Activation-induced and damage-induced cell death in aging human T cells

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A R T I C L E   I N F O

A B S T R A C T

In multicellular organisms the proper system functionality is ensured by the balance between cell division, differentiation, senescence and death. This balance is changed during aging. Immunosenescence plays a crucial role in aging and leads to the shrinkage of T cell repertoire and the propensity to apoptosis. The elimination of expanded T cells at the end of immune response is crucial to maintain homeostasis and avoid any uncontrolled inflammation. Resting mature T lymphocytes, when activated *via* their antigen-specific receptor (TCR) and CD28 co-receptor, start to proliferate and then undergo the so called activation induced cell death (AICD), which mechanistically is triggered by the death receptor and leads to apoptosis. T lymphocytes, like other cells, are also exposed to damage, which can trigger the so called damage-induced cell death (DICD). It was hypothesized that oxidative stress and chronic antigenic load increasing with age reduced lymphocyte susceptibility to DICD and enhanced a proinflamatory status leading to increased AICD. However, data collected so far are inconsistent and does not support this assumption. Systematic and comprehensive studies are still needed for conclusive elucidation of the role of AICD and DICD in human immunosenescence, including the role of autophagy and necroptosis in the processes.

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1. Introduction

In multicellular organisms, the proper system functionality is ensured by the balance of cell division, differentiation, senescence and death. This functionality changes during ontogenesis and aging. In the period of organism development, there is a dominance of cell growth and differentiation although cell death and senescence also take place. Programmed cell death, termed apoptosis, has been recognized many years ago as a process involved in morphogenesis (Lockshin and Zakeri, 2001). This is an evolutionarily conserved cell suicide program that is strictly regulated and executed through finely controlled signaling pathways. Recently, also programmed cellular senescence has been documented in mouse embryo (Munoz-Espin et al., 2013; Storer et al., 2013). Cellular senescence denotes a permanent proliferation arrest, which, like apoptosis, is controlled by signaling pathways, but does not result in cell death (Rodier and Campisi, 2011). Both cell fates seem to play the same role in morphogenesis ensuring the clearance of unneeded cells by the immune system.

Postnatal programmed cell death is a vital part of thymocyte maturation. Thymocytes undergo a process of positive and negative selection, which ensures the release to the periphery of properly differentiated non-autoreactive lymphocytes. Apoptosis is also necessary to keep homeostasis of the immune system by lymphocyte elimination. During the so called contraction period, which follows the antigenic load and is a critical element in the defense against autoimmune disease, lymphocytes are eliminated *via* apoptosis (Giovannetti et al., 2008). It was revealed that mice with mutations in *lpr* and *gld* genes encoding Fas (CD95) and Fas ligand (CD95L) proteins, respectively have autoimmune disease. (Takahashi et al., 1994; Watanabe-Fukunaga et al., 1992).

Apoptosis can be both beneficial, in ridding of pre-neoplastic damaged and mutated cells, but also harmful when cell loss exceeds the ability of stem cells to restore and maintain tissue homeostasis (Joaquin and Gollapudi, 2001).

Senescent cells are alive but metabolically altered. They secrete a lot of factors, such as proteases (*e.g.*, metaloproteases-MMP-1, 3, 10), chemokines (growth-related oncogene- GROα, IL-8, monocyte chemoattractant protein-1-MMC-1), cytokines (IL-1α, Il-6, IL-7) and many others (Freund et al., 2010). These factors can influence the tissue surveillance in a way which could promote tissue repair, prevent fibrosis, signal to the innate immune system to clear the senescent cells, but also reinforce senescence and promote cancer

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development and other age-related diseases associated with low grade inflammatory state (Hoare and Narita, 2013). On the other hand, senescence is a barrier to cancer as it stops division of damaged cells (Campisi, 2003). The number of senescent cells increase with age which can result in decreased tissue function, increased low grade inflammation (the so called inflammaging, (Franceschi et al., 2000)) and exhaustion of stem cells. In this way cell senescence can influence or even cause organismal aging (Sikora et al., 2011). Moreover, it is believed that senescence prevents cells from apoptosis and, indeed, resistance to apoptosis was documented for several types of cells *in vitro* (Salminen et al., 2011). Thus, accumulation of senescent cells with age, including senescent stem cells counteracts tissue regeneration.

