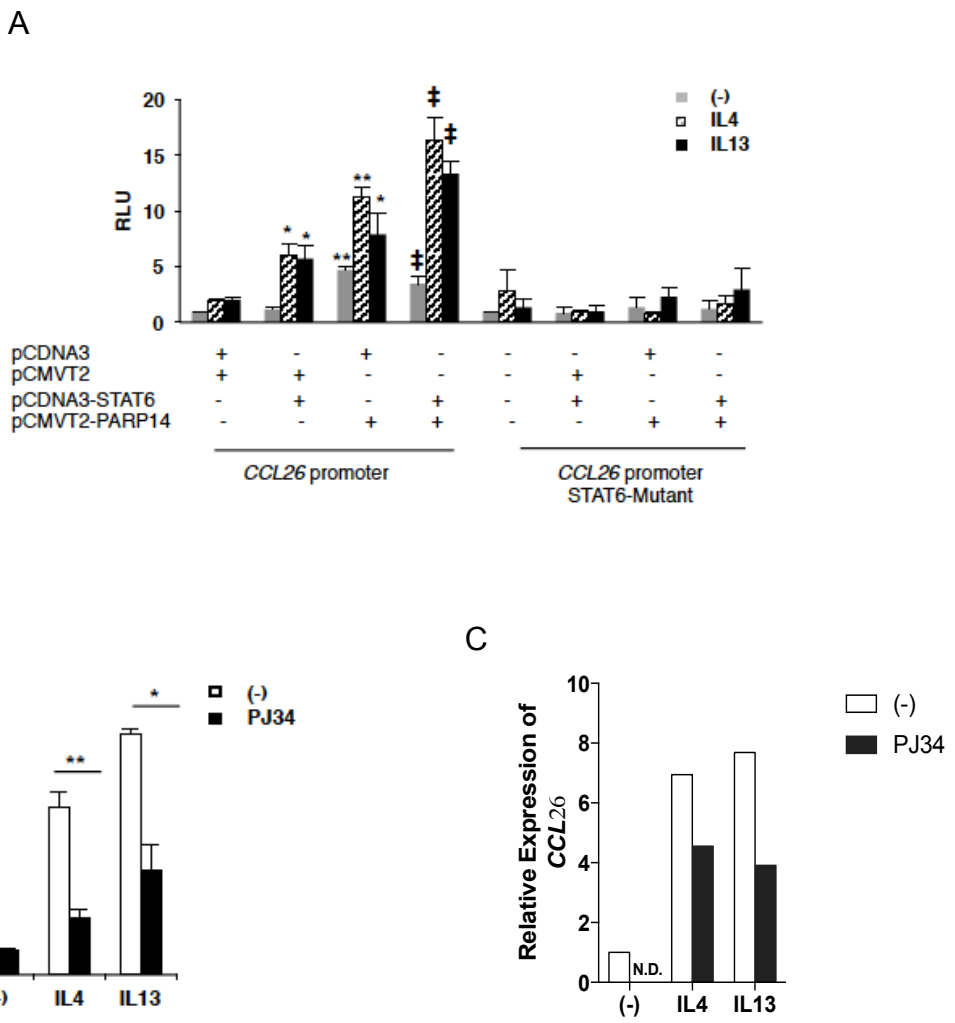

Figure 7. *PARP14* activates *CCL26* gene expression. (A) *CCL26* promoter reporter activity with co-transfection of STAT6 and/or PARP-14 expressing plasmids into TE-7 esophageal epithelial cells *p<0.05, **p<0.001, compared to transfection with control plasmids. **(B)** TE-7 esophageal epithelial cells were cultured with IL-4/IL-13 and PARP inhibitor, PJ34. RNA was isolated and CCL26 gene expression was assessed by RT-PCR. Results are an average of at least 3 experiments. *p<0.05, **p<0.001. **(C)** CCL26 expression in cytokine stimulated TE-1 esophageal cells incubated with PARP inhibitor, PJ34 (n=1).

Part II – PARP14 and allergic skin inflammation

Expression of Stat6VT in T cells of PARP14 deficient mice alters lymphocyte homeostasis

As PARP14 has been known to enhance STAT6-dependent transcription and *Parp14-/-* mice have markedly reduced allergen-induced airway disease (37), we wanted to determine the role of PARP14 in STAT6 mediated skin inflammation. To study the role of PARP14 in allergic skin inflammation we mated Stat6VT mice with *Parp14-/-* mice to generate Stat6VT transgenic mice (Stat6VTx*Parp14-/-*). As Stat6VT mice have altered lymphocyte homeostasis and the lymphocyte populations are normal in *Parp14-/-* mice, we wanted to determine if the absence of PARP14 had effects on lymphocyte populations in Stat6VTx*Parp14-/-* mice. We isolated spleens from wild-type, *Parp14-/-*, Stat6VT, Stat6VTx*Parp14-/-* mice and analyzed their lymphocyte populations by flow cytometry. Stat6VT mice have decreased percentage of splenic CD4+ cells compared to wild-type mice (99). We observed decreases in the frequency of CD4+ cells in the spleen of both STAT6VT and STAT6VTx*Parp14-/-* mice compared to wild-type mice (Figure 8A). Though the frequencies of CD4+ cells are similar in STAT6VT and STAT6VTx*Parp14-/-* mice, the number of CD4+ cells in Stat6VTx*Parp14-/-* mice is significantly lower compared to Stat6VT mice (Figure 8B). CD4+ T cells from Stat6VT mice have increased amounts of Th2 cytokines IL-4, IL-5 and IL-13 (99). To investigate cytokine expression, intracellular cytokine staining of splenocytes from wild-type, *Parp14-/-,* Stat6VT and Stat6VTx*Parp14-/-* mice were performed.

ICS showed that CD4+ cells from Stat6VTx*Parp14-/-* have lower percentage of IL-4 and IL-13 producing cells but a higher frequency of IL17A producing cells compared to Stat6VT CD4+ cells (Figure 8C). To further understand the effect of Stat6VT on PARP14 deficient T cells, splenocytes from wild-type, *Parp14-/-,* Stat6VT and Stat6VTx*Parp14-/-* mice were MACS enriched for CD4+ T cells and re-stimulated with anti-CD3 for 72 hours and cytokines measured by ELISA. Stat6VTx*Parp14-/-* T cells secreted significantly lower amounts of IL-4 and IFNγ compared to Stat6VT cells with a decrease in the production of IL-13 (Figure 8D). The amount of IL-17A produced by Stat6VTx*Parp14-/-* T cells was significantly increased compared to Stat6VT T cells (Figure 8D).

Stat6VT mice have an expanded B cell populations compared to wild-type mice. To analyze the B cell population in Stat6VTx*Parp14-/-* mice, we isolated spleens from wild-type, *Parp14-/-,* Stat6VT and Stat6VTx*Parp14-/-* mice and analyzed the B cell population by flow cytometry. We observed similar percentages of CD19+ cells in Stat6VT and Stat6VTx*Parp14-/-* mice (Figure 8E), while the total number of CD19+ cells in Stat6VTx*Parp14-/-* mice was significantly reduced compared to STAT6VT mice (Figure 8F).

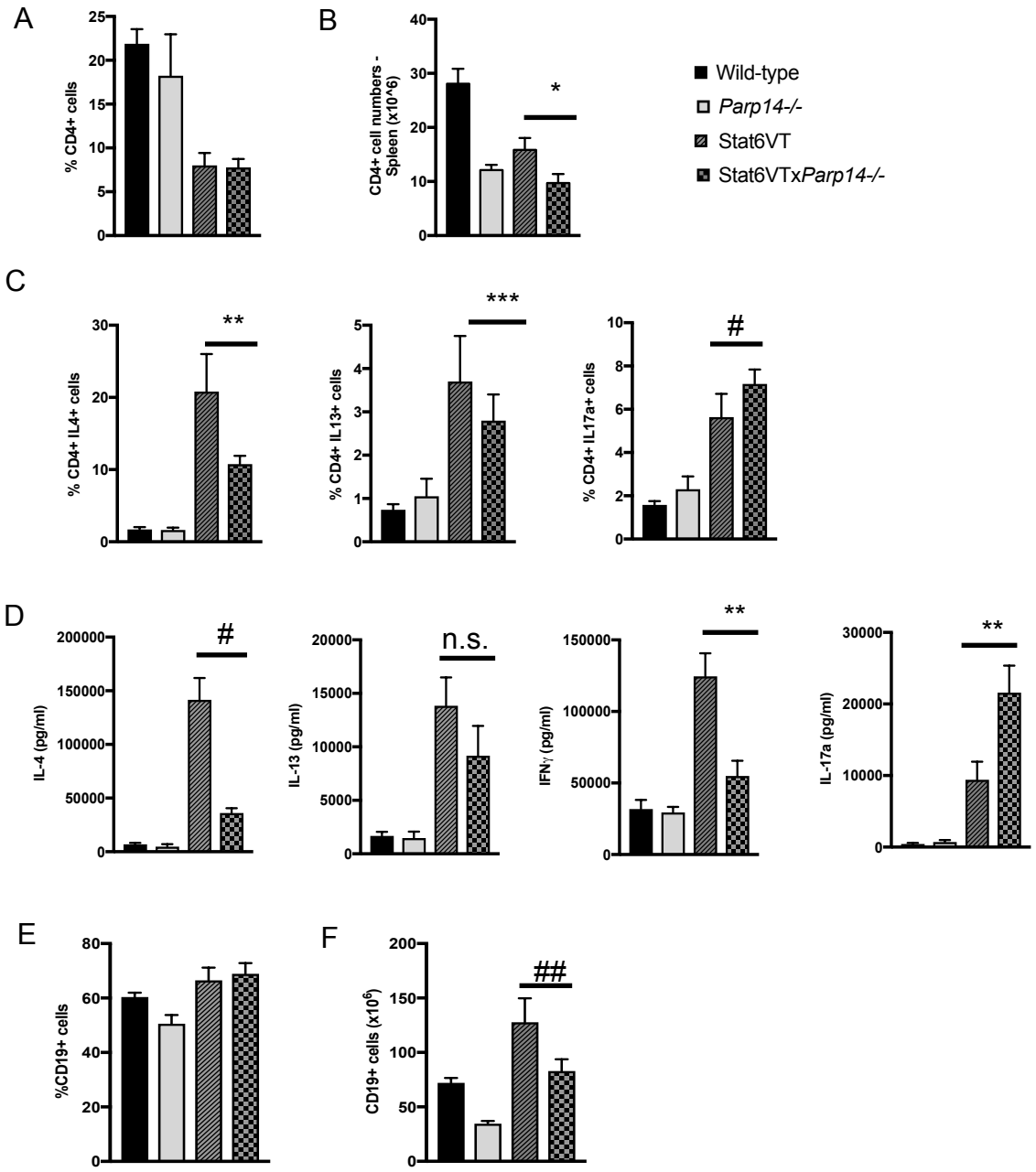

Figure 8. Altered lymphocyte homeostasis in PARP14 deficient Stat6VT mice. (A) Splenocytes from age matched wild-type, *Parp14-/-,* Stat6VT and Stat6VTx*Parp14-/-* mice were isolated and percentage of CD4+ T cells population was determined by flow cytometry **(B)** Cell numbers of CD4+ T cells in

the spleen of the indicated populations were determined **(C)** Splenocytes isolated from the indicated mice were restimulated with PMA/Ionomycin and stained for cytokines produced followed by flow cytometry analysis. **(D)** MACS sorted CD4+ T cells from the spleens of indicated mice were stimulated with plate bound anti-CD3 for 72 hours, the cell free supernatants were collected and cytokines produced were measured by ELISA. **(E)** Splenocytes isolated from the indicated mice were stained with antibody to CD19 and the percentage of CD19+ cells was determined by flow cytometry **(F)** Cell numbers of CD19+ cells in the spleen of the indicated populations. Data are mean ± SEM of 3 to 6 mice in each genotype (A-C) and mean ± SEM of 6 to 10 mice in each genotype (D). Statistical significance was determined by one-way ANOVA (A-C, F) and two-tailed test (D), is indicated as follows * p=0.05, ** p< 0.005, *** p<0.05, # p<0.0001, ## p<0.01

Analysis of cell surface marker expression on B lymphocytes

To study the expression of genes that are regulated by IL-4 and are Stat6 dependent, we studied the expression of MHC class II and CD86 on the surface of B cells. Percentages of CD19+ MHC-II expressing cells were similar between Stat6VT and Stat6VTx*Parp14-/-* mice, but Stat6VTx*Parp14-/-* mice have lower number of MHC-II expressing B cells compared to Stat6VT B cells (Figure 9A). The frequency and numbers of CD86 expressing B cells were reduced in Stat6VTx*Parp14-/-* mice compared to Stat6VT mice (Figure 9B).

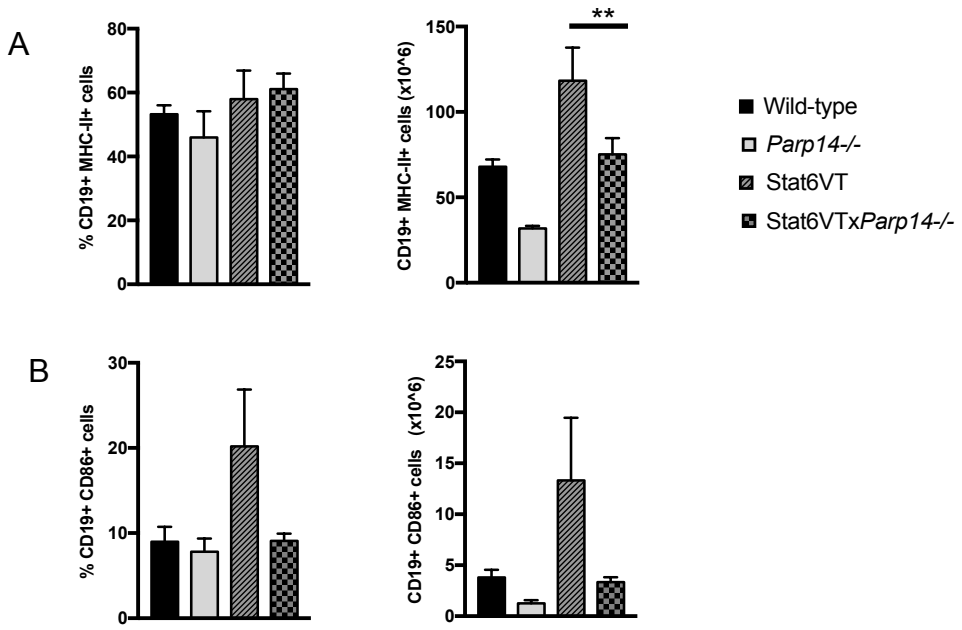

Figure 9. Cell surface marker expression on B cells. Splenocytes from age matched Stat6VT, Stat6VTx*Parp14-/-* mice and their litter-mate controls were isolated and stained with antibodies to CD19, MHC-II and CD86. The percentage and cell numbers of CD19+ MHC-II+ cells (A) and CD19+ CD86+ cells (B) were determined by flow cytometry. Data are mean ± SEM of 3 to 6 mice in each genotype. Statistical significance was determined by one-way ANOVA ** $p<0.01$

PARP14 deficient Stat6VT mice have increased serum IgE levels

IL-4 via Stat6 promotes class switching in activated B cells to IgG1 and IgE (110) and serum IgE levels are elevated in Stat6VT mice (99). Because Stat6VTx*Parp14-/-* mice produce decreased Th2 cytokines compared to Stat6VT mice, we wanted to examine serum IgE levels. To test this, we obtained sera from 4 month and 12 month old Stat6VT and Stat6VTx*Parp14-/-* mice and

analyzed IgE levels by ELISA. Serum IgE levels were significantly elevated in both 4 month and 12 month old Stat6VTx*Parp14-/-* transgenic mice compared to Stat6VT mice (Figure 10).

