Review Article

Immune Cells and Immunosenescence

(immune aging / immunomodulation / immunosenescence)

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Abstract. Aging is associated with progressive loss of physiological integrity, leading to impaired physical and mental functions as well as increased morbidity and mortality. With advancing age, the immune system is no longer able to adequately control autoimmunity, infections, or cancer. The abilities of the elderly to slow down undesirable effects of aging may depend on the genetic background, lifestyle, geographic region, and other presently unknown factors. Although most aspects of the immunity are constantly declining in relation to age, some features are retained, while e.g. the ability to produce high levels of cytokines, response to pathogens by increased inflammation, and imbalanced proteolytic activity are found in the elderly, and might eventually cause harm. In this context, it is important to differentiate between the effect of immunosenescence that is contributing to this decline and adaptations of the immune system that can be quickly reversed if necessary.

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Abbreviations: μHC – μ heavy chain, AEP – asparagine-endopeptidase, Aire – autoimmune regulator, APC – antigen-presenting cell, ATP – adenosine triphosphate, BCR – B-cell receptor, BM – bone marrow; Cat – cathepsin, CD – cluster of differentiation, CLP – common lymphoid progenitor, CR – complement receptor, CSR – class switch recombination, CTL – cytotoxic T lymphocytes, DC – dendritic cell, Fc – fragment crystallizable region, fMLP – N-formyl-methionyl-leucyl-phenylalanine, HSC – haematopoietic stem cell, IFN – interferon, IgM – immunoglobulin M, IL – interleukin, iNK – immature NK cell, LHRH – luteinizing hormone-releasing hormone, LPS – lipopolysaccharide, MAPK – mitogen-activated protein kinase, MHC – major histocompatibility complex, miRNA – microRNA, mNK – mature NK cell, mTOR – mechanistic target of rapamycin, NAD^+ – nicotinamide adenine dinucleotide (oxidized), NADPH – nicotinamide adenine dinucleotide phosphate (reduced), NET – neutrophil extracellular trap, NF-κB – nuclear factor 'κ-light-chain-enhancer' of activated B cells, NKP – NK cell precursor, PAMP – pathogen-associated molecular pattern, PBMC – peripheral blood mononuclear cell, pDC – plasmacytoid dendritic cell, PGE2 – prostaglandin E2, PI3K – phosphoinositide 3-kinase, ROS – reactive oxygen species, TCR – T-cell receptor, TGF-β – transforming growth factor β, Th – T helper cell, TLR – toll-like receptor, TM – memory T cells, TNF-α – tumour necrosis factor α, TREC – T-cell receptor excision circle, Treg – regulatory T cells.

1. Introduction

It has always been a human goal to strive for longevity, and in fact, the world population's lifespan has significantly prolonged over the last decades. The largest population of older people is found in industrialized countries. On the one hand, this shifted demographics of industrialized nations is caused by post-war patterns of immigration; on the other hand, this is due to the availability and high standards of medical care. After all, this positive trend comes along with new challenges. One of these issues is the cavity of immune aging, leading to higher susceptibility to infections (Nikolich-Zugich, 2008) and impaired response to vaccination (Cicin-Sain et al., 2010). Consequently, these circumstances are responsible for higher mortality rates from normally 'survivable' diseases. In addition, the risk of autoimmune diseases (Prelog, 2006) and the development of tumours increase (Fulop et al., 2011). As a whole, these trends are also defined as immunosenescence, gradual deterioration of the immune system simply resulting from advancement of age and being responsible for increased rates of morbidity and mortality among the elderly population.

Manifestations of immunosenescence can be found in several segments of the immune system. These include,

among others, changes of the lymphocyte cell pool, involving thymus involution, stem cell alterations, and finally reduced immunity. In the elderly, alterations of immune cell phenotype and activity result in impaired immunity affecting the health status. Furthermore, inflamm-aging, a state of low chronic systemic inflammation in the elderly, results in an impaired innate and adaptive immune response, which cannot clear infections efficiently and alters function of T regulatory cells (Garg et al., 2014). As a result, a weak and defective immune system is an entrance ticket for expansion of cancer cells (Franceschi et al., 2017; Pawelec, 2017; Xu and Larbi, 2017; Zinger et al., 2017). Since the elderly are more often affected by infections and tumour development than young people, closer understanding of the aging immune system is essentially needed in order to establish therapeutic strategies improving the severe burden of immunosenescence. In this review, we will summarize the findings regarding the involvement of immune cells and mediators in the process of immunosenescence.

2. T cells

It is well known that involution of the thymus limits the output of naïve T cells, which starts shortly after birth with progressive mass loss of about 3 % every year until the age of 50 and 1 % to the age of 85 (George and Ritter, 1996; Palmer, 2013). Progressively declining release of T cells from the thymus leads to a reduced repertoire of T cells in the peripheral lymphoid organs. On the other hand, high numbers of memory T cells or long-lived naïve T cells were detected in the elderly. These phenotypical changes result in the accumulation of deficiencies in T cell functions (Swain et al., 2005; Jones et al., 2008; Montecino-Rodriguez et al., 2013).

