

## REVIEW

## The aging of the immune system

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**Summary**

An age-related decline in immune functions, referred to as immunosenescence, is partially responsible for the increased prevalence and severity of infectious diseases, and the low efficacy of vaccination in elderly persons. Immunosenescence is characterized by a decrease in cell-mediated immune function as well as by reduced humoral immune responses. Age-dependent defects in T- and B-cell function coexist with age-related changes within the innate immune system. In this review, we discuss the mechanisms and consequences of age-associated immune alterations as well as their implications for health in old age.

**Introduction**

Age-related changes of the immune system contribute to the increased susceptibility of elderly persons to infectious diseases, vaccine failure, and possibly autoimmunity and cancer [1,2]. Immunosenescence affects various cell types in the bone marrow and the thymus, mature lymphocytes in the peripheral blood and secondary lymphatic organs, and also elements of the innate immune system. The immune system can be divided into an innate part, consisting mainly of monocytes, natural killer (NK), and dendritic cells (DC), and into an adaptive part, represented by B and T lymphocytes. Generally, the aging process affects both branches of the immune system (as summarized in Fig. 1). However, innate immunity seems to be better preserved, while more severe often detrimental age-dependent changes occur in the adaptive immune system [3].

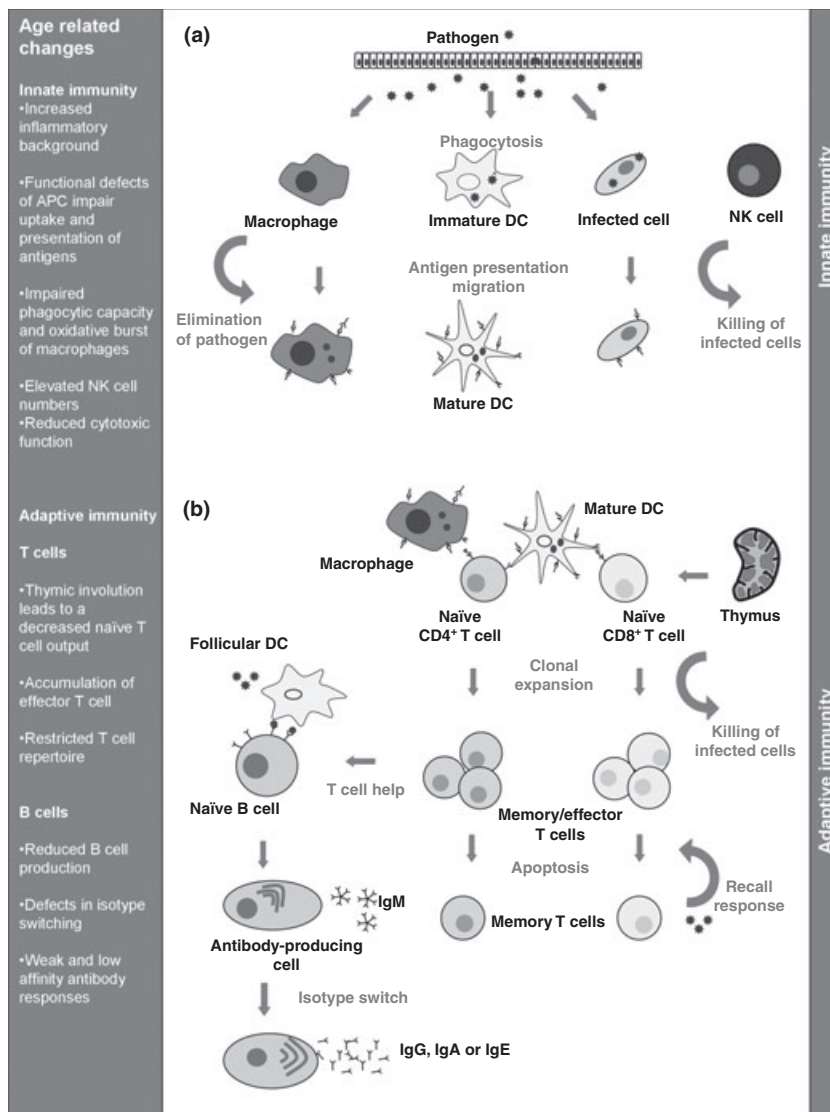
**Aging of the hematopoietic stem cell compartment and lymphoid progenitor cells**

