

The aging of the adaptive immune system

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Abstract

Adaptive immune responses are severely affected by the aging process as reflected by an increased morbidity and mortality from infectious diseases and a low efficacy of vaccination in elderly persons. Age-related changes within the bone marrow and thymus lead to an impaired generation of new T and B cells severely compromising the maintenance of a diverse and balanced T and B cell repertoire in old age. The maintenance of a balanced T cell repertoire is further challenged by latent persistent infections, such as Cytomegalovirus. Understanding the mechanisms of age-related alterations of the adaptive immune response may help to facilitate the development of more efficient vaccines for elderly persons and to envisage strategies to overcome immunosenescence.

Introduction

The increase in life expectancy will lead to a dramatic change in the population's age structure, with about 35% of the European population being older than 60 years of age in 2050 [1]. This will pose an enormous medical and socioeconomic burden on our future society because the aging process is accompanied by a decline of numerous physiological functions, including the immune system [2]. As a consequence infectious diseases, such as lower respiratory tract infections, urinary tract infections, skin and soft tissue infections, tuberculosis and herpes zoster, have a higher prevalence and are more severe in elderly persons [3]. In developed countries such as the United States, pneumonia, influenza and septicemia are ranked among the ten major causes of deaths in persons aged 65 years and older [4]. Furthermore, the development and progression of age-related diseases, such as certain cancers, atherosclerosis, diabetes, dementia, osteoporosis and rheumatoid arthritis have been associated with altered immune function in old age [5, 6]. Another consequence of the aging of the immune system is a low efficacy of vaccination in elderly persons [7, 8]. Longitudinal studies have also identified an immune risk phenotype predicting higher 2-year mortality in very old Swedish individuals [9, 10]. The immune risk phenotype includes a cluster of parameters such as CD4/CD8 ratio, increased frequency of highly differentiated $CD8^+CD28^-$ T cells and latent infection with Cytomegalovirus (CMV).

One important point regarding human studies is that age-related changes within the adaptive immune system have almost exclusively been documented for cells derived from peripheral blood, which only represent a small fraction of all lymphocytes in the body. Only limited data are available whether and how human aging affects the most abundant lymphocyte population which resides in gut-associated lymphoid tissue [11]. The bone marrow has also recently been attributed a key role in the homing and maintenance of potent memory T cells but the effect of aging has not been investigated [12]. It would thus be of great relevance to gain more information whether and how human aging affects the frequency, phenotype and function of lymphocyte subsets in different organs and which environmental factors play a role during aging.

This review aims to illustrate current knowledge of age-related changes within the adaptive immune system on a cellular and molecular level. Understanding the mechanisms of age-related alterations of the adaptive immune response may consequently help to facilitate the

development of more efficient vaccines for elderly persons and to envisage strategies to prevent or even reverse immunosenescence thereby improving the quality of life in old age.

Age-related alterations of the naive T cell compartment

The cells of the adaptive immune system originate from pluripotent hematopoietic stem cells (HSC), which are maintained in specialized niches in the bone marrow. However, deficiencies in DNA damage repair as well as a decreased capacity to generate committed lymphoid progenitors limit the function of HSC with age [13, 14]. It has been suggested that age-related changes of the stem cell niche may contribute to the decline of HSC function in old age [15]. A hallmark of immunosenescence is the age-related involution of the thymus, severely affecting the CD4⁺ and CD8⁺ T cell pool in old age. Beginning as young as one year of age, thymic tissue undergoes a continuous decline while adipose tissue increases during aging, with only about 10% residual functional thymic tissue at the age of 50 [16]. Consequently, thymic involution leads to a decreased output of antigen-inexperienced, naive T cells and to a dramatic shortage (up to -80%) of naive T cells in peripheral blood and lymph nodes of elderly persons [17, 18].

Studies in mice have demonstrated that naive CD4⁺ T cells from old mice exhibit defects related to T cell receptor (TCR)-mediated proliferation, interleukin (IL)-2 production and generation of long-term protective memory cells [19, 20]. Especially, the TCR-dependent recruitment of signal transduction complexes to the immunological synapse is impaired in CD4⁺ T cells from old mice [21, 22]. Age-related changes in lymphocyte membrane viscosity due to an altered cholesterol/phospholipid ratio may explain impaired immunological synapse formation [23]. Additionally, O-glycosylated forms of T cell surface molecules have been identified to contribute to age-related defects in TCR signaling, as O-sialoglycoprotein endopeptidase overcomes age-related defects in T cell activation [24]. Age-related defects in CD4⁺ T cell cognate helper function therefore lead to reduced humoral responses [25]. Using an adoptive transfer model and TCR transgenic mice, Haynes et al. [26] demonstrated that newly generated naive CD4⁺ T cells in aged animals do not exhibit age-related defects in response to antigen. Additionally, humoral responses were not impaired when naive CD4⁺ T cells from young mice were transferred into old hosts [25]. In this model, the microenvironment of an old host does not seem to play a major role, although long-term effects of an aged microenvironment on naive CD4⁺ T cells were not investigated.