Replicative senescence refers to the limit of population divisions due to telomere erosion (Hayflick, 2000). Another type of cell senescence is called stress- or-oncogene induced senescence (SIPS and OIS, respectively) and is telomere erosion-independent (Kuilman et al., 2010). Short telomeres, as a hallmark of cellular senescence, have been correlated with age and many age-dependent pathologies (Sikora et al., 2011). Senescent cells identified according to other markers, such as activity of SA-β-galactosidase, DNA damage foci or cell cycle inhibitors, p16 and p21, were shown to be present in various organs of mice and humans and their numbers were documented to increase with age (Jeyapalan and Sedivy, 2008; Wang et al., 2009). Logically thinking, if the number of senescent cells is increasing with age and senescent cells are resistant to apoptosis, thus the level of cell death should decrease with age. However, the results published so far are inconsistent as they report both increased and decreased apoptosis with age. Aberrations in apoptosis are observed in a number of age-related pathologies, such as osteoporosis, retinal degeneration, autoimmunity, ischemia, neurodegeneration, cardiovascular diseases, sarcopenia and the so called segmental aging syndromes for instance Werner's and Bloom syndrome (Warner, 1999, 2007).

Aging is an essential physiological phenomenon characterized by progressive accumulation of deleterious molecular damage in cells, that decreases cell viability to survive and increases the risk of death. There is an ongoing debate whether aging is a simple destruction, namely a stochastic or entropic process, or a continuation of the developmental program or even a quasi-program (Zimniak, 2012). Irrespective of which theory better describes the evolutionary origin of aging, the functionality of an organism is changing with age and the cell propensity to apoptosis and senescence is also a subject of age-dependent changes.

In this review, we will focus on lymphocyte propensity to undergo apoptosis and its relation to cell senescence and the aging process.

2. Cell death of T cells

Apoptosis serves to eliminate severely damaged cells, which acquired lesions mainly in DNA, due to the action of stress inducing factors such as reactive oxygen species, UVB, hyperproliferation and metabolism. This type of cell death can be called damaged-induced cell death (DICD). Immune cells, just like other cells, are exposed to the various insults (*e.g.*, DNA damaging factors, glucocorticoids, reactive oxygen species, cytokine deprivation) which can induce DICD (Ginaldi et al., 2004). However, in the immune system, apoptosis plays a unique role restricted only to this system. Namely, it ensures selection of T-cell repertoire in the thymus, deletion of self-reactive T and B lymphocytes both in the central and peripheral lymphoid compartments, the killing of target cells by cytotoxic T lymphocytes and natural killer cells as well as elimination of T (CD3+) cell clones resulted as a response to antigen (Osborne, 1996).

Precursors of CD3+ cells from the bone marrow enter the thymus, where they undergo negative or positive selection to produce CD4+ and CD8+ mature cells with diverse functions in the peripheral immune system (Palmer, 2003). In the periphery, T cells are resting until they encounter foreign antigens and gain the ability to proliferate, differentiate into effector cells, produce cytokines and eliminate target cells. T-cell activation is induced by signals received through the TCR (T Cell Receptor) activated by the antigen presented by APC (antigen presenting cells) in MHC (major histocompatibility complex) context, and by co-stimulatory molecules, including CD28, adhesion molecules and interleukins (IL) (IL-2, IL-13, IL-15). This clonal expansion phase is followed by the contraction phase in which T cell numbers decline to maintain homeostasis and avoid any uncontrolled inflammation. The majority of activated T cells die by apoptosis and only a few T cells that have been exposed to the antigen remain. These cells develop into memory T cells. The process in which expanded cells are eventually eliminated is called activation-induced cell death (AICD), which mechanistically occurs *via* the so called extrinsic apoptotic signaling pathway (Krueger et al., 2003; Sprent and Tough, 2001).

The extrinsic apoptotic pathway is triggered by signals originating from cell-surface death receptors belonging to the TNF (tumor necrosis factor) receptor (TNFR) superfamily that are activated by several ligands such as CD95L (FasL), TNF or TNF-related apoptosis-inducing ligand (TRAIL). Transduction of the apoptotic signal from the death receptors starts with the formation of a large protein complex at the cell membrane, known as the death inducing signaling complex – DISC. This complex facilitates in the assembly of pro-caspases 8 and 10 and their autoproteolytic activation followed by so called executor caspases activation. Upon stimulation of activated T cells, CD95L mRNA and protein expression are rapidly induced. CD95L is secreted and binds to the CD95 receptor on the same cell or on neighboring cells, and triggers CD95-dependent apoptosis. (Krammer et al., 2007). This signaling pathway should suffice to induce executor caspases and eventual cell death. However, the level of CD95, DISC and active caspase 8 may be too low and the signal requires an additional amplification loop involving the cleavage of a BH3-only BCL-2 (B-cell lymphoma 2) family protein, BID (BH3-interacting domain death agonist), by caspase 8 to form truncated BID (tBID). tBID, in turn, aggregates BAX (BCL-2 associated X protein) or BAK (BCL-2 agonist/killer), which leads to mitochondrial membrane permeabilization, cytochrome c release (Krammer et al., 2007) and activation of the second intrinsic apoptotic signaling pathway.