Cells of the immune system are constantly renewed from hematopoietic stem cells (HSCs). The overall capacity for renewal of these stem cells declines [4] and the total amount of hematopoietic tissue in the bone marrow

decreases with age [5]. Changes of the niche as well as alterations of hormone production may disturb self-renewal and lineage commitment of HSCs [6,7]. The ability of human HSCs to proliferate correlates inversely with age, possibly resulting from shortening of telomeres [8]. Interestingly, age-related changes do not affect erythroid and myeloid progenitors. In contrast, there are changes in B cell development. Fewer pro-B cells are generated and fewer of these cells transit into subsequent differentiation steps, which results in a lower number of mature B cells leaving the bone marrow [9]. Bone marrow-derived T lymphocyte precursors which migrate into the thymus seem to be less affected by aging. However, because of age-related involution of the thymus, the T cell compartment undergoes substantial changes with age.

**Aging of the adaptive immune system***T lymphocytes*

Maturation of T cells takes place in the thymus. The thymus is divided into multiple lobules, which consist of an outer cortex that is densely populated with thymocytes and an inner medulla that is sparsely populated with thymocytes. During differentiation, T cells migrate from the outer cortex to the medulla from where they exit the thymus and enter the periphery. One of the most striking



**Figure 1** The immune response and age-related alterations: (a) Invading pathogens induce innate immune responses at the site of infection. The pathogen is taken up by antigen-presenting cells, such as macrophages and dendritic cells (DCs). Macrophages process the antigen and present it in the lymph nodes in the context of MHC class II molecules accompanied by the secretion of pro-inflammatory cytokines. DCs mature, migrate to the lymph node, and present stable major histocompatibility class I and II molecule/peptide complexes. Infected cells are recognized and eliminated by natural killer cells. (b) Antigen-presenting cells induce activation and clonal expansion of naive CD4<sup>+</sup> and CD8<sup>+</sup> T cells. The activation and differentiation of naive B cells is induced by antigen and CD4<sup>+</sup> T cell help. Naive B cells differentiate into memory B cells and antibody-secreting cells. Long-term immunity is assured by memory T and B cells in the blood and lymph nodes.

changes in immunosenescence is the involution of the thymus, which is characterized by a reduction in the overall size of the organ and a replacement of the functional cortex and medulla tissue by fat. It is also of interest that the perivascular space (PVS) of human thymus increases in volume during aging, as thymopoiesis declines [10]. These changes start early in life and are almost complete by the age of 40–50 years [11]. As a consequence, the number of naive T cells exiting the thymus is dramatically decreased with age [12]. Although a diverse naive CD4<sup>+</sup> T cell compartment is maintained for decades in spite of thymic involution, a dramatic decline of diversity occurs after the age of 70, which results in a severely contracted repertoire [13]. Similar changes in the CD8<sup>+</sup> subpopulation take place even earlier in life [14]. By definition, naive T cells should proliferate well upon

stimulation with dendritic cells in combination with antigen, and contain a variety of cells of different antigen specificities as they have not encountered their specific antigen. Naive T cells from elderly persons exhibit numerous functional defects, including significantly shorter telomeres, a restricted T cell receptor repertoire, reduced interleukin (IL)-2 production, and impaired expansion and differentiation into effector cells, when compared with naive cells from young people [15–17]. As a consequence, their ability to mediate effective immune responses against new antigens is decreased.

In about 30% of healthy elderly individuals, a nonregulatory CD8<sup>+</sup>CD45RO<sup>+</sup>CD25<sup>+</sup> T cell population, which produces IL-2 and displays a highly diverse TCR repertoire, occurs [18]. This population shares phenotypic and functional characteristics with naive T cells from young

persons, and persons with a high percentage of CD8<sup>+</sup>CD25<sup>+</sup> cells can still raise a protective humoral immune response to vaccination [19]. CD8<sup>+</sup>CD45RO<sup>+</sup>CD25<sup>+</sup> T cells may compensate the loss of functional naïve T cells in old age and represent a good biomarker for immunological competence in elderly persons [20]. However, approximately 70% of the elderly population does not possess significant numbers of this specific cell type [19]. It is still imaginable that the few remaining CD8<sup>+</sup>CD45RO<sup>+</sup>CD25<sup>+</sup> T cells could be propagated by *in vitro* expansion. The resulting population could then be stored and re-transfused in case of immune deficiency, such as after immunosuppressive or radiation therapy.