But which factors may be responsible for the decreased function of naive CD4⁺ T cells in old age? Despite thymic involution, the number of naive CD4⁺ T cells declines slowly during aging [27]. Homeostatic proliferation has been identified to play a key role in the maintenance of peripheral naive T cell numbers [28]. However, homeostatic proliferation increases exponentially when naive T cell numbers drop below 4% of total T cells, thereby accelerating telomere shortening [28, 29]. Another mechanism to maintain peripheral naive T cell numbers is decreased apoptosis, leading to an increased lifespan of naive T cells in old age. An increased post-thymic lifespan of murine naive CD4⁺ T cells has recently been demonstrated, and seems to be independent of IL-7- or TCR-mediated signaling but mediated by a decreased expression of the pro-apoptotic molecule Bim [30, 31]. However, an increased post-thymic lifespan also prolongs the exposure time of naive CD4⁺ T cells to detrimental environmental factors which then leads to an increased accumulation of DNA damage, thereby contributing to the decreased function of naive CD4⁺ T cells in old age [32]. But mechanisms to maintain naive T cell numbers during aging seem to be limited, because a rapid loss of naive CD4⁺ T cells can be observed after the age of 75 [27, 33].

In humans, two distinct naive CD4⁺ T cell subsets have been described: CD31⁺ thymic-naive and CD31⁻ central-naive CD4⁺ T cells [34, 35]. CD31⁺ naive CD4⁺ T cells decline during aging, but still display a polyclonal TCR repertoire. In contrast, CD31⁻ naive CD4⁺ T cell numbers remain constant during aging but they exhibit increased TCR-mediated signaling and display a dramatically restricted TCR repertoire [34]. Evidence of premature immune aging has also been demonstrated for persons thymectomized during early childhood due to open heart surgery to correct congenital heart defects [36, 37]. About two decades after thymectomy, patients had decreased numbers of naive CD4⁺ and CD8⁺ T cells compared to healthy age-matched controls. Moreover, naive CD4⁺ T cells had lower numbers of TCR rearrangement excision circles (TREC) and a higher expression of the proliferation marker Ki-67 [36], indicating increased homeostatic proliferation. Of clinical relevance, thymectomized patients had lower tick-borne encephalitis virus (TBEV)-specific IgG antibody titers after TBEV vaccination compared to healthy controls [38], demonstrating the importance of the thymus in maintaining the integrity of adaptive immunity throughout life.

Thymic output and homeostatic proliferation are not the only determinants of peripheral naive T cell numbers. Latent infection with CMV, but not Epstein-Barr virus (EBV) or Varicella-

Zoster virus (VZV), has been shown to accelerate the age-related decline of naive CD4⁺ and CD8⁺ T cells and to facilitate the accumulation of highly differentiated CMV-specific effector-memory T cells [39-41]. In addition, thymectomized patients with latent CMV infection had lower naive T cell counts compared to CMV-seronegative patients [37]. In conclusion, the age-related decline of thymic output is likely to induce homeostatic proliferation and to prolong the post-thymic lifespan of naive CD4⁺ T cells to compensate the lack of new T cell production. Consequently, the naive CD4⁺ T cell compartment is dramatically restricted in diversity and functionality in old age, which is likely to diminish the capacity of the elderly to respond to novel pathogens. Latent infection with CMV even accelerates the loss of naive T cells and leads to the accumulation of highly differentiated effector-memory T cells.

In contrast to naive CD4⁺ T cells, the number of human naive CD8⁺ T cells in peripheral blood and lymph nodes declines more rapidly during aging [17, 18, 40]. Human naive CD8⁺ T cells have long been defined by the expression of the two surface molecules CD45RA and CD28. However, recent studies have demonstrated that the CD45RA⁺CD28⁺ CD8⁺ T cell population is heterogeneous in elderly persons and exhibits several phenotypic and functional alterations [42-44]. For example, CD45RA⁺CD28⁺ CD8⁺ T cells from elderly persons display decreased surface expression of CD62L and IL-6R α but up-regulate the senescence-associated molecule CD57 (Fig. 1A). CD45RA⁺CD28⁺ CD8⁺ T cells from elderly persons also produce larger amounts of the pro-inflammatory cytokine interferon (IFN)- γ after stimulation with OKT3 and IL-2 [45]. However, TCR-mediated *in vitro* proliferation of CD45RA⁺CD28⁺ CD8⁺ T cells does not seem to be impaired during aging [42]. When naive CD8⁺ T cells from elderly persons, characterized by a CD45RA⁺CD28⁺CD62L⁺ phenotype, were compared with young persons, they displayed a highly restricted TCR repertoire and shorter telomeres, suggesting increased homeostatic proliferation (Fig. 1B, C). Clonal expansions and loss of TCR diversity in the naive CD8⁺ T cell compartment have also been demonstrated in aged mice [46]. Altogether, these results indicate that healthy aging decreases the frequency and function of human naive CD8⁺ T cells, and latent infection with CMV further accelerates the loss of naive CD8⁺ T cells. Of clinical relevance, the decline in T cell diversity can lead to an impaired heterosubtypic immune response to influenza virus, as demonstrated in old as well as young thymectomized mice [47]. This has important implications for the design of vaccines for the elderly.