The intrinsic apoptotic pathway crucially depends on permeabilization of the outer mitochondrial membrane and mitochondria seem to be integrators of many apoptotic signals coming from the outside and inside of the cell. After receiving an apoptotic signal, mitochondria release a variety of molecules, including cytochrome c, which together with the cytoplasmic apoptotic-protease-activating factor 1 (APAF1) forms the apoptosome. In the apoptosome the initiator caspase 9 is activated. Mitochondrial membrane permeability is controlled primarily by a balance between the antagonistic actions of the pro-apoptotic and anti-apoptotic members of the BCL-2 family (Krammer et al., 2007). The intrinsic apoptotic pathway is the mechanism of DICD and can be triggered by DNA damage, thus DNA damage response pathway (DDR) with its key protein, p53 is involved in this type of apoptosis. The p53 protein acts as a transcriptional activator of pro-apoptotic or a suppressor of anti-apoptotic proteins from the BCL-2 family. P53 also directly interacts with the BCL-2 proteins influencing mitochondrial outer membrane permeabilization (Chipuk and Green, 2006).

One of the final events of the apoptotic signaling pathways is activation of specific endonucleases that cleave DNA into oligunucleosomal fragments: Endonuclease G and DFF/CAD. The latter

is activated by effector caspases, which cleave the DFF/CAD (DNA fragmentation factor/a caspase activated DNAse) inhibitory protein (Widlak and Garrard, 2005) and a number of cytoplasmic proteins.

AICD regulation is rather complex and involves both extrinsic and intrinsic mechanisms of apoptosis; it is also tightly connected with survival signaling pathways triggered by TCR and cytokines (Krammer et al., 2007). Studies performed on murine and human T cells suggested several transcription factors to be involved in activation of CD95L expression, such as Ap-1, NFAT, NFκB, c-Myc Erg1&3, and others. Cooperation between some of them is necessary for CD95 gene activation. Also, several tyrosine kinases, such as PKC, Lck ZAP-70 and MAPK, are known to be involved (Green et al., 2003; Hsu et al., 2005). TCR–mediated NFκB (nuclear factor kappa-light-chain-enhancer of activated B cells) signaling is critical for cell survival (death resistance in the phase of T cell activation) because it induces both pro-survival and anti-apoptotic genes (Arnold et al., 2006). It has been postulated that the hematopoetic progenitor kinase HPK1-C mediates sensitivity toward AICD through suppression of NFκB activity (Brenner et al., 2005).

Recently, caspase-independent cell death, namely necroptosis, or programmed necrosis, and autophagy gained attention as the types of cell death activated by death receptors and highly relevant to our understanding how adaptive immune responses are terminated.

For many years apoptosis was considered to be the only form of regulated cell death, whereas necrosis was seen as an unregulated accidental cell death process. Genetic, biochemical and functional evidence, and the discovery of specific chemical inhibitors of necrosis have redefined this process as a molecularly controlled regulated form of cell death, which gained the name necroptosis (Christofferson and Yuan, 2010). Necroptosis, mediated by receptor interacting protein kinase-1 and kinase-3 (RIPK1&3) and the substrate, mixed lineage kinase like (MLKL), is the best-characterized form of regulated necrosis (Pasparakis and Vandenabeele, 2015). The important physiological role of necroptosis was highlighted by a number of genetic studies showing that caspase 8 or Fas-associated protein with death domain (FADD) deficiency cause embryonic mouse lethality and trigger inflammation *in vivo* by sensitizing cells to RIPK3-mediated necroptosis. Despite advances in unraveling the pathways that regulate necroptosis, the precise mechanisms determining whether a cell will die by apoptosis or necroptosis remain poorly understood. However, several studies have suggested that expression levels of RIPK3 and MLKL correlate with sensitivity to necroptosis. Necroptosis has been recently implicated in the regulation of T cell proliferation and survival. Necroptosis regulates antigen-induced proliferation of T cells required for peripheral T cell homeostasis and T cell survival in response to activation stimuli. Mice lacking caspase 8 or expressing a dominantly interfering form of FADD unable to recruit caspase 8 (FADD DED deficient, FADDdd), displayed impaired T cell homeostasis and diminished peripheral T cell numbers. Defective accumulation of activated T cells in the absence of caspase 8 activity is due to the induction of necroptosis. Inhibition of necroptosis rescues the defective T cell proliferation in caspase 8−/− or FADDdd mice indicating that necroptotic signaling in T cells is regulated by caspase 8. Under physiological conditions, caspase 8 promotes survival of activated T cells by suppressing the necroptotic pathway. The interplay of these apoptotic and necroptotic pathways is critical in immune tolerance, as mice doubly deficient in both caspase 8-RIP1 and FADDdd-RIP3 develop lymphoproliferative disease and accumulate autoreactive lymphocytes. Necroptosis has been implicated in the elimination of excessive T cells during the contraction phase of viral infection as viruses possess inhibitors of caspase 8. Thus, necroptosis appears to serve as a backup death pathway for elimination of excess T cells when caspase 8-dependent apoptosis is blocked. Apoptosis and necroptosis must be tightly regulated in order to maintain immune homeostasis. However, this is not the end of the story, as there is also an interplay between necroptosis and autophagy (reviewed in Lu and Walsh, 2012).