The age-related reduction of naïve T cells is associated with an increase in the number of antigen-experienced memory and, in particular, effector cells. Impaired generation of protective antibody levels after vaccination in old age correlates with CD8<sup>+</sup> effector T cell accumulation [21,22]. These effector T cells display phenotypic changes, such as the loss of the co-stimulatory molecule CD28, which has been reported as a key predictor of immune incompetence in elderly persons [23]. In CD4<sup>+</sup> T cells, the loss of CD28 is accompanied by a concomitant defect in CD154 (CD40L) expression; hence, the capacity of CD4<sup>+</sup> T cells to help in B cell proliferation and antibody production is reduced. CD28<sup>-</sup> T cells produce pro-inflammatory cytokines [24]. Their proliferative capacity is limited, they have shortened telomeres, and they show increased resistance to programmed cell death and a restricted T cell diversity [23,25]. Thymic involution can also lead to a decreased output of regulatory T cells (Tregs). Treg-mediated suppression has been reported to decline after the age of 50, which might contribute to age-related phenomena such as increased inflammation and autoimmunity [26].

### Factors accelerating T cell aging

Repeated exposure to antigens directly shapes the T cell pool, and pathogens are directly contributing to immunosenescence [27]. Many viruses have developed various strategies with the final goal of coexistence with their hosts and establishment of persistent infections in humans [28]. Cytomegalovirus (CMV) is a ubiquitous, genetically stable herpes virus that infects 60–100% of the human population and establishes lifelong persistence [29]. Primary infections as well as latency normally occur asymptotically in immunocompetent hosts. Despite frequent reactivation of latent CMV in elderly persons, as suggested by increased anti-CMV antibodies and viral shedding in the urine [30], even very elderly individuals generally maintain an efficient CMV immunosurveillance and therefore do not experience clinical

symptoms. In immunocompromised persons, such as HIV-seropositive individuals or immunosuppressed transplant recipients [31], the balance between the virus and the immune system can be disturbed, leading to viral reactivation and disease associated with high morbidity in AIDS patients and transplant recipients [32,33]. Lifelong viral persistence in immunocompetent hosts shapes the immune system, and scientific evidence has accumulated that chronic CMV infection may accelerate the aging of the immune system and may lead to a high level of chronic subclinical inflammation [34]. T cells are essential for the control of viral replication, spread and disease [35,36]. Earlier reports have documented alterations in the surface phenotype of CD8<sup>+</sup> T cell subsets of CMV-seropositive individuals that are very similar to those associated with aging [29]. CMV infection as well as aging leads to characteristic changes in the CD8<sup>+</sup> T cell repertoire and to the accumulation of CD8<sup>+</sup> effector T cells with a CD28<sup>-</sup> phenotype [37,38]. CMV-specific CD8<sup>+</sup> effector T cells have been shown to occur as large expanded clones, which may dominate the repertoire [38,39] and affect the capacity of the immune system to respond to other pathogens. In CMV-seropositive elderly persons, up to 25% of the total CD8<sup>+</sup> T cell pool can be specific for CMV immunodominant epitopes [38]. The immune response to Epstein–Barr virus (EBV) seems for instance to be impaired as a result of CMV infection, as the number of EBV-specific cells increases with age only in CMV-negative but not in CMV-positive persons [40]. There are numerous human pathogens that persist after primary infection but the pathogen family that is linked most prominently to the expansion of T cells in elderly persons is the herpesviridae, with HSV, EBV and CMV as main contributors [41]. Among them, CMV stands out because it typifies the key features that are pertinent to T cell aging [42]. Other latent viruses may also be of interest in this context. Little information is presently available on how other classical latent virus infections such as those by other herpesviruses [human herpesvirus (HHV) 6 or 7] or polyomaviruses [e.g. BK virus (BKV), JC virus (JVC)] affect the aging of the immune system. For some of them, an increased virus shedding in elderly persons has been reported [43] but recent reports suggest that localized, niche limited, latent herpesvirus may not have an impact on immunosenescence [42]. It seems likely that lifelong episodes of reactivation may trigger similar but presumably less pronounced effects as CMV. However, several alterations of T cell characteristics have been reported in HIV-1-infected individuals [44]. These include the accumulation of highly differentiated CD8<sup>+</sup> or CD4<sup>+</sup> T cells (including the loss of the co-stimulatory receptor CD28); a reduced proliferation capacity of T cells associated with short telomere length and changes

in cytokine secretion capacity (in particular decrease in IL-2 production); and an increased susceptibility to activation-induced cell death. Altogether, these observations suggest that a decline of the T cell competence occurs in HIV-1 infection. Along these lines, rare HIV-infected individuals who are able to better control viral replication (so-called long-term non-progressors) were reported to have HIV-specific CD8<sup>+</sup> T cells that retained the capacity to proliferate upon antigenic stimulation and present pluri-functional capacities in terms of cytokine production capability [45]. Besides these latent and chronic infections, it is likely that premature immunosenescence can also be caused by other sources of chronic antigenic stress, such as cancer antigens or alloantigens [46–49].