The phenotypic characterization of human naive CD8⁺ T cells in elderly persons may, however, be complicated due to the proposed effects of homeostatic proliferation and the reexpression of CD45RA and CCR7 on memory T cells [48-50] (Fig. 2). In young adults, naive T cells have a doubling time of about 200 days and are maintained by IL-7 and TCR signaling via contact with self-peptide/MHC, which sustain the expression of anti-apoptotic molecules [51, 52]. In a lymphopenic environment, as demonstrated in recombination activating gene (RAG)-1^{-/-} mice, homeostatic proliferation of naïve T cells is enhanced [53, 54], which leads to a transient acquisition of a memory-like phenotype, characterized by the upregulation of CD44, IL-2R β and leukocyte functional antigen (LFA)-1, and to a rapid production of IFN- γ upon stimulation [53-55]. Cytokine-mediated signaling may also influence phenotype and function of post-thymic naive CD8⁺ T cells. For example, a IL-7R α ^{low} CD8⁺CD45RA⁺CD27⁺ T cell population with characteristic naive properties has been shown to accumulate in healthy elderly persons [44]. As common γ chain cytokines induce rapid down-regulation of IL-7R α , this new subset of naive T cells may encompass cells that have recently received homeostatic signals. However, there are only few studies available analyzing the effect of aging on intrinsic and extrinsic death pathways of naive T cells. Recently, it has been demonstrated, that naive CD4⁺ T cells from old mice have an increased post-thymic lifespan due to a decreased expression of Bim, decreased spontaneous apoptosis and decreased growth factor withdrawal-mediated apoptosis [31]. In contrast, naive CD8⁺ T cells from elderly persons seem to be more susceptible to death receptor-mediated apoptosis, triggered by tumor necrosis factor (TNF)- α or Fas, compared to naive CD8⁺ T cells from young persons [56, 57]. Differences in TNF- α -mediated apoptosis did not correlate with surface expression of TNFR1 or TNFR2, indicating age-related differences in TNFR downstream signaling, such as decreased NF- κ B activation [58].

In conclusion, the age-related involution of the thymus diminishes peripheral T cell renewal, decreases T cell diversity and thus reduces the capacity of elderly persons to mount efficient T cell responses to a variety of new pathogens and to efficiently respond to vaccinations with neoantigens, for example annual influenza virus vaccination.

Effect of aging on the generation, maintenance and exhaustion of antigen-experienced T cells

The ability to generate and maintain memory T cells after infection or vaccination is a hallmark of the adaptive immune response and ensures protection upon recurrent infection. Upon recognition of its specific antigen, the differentiation of a naive T cell into a memory T cell is determined by a multitude of parameters, such as signal strength, costimulation and cytokine milieu. After elimination of the pathogen, maintenance of the memory T cell pool depends on survival signals provided by IL-7 and/or IL-15. In old mice, the generation of functional CD8⁺ T cell memory is impaired [59]. Similarly, memory CD4⁺ T cells generated from naive CD4⁺ T cells from old mice proliferate less, produce reduced levels of cytokines (IL-2, IL-4, IL-5 but not IFN- γ), and exhibit reduced cognate helper function, compared to memory cells generated by using naive CD4⁺ T cells from young mice [60]. These results indicate that intrinsic defects of aged naive T cells are responsible for the generation of memory T cell populations that show a diminished recall response upon reencounter of the antigen.

In humans, three major classes of memory T cells can be distinguished based on their phenotypic and functional characteristics: central-memory T cells (T_{CM}) with a CD45RO⁺CD28⁺CD62L⁺CCR7⁺ phenotype, effector-memory T cells (T_{EM}) with a CD45RO⁺CD28⁺CD62L⁻CCR7⁻ phenotype and highly differentiated effector T cells (T_{EFF}) with a CD45RO^{+/+}CD28⁻CD62L⁻CCR7⁻ phenotype [61, 62]. T_{CM} can home to the lymph node, possess a diverse TCR repertoire and a high proliferative potential. T_{EM} can migrate to non-lymphoid tissues and exert immediate effector function. T_{EFF} are highly differentiated cells that display a highly restricted TCR repertoire, have short telomeres and an impaired TCR-mediated response but express high levels of cytotoxic molecules [63, 64]. During healthy aging, T_{EM} and T_{EFF} accumulate [39, 40, 65].