Autophagy is a highly conserved cellular recycle and maintenance mechanism that maintains cellular homeostasis by constant turnover of proteins and organelles. Autophagy either helps the cell to survive harsh conditions or directs it to the so called type-II/autophagic cell death. Autophagy plays many different roles in lymphocyte development and function by regulating naïve T lymphocyte homeostasis, specifically by controlling mitochondrial quality and turnover. It is also necessary for the proliferation of mature T cells. Autophagy also acts as a cellular death pathway in lymphocytes, both upon prolonged cytokine withdrawal and during acute antigen-receptor stimulation if improperly regulated (Valdor and Macian, 2012; Walsh and Edinger, 2010).

Interestingly, several groups observed hyper-autophagic phenotypes in FADDdd and caspase-8−/− T cells following antigenic stimulation that was associated with defective T cell survival and clonal expansion (Bell et al., 2008; Ch'en et al., 2008). This might suggest that necroptosis promotes autophagy. However, the role of autophagy in AICD and DICD of lymphocytes is still poorly recognized. To our knowledge there are no data concerning the level of necroptosis in aging lymphocytes and only one paper showing differences between autophagy of CD8+CD28+ and CD8+CD28− lymphocytes (Arnold et al., 2014), which is discussed below. Nonetheless, we can expect that the nearest future will bring new results revealing the importance of these processes in shaping the immune system during aging.

Some years ago Ginaldi and co-workers (De Martinis et al., 2007; Ginaldi et al., 2004, 2005) proposed that immunosenescence can be elicited by subtle remodeling of AICD and DICD, which contributes to the phenotypic and functional characteristic of the elderly immune system. It was hypothesized that both oxidative stress and chronic antigenic load decreased lymphocyte susceptibility to DICD and elicited a proinflamatory status leading to increased AICD. Indeed, antigenic stimulation and oxidative stress are correlated processes that increase with aging and underlie a persistent inflammatory status, "inflammageing". Accumulation of reactive oxygen species (ROS) leads to DNA and protein damage. As a result of a lifelong exposure to oxidative stress, cells of aged individuals develop a sort of oxidative stress adaptation and become less prone to DICD. In a consequence, senescent cells persist and accumulate, contributing to decreased stress responsiveness, increased resistance to spontaneous apoptosis, and accumulation of memory lymphocytes, which fill the immunological space making immune responses less efficient. Chronic immune stimulation (through hyperproduction of proinflammatory cytokines), increases production of activated cells and overexpression of death receptors on lymphocytes, all of which induce AICD upregulation. Because AICD intervenes in the down-modulation of clonal expansion following antigenic stimulation, the increased susceptibility of lymphocytes to AICD results in decreased immune responsiveness, less efficient clonal proliferation and impaired memory cell generation and survival (De Martinis et al., 2007).

We have done a search of the literature to find out evidence that could support the hypothesis that the extent of AICD and DICD of immune cells is changing with age. Below are presented published results which are both in favor and against the postulated decrease in AICD and increase in DICD with age. The data are summarized in Table 1.

3. Activation-induced cell death in aging T cells

The aging of the immune system, termed immunosenescence, contributes to the morbidity and mortality of elderly people. The

Table 1
Data directly and indirectly showing changes of AICD and DICD which occur in human lymphocytes during ageing/senescence.