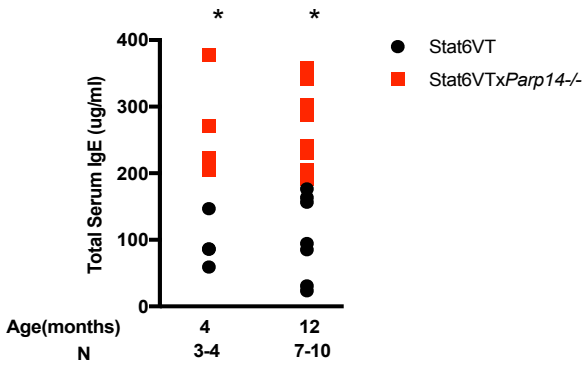

Figure 10. Increased serum IgE levels in Stat6VTx*Parp14-/-* mice. Serum from Stat6VT and Stat6VTx*Parp14-/-* mice were tested for IgE by ELISA. *p<0.01, t-test

CD4+ cells from Stat6VTxParp14-/- mice have decreased Th2 cytokines in the absence of inflammation

Since Stat6VT mice have increased cytokine production, we wanted to test whether the effects of PARP14 deficiency were intrinsic to the T cells or an effect of inflammation. To examine the effects of PARP14 deficiency in Stat6VT T cells in isolation, we transferred CD4+ cells from Stat6VT and Stat6VTx*Parp14-/-* mice to *Rag1-/-* mice. Though we observed an effective transfer of CD4+ cells, we did not observe skin inflammation in mice that received either Stat6VT or Stat6VTx*Parp14-/-* T cells. This suggested that T cells are not sufficient to cause disease, but also provided the opportunity to observe T cell function in the

absence of inflammation. We observed that 20 weeks post-transfer, Stat6VT CD4+ cell recipient mice have a significantly higher frequency of IL-4 and IL-13 producing cells compared to Stat6VTx*Parp14-/-* recipients (Figure 11A), though the frequency of IFNγ was similar between both groups of mice, the frequency of IFNγ was increased compared to 10 weeks post transfer (Figure 11B). Splenocytes isolated from the *Rag1-/-* recipient mice were re-stimulated with anti-CD3 for 72 hours and the levels of secreted cytokines were measured. At 20 weeks, IL-4 levels were significantly lower in Stat6VTx*Parp14-/-* recipient mice, IFNγ levels were also reduced (Figure 11C). The ratio of IFNγ to IL-4 is significantly higher in Stat6VTx*Parp14-/-* recipients at 10 weeks compared to Stat6VT recipients and further increased at 20 weeks compared to 10-week post-transfer Stat6VTx*Parp14-/-* recipient cells (Figure 11D). Taken together, these data suggest that the decreases of cytokine production caused by PARP14-deficiency are intrinsic to the T cells.

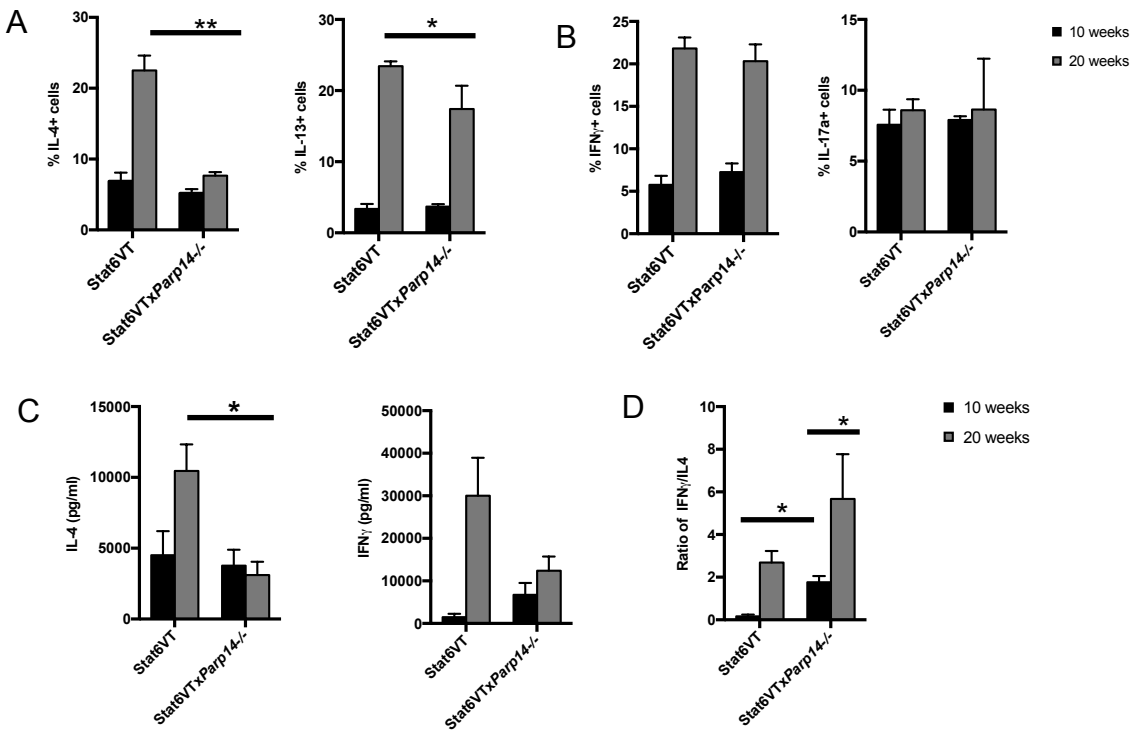

Figure 11. Decreased Th2 cytokines from Stat6VTx*Parp14-/-* CD4+ cells. (A-B) CD4+ cells from Stat6VT and Stat6VTx*Parp14-/-* mice were adoptively transferred to *Rag1-/-* mice. Splenocytes from the recipients were restimualted with PMA/Ionomycin and stained for IL-4, IL-13, IFNγ, IL-17a producing cells and analyzed by flow cytometry. **(C-D)** Cytokines produced from supernatants of anti-CD3 stimulated splenocytes were analyzed by ELISA. Data from 10-week experiment have 5-11 mice per group and 20-week experiment have 6-8 mice per group. *p<0.05, **p<0.001 – ANOVA with multiple comparisons, ***p<0.001 – t-test.

PARP14 deficiency in Stat6VT mice increases disease severity

As Stat6VTx*Parp14-/-* T cells have altered cytokine production, we next wanted to test the ability of PARP14 to modulate allergic skin inflammation. We monitored Stat6VT and Stat6VTx*Parp14-/-* mice for the development of AD like skin lesions, disease incidence and severity. Stat6VTx*Parp14-/-* mice developed severe allergic skin inflammation characterized by erythema, excoriation and lichenification (Figure 12A). We observed that a greater percentage of Stat6VTx*Parp14-/-* mice developed severe allergic skin inflammation compared to Stat6VT mice (Figure 12B). On comparing the percentage of mice that required euthanasia or died due to complications associated with severe AD lesions using the Kaplan-Meier morbidity analysis, we observed a higher percentage of Stat6VTx*Parp14-/-* mice requiring euthanasia because of severe disease compared to Stat6VT mice (Figure 12C).

EASI (Eczema Area Severity Index) is a clinical tool for scoring the extent and severity of atopic dermatitis lesions. Based on the EASI method of scoring human atopic dermatitis lesions, we developed a method to score mouse AD-like skin lesions, referred to as MEASI (Mouse Eczema Area Severity Index). AD-like skin lesions in mice were scored on characteristics such as erythema, excoriation, infiltration and lichenification on a scale of 0-5. Using MEASI, at 5 months a greater percentage of Stat6VTx*Parp14-/-* mice had severe (>5) MEASI score compared to Stat6VT mice (Figure 12D).

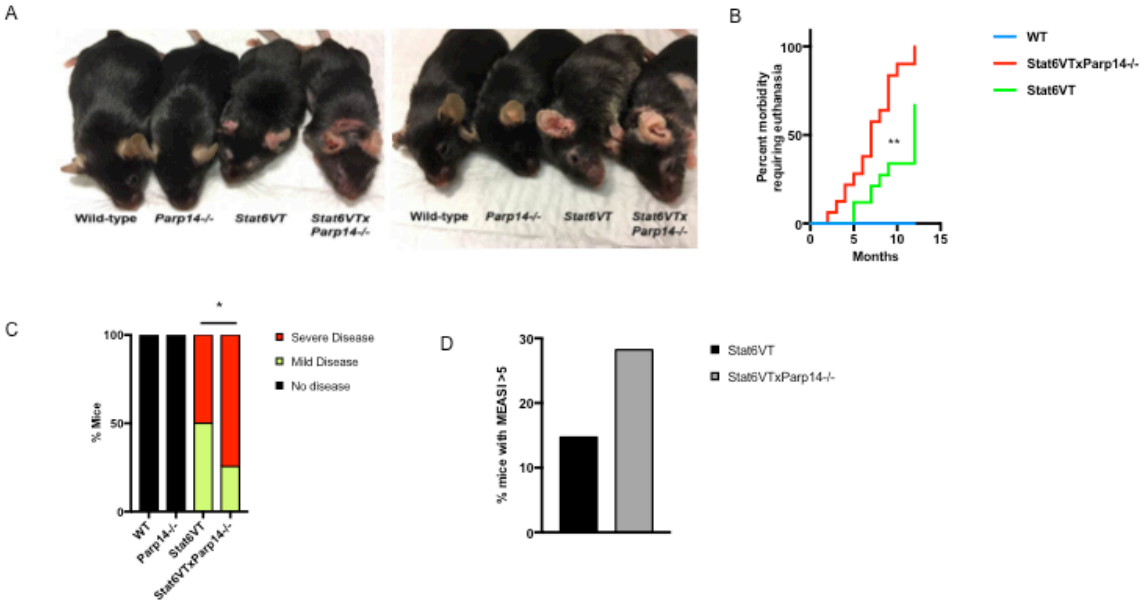

Figure 12. PARP14 deficiency in Stat6VT mice increases disease severity and morbidity. (A) Photographs of wild-type, *Parp14-/-*, Stat6VT and Stat6VTx*Parp14-/-* mice **(B)** Percent of mice with no disease, mild disease and severe disease (AD-like lesions), * p< 0.05, Fisher's exact test. **(C)** Percent morbidity graphed using Kaplan-Meier analysis, n= 34 ** p< 0.001, Mantel-Cox test. **(D)** Percentage of mice with MEASI score > 5 showing severe disease

Histological analysis of skin inflammation

Stat6VTx*Parp14-/-* mice had severe inflammation and a higher MEASI score compared to Stat6VT mice. To further characterize the histopathology of skin inflammation in Stat6VT and Stat6VTx*Parp14-/-* mice, we performed histological analysis of skin tissue from wild-type, Stat6VT and Stat6VTx*Parp14-/-* mice. We stained formalin fixed skin sections with hematoxylin and eosin and observed

increased dermal and epidermal thickening along with an increase in cellular infiltration in the skin of both Stat6VT and Stat6VTx*Parp14-/-* mice compared to wild-type mice (Figure 13A). Based on the percentage of inflammatory cells, the inflammation was scored on a scale of 0-5 (Figure 13B). The hematoxylin and eosin staining of the skin tissue sections and the inflammation score suggest increased cellular infiltrates in Stat6VTx*Parp14-/-* skin compared to Stat6VT skin.

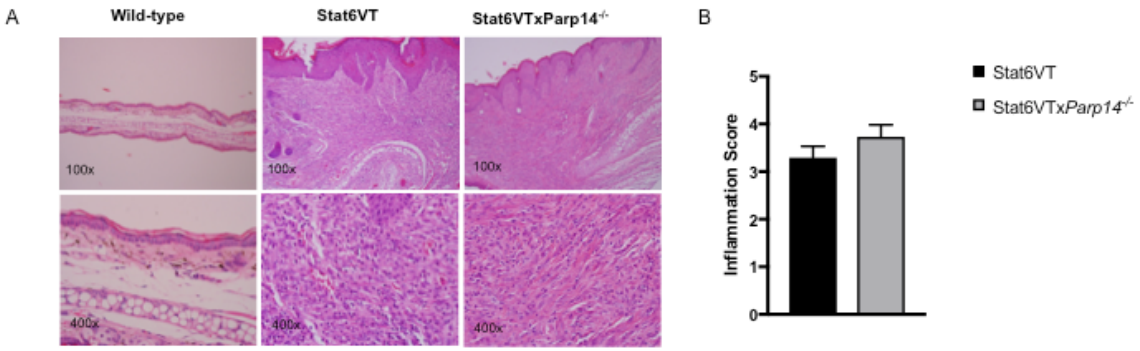

Figure 13. Skin histology of Stat6VT and Stat6VTx*Parp14-/-* mice. (A) Skin tissue collected from wild-type, Stat6VT and Stat6VTx*Parp14-/-* mice were embedded in paraffin and sectioned and stained with heamtoxylin and eosin. Images were taken at x100 and x400. **(B)** Inflammation was scored based on several pathophysiological parameters using a semi-quantitative scale of 0-5.

PARP14 deficiency in keratinocytes does not alter epidermal homeostasis associated gene expression

Th2 cytokines are increased in lesional skin and IL-4 is involved in the pathogenesis of AD. Skin biopsies from AD patients and primary human

keratinocytes differentiated with IL-4 demonstrate decreased expression of epidermal differentiation complex genes (87, 88). Based on the effects of PARP14 deficiency in allergic skin inflammation and in altering lymphocyte homeostasis, we next wanted to determine if the absence of PARP14 or its enzymatic activity affect keratinocyte function. To study the function of PARP14 in keratinocytes, we assessed the expression of IL-4 and STAT6 responsive genes that play a role in keratinocyte differentiation and barrier function. Human primary keratinocytes were cultured and differentiated in 2 mM calcium chloride for 2 days. Keratinocytes were stimulated with recombinant human IL-4 and treated with PARP inhibitor PJ34 for 24 hours. We assessed gene expression by qRT-PCR. We observed that on stimulation with IL-4 the expression of STAT6 dependent genes including *CISH, CA2, CCL26, HAS3, SERPINB3* and *SERPINB4* were increased compared to untreated keratinocytes. On treating keratinocytes with the PARP inhibitor PJ34 along with IL-4, we did not observe any difference in gene expression compared to cells that were treated with IL-4 alone (Figure 14). Although PARP14 functions in Stat6-dependent gene expression in T cells, B cells, macrophages and esophageal epithelial cells (37, 44, 47, 111), we observe that in keratinocytes PARP14 enzymatic activity is not required for IL-4 responses. Thus, the phenotype observed in Stat6VTx*Parp14-/-* mice is not likely to be due to altered function in keratinocytes.

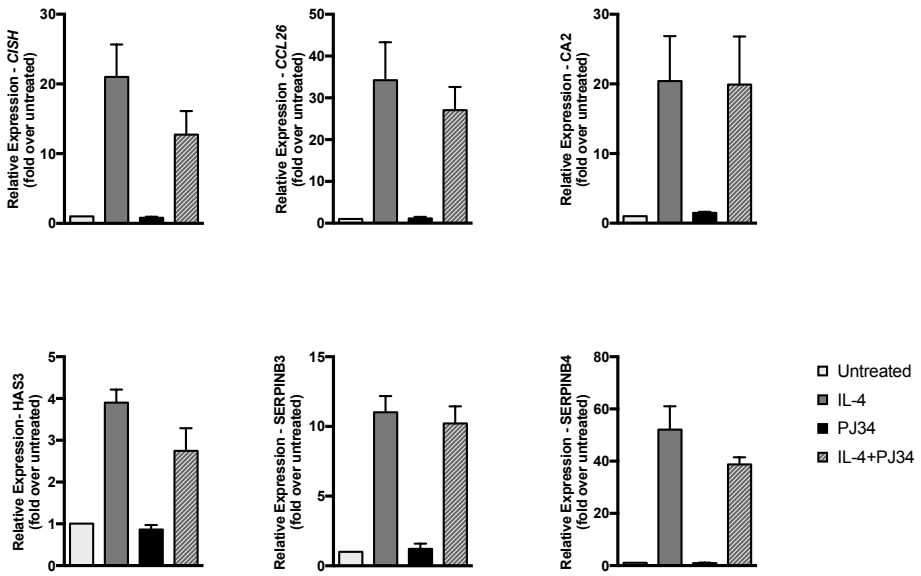

Figure 14. Indirect effects of PARP14 on epidermal homeostasis associated genes and barrier function. Human primary keratinocytes were differentiated and stimulated with human IL-4 and treated with PARP inhibitor, PJ34. Gene expression was measured by RT-PCR. Results are an average of at least 3 experiments.