The age-related exhaustion of the memory T cell pool, defined by the increase of highly differentiated CD8⁺CD28⁻ T cells, can be explained by three major mechanisms. Firstly, repeated TCR-mediated stimulation of naive or memory T cells, caused for instance by chronic infections or autoimmune diseases, leads to the loss of costimulatory molecules (CD28 and CD27) and cytokine receptors (IL-6R α and IL-7R α), and causes telomere erosion [66, 67]. (Fig. 2). Repeated TCR-mediated stimulation also explains loss of diversity due to clonal expansions within the memory T cell pool [68]. One of the most potent triggers of premature aging of the T cell pool is chronic infection with HIV, which can lead to a massive accumulation of exhausted CD8⁺CD28⁻ T cells already very early in life [62, 69]. Secondly, increased resistance to death

receptor-mediated apoptosis has been demonstrated in T_{EFF} , highlighting another mechanism for the increased persistence of T_{EFF} [58, 70]. Thirdly, long-term homeostatic proliferation of T_{CM} , mediated via IL-15 signaling, can down-regulate the expression of CD28, consequently decreasing CD28-mediated phosphorylation of Akt at Ser473, and leading to decreased telomerase activity and shortened telomeres in T_{EFF} [71-73]. However, IL-15-mediated proliferation of T_{CM} does not explain the loss of a diverse memory T cell pool, nor the accumulation of clonal expansions within the T_{EFF} population.

The accumulation of $CD8^+CD28^-$ T cells is a marker of chronic activated memory T cells and is included in a set of parameters defining the immune risk phenotype, which predicts higher 2-year mortality in very old persons [9, 10]. The age-related accumulation of $CD8^+CD28^-$ T cells has also been correlated with an imbalance in the production of Th1 and Th2 cytokines and with a lack of antibody production following immunization in old age [74]. Of utmost importance, latent infection with CMV, but not EBV or VZV, accelerates the age-related accumulation of T_{EFF} , thereby further restricting the memory T cell repertoire [39, 68] and compromising the response to a coresident EBV infection [75]. Additionally, CMV infection triggers production of bystander-secreted, differentiation-inducing factors, such as IFN- α , by CMV-infected plasmacytoid dendritic cells [66]. As a consequence, $CD4^+$ T cells of different specificities were significantly more differentiated in elderly CMV-seropositive individuals than the same cells in CMV-seronegative individuals [66]. The high frequency and persistence of CMV-specific T_{EFF} in old age may, in part, be explained by their high avidity and intact responsiveness to the homeostatic cytokine IL-15 [72, 76].

But what about markers of immunocompetence in old age? In humans, a novel, non-regulatory $CD45RO^+CD28^+IL-2R\alpha^{dim} CD8^+$ T cell population has been identified, which accumulates in healthy, CMV-seronegative elderly persons who characteristically still have a good humoral response after influenza vaccination [77, 78]. Interestingly, $IL-2R\alpha^{dim} CD8^+$ T cells from elderly persons share phenotype, gene expression and functional characteristics with naive $CD8^+$ T cells from young individuals [79]. For example, $IL-2R\alpha^{dim} CD8^+$ T cells express the lymph node homing markers CD62L and CCR7, the cytokine receptor IL-6R α , produce large amounts of IL-2, have long telomeres and a diverse TCR repertoire. Altogether, $IL-2R\alpha^{dim} CD8^+$ T cells may compensate for the age-dependent loss of antigen-inexperienced $CD45RA^+CD28^+ CD8^+$ T cells and represent a marker of immunocompetence in old age.

Effect of aging on the frequency and function of regulatory T cells

There are several types of regulatory/suppressor T cells, such as regulatory CD8⁺ T cells (CD8⁺ T_{reg}), IL-10-producing T regulatory-1 cells (Tr1), tumor growth factor (TGF)- β -secreting T helper 3 cells (Th3) and CD4⁺CD25⁺FOXP3⁺ cells (CD4⁺ T_{reg}) [80]. CD4⁺ T_{reg} cells consist of two indistinguishable subsets which can be either thymus-derived natural Treg (nT_{reg}) or induced Treg (iT_{reg}) derived from peripheral CD4⁺CD25⁻ T cells. But recent studies have also demonstrated that there might be common flexibility in T cell commitment, because IL-6, for example, could convert FOXP3⁺ nT_{reg} cells into FOXP3⁻ Th17 cells [81, 82].

The age-related involution of the thymus would suggest a decline in nT_{reg} cells, and indeed, an age-related decrease of human CD45RA⁺ CD4⁺FOXP3⁺ T cells has been described [83]. However, the total number of CD4⁺ T_{reg} cells does not change during aging due to an age-related increase of CD45RA⁻ CD4⁺FOXP3⁺ T cells. This study is in agreement with the majority of publications indicating that the frequency of total CD4⁺ T_{reg} cells does not change in healthy elderly persons [84]. However, CD4⁺ T_{reg} cell number and function may be altered in elderly persons suffering from inflammatory or autoimmune diseases [85, 86]. There may also be differences in the local distribution of human CD4⁺ T_{reg} cells. For example, an increased frequency of CD4⁺ T_{reg} cells in the skin may help to explain the decrease in cutaneous immune responses during aging [87].