Data showing **increased AICD** in ageing	References
The number of CD95-positive cells increases with age	Brzezinska et al. (2003), Fagnoni et al. (2000), Potestio et al. (1999)
Increased expression of CD95 mRNA in the elderly, in comparison with young subjects	Aggarwal and Gupta (1998)
Correlation between increased CD95 expression and increased AICD in T cells from elderly subjects	Lechner et al. (1996), Phelouzat et al. (1997), Potestio et al. (1998)
A slight but statistically significant increase of AICD in PBMCs from elderly in comparison to young individuals	Schindowski et al. (2000)
Increased susceptibility to AICD in a late-passage in comparison to an early-passage cultured CD4+ T cell clone	Pawelec et al. (1996)
Increased apoptosis of CD4+ and CD8+ T cells induced by CD95 in old subjects in comparison with young ones	Aggarwal and Gupta (1998)
TNF-α-induced apoptosis of CD4+ and CD8+ T cells increased in old subjects in comparison with young	Aggarwal et al. (1999)

Data showing **unchanged AICD** in ageing	References
No differences in the level of caspase3 between CD8+CD28- and CD8+CD28− cells	Brzezinska et al. (2004)
The same level of Fas-induced apoptosis in young, middle aged and centenarians	Pinti et al. (2004)
No differences in AICD of the total CD3+ cell population from young and old donors.	Herndon et al. (1997)

Data showing **decreased AICD** in ageing	References
AICD level analyzed in CD4+ naïve (CD62L+CD95-) and memory (CD62L-CD95+) cells, did not correlate with age	Donnini et al. (2005)
CD8+CD28− cells activated with a superantigen were less susceptible to apoptosis than their CD8+CD28+ counterparts	Posnett et al. (1999)
T cells reactivated after achieving in vitro the state of replicative senescence acquired resistance do apoptosis induced with different stimuli, including antiCD3 and antiFas	Spaulding et al. (1999)
CD4+CD28− cells, unlike their CD28+ counterparts, were protected from AICD due to high expression of cFLIP	Vallejo et al. (2000)
Within a superantigen-activated T cell population, cells sensitive to Fas ligation were characterized by low CD28 expression (prior to treatment with Fas)	Walker et al. 1998
T cells undergoing AICD, when co-stimulated by CD28, showed, beside strong up-regulation of BCL-X$_L$, down-regulation of CD95L mRNA and strong up-regulation of cFLIPs	Kirchhoff et al. 2000
Low CD28 expression predisposed activated T cells to CD95L mediated apoptosis and CD28 ligation protected from apoptosis.	Dennett et al. 2002
Decreased ability of CD8+CD28−, in comparison to CD8+CD28+ cells, to induce autophagy as a survival mechanism following TCR-activation	Arnold et al. 2014
Age-related accumulation of CD28−CD95+ T cells was associated with a resistance to AICD	Hsu et al. 2005

Data showing **decreased DICD** in ageing	References
Lower propensity of PBMC to undergo oxidative stress-induced apoptosis in the elderly and centenarians in comparison to young subjects	Monti et al. 2000
Lower sensitivity of human in vitro senescing non-proliferating lymphocytes (CD8+CD28−) than young proliferating ones (CD8+CD28+) to UV exposure	Radziszewska et al. 1999

Data showing **unchanged DICD** in ageing	References
H_2O_2-induced cell death of lymphocytes was dose-dependent but not age-dependent	Brunner et al. 2012

Data showing **increased DICD** in ageing	References
Increased susceptibility to etoposide-induced apoptosis of CD8+CD28− in comparison with CD8+CD28+ cells	Behrens et al. (2011)
Higher level of H_2O_2-induced cell death in lymphocytes from patient with AD than those with mild dementia and healthy donors	Behrens et al. (2012), Ponce et al. (2014)

main aspects of immunosenescence are: (i) involution of the thymus and exhaustion of naïve T cells; (ii) diminution of the T cell repertoire and accumulation of oligoclonal expansions (megaclones) of memory/effector cells; and (iii) a chronic inflammatory state called inflammaging (Capri et al., 2006).

Alterations in the process of apoptosis in aging T cells have been reported, however data are inconsistent. Generally, the literature guides us to three possibilities concerning the level of AICD in the elderly. The first is that T cell susceptibility to AICD is increased.

The main indicator of the cell ability to undergo AICD is the expression of the CD95 receptor. While T cells from the cord blood are CD95-negative, the proportion of CD95-positive cells is growing with age (Fagnoni et al., 2000; Potestio et al., 1999). Also increased expression of CD95 mRNA in the elderly, in comparison with young subjects, was reported (Aggarwal and Gupta, 1998). Fagnoni et al. (2000) postulated that the CD95-negative cells that disappeared with age were unprimed, naïve cells. We also found a dramatic decrease in the percentage of CD95-negative cells with age, from virtually 100% in the cord blood to an almost undetectable level in peripheral blood of centenarians (Brzezinska et al., 2003). However, PBMC cultures derived from young and old individuals alike show an increase in CD95 expression upon activation, the cells