Gene expression in the skin

AD skin biopsies are characterized by over-expression of IL-4 and IL-13 and a deficiency in filaggrin gene expression (87). Keratinocytes differentiated in the presence of IL-4 and IL-13 also show reduced filaggrin, involucrin and loricrin gene expression (87, 88). Skin from Stat6VT mice had decreased expression of EDC genes *Flg, Ivl, Lor* (100). Based on the observation that T cells from Stat6VTx*Parp14-/-* mice produce decreased amounts of cytokines IL-4, IL-13 and IFNγ compared to Stat6VT T cells but had severe skin inflammation, we tested

the expression of EDC genes from lesional and non-leisonal skin tissue. We obtained skin biopsies from wild-type, Stat6VT and Stat6VTx*Parp14-/-* mice and measured EDC gene expression by qRT-PCR (Figure 15A). *Flg* expression in Stat6VTx*Parp14-/-* lesional skin showed a trend towards an increase in expression compared to Stat6VT lesional skin. Similar expression of *Ivl* and *Lor* were observed in Stat6VT and Stat6VTx*Parp14-/-* lesional skin.

As Stat6VTx*Parp14-/-* mice have severe skin inflammation compared to Stat6VT mice, we next wanted to determine the expression of chemokines in the skin. We did not observe differences in *Ccl11, Ccl22 and Cxcl10* expression between Stat6VT and Stat6VTx*Parp14-/-* lesional skin (Figure 15B). Expression of *Ccl24* was higher in non-lesional and lesional Stat6VT skin compared to Stat6VTx*Parp14-/-* skin.

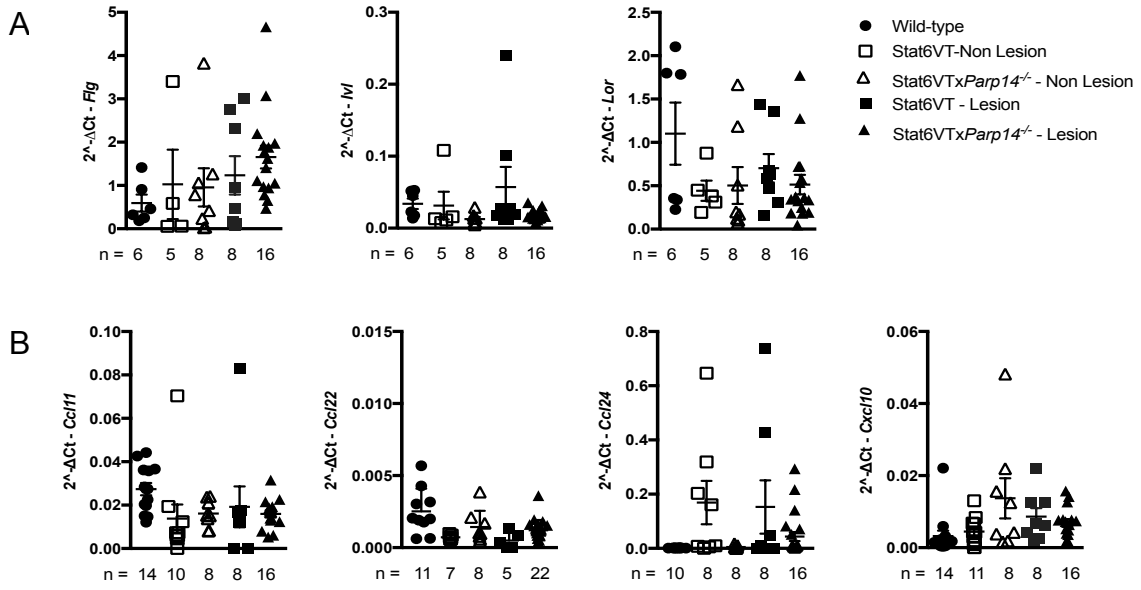

Figure 15. Expression of EDC genes and cytokines in skin. RNA was isolated from wild-type, lesional and non-lesional Stat6VT and Stat6VTx*Parp14-/-* skin tissue. Expression of the indicated genes were measured by qRT-PCR, samples were normalized to the expression of β2-microglobulin mRNA.

Th2 cytokines are important in altering EDC gene expression; hence we tested the expression of cytokines in the skin tissue. The expression of *Il4* was higher in lesional Stat6VT and Stat6VTx*Parp14-/-* skin compared to wild-type and non-lesional Stat6VT and Stat6VTx*Parp14-/-* skin. Expression of *Il13* was similar between wild-type, Stat6VT lesional and non-lesional skin and Stat6VTx*Parp14-/-* non-lesional skin, though a higher expression was observed in lesional skin from Stat6VTx*Parp14-/-* mice (Figure 16A).

No differences in the expression of *Il17a* between the lesional and non-lesional skin of Stat6VT and Stat6VTx*Parp14-/-* were observed. The expression of *Ifnγ* showed increases in lesional skin from Stat6VTx*Parp14-/-* mice (Figrure 16A). Lesional skin from Stat6VT and Stat6VTx*Parp14-/-* mice displayed similar levels of *Il12 and Il6* expression, though the expression of *Il1b* was higher in lesional skin compared to non-lesional skin from both Stat6VTand Stat6VTx*Parp14-/-* mice (Figure 16B).

To determine T cell recruitment to the skin, we tested the expression of CD3. *Cd3* expression decreased in non-lesional skin from Stat6VT and Stat6VTx*Parp14-/-* mice compared to wild-type skin, however lesional skin from Stat6VT and Stat6VTx*Parp14-/-* showed increased *Cd3* expression (Figure 16C).

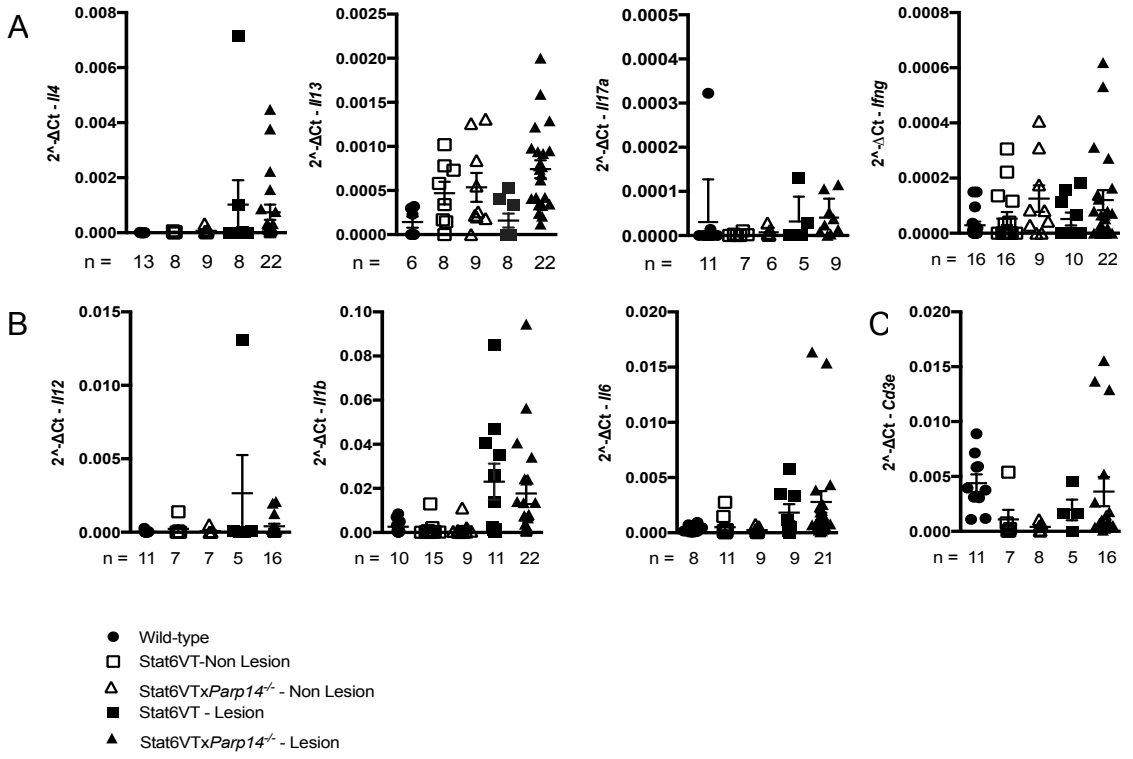

Figure 16. Chemokine and cytokine gene expression in skin. RNA was isolated from wild-type, lesional and non-lesional Stat6VT and Stat6VTx*Parp14-/-* skin tissue. Expression of the indicated chemokine (A) and cytokine (B) genes were measured by qRT-PCR, samples were normalized to the expression of β2-microglobulin mRNA.

Skin infiltrating cells in Stat6VT and Stat6VTxParp14-/- mice

We observed that Stat6VTx*Parp14-/-* have severe skin inflammation and to determine if there was a difference in cellular infiltrate in the skin of Stat6VT versus Stat6VTx*Parp14-/-* mice, we wanted to determine the cell types that infiltrate the skin of Stat6VT and Stat6VTx*Parp14-/-* mice. To investigate this, we

digested the ear skin of wild-type, *Parp14-/-,* Stat6VT and Stat6VTx*Parp14-/-* mice and analyzed the cells infiltrating the skin by flow cytometry. In skin from wild-type mice, T cells form 67.3% of the infiltrating cells; mast cells, basophils, eosinophils and neutrophils constitute 15.6%, 13.6%, 1.8% and 1.4% of cells respectively (Figure 17A). The composition of cells infiltrating the skin of *Parp14-/-* mice was similar to wild-type mice, where T cells formed the majority of infiltrating cells (68.1%); mast cells, basophils, eosinophils and neutrophils made up 14.4%, 14.3%, 2.0% and 1.2% of cells respectively. Skin from Stat6VT mice had lower percentage of T cells (35.4%) compared to wild-type or *Parp14-/-* skin, with an increase in the percentage of infiltrating eosinophils and neutrophils (15.4% and 18.5% respectively) and a decrease in percentage of basophils (7.1%) and mast cells (7.4%) compared to wild-type or *Parp14-/-* skin. In Stat6VT skin we also observed the infiltration of cells (16.1%) that were CD45+ but were CD3-, CD19-, NK1.1-, CD117-, CD11b-, CD11c-, CD49b-, FcεRIa-, Gr1- and SiglecF- (cells that are not keratinocytes; shown in white in Figure 17A).

Similar to what was observed in Stat6VT skin, Stat6VTx*Parp14-/-* mice had a decrease in the percentage of T cells (34.7%) infiltrating the ear skin tissue compared to wild-type and *Parp14-/-* mice. The percentage of eosinophils and neutrophils (15.4% and 10.5% respectively) were increased compared to wild-type and *Parp14-/-* skin and the percentage of neutrophil decreased compared to Stat6VT skin. The percentage of basophils and mast cells (4.2% and 5.6%) were also reduced in comparison to wild-type, *Parp14-/-* mice and Stat6VT mice.

Similar to what was observed in Stat6VT skin, Stat6VTx*Parp14-/-* skin also had infiltration of CD45+ cells that are CD3-, CD19-, NK1.1-, CD117-, CD11b-, CD11c-, CD49b-, FcεRIα-, Gr1- and SiglecF-. These cells constituted 29.7% of the cells infiltrating the skin of Stat6VTx*Parp14-/-* mice, twice as much as that was observed in Stat6VT skin (Figure 17A). Though the frequencies of infiltrating cells were altered in Stat6VTx*Parp14-/-* skin compared to Stat6VT skin, the total cell numbers were reduced compared to Stat6VT skin (Figure 17B).

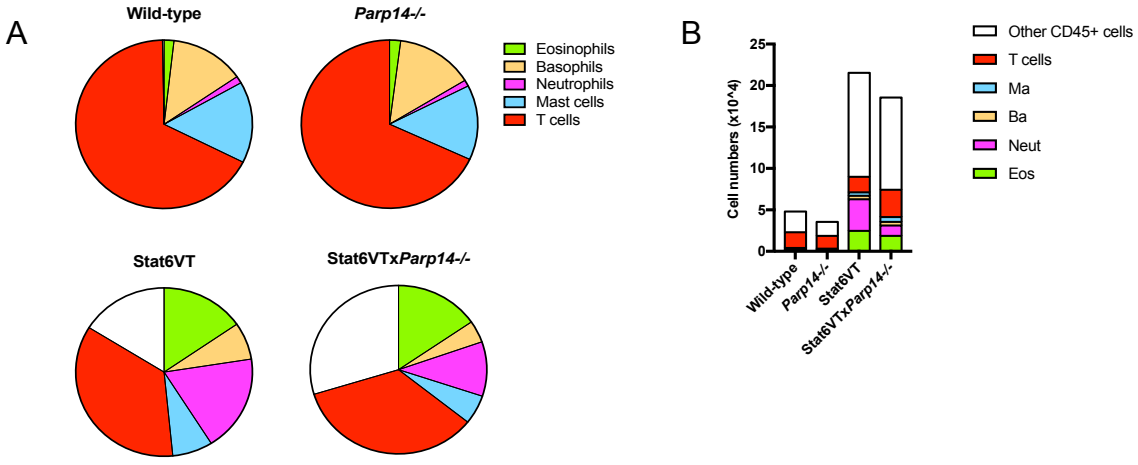

Figure 17. Skin infiltrating cells. (A) Ear skin isolated from wild-type, *Parp14-/-*, Stat6VT and Stat6VTx*Parp14-/-* mice were digested in Liberase and the dissociated cells were stained with antibodies to infiltrating cells and percentages of CD45+ cells infiltrating the skin of indicated mice were determined by flow cytometry. **(B)** Total number of cells infiltrating the ear skin of the indicated populations.

Stat6VT interaction with Parp14-/- T cells

Since PARP14 deficient Th2 cells secreted significantly reduced amounts of Th2 cytokines and PARP14 was required for ambient expression of *Gata3* (37), we wanted to determine the effect of constitutively active Stat6 (Stat6VT) in PARP14 deficient T cells. To study how Stat6VT impacts T cells that are deficient in PARP14, we retrovirally transduced wild-type and *Parp14-/-* Th0 and Th2 cells with MIEG-Stat6VT or MIEG-GFP (Control) and the resulting GFP+ cells were analyzed by flow cytometry. Transduction of Stat6VT retrovirus into either wild-type or *Parp14-/-* Th0 or Th2 cells resulted in an increase in the frequency of phospho-Stat6 (Figure 18A) and Gata-3 (Figure 18B) and this was similar between wild-type and *Parp14-/-* cells. At the end of 5 days of culture, intracellular cytokine staining was performed to study the effect of Stat6VT on cytokine expression. In Th0 cells, we observed that transduction of Stat6VT virus resulted in an increase in the percentage of IL-4, IL-5 and IL-13 producing *Parp14-/-* cells compared to wild-type cells (Figure 18C). Surprisingly in Th2 cells, we observed that transducing wild-type or *Parp14-/-* cells with Stat6VT virus reduced the percentage of IL-4 producing cells (Figure 18D). This reduction was also observed in the percentage of IL-5 and IL-13 producing cells (Figure 18D). Our data shows that the presence of Stat6VT in *Parp14-/-* Th0 culture conditions increases Th2 cytokine production, suggesting an altered function of PARP14 in the presence of Stat6VT in Th0 cells.