The rapid turnover of peripheral human CD4⁺ T_{reg} cells may necessitate constant renewal and/or homeostatic proliferation of the CD4⁺ T_{reg} cell pool [88]. Although the TCR repertoire of human CD4⁺ T_{reg} cells is highly diverse [89], age-related thymic involution may endanger CD4⁺ T_{reg} cell diversity, similarly to what is observed within the total memory T cell pool during aging. In addition, aging may also affect the immunosuppressive function of CD4⁺ T_{reg} cells. However, several studies demonstrated that the suppressive function of CD4⁺ T_{reg} cells did not change during aging [83, 90]. In conclusion, the frequency and function of human CD4⁺ T_{reg} cells in the peripheral blood does not seem to be altered during healthy aging, although age-related differences in the local distribution and/or in subsets of regulatory T cells may be evident.

There has been long-lasting evidence that CD8⁺ T_{reg} cells play an important role in the regulation and suppression of immune responses to self- and foreign antigens [91]. In persistent viral infections caused for example by human immunodeficiency virus (HIV) or hepatitis C virus

(HCV), TGF- β - as well as IL-10-producing CD8⁺ T_{reg} cells have been identified [92]. But also tumors can induce CD8⁺ T_{reg} cells, which display a CD28⁻ phenotype [93]. The suppressive activity of CD8⁺CD28⁻ T_{reg} cells requires cell-to-cell contact and renders antigen-presenting cells to become tolerogenic [94]. Although CD8⁺CD28⁻ T cells generally accumulate during aging and correlate with decreased vaccine efficacy [74], nothing is yet known about human regulatory CD8⁺CD28⁻ T cells in elderly persons.

Effect of aging on Th17 differentiation and function

The memory CD4⁺ T cell pool is characterized by great phenotypic and functional heterogeneity, comprising Th1, Th2, T_{reg}, and recently also IL-21-producing follicular Th cells and Th17 [95]. The effect of aging on the frequency, differentiation and function of Th17 cells is of great relevance, because Th17 differentiation is induced by IL-1 β and IL-23, and Th17 cells can mediate pro-inflammatory (IL-17 and IL-6) as well as humoral responses (IL-21) [96]. In mice, an impaired differentiation of aged naive CD4⁺ T cells into functional Th1 and Th2 cells has already been demonstrated. However, naive CD4⁺ T cells from old mice have retained or even amplified their capacity to differentiate into functional Th17 effector cells [97, 98]. Th17 effector cells exhibited an even better helper function in old mice leading to a good expansion of antigen-specific B cells and differentiation to a GC phenotype. In addition, aged CD4⁺ T cell effectors generated in the presence of proinflammatory cytokines or an adjuvant that induces these cytokines, also produce high levels of IL-17 and IL-21 and exhibit significantly enhanced B cell helper activity, highlighting a possible strategy to induce potent humoral responses by vaccines in old age [97]. Whether the results from old mice reflect the human situation has still to be determined.

Age-related alterations of the B cell compartment

B cells play a crucial role in the establishment and maintenance of protective immune responses. Their main function is the production of antibodies; however, they are also potent antigen presenting cells and have regulatory functions. Aging affects developing B cell precursors, the composition of the peripheral B cell repertoire and the generation and maintenance of plasma cells [99, 100]. As a consequence, a decrease in specific antibody production and a concomitant increase in autoreactive and cross-reactive antibodies can be observed in the elderly [101, 102].

It has to be taken into considerations that the vast majority of studies on B cell aging are performed in aged mice, whereas data on human B cells are scarce.

B cell precursors are generated continuously from HSC in the bone marrow. However, changes in the numbers and functional properties of developing B cell subsets have been reported with age. HSCs give rise to multipotent progenitor cells, which then become determined to the B cell lineage by expressing lineage-specific transcription factors, such as PU.1, Ikaros, ID-1, E2A, EBF and PAX-5 [103, 104]. The number of early B cell progenitors is reduced in old age [105] and the expression of transcriptional regulators required for the generation of pro-B cells is decreased [106]. Differentiation and expansion of B-lineage precursors into pro-B cells is mediated by IL-7 [107]. The next steps of B cell development include recombination of the immunoglobulin heavy (IgH) and light (IgL) chain loci and further differentiation into pre-B cells [108]. Progression from the pro- to the pre-B cells stage is also disturbed in old age due to reduced expression of genes required for Ig recombination, such as RAG [109] and decreased responsiveness to IL-7 [110]. It is difficult to assess the extent to which age-related changes of the microenvironment contribute to defects in B cell generation, but it seems clear that both, intrinsic alterations of B cells as well as changes of the microenvironment play a role. For example, the consequences of the decreased responsiveness of B cell progenitors from aged mice to IL-7 [110] is further aggravated by aged BM stromal cells, which fail to provide sufficient IL-7 [111]. Only B cells with functional immunoglobulin chains survive and continue differentiation into immature B cells expressing IgM on the cell surface. Immature B cells expressing self-reactive IgM undergo further rearrangement of the light chain (receptor editing) to reduce self-reactivity [112]. Mature B cells co-express IgM and IgD and migrate into the periphery, where they constitute the majority of the B cell pool in secondary lymphoid organs. In summary, the age-related disturbance of the production of pro-, pre- and immature B cells leads to a decreased output of mature B cells from the bone marrow into the periphery. As a consequence, the number of naïve, CD27⁻ B cells declines with age [113, 114].