from old individuals reaching even higher CD95 levels than those from young individuals (McLeod et al., 1998; Phelouzat et al., 1997; Potestio et al., 1998). Therefore, T cells from the elderly are able to respond to activation with CD95 engagement. Increased expression of CD95 mRNA in the elderly, in comparison with young subjects, has also been reported (Aggarwal and Gupta, 1998) Under long-term cultivation, namely during replicative senescence in vitro, an increase in AICD rate was observed and it was more pronounced in T cell populations from old than from young individuals. A more pronounced depletion of T cells upon stimulation in old than in young PBMC was shown to be due to apoptosis (Potestio et al., 1998). Others (Schindowski et al., 2000) described a slight but statistically significant increase of AICD in elderly in comparison to young individuals. Pawelec et al. (1996) reported on increased susceptibility to AICD in a late-passage in comparison to an early-passage CD4+ cultured T cell clone. Greater CD95-induced apoptosis was found in anti-CD3 stimulated CD4+ than in CD8+ cells derived from healthy donors, and both CD4+ and CD8+ T cells from the elderly were more sensitive than those from young individuals (Aggarwal and Gupta, 1998). Similarly, CD4+ and CD8+ lymphocytes from the elderly were more sensitive to TNF-α-induced AICD than cells of young people (Aggarwal et al., 1999).

On the other hand, the literature also shows evidence in favor of other possibilities, namely that T-cell susceptibility to AICD is unchanged or even decreased with age. The level of AICD was the same irrespective of the age of donors (young, middle-aged, centenarians) (Pinti et al., 2004). This is in agreement with our results showing no differences in AICD levels in T lymphocytes derived from young in comparison to old donors (Brzezinska et al., 2004). The results obtained by others indicated no differences in AICD of the total CD3+ cell population in young and old donors. (Herndon et al., 1997). Also, Donnini et al. clearly showed that the AICD level analyzed in CD4+ cell subsets, namely naïve (CD62L+CD95−) and memory (CD62L-CD95+), did not correlate with age (Donnini et al., 2005).

The recognition of MHC-bound antigen by TCR is a low-affinity interaction unable to sustain activation of T cells; productive activation requires co-stimulation with CD28 which serves as an amplifier of the TCR signal (Vallejo, 2005). By activating Akt CD28 acts as a typical transducer of the pro-survival pathway (Hsu et al., 2005).

It is known that T-cell activation leads to CD28 down-regulation. Indeed, various models of T-cell replicative senescence show that subsequent rounds of cell divisions eventually lead to accumulation of CD28-negative cells, which are the progeny of CD28-positive ones (Brzezinska, 2005; Effros et al., 2005; Posnett et al., 1999). We showed a gradual replacement of CD8+CD28+ cells by CD28− cells in long-term cultures of T cells derived from both the cord blood and the peripheral blood of donors of different age, including centenarians (Brzezinska, 2005). It was also shown that purified human CD28+ T cells progressively lose CD28 during each successive stimulation, with CD8+ T cells losing CD28 more rapidly than CD4+ cells (Vallejo, 2005). Also, in vivo, the accumulation with age of CD8+CD28− and, to a lesser extent CD4+CD28−, is observed (Brzezinska, 2005; Effros et al., 2005; Fagnoni et al., 2000; Hsu et al., 2005; Vallejo, 2005; Wikby et al., 2005). CD28-negative cells are highly oligoclonal and have very short telomeres (Vallejo, 2005). It is believed that they are unable to proliferate, we found however, that this is only true in the case of cells undergoing replicative senescence in vitro, but not for those aging in vivo (Brzezinska, 2005).

As they accumulate progressively through life and large clones persist for years, CD28-negative cells are considered to be resistant to AICD. Indeed, it was demonstrated that CD8+CD28− cells activated with a superantigen were less susceptible to apoptosis than their CD8+CD28+ counterparts (Posnett et al., 1999). Others showed that T cells reactivated after achieving in vitro the state of replicative senescence acquired resistance to apoptosis induced with different stimuli, including antiCD3 and antiFas (Spaulding et al., 1999). Hsu et al. (2005) documented that age-related accumulation of CD28-CD95+ T cells was associated with resistance to AICD.

Many of the CD8+ and CD28− expanded clones seem to result from previous infections by persistent viruses, especially CMV and to a lesser extent, EBV (Epstein–Barr Virus) and other herpesviruses (Vasto et al., 2007). Thus it seems that accumulation of long-living CD8+CD28− cells with age can actually be explained by their relative resistance to AICD. Also CD4+CD28− cells, unlike their CD28+ counterparts, were shown to be protected from AICD due to high expression of cFLIP (Vallejo et al., 2000).