### B lymphocytes

Aging is accompanied by substantial changes in all B cell compartments and – as a consequence – humoral immune function. While peripheral B cell numbers do not decline with age, the composition of the compartment changes. Similar to the T cell pool, the peripheral B cell pool fills up with antigen-experienced memory cells at the expense of a concurrent displacement of naïve B cells. The percentage of naïve B cells, which are defined by the absence of CD27, is significantly reduced in aged individuals. In contrast, memory B cells which show a decreased susceptibility to apoptosis [50] accumulate in elderly persons, leading to clonal expansions of certain B cell specificities [51]. These expansions may limit the diversity of the repertoire and influence the outcome of vaccinations in elderly individuals. Although serum immunoglobulin levels are stable during aging, the antibodies generated in old age are of lower affinity because of a shift in antibody isotypes from IgG to IgM [52].

Interactions with other immune cells are essential for B cell activation and antibody production. Therefore, age-related differences in the B cell compartment are likely to result from a combination of intrinsic age-related defects in the generation and maturation of B cells, and dysregulated interactions with other cell types of the immune system. Of particular importance, B cells from elderly individuals are stimulated 70% less efficiently by follicular dendritic cells than B cells from young subjects [53], suggesting loss of B cell function, as a result of the decreased expression of co-stimulatory molecules, such as CD40 or CD27 [54]. As described above, aged CD4<sup>+</sup> T cells produce less IL-2 and express less CD40L, which is crucial in the interaction of B and T cells. Less efficient T cell help and the altered cytokine environment contribute to defects in antibody production [55]. Disturbed T cell/B cell communication is likely to lead to a reduced B cell expansion and differentiation in response to antigens as

**Table 1.** Age-related changes in the adaptive immune system.

Cell type	Age-related increase	Age-related decrease
T lymphocytes	Number of memory and effector cells Expanded clones of effector cells Release of pro-inflammatory cytokines	Number of naïve T cells Diversity of the T cell repertoire Expression of co-stimulatory molecules (CD28, CD27, CD40L). Proliferative capacity
B lymphocytes	Autoreactive serum antibodies	Generation of B cell precursors Number of naïve B cells Diversity of the B cell repertoire Expression of costimulatory molecules (CD27, CD40) Antibody affinity Isotype switch

well as to decreased antibody production and germinal center formation [55,56]. Age-related loss of B cell function is thus also the result of age-related weakened interactions among immune cells. The age-related changes which occur in the adaptive immune response are summarized in Table 1.

### Aging of the innate immune system

Innate immunity represents the first line of host defense and provides the basis for an adequate response to pathogens. Aging is frequently associated with a decreased function of epithelial barriers of the skin, lung, or gastrointestinal tract, which enables pathogenic organisms to invade mucosal tissues, resulting in an increased challenge for the aged innate immune system [57,58].

Besides phagocytic cells [neutrophils, monocytes, macrophages and DCs] and NKs, soluble mediators such as cytokines, chemokines, hormones, and/or oxygen-free radicals are also of importance within the innate immune system. Elevated plasma concentrations of interleukin 6 (IL-6), IL-1 $\beta$ , and tumor necrosis factor-alpha (TNF $\alpha$ ) have been described in elderly populations and were postulated as predictive markers of functional disability, frailty and mortality [59–61]. These factors are thought to contribute to a lifelong continuous stimulation of the immune system, resulting in a subclinical inflammatory status defined as inflamm-aging [62]. Chronic inflammation supports the development and progression of age-related diseases, such as osteoporosis, neurodegeneration and atherosclerosis [63–65]. Subclinical inflammation may be caused by chronic stimulation of the innate immune system by degradation products and/or by the partial inability of the aged immune system to eliminate certain pathogens [66]. The age-related changes which

**Table 2.** Age-related changes in the innate immune system.

Cell type	Age-related increase	Age-related decrease
Neutrophils		Oxidative burst Phagocytic capacity Bactericidal activity
Macrophages		Oxidative burst Phagocytic capacity
NK cells	Total number of cells	Proliferative response to IL2 Cytotoxicity
Dendritic cells		Capacity to stimulate antigen specific T cells Lymph node homing
Cytokines and Chemokines	serum levels of IL6, IL1 $\beta$ and TNF $\alpha$	

occur in the innate immune response are summarized in Table 2.