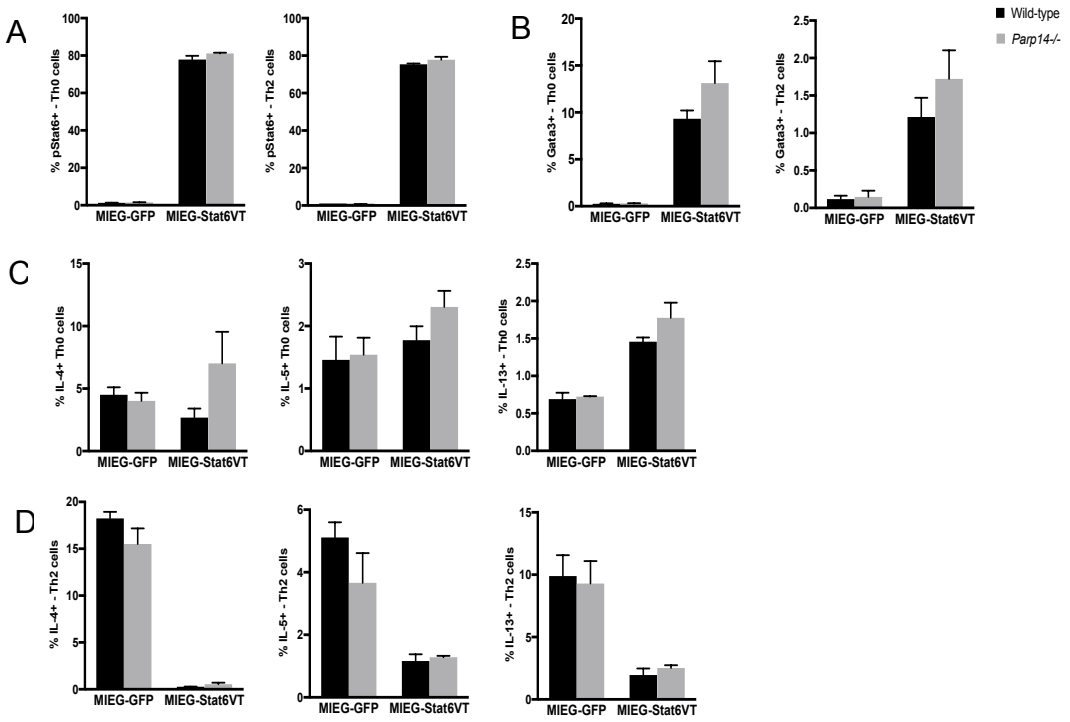

Figure 18. Stat6VT interaction with PARP14. (A) Naïve CD4+ T cells from Wild-type and *Parp14-/-* mice were cultured under Th0 and Th2 conditions. On day 2 of culture, the cells were infected with either MIEG-GFP (Control) or MIEG-Stat6VT retrovirus and on day 5, percentage of phospho-Stat6 expressing cells were analyzed by flow cytometry. **(B)** Percentage of Th0 and Th2 cells expressing Gata-3. **(C, D)** On day 5, cells were stimulated with anti-CD3 and stained for intracellular cytokine production and analyzed by flow cytometry.

Induced allergen specific skin inflammation

To investigate the role of PARP14 in a model of allergen specific skin inflammation, we performed three different protocols to epicutaneously sensitize wild-type and *Parp14-/-* mice (Figure 19A-C). For all protocols we shaved the

backs of mice, tape-stripped the skin and sensitized the mice epicutaneously (EC) with Ova. As per protocol in Figure 19C, mice were sensitized on days 0 and 7 with Ova with the adjuvant alum or PBS (control). A patch of Ova or PBS was taped to the backs of mice on days 14, 18, 22, 26 and 30, removed for two days in between and mice were euthanized two days after the last Ova or PBS patch was removed on day 35.

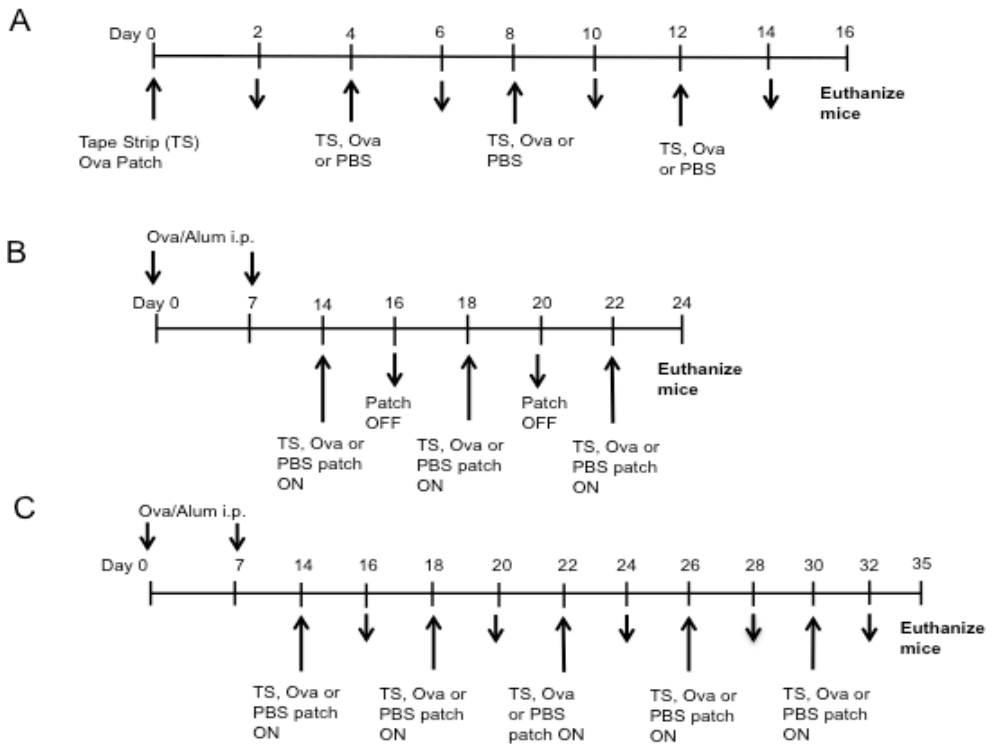

Figure 19 (A-C). Models of epicutaneous sensitization. Schematic diagram showing experimental setup of 3 protocols for epicutaneous sensitization.

We observed that the percentage of CD4+IL4+ cells and CD4+IL13+ cells were significantly lowered in Ova sensitized *Parp14-/-* mice and were similar to the

PBS treated mice (Figure 20A). *Parp14-/-* mice sensitized with Ova had lower total serum IgE levels compared to wild-type mice, though we do not observe a similar decrease in Ova-Specific IgE in *Parp14-/-* mice (Figure 20B). Th2 cytokines, IL-4 and IL-13 from the draining lymph nodes were not different between wild-type and *Parp14-/-* Ova sensitized mice (Figure 20C). Expression of EDC genes- *Flg, Ivl, Lor* and chemokines *Ccl11, Ccl22, Ccl24* from the epicutaneously sensitized skin were also similar between wild-type and *Parp14-/-* Ova sensitized mice (Figure 20D, E). Though we begin to see an immune response upon epicutaneous sensitization, we did not observe robust responses. From our studies we observe that upon epicutaneous sensitization, the absence of PARP14 does decrease Th2 cytokine production by CD4+ T cells and total serum IgE, but does not alter EDC gene expression in the skin.

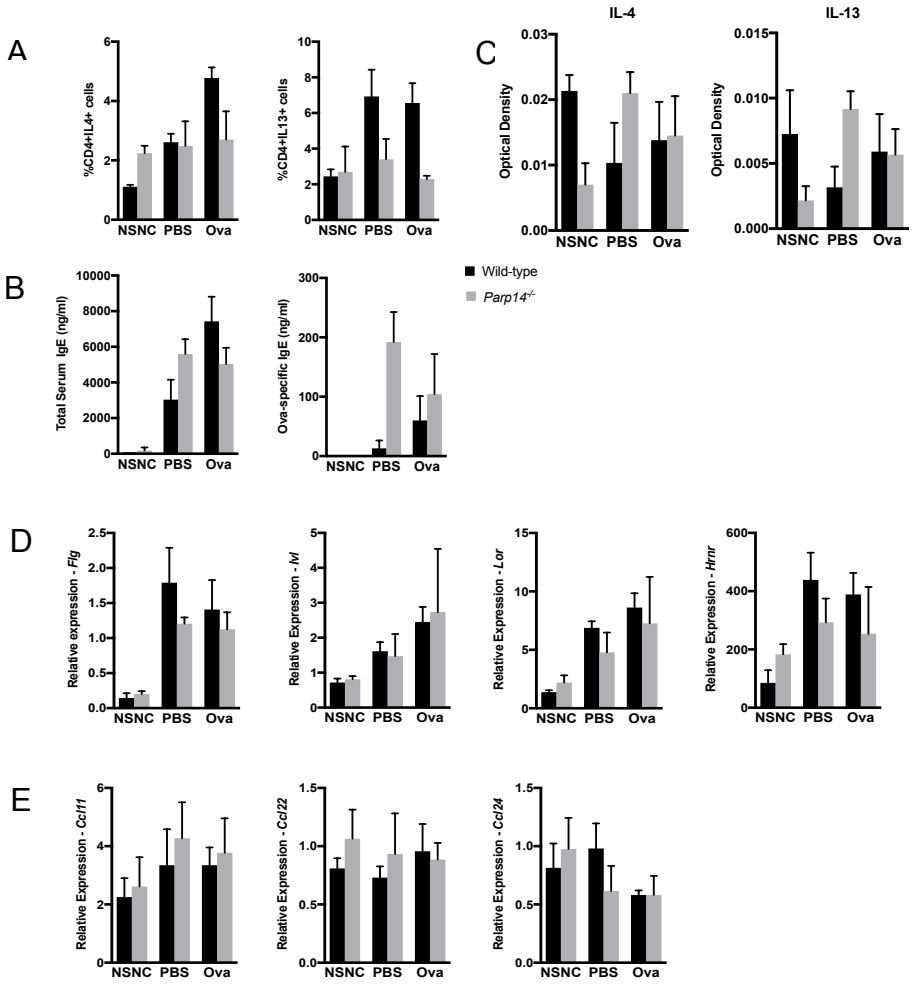

Figure 20. Induced allergen specific skin inflammation. (A) Wild-type and *Parp14-/-* mice were sensitized with Ova/alum i.p. and challenged epicutaneously with Ova for five 2-day challenges. Splenocytes were restimulated with PMA/Ionomycin and stained with antibodies to detect cells producing Th2 cytokines IL-4 and IL-13 and analyzed by flow cytometry. **(B)** Serum from the indicated mice were tested for total IgE and Ova-specific IgE by ELISA **(C)** Cells isolated from the draining lymph nodes were stimulated with anti-CD3 for 72 hours and the cytokines produced were detected by ELISA **(D, E)** Epicutaneously sensitized skin from the indicated mice were used to generate mRNA and gene

expression was measured by RT-PCR. Data are a representative of 3-5 mice per group.

DISCUSSION

PARP14 is required to modulate Th2 differentiation through its catalytic activity. In a murine model of allergic airway disease, PARP14 is critical to regulate disease pathogenesis. Though the function of PARP14 in T cells and in airway inflammation has been studied, its function in non-hematopoietic cells and in other models of inflammation has not yet been investigated. Here in part I, we identified that PARP14 expression is elevated in EoE biopsies from children. *CCL26* expression is dramatically increased in EoE biopsies and we demonstrate a positive correlation between *PARP14* and *CCL26* expression. PARP14 and PARP catalytic activity also regulate *CCL26* expression in esophageal cells. In Part II to study allergic skin inflammation, we used Stat6VT model of spontaneous allergic inflammation and demonstrate that PARP14 deficiency in Stat6VT mice altered cytokine production and Stat6VTx*Parp14-/-* mice developed severe allergic skin inflammation. However in keratinocytes, PARP14 was not required for the induction of IL-4 responsive genes. Adoptive transfer of Stat6VTx*Parp14-/-* CD4+ cells established that decreases in cytokine production were due to a T cell intrinsic function of PARP14. In addition, an altered cellular infiltrate was observed in the skin of Stat6VTx*Parp14-/-* mice compared to Stat6VT mice. Together our findings provide insight into the role of PARP14 in allergic inflammation, carrying out different functions depending on the target organ.

PARP14 and Eosinophilic esophagitis

EoE is an emerging chronic allergic inflammatory disorder of the esophagus that is characterized by esophageal eosinophilia and symptoms related to esophageal dysfunction. There is limited information about EoE disease pathogenesis and its treatment options. Early studies have demonstrated that esophageal eosinophilia occurs in association with Th2 allergic responses (112, 113, 114). IL-5 and IL-13 promote eosinophil infiltration in to the esophagus. A genome wide microarray expression analysis of EoE biopsies revealed the differential expression of 574 genes, of which eotaxin-3 was the most overexpressed gene (45). The human eotaxin-3 promoter carries a STAT6 consensus binding motif (53). In the current study we focused on identifying the function of PARP14 in regulating CCL26 expression in esophageal epithelial cells. Prior studies have demonstrated PARP14 functions as a co-activator of STAT6 under stimulating conditions in T cells, B-cells and in airway epithelium (37, 44). Here we demonstrated the expression of macro-PARPs in esophageal biopsy samples from pediatric patients and observed an increase in the expression of PARP1 and PARP14. We demonstrated a correlation between the expression of *PARP14* and *CCL26* in patient biopsy samples. Though PARP1 has not been implicated in EoE, PARP1 has been extensively studied in other models of allergic inflammation such as asthma. In asthma, PARP1 deficiency decreases cell infiltration into the lung, airway hyperresponsivess and mucous secretion (25, 26, 27 & 28). PARP1 deficiency decreases IL-5 production and

thus eosinophil infiltration into the lung (24). This suggests that PARP1 could also play a role in recruiting eosinophils to esophageal epithelial cells and hence in EoE. Though a significant correlation with CCL26 expression was not determined, PARP1 could regulate the expression of other eosinophil chemoattractants (CCL11, CCL24).

Intestinal epithelial cells stimulated with IL-4 or IL-13 increased the expression of eotaxin-3 in a dose dependent manner (53). STAT6 signaling via IL-4/IL-13 was sufficient to activate transcription of eotaxin-3 (53). Our studies provide evidence for the ability of PARP14 to directly regulate eotaxin-3 expression via STAT6 pathway in esophageal epithelial cells. Effects of PARP14 were entirely dependent on STAT6 binding to eotaxin-3 promoter. Inhibiting PARP activity using a global PARP inhibitor (PJ34) decreased the expression of eotaxin-3. However, PARP14 could also be expressed by and function in other cells that contribute to the pathology of EoE. Mucosal layers of the esophagus and skin show similarity and altered expression of epidermal differentiation complex (EDC) genes have been observed in EoE and in AD. Filaggrin and involucrin expression was downregulated in EoE biopsies and increasing concentrations of IL-13 decreased filaggrin and involucrin expression. IL-13 via STAT6 regulates the expression of involucrin in TE-7 cells. This suggests that PARP14 could regulate the expression of involucrin in esophageal epithelial cells and hence in EoE (60).

PARP14 and allergic skin inflammation

The function of PARP14 in allergy is emerging, though its role in allergic skin inflammation and in keratinocytes is still unclear. In this study we demonstrate that decreases in cytokine production by Stat6VTx*Parp14-/-* mice are intrinsically due to PARP14 deficiency in T cells. The absence of PARP14 in Stat6VT mice escalates the severity of spontaneous allergic skin inflammation. In keratinocytes, PARP14 is not required for the induction of IL-4 and Stat6 responsive genes that are important for maintaining skin barrier function. In addition, altered immune responses also resulted in altered cellular infiltrate in the skin of Stat6VTx*Parp14-/-* mice compared to skin from Stat6VT mice. Together, these data suggest PARP14 deficiency alters immune responses thus altering cellular infiltrate in the skin in a model of spontaneous allergic skin inflammation.