On the other hand, data showing quite opposite correlation between CD28 and AICD cannot be neglected. We showed no differences between CD28+ and CD28− in susceptibility to undergo AICD (Brzezinska et al., 2004), but there are results providing evidence that maintenance of CD28 expression on T cells may be crucial for prevention of Fas-mediated apoptosis during the course of antigen engagement. Indeed, it was documented that within a superantigen-activated T cell population, cells which were sensitive to Fas ligation were characterized by low CD28 expression prior to treatment with Fas (Walker et al., 1998). It was also shown that CD28-mediated signaling increased the expression of anti-apoptotic BCL-X$_L$ and thereby promoted cell survival implying anti-apoptotic activity of CD28. Indeed, Krammer's group reported that activated T cells and cultured in the presence of IL-2 (undergoing AICD), when co-stimulated by CD28 showed, beside strong up-regulation of BCL-X$_L$, down-regulation of CD95L mRNA and strong up-regulation of cFLIPs (Kirchhoff et al., 2000). In agreement with these results are data presented by others showing that low CD28 expression predisposed activated T cells to CD95L mediated apoptosis and that CD28 ligation protected from apoptosis (Dennett et al., 2002).

This apparent controversy probably stems from different experimental approaches and highly fragmentary data, especially those concerning human studies. Systematic and comprehensive studies are still needed for conclusive elucidation of the role of AICD in human aging.

Recently, induction of autophagy has been shown in human CD8+ T cells following TCR engagement (Arnold et al., 2014). Moreover, a decreased ability of CD8+CD28−, in comparison to CD8+CD28+ cells, to induce autophagy was evidenced. This suggests that the former cells cannot meet the metabolic needs of antigen receptor-mediated activation and are therefore, unlikely to survive when confronted by specific antigens (Arnold et al., 2014).

4. Damage-induced cell death in aging lymphocytes

Severe damage to vital cellular ingredients directly induces intrinsic (mitochondrial) or, indirectly, autophagic cell death. The cells of our bodies are constantly exposed to different kinds of insults from which oxidative stress is the most common. Oxidative stress denotes imbalance between prooxidants and antioxidants. Reactive oxygen species (ROS) produced by mitochondria during respiration as well as many extrinsic agents, such as toxins and irradiations, are the main culprit of oxidative stress. ROS-induced damage affects lipids, proteins and DNA and results in the deterioration of intracellular organelles.

According to stochastic theories, aging is caused by gradual accumulation of damage, which leads to cell and tissue deterioration and altered functionality (Kirkwood, 2008). DNA damage-induced cellular senescence has got a very strong support from experimental results (d'Adda di Fagagna, 2008). During the lifespan the antigenic load, like other stressors, leads to an increase in oxidative stress, cell senescence and inflammaging (Franceschi et al., 2000). Lymphocytes, similarly to other cells exposed to oxidative damage are equipped with defense mechanisms including the apoptotic response. DICD in aging lymphocytes was postulated to be not as effective as in young cells (Ginaldi et al., 2005). Indeed, Monti et al. (2000) showed lower propensity of PBMC to undergo oxidative stress-induced apoptosis in the case of the elderly and centenarians in comparison with young subjects. We showed that spelocytes from old rats were resistant to UV-induced apoptosis in comparison to young animals (Radziszewska et al., 2000). Similarly, human in vitro senescing non-proliferating lymphocytes (CD8+CD28−) were less sensitive to UV exposure than young proliferating ones (CD8+CD28+) (Radziszewska et al., 1999). However, in the presence of low concentration of etoposide, which is a topoisomerase II inhibitor, it appeared that in vitro senescing lymphocytes were much more susceptible to apoptosis than young ones (Z. Korwek, personal communication).

Considering the fact that senescent CD3+ do not express the CD28 co-receptor, it can be assumed that CD28-negative cells are more prone to DNA damage-induced apoptosis which is in contrast to Ginaldi's hypothesis. The so far published data concerning

damage (including DNA damage)-induced cell death are very scarce. However, a recently published paper reports on the increased susceptibility to etoposide-induced apoptosis of CD8+CD28− in comparison with CD8+CD28+ cells (Brunner et al., 2012). The postulated reason is decreased DNA damage response in CD28-neagative cells, associated with upregulation of miR-24. As it was stated before there is accumulation of CD8+CD28− cells with age, thus it can be assumed that there must be a factor which favors CD8+CD28− cell survival. Such a role can be attributed to Interleukin-15 (Brunner et al., 2012). Another work was performed on PBMCs isolated from healthy donors of different age. When treated with H_2O_2, the survival of lymphocytes was dose-dependent but not age-dependent. Interestingly, H_2O_2-treatment induced high levels of necrosis in cells from young donors (≤30 years), which progressively was substituted by apoptosis (Behrens et al., 2011). The same authors have recently published results showing that the level of H_2O_2-induced cell death is higher in lymphocytes of patient with AD than those with mild dementia and healthy donors. For this differences apoptotic death was responsible (Behrens et al., 2012; Ponce et al., 2014).