### Neutrophils

Neutrophils are short-lived cells that play an important role in host defense to both bacterial and fungal infections and during acute inflammation. Neutrophil numbers in the blood and neutrophil precursors in the bone marrow are well-preserved in healthy elderly persons [67]. Neutrophils are recruited from the periphery along a gradient of chemotactic factors produced at the site of infection. This includes adhesion to vascular endothelial cells and migration into the affected tissue. Neither chemotaxis nor adhesion processes seem to be affected during aging [68]. However, studies measuring phagocytosis of opsonized bacteria (*Escherichia coli* and *Staphylococcus aureus*) as the neutrophil target have shown a significant reduction in phagocytic ability in the elderly population [69]. The reduced response of neutrophils to *S. aureus* is of particular clinical importance bearing in mind the increased susceptibility to this pathogen in elderly persons. Interestingly, phagocytosis of unopsonized bacterial targets occurs at the same level in young and old subjects [70]. This suggests that receptors for innate recognition of bacterial components (e.g. the LPS receptor CD14) are not affected by aging. In contrast, the expression of the Fc $\gamma$  receptor CD16 is significantly reduced in neutrophils from elderly donors [71]. Additionally, it has been shown that Fc receptor-mediated superoxide production is significantly reduced in elderly persons [72]. Thus, both Fc receptor-mediated superoxide generation and phagocytosis are decreased in elderly persons, suggesting that a decline in Fc receptor-triggered effector responses is of particular importance in age-related neutrophil dysfunction.

### Macrophages

Macrophages function as 'pathogen sensors' and play an important role in the initiation of inflammatory responses, elimination of pathogens, regulation of the adaptive immune response, and repair of damaged tissue. They can act through several mechanisms, either directly by destroying invading pathogens or tumor cells, or indirectly, by releasing mediators (e.g. IL-1, TNF $\alpha$ ) which can activate other inflammatory cells. Additionally, they are able to process antigens and present peptides to T cells. An increasing number of studies confirm that macrophage function is compromised with age [73]. Aging human and rodent macrophage populations appear to have reduced levels of MHC class II molecules [74,75], which may contribute to poor CD4<sup>+</sup> T cell responses. Upon stimulation with saturating amounts of IFN $\gamma$ , macrophages from aged mice expressed half of the MHC class II molecules at the cell surface than macrophages from young mice [76].

The number of blood monocytes in elderly persons appears to be similar compared to that in young subjects. However, a significant decrease in macrophage precursors as well as macrophages in the bone marrow of elderly persons has been described [75]. Macrophages can destroy microbes via products of the respiratory burst induced by IFN $\gamma$ . Studies on rats have demonstrated a 75% decrease in the ability of macrophages from aged animals to produce superoxide anion following incubation with IFN $\gamma$  [77]. As a consequence of decreased respiratory burst in elderly individuals, the intercellular killing of bacteria is impaired and may cause longer duration of infections as a consequence [77]. The phagocytic function of macrophages is also impaired with age. Furthermore, it has been observed that the phagocytic ability of macrophages from aged individuals declines in parallel with the decreased levels of macrophage-derived chemokines, such as MIP-1 $\alpha$ , MIP-1 $\beta$ , MIP-2 and eotaxin [78]. An age-related decline in macrophage function may thus have an impact on both innate and adaptive immunity.

### Dendritic cells

Dendritic cells play a critical role in linking the innate and the adaptive immune system. As professional antigen-presenting cells, they play a key role in initiating immune responses, being capable of capturing and processing antigens and of secreting a variety of cytokines. How DCs are affected by age is still not fully understood. *In vitro* generated DCs, originating from peripheral blood monocytes (monocyte-derived dendritic cells, MODCs) from elderly persons, are functionally intact regarding differentiation and maturation [79,80]. However, studies