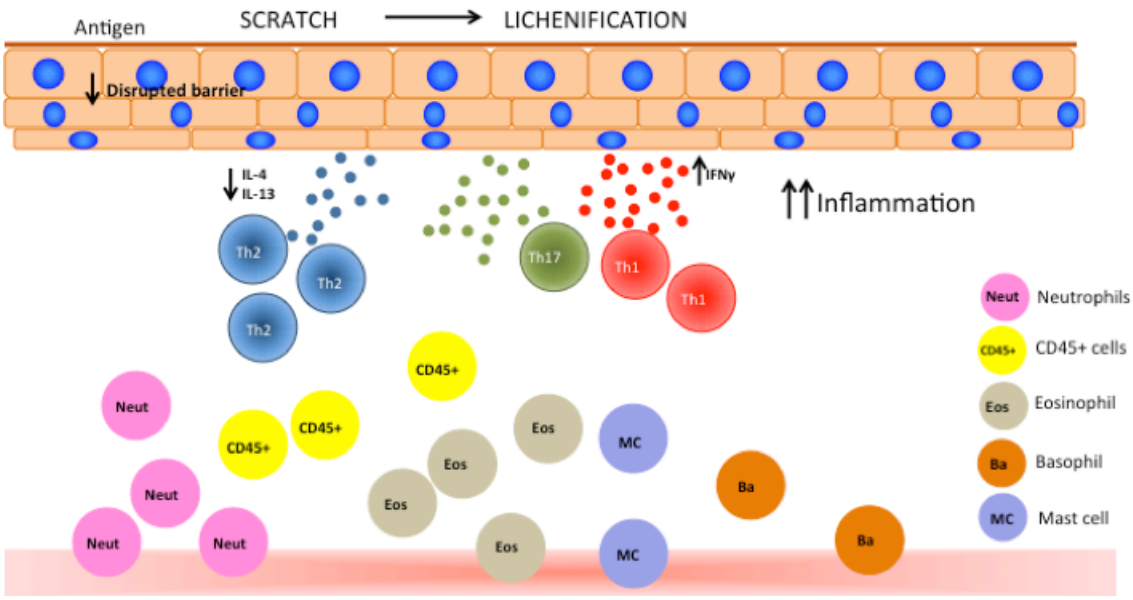

Figure 21. Summary showing the effect of PARP14 deficiency in Stat6VT mice and hence in allergic skin inflammation.

Previous studies on Stat6VT mice have demonstrated decreased percentages of T cells in the periphery and Stat6VT T cells are predisposed to differentiate into Th2 cells (98). In this report, we showed that PARP14 deficiency preserves the effects of Stat6VT expression with similar percentages of T cells in Stat6VT and Stat6VTx*Parp14-/-* spleens. Though the frequency of T cells was similar, T cell numbers were significantly reduced in Stat6VTx*Parp14-/-* mice. T cells from Stat6VT mice secreted increased levels of the Th2 cytokines IL-4 and IL-13. We demonstrated a decrease in IL-4 and IL-13 production in T cells from Stat6VTx*Parp14-/-* mice. This is in agreement with previous studies where PARP14 has been shown to affect Th2 differentiation (37). Adoptive transfer of CD4+ T cells into *Rag1-/-* mice resulted in an increased IFNγ/IL-4 ratio. This could be attributed to the ability of PARP14 to negatively regulate *Stat1* and

positively regulate *Stat6* expression (42). *In vitro*, *Parp14-/-* Th17 cells had significantly reduced frequency of IL-17A+ cells and *in vivo*, in a model of allergic airway disease, *Parp14-/-* mice had decreased numbers of IL-17A producing CD4+ cells and reduced concentration of IL-17A from antigen-stimulated splenocytes (43). However, in Stat6VTx*Parp14-/-* mice, we observed an overall increase in IL-17A-producing CD4+ cells. IL-4 has been shown to suppress IL-23 and IL-17 (115), suggesting that increase in IL-17A in Stat6VTx*Parp14-/-* T cells could be due to decreases in IL-4. Increase in IL-17A could also represent increased bacterial infections in the AD-like lesions (116). Studies in AD patients revealed a correlation of AD severity with increased IL-17 producing T cells in the peripheral blood and in acute lesional skin (117). Adoptive transfer of CD4+ T cells into *Rag1-/-* mice did not result in altered IL-17A levels and these mice did not develop skin inflammation, suggesting that elevated IL-17A could cause allergic skin inflammation. Thus, IL-17 producing cells may contribute in Stat6VTx*Parp14-/-* disease severity.

In the presence of IL-4, PARP14 promotes Stat6 binding to target gene promoters in B-cells and potentiates IL-4 induced Stat6 dependent transcription (34, 36). Stat6VT mice have an expanded B cell population in the periphery (98). This increased B cell population is due to IL-4/Stat6 dependent increase in Bcl-x expression (98). Though we observe similar increases in peripheral B cell population in Stat6VTx*Parp14-/-* mice, we observed a significant reduction in B cell numbers in the spleen compared to Stat6VT mice. PARP14 has been shown

to regulate genes that promote B cell survival. Decreases in the expression of anti-apoptotic genes Bcl-x and Mcl-1 in *Parp14-/-* B cells increases their susceptibility to apoptosis (44). Thus, the decrease in B cell numbers in Stat6VTx*Parp14-/-* mice could be due to altered expression of B cell survival genes. Cell surface markers like MHC Class II and CD86 that are up-regulated upon IL-4 stimulation, were elevated in B cells from Stat6VT mice (98). In line with reduced IL-4 produced by Stat6VTx*Parp14-/-* T cells, we observed decreased number of MHC class II and CD86 positive B cells. Although Th2 cytokines were reduced in Stat6VTx*Parp14-/-* mice, we observed highly elevated serum IgE levels. This could be attributed to altered isotype class switching to IgE. As described above, we observed increased levels of IL-17A in Stat6VTx*Parp14-/-* mice. It has been reported that IL-17A promotes IgE production in human B cells *in vitro* (118), suggesting a pro-allergic role for IL-17A.

PARP14 regulates transcriptional function of Stat6 in T cells and B cells (36), however in keratinocytes we observe that inhibiting PARP14 using PARP inhibitor, PJ34 does not affect IL-4 responsive gene expression. In contrast, in esophageal cells we demonstrated PARP14 regulates Stat6 responsive gene expression of *CCL26* and blocking PARP catalytic activity of PARP14 using PJ34 decreased *CCL26* expression. Though PJ34 is a global PARP inhibitor, previous studies in a model of allergic airway disease demonstrate that using PJ34 in *Parp14-/-* mice did not further alleviate disease, suggesting that PARP14 is the

major PARP in allergic inflammation (37). Thus, though PARP14 alters IL-4 responsive gene expression in esophageal cells, in keratinocytes inhibiting PARP14 did not alter IL-4 responsive gene expression. This suggests that the skin inflammation observed in Stat6VTx*Parp14-/-* mice was not likely due to an altered function in keratinocytes.

One of the questions we have tried to address is whether the skin infiltrating cells were altered in Stat6VTx*Parp14-/-* mice. We characterized the cells infiltrating the skin from Stat6VT and Stat6VTx*Parp14-/-* mice and observed that the largely altered cell infiltrate had a decrease in the frequency of neutrophils, basophils and mast cells in Stat6VTx*Parp14-/-* skin compared to Stat6VT skin. Although there was a decrease in the frequency of mast cells, we observed an increase in the absolute number of mast cells in the skin of both Stat6VT and Stat6VTx*Parp14-/-* mice (119). Decreased percentages and cell numbers of neutrophils in Stat6VTx*Parp14-/-* skin could be attributed to impaired neutrophil migration from bone marrow to the periphery as observed in allergic inflammation (120, 121). Moreover, we observed the infiltration of hematopoietic cells (CD45+) that were negative for CD3, CD19, CD117, NK1.1, CD11c, CD11b, CD49b, GR1, FcεRIα and SiglecF in Stat6VT skin. This cell population was further elevated in Stat6VTx*Parp14-/-* skin. Looking at other CD45+ cells, studies on leisonal skin of transgenic mouse models of AD show an increase in macrophage infiltration (122, 123). Other studies in human AD have also shown the involvement of macrophages in both acute and chronic lesions. In chronic lesions macrophages

participate in skin remodeling and dominate the dermal mononuclear cell infiltrate (124, 125). Skin from mast cell deficient Stat6VT mice has shown an increase in tissue macrophages (119). Thus, it is possible that CD45+ cells that were negative for CD3, CD19, CD117, NK1.1, CD11c, CD11b, CD49b, GR1, FcεRIα and SiglecF could be macrophages. Taken together, our data suggests that the altered immune response observed in the Stat6VT transgenic mice result in altered cellular infiltrate and consequently a more severe disease phenotype.

On comparing PARP14 function in skin inflammation and allergic airway disease, we observed that PARP14-deficiency results in a distinct outcome in skin inflammation compared to what was previously observed during allergic airway disease. As observed in the allergic airway disease model, the absence of PARP14 affects Th2 cytokine production in Stat6VTx*Parp14-/-* mice (37). Here we demonstrated that inhibiting PARP activity of PARP14 does not modify IL-4 responsive gene expression in keratinocytes. This is distinct from what has previously been observed in airway inflammation where PARP14 deficiency decreased the expression of inflammatory mediators. PARP14 is expressed both in mouse skin tissue and in human keratinocytes. It is not clear if the differences in PARP activity in keratinocytes could be due to altered ability of PARP14 to bind Stat6 responsive gene promoters or altered enzymatic activity affecting ADP-ribosylating ability of PARP14.

AD is predominantly a Th2 mediated disease. However, Stat6VTx*Parp14-/-* mice develop severe AD like skin inflammation even with significant decreases in Th2 cytokines compared to Stat6VT mice. In other models of AD like Nc/Nga mice, absence of Stat6 still resulted in the development of AD like lesions similar to that observed in Stat6 competent NC/Nga mice (126). Similarly, mice that are transgenic for IL-18 or Caspase-1 develop AD like skin lesions even on a Stat6 deficient background (127). In both these studies, the authors observe an IFNγ-favored skin microenvironment that is the likely cause of skin pathology. Similar to these studies, upon adoptive transfer of Stat6VTx*Parp14-/-* CD4+ cells into *Rag-/-* mice we observed an increase in the IFNγ/IL-4 ratio. Stat6VTx*Parp14-/-* mice also have elevated levels of IL-17A producing CD4+ cells. Altered IFNγ/IL-4 ratio and increased IL-17A levels could contribute to disease pathology in Stat6VTx*Parp14-/-* mice. These studies together with our observations suggest that absence of Th2 responses could also result in skin inflammation.

It should be noted that the Stat6VTx*Parp14-/-* mice lack PARP14 expression in all cells. PARP14 function has been investigated in T cells, B cells, macrophages, airway and esophageal epithelial cells (37, 44, 47, 111) though its function in other immune cells are yet to be explored. Several genes have been identified that are PARP14 dependent and Stat6 independent (42), suggesting that PARP14 could impact are other pathways in distinct cell types. Together, our findings demonstrate that PARP14 has similar effects in cytokine production in

multiple models of allergic inflammation, though the consequence of this altered cytokine response is distinct depending on the target organ.

Stat6VT mice have a T cell-specific expression of constitutively active Stat6 (98). Our studies demonstrated that PARP14 deficiency in Stat6VT mice resulted in altered cytokine production with a decrease in Th2 cytokines and increase in IL-17A (Figure 21). In B cells, the altered phenotype observed may be due to altered cytokines produced by T cells and also due to intrinsic PARP14 deficiency. An impact of altered cytokine environment was increased serum IgE levels in Stat6VTx*Parp14-/-* mice. Increased serum IgE produced could have an effect on allergic skin inflammation. Another impact of altered cytokine production is increased skin infiltration of monocyte/macrophage population. Together, our data suggests that the altered skin inflammation observed in Stat6VTx*Parp14-/-* mice likely results from a combination of altered cytokines produced by T cells, (which includes reduced Th2 cytokines, increased IFNγ and IL-17A) along with elevated serum IgE levels and an increase in the monocyte/macrophage infiltration in the skin (Figure 21).

FUTURE DIRECTIONS

PARP14 in eosinophilic esophagitis

The data presented in this dissertation provides evidence and contributes to further our understanding of the function of PARP14 in esophageal epithelial cells. Here we determined the expression of PARP14 in EoE biopsies and correlated the expression of PARP14 with the expression of eotaxin-3. It will be important to look at the expression of PARP14 in T cells from patients with EoE, to establish if the expression of PARP14 is altered in immune cells along with esophageal cells. Moreover, we observed the expression of other PARPs in EoE biopsies and identified a decrease in the expression of PARP15 compared to control biopsies. A further study of the effects of PARP15 in regulating the expression of eotaxin-3 is essential in understanding the role of PARP15 in EoE.

Our studies here establish that PARP14 regulates the expression of eotaxin-3 in esophageal epithelial cells though the mechanism by which PARP14 functions needs to be established. It is possible PARP14 functions in a similar manner in esophageal epithelial cells as it does in Th2 cells, where PARP14 is required for the binding of STAT6 to the Gata3 promoter and regulate its expression (37). This can be tested by performing chromatin immunoprecipitation (ChIP) assays by treating esophageal epithelial cells with PARP inhibitor or siRNA to PARP14.

From our initial studies on increased PARP14 and eotaxin-3 expression in EoE biopsies, in vivo models to further understand the mechanism of PARP14 in EoE are necessary. Experimental model of EoE can be induced by allergen challenge after epicutaneous (128) or respiratory (129) sensitization in *Parp14-/-* mice. As IL-17A is elevated in our model of allergic skin inflammation, it would also be key to look at the expression of IL-17A in EoE both locally in the esophageal tissue and systemically, in the blood.

Current therapy for children with EoE is elimination diet, the most effective being elemental amino acid based diets (130). Proton pump inhibitors (PPI) are commonly prescribed to EoE patients to reduce acid production or for their anti-inflammatory effects, the function of which is still not understood. Omeprazole (PPI) decreases IL-4 stimulated eotaxin-3 expression in esophageal cells from patients with EoE by affecting the binding of Stat6 to the eotaxin-3 promoter (58) and JAK-STAT6 inhibitors block eotaxin-3 expression in esophageal fibroblasts and epithelial cells (131). These studies suggest a potential for PARP14 inhibitors to be tested as therapy for EoE as they could target the Th2 responses and eotaxin-3 expression. Therapeutic effects of PARP14 inhibition can be established by treating the experimental murine models of EoE with PARP14 inhibitor PJ34 during the establishment of disease and after disease.

PARP14 and allergic skin inflammation

Our study identifies a novel role of PARP14 in allergic skin inflammation where in contrast to what has previously been observed in allergic airway disease and in EoE, we demonstrate that PARP14 is required to limit severity of allergic skin inflammation. In our studies, we see worse disease even with a decrease in Th2 cytokines. Although atopic dermatitis is recognized predominantly as a Th2 mediated disease, there are increasing studies demonstrating the involvement of other T helper subsets in disease pathogenesis. More recent evidence suggests the additional involvement of Th17 and Th22 cells in disease pathogenesis (132). Though IL-17 was initially identified to be present in the chronic phase of the disease, recent evidence on early-onset pediatric patient biopsies suggests both a Th2 and Th17 polarization early on (133). IL-17 could possibly play a role in inducing Th2 type immune responses (134). In peripheral blood from allergic asthma patients, a subset of Th2 cells capable of producing IL-4 and IL-17a was identified (135). Based on these studies, we could identify the IL-17 producers and determine if Th2 cells express IL-17a in our mouse model. It will also be important to assess the expression of other cytokines like IL-6 and IL-23 that promote IL17 production and demonstrate how Stat6VT T cells in the absence of PARP14 alter their cytokine expression.

Stat6VTx*Parp14-/-* mice have the limitation that mice lack PARP14 in all cells. Macrophages accumulate in the skin of both acute and chronic AD lesions (136).

Mast cell deficient Stat6VT mice have an increase in tissue macrophages (119). As it has been reported, PARP14 induces IL-4 triggered responses and has a protective effect in vascular disease and PARP14 deficiency in hematopoietic cells increases artherogenesis (111). Further understanding of the function of PARP14 in macrophages in our model of allergic inflammation would be beneficial in understanding disease pathology. To determine this we could induce allergic skin inflammation in *Parp14-/-* mice and determine the involvement of macrophages in the skin lesions. This model would also enable us to further study and delineate the specific subset – CD45+ CD3-, CD19-, NK1.1-, CD117-, CD11b-, CD11c-, CD49b-, FcεRIα-, Gr1- and SiglecF- that we identified in the skin of Stat6VT and Stat6VTx*Parp14-/-* mice.