Total numbers of B cells are relatively constant with age. Therefore, alterations in the dynamics and turn-over of mature B cells are necessary to compensate for the decreased output of freshly generated B cells. Indeed, peripheral B lymphocytes in aged mice show reduced turnover rates and therefore have a longer lifespan [115, 116]. With advancing age the peripheral B cell pool becomes dominated by antigen-experienced, memory B cells expressing CD27. In

addition there is a shift from B2 cells, which are stimulated by protein antigens and require T cell help, to B1 lymphocytes, which are activated by carbohydrate antigens and autoantigens [117].

The B cell receptor (BCR) repertoire is altered in aged mice [118] and humans and it has been shown that decreased B cell diversity is correlated with poor health status and frailty [119]. Similar to the T cell pool, clonal expansions are appearing within the B cell compartment. Repertoire skewing impacts the extent and quality of humoral responses to pathogens. Age-related differences in the usage of the immunoglobulin heavy chain variable gene region have been observed in human B cells specific for pneumococcal polysaccharides [120]. It has to be taken into account that not only intrinsic defects of B cells and an altered B cell repertoire, but also impaired CD4⁺ T cell help is contributing to age-related changes of humoral immune responses. A severe reduction in germinal center reactions has been shown in aged mice [121]. T cell help is crucial for the formation of germinal centers, which are needed for antibody maturation and isotype switch.

In summary, age-related changes in the B cell pool lead to impaired humoral immune responses in the elderly. Primary antibody responses, e.g. after vaccination with neoantigens, are generally lower in the elderly compared to young adults [122, 123].

Strategies to reverse or delay immunological aging

The age-related involution of the thymus is the most important event contributing to the aging of the adaptive immune system. Thymic reconstitution would therefore be a promising approach to prevent the decline of naive T cell output and the accumulation of highly differentiated, pro-inflammatory CD8⁺CD28⁻ T cells. IL-7, IL-15, growth hormone, insulin-like growth factor 1, keratinocyte growth factor (KGF), ghrelin and ablation of sex steroids have all been demonstrated to be involved in thymopoiesis and could be used for therapeutic thymus restoration [124-127]. For example, IL-7 treatment of old rhesus macaques increased the number of naive CD4⁺ and CD8⁺ T cells, increased TREC levels per T cell and improved the response to influenza vaccination [128]. To decrease dosage and systemic side effects of IL-7, a chimeric IL-7-CCR9 cytokine has been generated which showed enhanced retention to the thymus, improved thymic output and reduced viral load in mice after challenge with influenza virus [129]. Although the use of hormones or cytokines to enhance thymopoiesis has yielded promising results in animals, side effects, continuous treatment and cost-effectiveness may limit this

approach in humans. Of great interest will be how the progress in the field of thymus and stem cell biology will be applicable for the reconstitution of thymic tissue in old age [130, 131].

It has also been shown that obesity accelerates thymic aging while caloric restriction inhibits thymic adipogenesis and thereby reduces the age-related involution of the thymus [132, 133]. For example, caloric restriction initiated in early adulthood in non-human primates, was shown to improve the maintenance and/or production of naive T cells and consequently preserved TCR repertoire diversity [134]. Caloric restriction also improved T cell function and reduced the production of inflammatory cytokines. However, initiation of caloric restriction before puberty can be disadvantageous, as it accelerated the loss of naive T cells and reduced TCR repertoire diversity [135].

The prevention of life-long stimulation of the immune system by chronic bacterial and viral infections, especially CMV, could reduce the accumulation of highly differentiated CD8⁺CD28⁻ T cells which restrict T cell diversity and contribute to subclinical inflammatory processes. Early childhood vaccination against CMV might be one option to prevent CMV infection and CMV-associated acceleration of immunosenescence. Although no CMV vaccines are yet available on the market, several CMV vaccine candidates are being tested in clinical trials [136].

Another strategy to reverse immunosenescence would be the depletion of highly differentiated, pro-inflammatory CD8⁺CD28⁻ T cells. Recent studies have shown that the same clonotypes occur in both, early memory CD8⁺ T cells and highly differentiated CD8⁺CD28⁻ T cells, indicating no loss of unique T cell specificities when depleting CD8⁺CD28⁻ T cells [137, 138]. For example, the depletion of autoreactive immunologic memory followed by autologous hematopoietic stem cell transplantation in patients with refractory systemic lupus erythematosus induced long-term remission through *de novo* generation of a juvenile and tolerant immune system [139].