Altogether, some data published so far indicate AICD changes in the elderly. Nonetheless, these results are inconclusive, some showing an increased, some a decreased, propensity of T cells to AICD in the elderly. There are also reports of unchanged AICD. Immune cells, just like other cells, are continuously exposed to damage, including DNA lesions. The data showing T cells propensity to undergo DICD with age are very scarce and also inconclusive. This apparent controversy probably stems from different experimental approaches and highly fragmentary data, especially those concerning human studies. Some data come from in vitro senescence studies while other emerge from ex vivo analysis. Although we believe that in vitro senescence reflects in vivo aging this assumption is not fully justified as the microenvironment plays a critical role in cell death and survival. This has been shown at least in the context of T cell propensity to undergo proliferation (Brzezinska, 2005). Moreover, it seems that, in addition to apoptosis, autophagy and programmed necrosis can play significant roles in termination of the T-cell-dependent immune response. Ginaldi's hypothesis is very attractive, it is not however supported by consistent results. Systematic and comprehensive studies are still needed for conclusive elucidation of the role of AICD and DICD in human immunosenescence. Moreover, in the light of recent discoveries concerning autophagy and necroptosis the studies of their involvement in T cell death in the elderly are urgently needed.

5. Conclusions

Immunosenscence is believed to be driven by thymus involution, continuous pathogen load and common damaging insults. All these phenomena lead to the shrinkage of T cell repertoire due to reduction in the number of naïve cells and accumulation of oligoclonal CD8+ and, to a lesser extent, CD4+ cells which are CD28−. Although so far collected data do not support the lesser propensity of CD28- T cells to undergo either apoptotic, necroptotic or autophagic cell death upon different stressors their prevalence in aging organisms is unquestionable. Moreover, these cells dominate not only in elderly people, especially in those who are also infected with cytomegalovirus (CMV) (Pawelec, 2014), but their presence has also been linked to enhanced progression of age-related diseases such as Alzheimer's disease (Lurain et al., 2013), atherosclerosis (Dumitriu et al., 2009), or decreased efficacy of vaccination (Chen et al., 2009) observed in the elderly. Moreover, high numbers of CD8+ CD28− cells are found in autoimmune diseases, such as rheumatoid arthritis (Dejaco et al., 2010), and ankylosing spondylitis (Schirmer et al., 2002). Significantly higher counts of

CD8+ and CD8+ CD28− T cells were found in frail compared to non-frail older women matched by age and major comorbidities (cancer, arthritis, diabetes, cardiovascular disease, hypertension, and hormone replacement therapy) (Semba et al., 2005). While no difference was observed in CD4+ T cell frequencies between the two study groups, the frail group had significantly lower CD4+:CD8+ ratio compared to the non-frail group (Semba et al., 2005). Frailty is a geriatric syndrome in which ageing disorders are accelerated and homeostasis mechanisms start failing. It is characterized by multisystem dysregulation manifested by maladaptive response to stressors leading to a vicious cycle toward functional decline and other serious adverse health outcomes (Li et al., 2011).

CD8+CD28− cells display many markers of senescence characteristic also for other cells, such as impaired propensity to proliferate, increased expression of cyclin-dependent inhibitor, p16, telomere erosion and secretory phenotype (SASP) (Pallis et al., 2014). Latter conditions generates a low grade inflammatory state. Although it arises from the overproduction of proinflammatory cytokines, such as IL-6, tumor necrosis factor alpha, and IL-1, mainly by the innate system, there are some data showing a different profile of produced cytokines by CD8+CD28− in comparison with CD8+CD28+ cells (Zanni et al., 2003). Low grade inflammation state is associated with many age-related disorders, such as diabetes II, cardiovascular disorders, obesity and cancer (Sikora, 2013). Thus, paradoxically, even if senescent cells are considered as a barrier to cancer due to their disability to proliferate, they can create a milieu that promotes cancer development. Indeed, with aging, the prevalence and incidence of cancer increase while at the same time immunity is compromised (Pawelec et al., 2014). Interestingly, the tumor mass is not only an immune suppressive environment, but is also a source of a large amount of antigens that chronically stimulate T cells that infiltrate it leading to AICD (Lu and Finn, 2008). Indeed, it was documented for example that chronic presence of lung tumors induces dysfunctions in CD8+ cells and sensitizes them to AICD (Prado-Garcia et al., 2012).

Altogether, accumulation of CD8+CD28− cells with age can suggest that, even if they are not particularly resistant to cell death in a given experimental setting, their survival in organism can be assured by a microenvironment, which the experimental approach never fully reflects.

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