examining the ability for micropinocytosis revealed a decreased uptake of FITC dextran by dendritic cells derived from aged monocytes compared with MODCs from young subjects [81]. The reduced uptake of antigen may also affect antigen processing and presentation, and thus effective T cell responses in aging. Additionally, it has been shown that MODCs from aged subjects display impaired capacity to phagocytose apoptotic cells compared to that of young MODCs [81]. As phagocytosis of apoptotic cells results in an anti-inflammatory response, [82] this decreased ability to phagocytose may contribute to the pro-inflammatory background observed in elderly individuals. *In vivo* data from mice suggest that aged dendritic cells have an impaired capacity to stimulate antigen-specific T cells and that DC *in vivo* trafficking to drain lymph nodes is affected by aging, as a result of an impaired expression of the lymph node homing marker CCR7. [83]. MODCs from aged persons also display impaired migration. However, in humans, the cell surface receptors are comparable between young and aged, thus the defect appears to be in downstream signaling pathways [81]. Phosphoinositide 3 kinase (PI3K) has been demonstrated to play a critical role in both phagocytosis and migration of DCs [84,85], and a decreased activation of PI3K, as evidenced by impaired phosphorylation of AKT shown in MODCs of aged subjects [81].

These age-related defects in micropinocytosis, phagocytosis and migration could contribute to impairments in immunity in old age. They could specifically have implications on the use of DC-based immunotherapy against cancer in elderly persons.

### Natural killer cells

NK cells are cytotoxic cells that play a major role in the MHC unrestricted recognition of virally infected cells and in the rejection of tumors. Recent studies support the hypothesis that high NK cytotoxicity associates with healthy aging and longevity, whereas low NK cytotoxicity associates with increased morbidity and mortality as a result of infections, atherosclerosis, and poor response to influenza vaccination [86–89]. Unlike T and B cells, the absolute number of NK cells is increased in aged individuals [90,91]. However, NK cell cytotoxicity and IFN- $\gamma$  production, on a 'per cell' basis, are decreased in old age [91]. NK cells are able to directly kill cells by releasing perforin and granzymes. These enzymes activate caspases and induce apoptosis of the target cell. NK cell proliferation, expression of CD69, and killing of NK-resistant cell lines in response to IL-2 are also decreased in aging, whereas other NK cell functions such as TNF- $\alpha$  production or perforin synthesis are not significantly altered [92]. Taken together, these results indicate that senescence

is associated with a defective functional capacity of NK cells that is partially compensated by an increased number of mature NK cells.

### Clinical consequences and conclusions

The elderly population is particularly susceptible to infection and vulnerable in case of disease [93,94]. Deaths resulting from influenza and pneumonia, for example, represent the sixth leading cause of death among persons aged 65 or older in developed countries, such as the United States [95]. Vaccinations could help to overcome this increased risk of infectious death in elderly persons and specifically in transplant recipients. However, the protective effect of vaccination is partially lost in the elderly population [96]. Declining immune function with age substantially contributes to the decreased efficacy of vaccines in elderly persons. Thus, the age-related decreased output and functional deficiency of naïve T cells hampers the induction of adaptive immune responses to neoantigens. In the context of primary vaccinations, this leads to reduced response rates. This may specifically hamper the success of novel vaccines, such as vaccinations against yellow fever or rabies. The accumulation of CD28<sup>-</sup> effector T cells has been shown to accompany an impaired response to influenza vaccination [22]. Age-related impaired immunity may thus partly explain that influenza vaccinations have a protection rate of only 56% in elderly persons [97]. Antibody titers following classical booster vaccinations such as against tetanus or TBE are also lower in elderly persons than in the young [98], decline faster, and the function of the antibodies produced is also diminished [99–101]. Improved vaccination strategies, new adjuvants, and new vaccines that specifically target the aged immune system will help to overcome the limitations of immunosenescence and ensure a sufficient immune response in elderly persons.

In the context of transplantation, it is now well-understood that the co-stimulatory pathways of allorecognition are impaired in elderly individuals [102]. Both the CD28–CTLA4 and CD40–CD40L pathways become dysfunctional with aging. It has been shown that the levels of CD28, CD40, and CD40L are reduced, whereas the level of CTLA-4 is increased [103]. Co-stimulation is a critical step in fully activating T cells and has a paramount importance in transplantation, because it is necessary for both rejection and tolerance [104]. The chronic antigenic stress together with the high inflammatory background in the elderly persons, as described above, may also contribute to chronic allograft deterioration (reviewed in [105]). In conclusion, a better insight into the basic mechanisms of immune dysfunction that occur with age will help to delay or even reverse the detrimen-

tal effects of immunosenescence, and thereby ensure a better protection of the vulnerable elderly population from disease, subsequent loss of independence, and death.

### Authorship

DW and BW: wrote and approved the paper. BG-L: supervised the preparation of the manuscript.

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