In EoE patient biopsies, we identified an increase in the expression of PARP14 compared to control biopsy samples. Looking forward, it would be important to analyze PARP1 and PARP14 expression in AD patient biopsy samples. PARP1 has been implicated in allergic lung inflammation, contact hypersensitivity, EAE and arthritis (24, 28, 49, 50, 137 & 138). PARP1 delays wound healing by slowing down the migration of keratinocytes (139). The question of redundancy or compensatory mechanism among PARP family members is not well understood. PARP14 deficiency could alter the expression of other PARP family members. In the case of PARP1, an increase in the expression could delay wound closure, leaving AD like lesions prone to increased infections. In a recent study from our laboratory, we demonstrate that IL-4 by repressing fibronectin

expression delays reepithelialization and hence impairs wound healing (140). These studies suggest that healing of AD wounds could further be impaired in a system that lacks PARP14 expression. We could use a similar approach as shown by Serezani et al and punch ears of Stat6VTx*Parp14-/-* mice and monitor wound healing and reepithelialization.

REFERENCES

1. Bieber, T. 2008. Atopic Dermatitis. *N Engl J Med*. 358:1483-94.
2. Murphy, K., P. Travers and Walport M. 2007. *Janeway's Immunobiology*. 7th ed.
3. Chaplin, D.D. 2010. Overview of the immune response. *J. Allergy Clin. Immunol.*125:S3-23.
4. Luckheeram, R. V., R. Zhou, A. D. Verma and B. Xia. 2012. CD4+ T cells: Differentiation and fucntions. *Clin and Dev Immunol*. 2012.
5. Galli, S. J., M. Tsai. 2012. IgE and mast cells in allergic disease. *Nat. Med.* 18:693-704.
6. Stritesky, G. L., R. Muthukrishnan, S. Sehra, R. Goswami, D. Pham, J. Travers, E. T. Nguyen, D. E. Levy, M. H. Kaplan. 2011. The transcription factor STAT3 is required for T helper 2 cell differentiation. *Immunity*. 34: 39-49.
7. Paul, W. E., and J. Zhu. 2010. How are TH2-type immune responses initiated and amplified?. *Nat. Rev. Immunol.* 10(4): 225-35.
8. Levy, D. E., and J. E. Darnell. 2002. Stats: transcriptional control and biological impact. *Nat. Rev. Mol. Cell Biol.* 3: 651–662.
9. Galli, S. J., M. Tsai, and A. M. Piliponsky. 2008. The development of allergic inflammation. *Nature* 454: 445–454.
10. Quelle, F. W., K. Shimoda, W. Thierfelder, C. Fischer, A. Kim, S. M. Ruben, J. L. Cleveland, J. H. Pierce, A. D. Keegan, K. Nelms, W. E. Paul, and J. N. Ihle. 1995. Cloning of murine Stat6 and human Stat6, Stat

proteins that are tyrosine phosphorylated in responses to IL-4 and IL-3 but are not required for mitogenesis. *Mol. Cell. Biol.* 15: 3336–3343.

11. Mikita, T., D. Campbell, P. Wu, K. Williamson, and U. Schindler. 1996. Requirements for interleukin-4-induced gene expression and functional characterization of Stat6. *Mol. Cell. Biol.* 16: 5811–5820.
12. Wurster, A. L., T. Tanaka, and M. J. Grusby. 2000. The biology of Stat4 and Stat6. *Oncogene* 19: 2577–2584.
13. Hebenstreit, D., G. Wirnsberger, J. Horejs-hoeck, and A. Duschl. 2006. Signaling mechanisms , interaction partners , and target genes of STAT6. *Cytokine Growth Factor Rev.* 17: 173–188.
14. Takeda, K., T. Tanaka, W. Shi, M. Matsumoto, M. Minami, S. Kashiwamura, K. Nakanishi, N. Yoshida, T. Kishimoto, and S. Akira. 1996. Essential role of Stat6 in IL-4 signalling. *Nature* 380: 627–630.
15. Shimoda, K., J. van Deursen, M. Y. Sangster, S. R. Sarawar, R. T. Carson, R. A. Tripp, C. Chu, F. W. Quelle, T. Nosaka, D. A. A. Vignali, P. C. Doherty, G. Grosveld, W. E. Paul, and J. N. Ihle. 1996. Lack of IL-4-induced Th2 response and IgE class switching in mice with disrupted Stat6 gene. *Nature* 380: 630–633.
16. Kaplan, M. H., U. Schindler, S. T. Smiley, and M. J. Grusby. 1996. Stat6 is required for mediating responses to IL-4 and for the development of Th2 cells. *Immunity* 4: 313–319.
17. Ouyang, W., M. Löhning, Z. Gao, M. Assenmacher, S. Ranganath, A Radbruch, and K. M. Murphy. 2000. Stat6-independent GATA-3

autoactivation directs IL-4-independent Th2 development and commitment. *Immunity* 12: 27–37.

18. Hottiger, M. O., P. O. Hassa, B. Lüscher, H. Schüler, and F. Koch-Nolte. 2010. Toward a unified nomenclature for mammalian ADP-ribosyltransferases. *Trends Biochem. Sci.* 35: 208–219.

19. Schreiber, V., F. Dantzer, J.-C. Ame, and G. de Murcia. 2006. Poly(ADP-ribose): novel functions for an old molecule. *Nat. Rev. Mol. Cell Biol.* 7: 517–528.

20. Yélamos, J., Y. Monreal, L. Saenz, E. Aguado, V. Schreiber, R. Mota, T. Fuente, A. Minguela, P. Parrilla, G. de Murcia, E. Almarza, P. Aparicio, and J. Ménissier-de Murcia. 2006. PARP-2 deficiency affects the survival of CD4+CD8+ double-positive thymocytes. *EMBO J.* 25: 4350–4360.

21. Rosado, M. M., E. Bennici, F. Novelli, and C. Pioli. 2013. Beyond DNA repair, the immunological role of PARP-1 and its siblings. *Immunology* 139: 428–437.

22. Saenz, L., J. J. Lozano, R. Valdor, A. Baroja-Mazo, P. Ramirez, P. Parrilla, P. Aparicio, L. Sumoy, and J. Yélamos. 2008. Transcriptional regulation by poly(ADP-ribose) polymerase-1 during T cell activation. *BMC Genomics* 9: 171.

23. Kim, M. Y., T. Zhang, and W. L. Kraus. 2005. Poly (ADP-ribosyl) ation by PARP-1 : “ PAR-laying ” NAD + into a nuclear signal. 1951–1967.

24. Oumouna, M., R. Datta, K. Oumouna-Benachour, Y. Suzuki, C. Hans, K. Matthews, K. Fallon, and H. Boulares. 2006. Poly(ADP-ribose)

polymerase-1 inhibition prevents eosinophil recruitment by modulating Th2 cytokines in a murine model of allergic airway inflammation: a potential specific effect on IL-5. *J. Immunol.* 177: 6489–6496.

25. Boulares, A. H., A. J. Zoltoski, Z. A. Sherif, P. Jolly, D. Massaro, and M. E. Smulson. 2003. Gene knockout or pharmacological inhibition of poly(ADP-ribose) polymerase-1 prevents lung inflammation in a murine model of asthma. *Am. J. Respir. Cell Mol. Biol.* 28: 322–329.

26. Suzuki, Y., E. Masini, C. Mazzocca, S. Cuzzocrea, A. Ciampa, H. Suzuki, and D. Bani. 2004. Inhibition of Poly (ADP-Ribose) Polymerase Prevents Allergen- Induced Asthma-Like Reaction in Sensitized Guinea Pigs. *Pharmacology* 311: 1241–1248.

27. Virág, L., P. Bai, I. Bak, P. Pacher, J. G. Mabley, L. Liaudet, E. Bakondi, P. Gergely, M. Kollai, and C. Szabó. 2004. Effects of poly(ADP-ribose) polymerase inhibition on inflammatory cell migration in a murine model of asthma. *Med. Sci. Monit.* 10: BR77-R83.

28. Naura, A. S., C. P. Hans, M. Zerfaoui, D. You, S. a Cormier, M. Oumouna, and A. H. Boulares. 2008. Post-allergen challenge inhibition of poly(ADP-ribose) polymerase harbors therapeutic potential for treatment of allergic airway inflammation. *Clin. Exp. Allergy* 38: 839–846.

29. Olabisi, O. A., N. Soto-Nieves, E. Nieves, T. T. C. Yang, X. Yang, R. Y. L. Yu, H. Y. Suk, F. Macian, and C.-W. Chow. 2008. Regulation of transcription factor NFAT by ADP-ribosylation. *Mol. Cell. Biol.* 28: 2860–2871.

30. Datta. R., A. S. Naura, M. Zerfaoui, Y. Errami, M. Oumouna, H. Kim, J. Ju, V. P. Ronchi, A. L. Haas, and A. H. Boulares. 2011. PARP-1 deficiency bocks IL-5 expression through calpain-dependent degradation of STAT-6 in a murine asthma model. *Allergy.* 66: 853-861.

31. Pehrson, J. R., V. A. Fried. 1992. MacroH2A, a core histone containing a large nonhistone region. *Science.* 5075: 1398-1400.

32. Ladurner, A. G. 2003. Inactivating chromosomes: A macro domain that minimizes transcription. *Mol. Cell*. 12: 1-4.

33. Vyas, S., I. Matic, L. Uchima, J. Rood, R. Zaja, R. T. Hay, I. Ahel, and P. Chang. 2014. Family-wide analysis of poly(ADP-ribose) polymerase activity. *Nat. Commun.* 5: 4426.

34. Goenka, S., and M. Boothby. 2006. Selective potentiation of Stat-dependent gene expression by collaborator of Stat6 (CoaSt6), a transcriptional cofactor. *Proc. Natl. Acad. Sci. U. S. A.* 103: 4210–4215.

35. Goenka, S., H. C. Sung, and M. Boothby. 2007. Collaborator of Stat6 (CoaSt6)-associated poly(ADP-ribose) polymerase activity modulates Stat6-dependent gene transcription. *J. Biol. Chem.* 282: 18732–18739.

36. Mehrotra, P., J. P. Riley, R. Patel, F. Li, L. Voss, and S. Goenka. 2011. PARP-14 functions as a transcriptional switch for Stat6-dependent gene activation. *J. Biol. Chem.* 286: 1767–1776.

37. Mehrotra, P., A. Hollenbeck, J. P. Riley, F. Li, R. J. Patel, N. Akhtar, and S. Goenka. 2013. Poly (ADP-ribose) polymerase 14 and its enzyme activity regulates TH2 differentiation and allergic airway disease. *J. Allergy*

Clin. Immunol. 131: 521–531.

38. Bettelli, E., T. Korn, and V. K. Kuchroo. 2007. Th17 : the third member of the effector T cell trilogy. *Curr. Opin. Immunol.* 19: 652–657.

39. Alcorn, J. F., C. R. Crowe, and J. K. Kolls. 2010. T H 17 Cells in Asthma and COPD. *Annu. Rev. Physiol.* 72: 495–516.

40. Kemeny, D. M. 2012. The role of the T follicular helper cells in allergic disease. *Cell. Mol. Immunol.* 9: 386–389.

41. Crotty, S. 2014. Review T Follicular Helper Cell Differentiation , Function , and Roles in Disease. *Immunity* 41: 529–542.

42. Riley, J. P., A. Kulkarni, P. Mehrotra, B. Koh, N. B. Perumal, M. H. Kaplan, and S. Goenka. 2013. PARP-14 Binds Specific DNA Sequences to Promote Th2 Cell Gene Expression. *PLoS One* 8: e83127.

43. Mehrotra, P., P. Krishnamurthy, J. Sun, S. Goenka, and M. H. Kaplan. 2015. Poly-ADP-ribosyl polymerase-14 promotes T helper 17 and follicular T helper development. *Immunology* 146: 537–546.

44. Cho, S. H., S. Goenka, T. Henttinen, P. Gudapati, A. Reinikainen, C. M. Eischen, R. Lahesmaa, and M. Boothby. 2009. PARP-14 , a member of the B aggressive lymphoma family, transduces survival signals in primary B cells. *Blood* 113: 2416–2425.

45. Blanchard, C., N. Wang, K. F. Stringer, A. Mishra, P. C. Fulkerson, J. P. Abonia, S. C. Jameson, C. Kirby, M. R. Konikoff, M. H. Collins, M. B. Cohen, R. Akers, S. P. Hogan, A. H. Assa'ad, P. E. Putnam, B. J. Aronow, and M. E. Rothenberg. 2006. Eotaxin-3 and a uniquely conserved gene

expression profile in eosinophilic esophagitis. *J. Clin. Invest.* 116: 536–47.

46. Gupta, S. K., J. F. Fitzgerald, T. Kondratyuk, and H. HogenEsch. 2006. Cytokine expression in normal and inflamed esophageal mucosa: a study into the pathogenesis of allergic eosinophilic esophagitis. *J. Pediatr. Gastroenterol. Nutr.* 42: 22–26.

47. Krishnamurthy, P., J. D. Sherrill, K. Parashette, S. Goenka, M. E. Rothenberg, S. Gupta, and M. H. Kaplan. 2014. Correlation of increased PARP14 and CCL26 expression in biopsies from children with eosinophilic esophagitis. *J. Allergy Clin. Immunol.* 133: 577–580.e2.

48. Nasta, F., F. Laudisi, M. Sambucci, M. M. Rosado, and C. Piolo. 2010. Increased Foxp3+ regulatory T cells in poly (ADP-ribose) polymerase-1 deficiency. *J. Immunol*. 184: 3470-3477

49. Bai, P., C. Hegedus, E. Szabo, L. Gyure, E. Bakondi, A. Brunyanszki, S. Gergely, C. Szabo, and L. Virag. 2009. Poly (ADP-Ribose) Polymerase Mediates Inflammation in a Mouse Model of Contact Hypersensitivity. *J. Invest. Dermatol.* 129: 234–238.

50. Brunyanszki, A., C. Hegedus, M. Szanto, K. Erdelyi, K. Kovacs, V. Schreiber, S. Gergely, B. Kiss, E. Szabo, L. Virag, and P. Bai. 2010. Genetic Ablation of PARP-1 Protects Against Oxazolone-Induced Contact Hypersensitivity by Modulating Oxidative Stress. *J. Invest. Dermatol.* 130: 2629–2637.

51. Yazaki, E., and D. Sifrim. 2012. Anantomy and physiology of the esophageal body. *Dis. Esophagus*. 25(4): 292-8.

52. Orlando, R. C. 2006. Esophageal mucosal defense mechanisms, PART 1 Oral cavity, pahrynx and esophagus. *GI Motility online.*

53. Blanchard, C., S. Durual, M. Estienne, S. Emami, S. Vasseur, and J. C. Cuber. 2005. Eotaxin-3/CCL26 gene expression in intestinal epithelial cells is up-regulated by interleukin-4 and interleukin-13 via the signal transducer and activator of transcription 6. *Int. J. Biochem. Cell Biol.* 37: 2559–2573.

54. Furuta, G. T., and D. A. Katzka. 2015. Eosinophilic Esophagitis. *N. Engl. J. Med.* 373: 1640–8.

55. Kagami, S., H. Saeki, M. Komine, T. Kakinuma, Y. Tsunemi, K. Nakamura, K. Sasaki, A. Asahina, and K. Tamaki. 2005. Interleukin-4 and interleukin-13 enhance CCL26 production in a human keratinocyte cell line, HaCaT cells. *Clin. Exp. Immunol.* 141: 459–466.

56. Hoeck, J., and M. Woisetschläger. 2001. Activation of eotaxin-3/CCLl26 gene expression in human dermal fibroblasts is mediated by STAT6. *J. Immunol.* 167: 3216–3222.

57. Mishra, A., and M. E. Rothenberg. 2003. Intratracheal IL-13 Induces Eosinophilic Esophagitis by an IL-5, Eotaxin-1, and STAT6-Dependent Mechanism. *Gastroenterology* 125: 1419–1427.