Aging is also associated with a decreased efficacy of vaccinations [140]. Improved vaccination strategies, new adjuvants, alternative routes of vaccine application, and new vaccines that specifically target the aged immune system could help to ensure a better protection and decrease morbidity and mortality caused by infectious diseases in the elderly population. This has been reviewed extensively by Kovaïou et al. [141] and McElhaney [142].

Because the aging process varies widely between individuals it will be important to identify those persons who would benefit most from immunomodulatory treatments. Several biomarkers have already been suggested, such as the frequency of pro-inflammatory CD8⁺CD28⁻ T cells, but have yet to be validated in large cohorts.

Conclusions

Age-related changes within the bone marrow and thymus lead to an impaired generation of new, naive T and B cells which severely compromises the maintenance of a diverse T and B cell repertoire in old age. In addition, life-long stimulation of the immune system by chronic infectious diseases leads to the exhaustion of the memory pool and negatively correlates with intact humoral immune responses after vaccination. Promising strategies to reverse or delay immunosenescence are currently being validated in animal studies. Furthermore, improved vaccination strategies and the development of more efficient vaccines should help to decrease morbidity and mortality caused by infectious diseases in the elderly.

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Figure legends

Figure 1 Effect of aging on phenotype and function of human naive CD8⁺ T cells. (A) Flow cytometric analysis of the expression of the lymph node homing marker CD62L, the cytokine receptor IL-6R α and the senescence marker CD57 on CD45RA⁺CD28⁺ CD8⁺ T cells from young and elderly persons. Results are expressed as mean \pm SEM. (B) Analysis of the TCR repertoire of CD45RA⁺CD28⁺CD62L⁺ CD8⁺ T cells from five young and five elderly persons by TCR V β CDR3 spectratyping as described elsewhere [78]. (C) Analysis of the relative telomere length of CD45RA⁺CD28⁺CD62L⁺ CD8⁺ T cells from five young and five elderly persons using flow FISH. The tetraploid human T cell leukaemia cell line 1301 with a telomere length of approximately 25 kbp was used as an internal standard. Results are expressed as mean \pm SEM.

Figure 2 Effect of aging on the peripheral CD8⁺ T cell composition. Peripheral naive T cells are maintained by TCR – self-peptide/MHC- and IL-7-signaling, with a mean doubling time of about 200 days [143]. They are constantly replaced by new, naive T cells from the thymus, thus guaranteeing a diverse and balanced peripheral naive T cell pool. Upon antigenic contact, naive CD8⁺ T cells are activated, expand and kill infected host cells. After elimination of the pathogen, 90-95% of the expanded effector T cells go into apoptosis, while the surviving cells differentiate into memory T cells which are maintained by homeostatic signaling via IL-7 and IL-15. In old age, decreased thymic output leads to an enhanced homeostatic proliferation and an increased post-thymic lifespan of naive T cells. Nevertheless, naive T cell numbers and diversity decline during aging and naive T cells have shorter telomeres and acquire TCR signaling defects. Naive T cell numbers are further decreased by life-long contact with pathogens. However, memory T cells generated from aged naive T cells show an impaired recall response. Repeated activation of memory T cells because of chronic infections or long-term homeostatic proliferation leads to the accumulation of highly differentiated CD57⁺CD28⁻ CD8⁺ T cells which have short telomeres and produce large amounts of INF- γ and TNF- α .