58. Zhang, X., E. Cheng, X. Huo, C. Yu, Q. Zhang, T. H. Pham, D. H. Wang, S. J. Spechler, and R. F. Souza. 2012. Omeprazole Blocks STAT6 Binding to the Eotaxin-3 Promoter in Eosinophilic Esophagitis Cells. *PLoS One* 7: e50037.

59. Niranjan, R., M. Rayapudi, A. Mishra, P. Dutt, S. Dynda, and A. Mishra. 2013. Pathogenesis of allergen-induced eosinophilic esophagitis is independent of interleukin (IL)-13. *Immunol. Cell Biol.* 91: 408–15.

60. Blanchard, C., E. M. Stucke, K. Burwinkel, J. M. Caldwell, M. H. Collins, A. Ahrens, B. K. Buckmeier, S. C. Jameson, A. Greenberg, A. Kaul, J. P. Franciosi, J. P. Kushner, L. J. Martin, P. E. Putnam, J. P. Abonia, S. I. Wells, and M. E. Rothenberg. 2010. Coordinate Interaction between IL-13 and Epithelial Differentiation Cluster Genes in Eosinophilic Esophagitis. *J. Immunol.* 184: 4033–4041.

61. Sherrill, J. D., K. Kc, D. Wu, Z. Djukic, J. M. Caldwell, E. M. Stucke, K. A. Kemme, M. S. Costello, M. K. Mingler, C. Blanchard, M. H. Collins, J. P. Abonia, P. E. Putnam, E. S. Dellon, R. C. Orlando, S. P. Hogan, and M. E. Rothenberg. 2014. Desmoglein-1 regulates esophageal epithelial barrier function and immune responses in eosinophilic esophagitis. *Mucosal Immunol.* 7: 718–29.

62. McLafferty, E., C. Hendry, and A. Farley. 2012. The integumentary system : anatomy , physiology and function of skin. *Nurs. Stand.* 27: 35–42.

63. Fuchs, E. 2007. Scratching the surface of skin development. *Nature* 445: 834–842.

64. Candi, E., R. Schmidt, and G. Melino. 2005. The Cornified Envelope : A model of cell death in the skin. *Nat. Rev. Mol. Cell Biol.* 6: 328–340.

65. Fuchs, E., and H. Green. 1980. Changes in Keratin Gene Expression

Differentiation of the Keratinocyte during Terminal Differentiation of the Keratinocyte. *Cell* 19: 1033–1042.

66. Kypriotou, M., M. Huber, and D. Hohl. 2012. The human epidermal differentiation complex : cornified envelope precursors , S100 proteins and the “ fused genes ” family. *Exp. Dermatol.* 21: 643–649.

67. Nestle, F. O., P. Di Meglio, J.-Z. Qin, and B. J. Nickoloff. 2009. Skin immune sentinels in health and disease. *Nat. Rev. Immunol.* 9: 679–691.

68. Pasparakis, M., I. Haase, and F. O. Nestle. 2014. Mechanisms regulating skin immunity and inflammation. *Nat. Rev. Immunol.* 14: 289–301.

69. Levin, J., S. F. Friedlander, and J. Q. Del Rosso. 2013. Atopic Dermatitis and the Stratum Corneum Part 1 : The Role of Filaggrin in the Stratum Corneum Barrier and Atopic Skin. *J. Clin. Aethetic Dermatology* 6: 16–22.

70. Kamsteeg, M., P. L. J. M. Zeeuwen, D. Rodijk-Olthuis, M. E. J. Zeeuwen-Franssen, P. E. J. van Erp, and J. Schalkwijk. 2007. Increased Expression of Carbonic Anhydrase II (CA II) in Lesional Skin of Atopic Dermatitis : Regulation by Th2 Cytokines. *J. Invest. Dermatol.* 127: 1786–1789.

71. Federici, M., M. L. Giustizieri, C. Scarponi, G. Girolomoni, and C. Albanesi. 2002. Impaired IFN- γ -Dependent Inflammatory Responses in Human Keratinocytes Overexpressing the Suppressor of Cytokine Signaling 1. *J. Imunol.* 169: 434–442.

72. Yamasaki, K., Y. Hanakawa, S. Tokumaru, Y. Shirakata, K. Sayama, T. Hanada, A. Yoshimura, and K. Hashimoto. 2003. Suppressor of Cytokine Signaling 1/JAB and Suppressor of Cytokine Signaling 3/Cytokine-

Inducible SH2 Containing Protein 3 Negatively Regulate the Signal Transducers and Activators of Transcription Signaling Pathway in Normal Human Epidermal Keratinocytes. *J. Invest. Dermatol.* 120: 571–580.

73. Sayo, T., Y. Sugiyama, Y. Takahashi, N. Ozawa, S. Sakai, O. Ishikawa, M. Tamura, and S. Inoue. 2002. Hyaluronan Synthase 3 Regulates Hyaluronan Synthesis in Cultured Human Keratinocytes. *J. Invest. Dermatol.* 118: 43–48.

74. Malaisse, J., V. Bourguignon, E. De Vuyst, C. Lambert de Rouvroit, A. F. Nikkels, B. Flamion, and Y. Poumay. 2014. Hyaluronan Metabolism in Human Keratinocytes and Atopic Dermatitis Skin Is Driven by a Balance of Hyaluronan Synthases 1 and 3. *J. Invest. Dermatol.* 134: 2174–2182.

75. Ohtani, T., A. Memezawa, R. Okuyama, T. Sayo, Y. Sugiyama, S. Inoue, and S. Aiba. 2009. Increased Hyaluronan Production and Decreased E-Cadherin Expression by Cytokine-Stimulated Keratinocytes Lead to Spongiosis Formation. *J. Invest. Dermatol.* 129: 1412–1420.

76. Sivaprasad, U., D. J. Askew, M. B. Ericksen, A. M. Gibson, M. T. Stier, E. B. Brandt, S. A. Bass, M. O. Daines, J. Chakir, K. F. Stringer, S. E. Wert, J. A. Whitsett, T. D. Le Cras, M. Wills-karp, G. A. Silverman, and G. K. Khurana Hershey. 2011. A nonredundant role for mouse Serpinb3a in the induction of mucus production in asthma. *J. Allergy Clin. Immunol.* 127: 254–261.

77. Sivaprasad, U., K. G. Kinker, M. B. Ericksen, M. Lindsey, A. M. Gibson, S. A. Bass, N. S. Hershey, J. Deng, M. Medvedovic, and G. K. Khurana

Hershey. 2015. SERPINB3 / B4 Contributes to Early Inflammation and Barrier Dysfunction in an Experimental Murine Model of Atopic Dermatitis. *J. Invest. Dermatol.* 135: 160–169.

78. Hamid, Q., M. Boguniewicz, and D. Y. M. Leung. 1994. Differential in situ cytokine gene expression in acute versus chronic atopic dermatitis. *J. Clin. Invest.* 94: 870–6.

79. Tokura, Y. 2010. Extrinsic and intrinsic types of atopic dermatitis. J Dermatol Sci. 58 (1): 1-7

80. Palmer, C. N. A., A. D. Irvine, A. Terron-Kwiatkowski, Y. Zhao, H. Liao, S. P. Lee, D. R. Goudie, A. Sandilands, L. E. Campbell, F. J. D. Smith, G. M. O'Regan, R. M. Watson, J. E. Cecil, S. J. Bale, J. G. Compton, J. J. DiGiovanna, P. Fleckman, S. Lewis-Jones, G. Arseculeratne, A. Sergeant, C. S. Munro, B. El Houate, K. McElreavey, L. B. Halkjaer, H. Bisgaard, S. Mukhopadhyay, and W. H. I. McLean. 2006. Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. *Nat. Genet.* 38: 441–446.

81. Baurecht, H., A. D. Irvine, N. Novak, T. Illig, B. Bühler, J. Ring, S. Wagenpfeil, and S. Weidinger. 2007. Toward a major risk factor for atopic eczema: Meta-analysis of filaggrin polymorphism data. *J. Allergy Clin. Immunol.* 120: 1406–1412.

82. Henderson, J., K. Northstone, S. P. Lee, H. Liao, Y. Zhao, M. Pembrey, S. Mukhopadhyay, G. D. Smith, C. N. A. Palmer, W. H. I. Mclean, and A. D. Irvine. 2008. The burden of disease associated with filaggrin mutations : A

population-based, longitudinal birth cohort study. *J. Allergy Clin. Immunol.* 121: 872–877.

83. Brown, S. J., K. Kroboth, A. Sandilands, L. E. Campbell, E. Pohler, S. Kezic, H. J. Cordell, W. H. I. Mclean, and A. D. Irvine. 2012. Intragenic Copy Number Variation within Filaggrin Contributes to the Risk of Atopic Dermatitis with a Dose-Dependent Effect. *J. Invest. Dermatol.* 132: 98–104.

84. Fallon, P. G., T. Sasaki, A. Sandilands, L. E. Campbell, S. P. Saunders, N. E. Mangan, J. J. Callanan, H. Kawasaki, A. Shiohama, A. Kubo, J. P. Sundberg, R. B. Presland, P. Fleckman, N. Shimizu, J. Kudoh, A. D. Irvine, M. Amagai, and W. H. I. Mclean. 2009. A homozygous frameshift mutation in the mouse Flg gene facilitates enhanced percutaneous allergen priming. *Nat. Genet.* 41: 602–608.

85. Oyoshi, M. K., G. F. Murphy, and R. S. Geha. 2009. Filaggrin-deficient mice exhibit T H 17-dominated skin inflammation and permissiveness to epicutaneous sensitization with protein antigen. *J. Allergy Clin. Immunol.* 124: 485–493.

86. Saunders, S. P., T. Moran, A. Floudas, F. Wurlod, A. Kaszlikowska, M. Salimi, E. M. Quinn, C. J. Oliphant, G. Nunez, R. McManus, E. Hams, A. D. Irvine, A. N. J. Mckenzie, G. S. Ogg, and P. G. Fallon. 2016. Spontaneous atopic dermatitis is mediated by innate immunity, with the secondary lung inflammation of the atopic march requiring adaptive immunity. *J. Allergy Clin. Immunol.* 137: 482–491.

87. Howell, M. D., B. E. Kim, P. Gao, A. V. Grant, M. Boguniewicz, A. DeBenedetto, L. Schneider, L. A. Beck, K. C. Barnes, and D. Y. M. Leung. 2007. Cytokine modulation of atopic dermatitis filaggrin skin expression. *J. Allergy Clin. Immunol.* 120: 150–155.

88. Kim, B. E., D. Y. M. Leung, M. Boguniewicz, and M. D. Howell. 2008. Loricrin and involucrin expression is down-regulated by Th2 cytokines through STAT-6. *Clin. Immunol.* 126: 332–337.

89. Nomura, I., E. Goleva, M. D. Howell, Q. A. Hamid, P. Y. Ong, C. F. Hall, M. A. Darst, B. Gao, M. Boguniewicz, J. B. Travers, and D. Y. M. Leung. 2003. Cytokine milieu of atopic dermatitis, as compared to psoriasis, skin prevents induction of innate immune response genes. *J. Immunol.* 171: 3262–3269.

90. Hussein, Y. M., S. M. Shalaby, A. Nassar, S. S. Alzahrani, A. S. Alharbi, and M. Nouh. 2014. Association between genes encoding components of the IL-4 / IL-4 receptor pathway and dermatitis in children. *Gene* 545: 276–281.

91. Kayserova, J., K. S. I. Zentsova-jaresova, S. Katina, E. Vernerova, A. Polouckova, S. Capkova, V. Malinova, I. Striz, and A. Sediva. 2012. A Prospective Study in Children With a Severe Form of Atopic Dermatitis : Clinical Outcome in Relation to Cytokine Gene Polymorphisms. *Joural Investig. Allergol. Cinical Immunol.* 22: 92–101.

92. Hummelshoj, T., U. Bodtger, P. Datta, H. J. Malling, A. Oturai, L. K. Poulsen, L. P. Ryder, P. S. Sorensen, E. Svejgaard, and A. Svejgaard.

2003. Association between an interleukin-13 promoter polymorphism and atopy. *Eur. J. Immunogentics* 30: 355–359.

93. Tsunemi Y, K. Saeki, K. Nakamura, T. Sekiya, T. Kakinuma, H. Fujita, N. Asano, Y. Tanida, M. Wakugawa, H. Torii, K. Tamaki. 2002. interleukin-13 gene polymorphisms G4257A is associated with atpic dermatitis in Japanese patients. *J Dermatol Sci.* 30(2): 100-107

94. Hosomi, N., K. Fukai, N. Oiso, A. Kato, M. Ishii, H. Kunimoto, and K. Nakajima. 2004. Polymorphisms in the Promoter of the Interleukin-4 Receptor a Chain Gene Are Associated with Atopic Dermatitis in Japan. *J. Invest. Dermatol.* 122: 843–845.

95. Callard, R. E., R. Hamvas, C. Chatterton, C. Blanco, M. Pembrey, R. Jones, A. Sherriff, J. Henderson, and A. study Teams. 2002. An interaction between the IL-4R a gene and infection is associated with atopic eczema in young children. *Clin. Exp. Allergy* 32: 990–993.

96. Oiso, N., K. Fukai, and M. Ishii. 2000. Interleukin 4 receptor a chain polymorphism Gln551Arg is associated with adult atopic dermatitis in Japan. *Br. J. Dermatol.* 142: 1003–1006.

97. Tamura. K, M. Suzuki, H. Arakawa, K. Tokuyama, A. Morikawa. 2003. Linkage and association studies of STAT6 gene polymorphisms and allergic diseases. *Int Arch Allergy Immunol*. 131(1): 33-38

98. Bruns, H. A., U. Schindler, and M. H. Kaplan. 2003. Expression of a constitutively active Stat6 in vivo alters lymphocyte homeostasis with distinct effects in T and B cells. *J. Immunol.* 170: 3478–3487.

99. Sehra, S., H. A. Bruns, A.-N. N. Ahyi, E. T. Nguyen, N. W. Schmidt, E. G. Michels, G.-U. von Bülow, and M. H. Kaplan. 2008. IL-4 is a critical determinant in the generation of allergic inflammation initiated by a constitutively active Stat6. *J. Immunol.* 180: 3551–3559.

100. Sehra, S., Y. Yao, M. D. Howell, E. T. Nguyen, G. S. Kansas, D. Y. M. Leung, J. B. Travers, and M. H. Kaplan. 2010. IL-4 regulates skin homeostasis and the predisposition toward allergic skin inflammation. *J. Immunol.* 184: 3186–3190.

101. Turner, M. J., S. DaSilva-Arnold, N. Luo, X. Hu, C. C. West, L. Sun, C. Hall, J. Bradish, M. H. Kaplan, J. B. Travers, and Y. Sun. 2014. STAT6-Mediated Keratitis and Blepharitis : A Novel Murine Model of Ocular Atopic Dermatitis. *Invest. Ophthalmol. Vis. Sci.* 55: 3803–3808.

102. DaSilva, S. C., R. P. Sahu, R. L. Konger, S. M. Perkins, M. H. Kaplan, and J. B. Travers. 2012. Increased skin barrier disruption by sodium lauryl sulfate in mice expressing a constitutively active STAT6 in T cells. *Arch. Dermatol. Res.* 304(1): 65-71.

103. Turner, M. J., S. C. DaSilva-Arnold, Q. Yi, P. Mehrotra, M. H. Kaplan, and J. B. Travers. 2013. Topical application of vitamin D analogue exacerbates atopic dermatitis-like in phenotype Stat6VT mice. *Pediatr. Dermatol*. 30(5): 574-578.

104. Howell, M. D., P. Gao, B. E. Kim, L. J. Lesley, J. E. Streib, P. A. Taylor, D. J. Zaccaro, M. Boguniewicz, L. A. Beck, J. M. Hanifin, L. C. Schneider, T. R. Hata, R. L. Gallo, M. H. Kaplan, K. C. Barnes, and D. Y. M. Leung.

2011. The signal transducer and activator of trasncription 6 gene (STAT6) increases the propensity of patients with atopic dermatitis towards disseminated viral skin infections. 128: 1006-14.