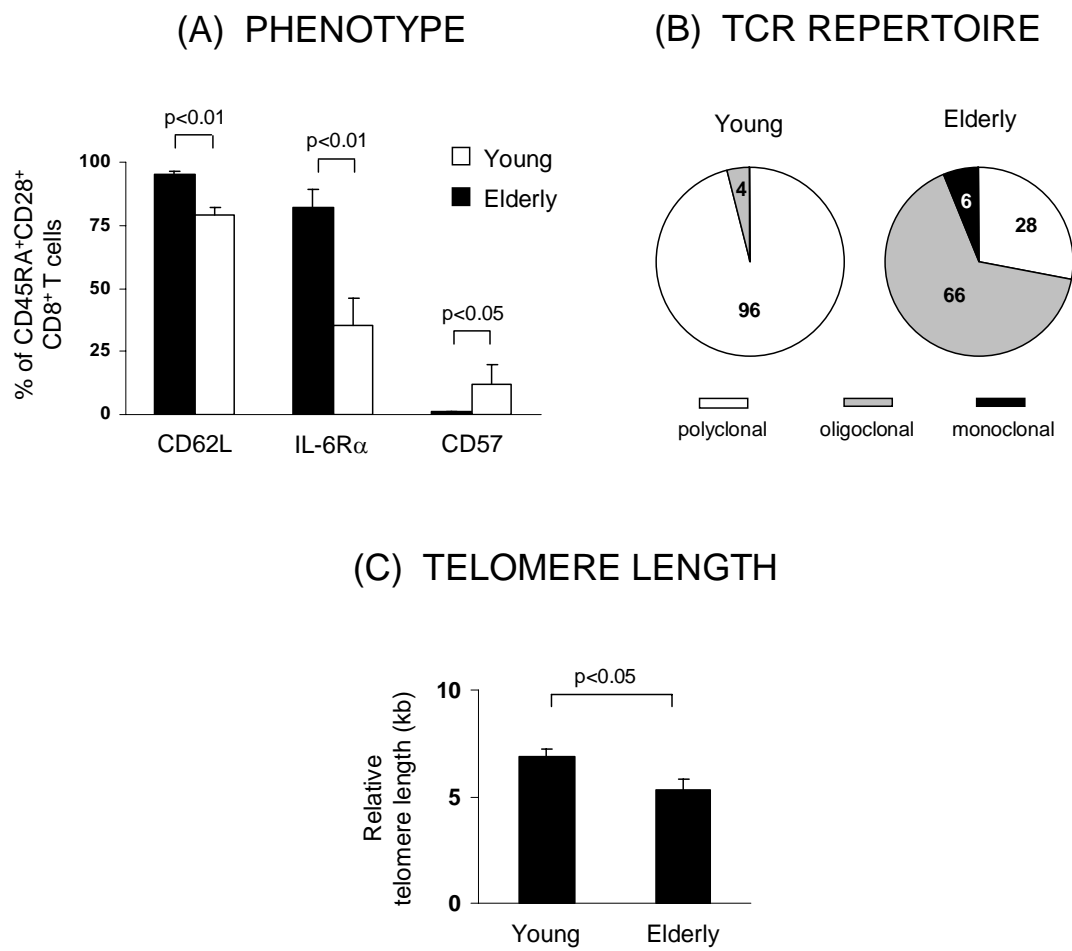


Fig. 1

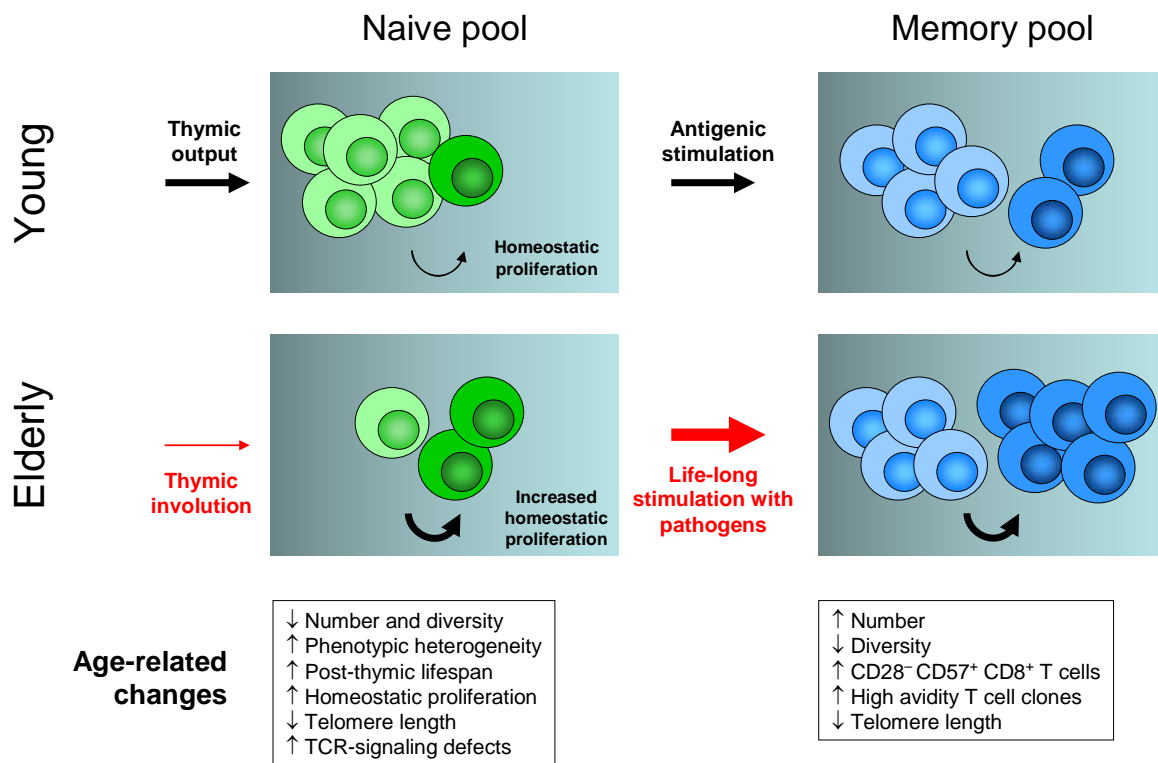


Fig. 2