105. Chan, L. S., N. Robinson, and L. Xu. 2001. Expression of interleukin-4 in the epidermis of transgenic mice results in a pruritic inflammatory skin disease: an experimental animal model to study atopic dermatitis. *J. Invest. Dermatol.* 117: 977–983.

106. Zheng, T., M. H. Oh, S. Y. Oh, J. T. Schroeder, A. B. Glick, and Z. Zhu. 2009. Transgenic Expression of Interleukin-13 in the Skin Induces a Pruritic Dermatitis and Skin Remodeling. *J. Invest. Dermatol.* 129: 742–751.

107. Kuhn, C., S. A. Hurwitz, M. G. Kumar, J. Cotton, D. F. Spandau. 1999. Activation of insulin-like growth factor-1 receptor promotes the survival of human keratinocytes following ultraviolet B irradiation. *Int. J. Cancer.* 80: 431-438.

108. Spergel, J. M., E. Mizoguchi, J. P. Brewer, T. R. Martin, A. K. Bhan, R. S. Geha. 1998. Epicutaneous sensitization with protei antigen induces localized allergic dermatitis and hyperresponsivenessto mehtacholine after single exposure to aerosolized antigen in mice. *J. Clin. Invest.* 101(8): 1614-1622.

109. Lim, E. J., T. X. Lu, C. Blanchard, and M. E. Rothenberg. 2011. Epigenetic regulation of the IL-13 induced Human Eotaxin-3 Gene by CREB-Binding Protein-mediated Histone 3 Acetylation. *J. Biol. Chem.* 296 (15): 13193-

13204

110. Burnstein, H. J., R. I. Tepper, P. Leder and A. K. Abbas. 1991. Humoral immune functions in IL-4 transgenic mice. *J. Immunol.* 147:2950-2956

111. Iwata, H., C. Goettsch, A. Sharma, P. Ricchiuto, W. Wen Bin Goh, A. Halu, I. Yamada, H. Yoshida, T. Hara, M. Wei, N. Inoue, D. Fukuda, A. Mojcher, P. C. Mattson, A. L. Barabasi, M. Boothby, E. Aikawa, S. A. Singh, and M. Aikawa. 2016. PARP9 and PARP14 cross-regulate macrophage activation via STAT1 ADP-ribosylation. *Nat. Comm*. 7:12849

112. Mishra, A., S. P. Hogan, E. B. Brandt, and M. E. Rothenberg. 2001. An etiological role for aeroallergens and eosinophils in experimental esophagitis. *J. Cli. Invest.* 107: 83-90.

113. Mishra, A., S. P. Hogan, E. B. Brandt, and M. E. Rothenberg. 2002. IL-5 promotes eosinophil trafficking to the esophagus. *J. Immunol.* 168: 2464-2469.

114. Mishra, A., and M. E. Rothenberg. 2003. Intratracheal IL-13 induces eosinophilic esophagitisby an IL-5, Eotaxin-1, and STAT-6-dependent mechanism. *Gastroenterology.* 125: 1419-1427.

115. Guenova, E., Y. Skabytska, W. Hoetzenecker, G. Weindl, K. Sauer, M. Tham, K. W. Kim, J. H. Park, J. H. Seo, D. Ignatova, A. Cozzio, M. P. Levesque, T. Volz, M. Koberle, S. Kaesler, P. Thomas, R. Mailhammer,K. Ghoreschi, K. Schakel, B. Amarov, M. Eichner, M. Schaller, R. A. Clark, M. Rocken, and T. Biedermann. 2015. IL-4 abrogates TH-17 cell-mediated inflammation by selesctive silencing of IL-23 in antigen-presenting cells.

Proc. Natl. Acad. Sci. U. S. A. 112(7): 2163-8.

116. Eyerich, K., D. Pennino, C. Scarponi, S. Foerster, F. Nasorri, H. Behrendt, J. Ring, C. Traidl-Hoffmann, C. Albanesi, and A. Cavani. 2009. IL-17 in atopic eczema: Linking allergen-specific adaptive and microbial-triggered innate immune response. *J. Allergy. Clin. Immunol.* 123: 59-66.

117. Koga, C., K. Kabashima, N. Shiraishi, M. Kobayashi, and Y. Tokura. 2008. Possible pathogenic role of TH17 cells for atopic dermatitis. *J. Invest. Dermatol.* 128(11): 2625-30.

118. Milovanovic, M., G. Drozdenko, C. Weise, M. Babina, and M. Worm. 2010. Interleukin-17A promotes IgE production in human B cells. *J. Invest. Dermatol.* 130(11): 2621-8.

119. Sehra, S., A. P. Serezani, J. A. Ocana, J. B. Travers, and M. H. Kaplan. 2016. Mast cells regulate epidermal barrier function and the development of allergic skin inflammation. *J. Invest. Dermatol.* 136: 1429-1437.

120. Benedetto, A. D., R. Agnihothri, L. Y. McGirt, L. G. Bankova, and L. A. Beck. 2009. Atopic dermatitis: A disease caused by innate immune defects? *J. Invest. Dermatol.* 129: 14-30.

121. Woytschak, J., N. Keller, C. Krieg, D. Impellizzieri, R. W. Thompson, T. A. Wynn, A. S. Zinkernagel, and O. Boyman. 2016. Type 2 interleukin 4-receptor signaling in neutrophils antagonizes their expansion and migration during infection and inflammation. *Immunity.* 45: 172-184.

122. Yoo, J., M. Omori, D. Gyarmati, B. Zhou, T. Aye, A. Brewer, M. R. Comeau, D. J. Campbell, and S. F. Ziegler. 2005. Spontaneous atopic

dermatitis in mice expressing an inducible thymic stromal lymphopoietin transgene specifically in the skin. *J. Exp. Med*. 202(4): 541-9.

123. Tsukuba. T., K. Okamoto, Y. Okamoto, M. Yanagawa, K. Kohmura, Y. Yasuda, H. Uchi, T. Nakahara, M. Furue, K. Nakayama, Y. Kadowaki, K. Yamamoto, and K. I. Nakayama. 2003. Association of cathepsin E deficiency with development of atopic dermatitis. *J. Biochem.* 134(6): 893-902.

124. Akdis. C. A, M. Akdis, T. Bieber, C. Bindslev-Jensen, M. Boguniewicz, P. Eigenmann, Q. Hamid, A. Kapp, D. Y. M. Leung, J. Lipozencic, T. A. Luger, A. Muraro, N. Novak, T. A. E. Platts-Mills, L. Rosenwasser, A. Scheynius, E. R. Simons, J. Spergel, K. Turjanmaa, U. Wahn, S. Weidinger, T. Werferl, and T. Zuberbier. 2006. Diagnosis and treatment of atopic dermatitis in children and adults: European academy of allegology and clinical immunology / American academy of allergy, asthma, and immunology/ PRACTALL consensus report. *J. Allergy. Clin. Immunol.* 118: 152-69.

125. Kiekens, R. C. M., T. Thepen, A. J. Oosting, J. G. J. Van de Winkel, C. A. F. M. Bruijnzeel-Koomen, and E. F. Knol. 2001. Heterogeneity within tissue-specific macrophage and dendritic cell populations during cutaneous inflammation in atopic dermatitis. *Br. J. Dermatol.* 145(6): 957-65.

126. Yagi, R., H. Nagai, Y. Iigo, T. Akimoto, T. Arai, and M. Kubo. 2002. Development of atopic dermatitis-like skin lesions in STAT6 deficient

NC/Nga mice. *J. Immunol.* 168: 2020-2027.

127. Konishi, H., H. Tsutsui, T. Murakami, S. Yumikura-Futatsugi, K. Yamanaka, M. Tanaka, Y. Iwakura, N. Suzuki, K. Takeda, S. Akira, K. Nakanishi, and H. Mizutani. 2002. IL-18 contributes to the spontaneous development of atopic dermatitis-like inflammatory skin lesion independently of IgE/Stat6 under specific pathogen-free conditions. *Proc. Natl. Acad. Sci. U. S. A.* 99: 11340-113345.

128. Akei, H. S., A. Mishra, C. Blanchard, and M. E. Rothenberg. 2005. Epicutaneous antigen exposure primes for experitmental eosinophilic esophagitis in mice. *Gastroenterology.* 129: 985-994.

129. Mishra, A., J. Schlotman, M. Wang, and M. E. Rothenberg. 2007. Critical role for adaptive T cell immunity in experimental eosinophilic esophagitis in mice. *J. Leukoc. Biol.* 81: 916-924.

130. Blatman, K. H., A. M. Ditto. 2012. Chapter 26: Eosinophilic esophagitis. *Allergy Asthma Proc.* 33 Suppl 1: S88-90.

131. Cheng, E., X. Zhang, K. S. Wilson, D. H. Wang, J. Y. Park, X. Huo, C. Yu, Q. Zhang, S. J. Spechler, R. F. Souza. 2016. JAK-STAT6 pathway inhibitors block eotaxin-3 secretion by epithelial cells and fibroblasts from esophageal eosinophilia patients: promising agents to improve inflammation and prevent fibrosis in EoE. *PLoS. One.* 11(6): e0157376.

132. Noda. S., M. Suarez-Farinas, B. Ungar, S. J. Kim, C. de Guzzman Strong, H. Xui, X. Peng, Y. D. Estrada, S. Nakajima, T. Honda, J. U. Shin, H. Lee, J. G. Krueger, K. H. Lee, K. Kabashima, and E. Guttman-Yassky. 2015.

The asian atopic dermatitis phenotype combines features of atopic dermatitis and psoriasis with increased TH17 polarization. *J. Allergy. Clin. Immunol.* 136: 1254-64.

133. Esaki. H., P. M. Brunner, Y. Renert-Yuval, T. Czarnowicki, T. Huynh, G. Tran, S. Lyon, G. Rodriguez, S. Immaneni, D. B. Johnson, B. Bauer, J. Fuentes-Duculan, X. Zheng, X. Peng, Y. D. Estrada, H. Xu, C. de Guzzman Strong, M. Suarez-Farinas, J. G. Krueger, A. S. Paller, and E. Guttman-Yassky. 2016. Early-onset pediatric atopic dermatitis is TH2 but not TH17 polarized in skin. *J. Allergy. Clin. Immunol.* 138:1639-51.

134. Nakajima. S., A. Kitoh, G. Egawa, Y. Natsuaki, S. Nakamizo, C. S. Moniaga, A. Otsuka, T. Honda, S. Hanakawa, W. Amano, Y. Iwakura, S. Nakae, M. Kubo, Y. Miyachi, and K. Kabashima. 2014. IL-17A as an inducer for TH2 immune responses in muring atopic dermatitis models *J. Invest. Dermatol.* 134: 2122-2130.

135. Wang. Y. H., K. S. Voo, B. Liu, C. Y. Chen, B. Uygungil, W. Spoede, J. A. Bernstein, D. P. Huston, Y. J. Liu. 2010. A novel subset of CD4+ TH2 memory/effector cells that produce inflammatory IL-17 cytokine and promote the exacerbation of chronic allergic asthma. *J. Exp. Med.* 207(11): 2479-91.

136. Kasraie, S., and T. Werfel. Role of macrophages in the pathogenesis of atopic dermatitis. 2013. *Mediators. Inflamm.* 2013: 942375.

137. Scott, G. S., R. B. Kean, T. Mikheeva, M. J. Fabis, J. G. Mabley, C. Szabo, and D. C. Hooper. 2004. The therapeutic effects of PJ34 [N-(6-

Oxo-5,6-dihydrophenanthridin-2-yl)-N,N-dimethylacetamide.HCl], a selective inhibitor of Poly (ADP-Ribose) Polymerase, in experimental allergic encephalomyelitis are associated with immunomodulation. *J. Pharmacol. Exp. Ther.* 310: 1053-1061.

138. Gonzalez-Rey, E., R. Martinez-Romero, F. O'Valle, R. Aguilar-Quesada, C. Conde, M. Delgado, and F. J. Oliver. 2007. Therapeutic effect of a poly (ADP-Ribose) polymerase-1 inhibitor on experimental arthritis by downregulating inflammation and Th1 response. *PLoS One.* 10: e1071.

139. El-Hamoly, T., C. Hegedus, P. Lakatos, K. Kovacs, P. Bai, M. A. El-Ghazaly, E. S. El-Denshary, E. Szabo, and L. Virag. 2014. Activaton of poly (ADP-Ribose) polymerase-1 delays wound healing by regulating keratinocyte migration and production of inflammatory mediators. *Mol. Med.* 20: 363-371.

140. Serezani, A. P., G. Bozdogan, S. Sehra, D. Walsh, P. Krishnamurthy, E. A. Sierra Potchanant, G. Nalepa, S. Goenka, M. J. Turner, D. F. Spandau, and M. H. Kaplan. 2016. IL-4 impairs wound healing potential in the skin by repressing fibronectin expression. *J. Allergy. Clin. Immunol.* 2016. Aug 20. pii: S0091-6749(16)30794-1.

CURRICULUM VITAE

Purna Krishnamurthy

Education

2006	**B.Sc.,** Biotechnology Manipal University	Manipal, India
2008	**M.Sc.,** Medical Biotechnology Manipal University	Manipal, India
2017	**Ph.D.** Microbiology & Immunology Indiana University	Indianapolis, IN

Awards

2016 AAI Trainee Abstract Award for an oral presentation at American Association of Immunologist Annual Meeting

Abstracts

2012 Krishnamurthy P, Parashette K, Gupta S, Goenka S. Role of PARP14 in Eosinophilic esophagitis. Autumn Immunology Conference

2015 Krishnamurthy P, Serezani AP, Kaplan MH. The transcriptional co-factor PARP14 limits the severity of allergic skin inflammation. American Association of Immunologist Annual Meeting

2016 Krishnamurthy P, Serezani AP, Kaplan MH. The transcriptional co-factor PARP14 limits the severity of allergic skin inflammation. American Association of Immunologist Annual Meeting

Peer reviewed publications

2014 Krishnamurthy P, Sherrill JD, Parashette K, Goenka S, Rothenberg ME, Gupta S, Kaplan MH. Correlation of increased PARP14 and CCL26 expression in biopsies from children with eosinophilic esophagitis. J*. Allergy. Clin. Immunol.*133(2): 577-80.

2015 Burnett RM, Craven KE, Krishnamurthy P, Goswami CP, Badve S, Crooks P, Mathews WP, Bhat-Nakshatri P, Nakshatri H. Organ-specific adaptive signaling pathway activation in metastatic breast cancer cells. *Oncotarget.* 6(14): 12682-96.

Mehrotra P, Krishnamurthy P, Sun J, Goenka S, Kaplan MH.Poly-ADP-ribosyl polymerase-14 promotes T helper 17 and follicular T helper development. *Immunology.* 146(4): 537-46.

Taghavie-Moghadam PL, Gjurich BN, Jabeen R, Krishnamurthy P, Kaplan MH, Dobrian AD, Nadler JL, Galkina EV. STAT4 deficiency reduces the development of atherosclerosis in mice. *Atherosclerosis*. 243(1): 169-78.

2016 Krishnamurthy P, Kaplan MH. STAT6 and PARP family members in the development of T cell-dependent allergic inflammation. *Immune. Netw.* 16(4): 201-10.

Serezani AP, Bozdogan G, Sehra S, Walsh D, Krishnamurthy P, Sierra Potchanant EA, Nalepa G, Goenka S, Turner MJ, Spandau DF, Kaplan MH. IL-4 impairs wound healing potential in the skin by repressing fibronectin expression. *J. Allergy. Clin. Immunol.*

Sehra S, Krishnamurthy P, Koh B, Zhou HM, Seymour L, Akhtar N, Travers JB, Turner MJ, Kaplan MH. Increased Th2 activity and diminished skin barrier function cooperate in allergic skin inflammation. *Eur. J. Immunol.* 46(11): 2609-2613.

2017 Krishnamurthy P, DaSilva-Arnold S, Turner MJ, Travers JB, Kaplan MH. PARP14 limits severity of allergic skin inflammation. *Submitted*