One possible therapy aimed to delay the aging process could involve inhibition of thymus shrinking. Sex hormones are one of the factors triggering the thymus involution. Sutherland et al. (2005) investigated sex steroid ablation in humans and mice by castrating mice either surgically or chemically. Chemical castration was performed by luteinizing hormone-releasing hormone (LHRH) agonists, which decrease testosterone levels. Both methods of castration in aged mice led to reconstitution of the thymus and increased expression of major histocompatibility complex II (MHC II) which was comparable to that of young mice. Moreover, thymocyte differentiation and proliferation were equal to those found in young mice. In addition, T cell numbers in lymph nodes and spleen were restored and presented a ratio similar to that of young mice. Lastly, the T cell receptor (TCR) repertoire and T cell function against herpes simplex virus 1 were also positively affected. In humans, a castration study by using LHRH agonists was performed in aged (> 60 years) prostate cancer patients. The chemical castration led to a significant increase of lymphocytes and natural killer (NK) cells. A higher number of T cells was mainly found within a T helper cell pool, although numbers of naïve and memory cytotoxic T cells were markedly increased. Blocking of sex hormones by LHRH agonists to reconstitute T cell numbers in elderly patients should be considered as a therapeutic option, given the advantage of reversibility of LHRH agonists (Sutherland et al., 2005). However, there is the downside of muscle mass loss after blocking testosterone in older adults (Cheung et al., 2014).

Aging of T cells, and consequently the function of naïve and differentiated T cells, can be regulated epigenetically by changes of histone expression, histone modifications, or DNA methylation. Interestingly, age-associated modifications of chromatin structures are among others driven by transcription factor networks normally driving T cell differentiation. Therefore, aging of T cells is directly linked to modulation of the activity of T cell differentiation pathways (reviewed by Goronzy et al., (2018)).

Several studies revealed that age-related dysfunction of naïve peripheral T cells is related to reduced proliferation and reduced IL-2 secretion (Nagel et al., 1988; Salam et al., 2013). Prostaglandin E_2 (PGE_2), a tissue mediator with T cell inhibitory capacity, suppresses IL-2 secretion and is highly expressed in peripheral blood mononuclear cells (PBMCs) from elderly people (Phipps et al., 1991). This explains, at least in part, the dysfunction of naïve T cells.

T cells can be divided into different subsets: (i) T helper (Th) cells ($CD4^+$ T cells, mainly subdivided to Th1, Th2, and Th17), which are required for initiation and promotion of adaptive immune responses, (ii) cytotoxic T cells ($CD8^+$ T cells, CTLs), which can eliminate infected and transformed cells, (iii) memory T cells (TM), which have already encountered antigen during previous infection, and (iv) T regulatory (Treg) cells, which are suppressive and required for self-tolerance.

In addition, T cells in aged individuals tend to differentiate into specific subsets, limiting the diverse repertoire. One of the more important phenotypical changes is associated with the expression of CD28, which serves as a hallmark for the age-related decline of T cell function (Godlove et al., 2007). This change can preferentially be detected in $CD8^+$ T cells that expand to $CD8^+CD28^-$ cells, while $CD4^+$ T cells acquire more of a Th17 cell phenotype (Montecino-Rodriguez et al., 2013). Undeterred by the fact that $CD4^+$ T cells seem to be more resistant to phenotypical and functional changes associated with age (Weinberger et al., 2007), $CD28^-$ $CD4^+$ T cells progressively emerge during the aging process (Goronzy et al., 2007). High numbers of Th17 cells result in an increased Th17/Treg ratio (Schmitt et al., 2013). This increased ratio is typical of an inflammatory environment. The described change in the altered function of Th17/Treg cells can be linked to an increased risk of autoimmunity (Dejaco et al., 2006).

Beside expressing a reduced diversity of antigen receptors, defective proliferation after antigen engagement, leading to an impairment of memory cell function, was described (Weng et al., 2009) and is regarded

as one of the reasons why elderly people fail to respond to vaccination (Saurwein-Teissl et al., 2002). Moreover, $CD8^+CD28^-$ T cells show different gene expression. Remarkably, this altered gene expression profile turns into a shift of protein homeostasis in T cells, thereby changing their response to co-stimulation, signalling, and transcription signals (Adibzadeh et al., 1995). Furthermore, T cells acquire natural killer receptors and show high levels of serine proteases (granzymes) and cytolytic proteins (perforins), explaining the increased cytotoxicity of these cells (Fann et al., 2005). In general, granzymes are pro-apoptotic enzymes, such as for instance granzyme B (Waugh et al., 2000), and are capable of cleaving proteins after acidic amino acid residues, particularly aspartic acid (Trapani, 2001). Granzymes are usually expressed by $CD8^+$ T cells and natural killer cells. $CD8^+$ T cells were shown to up-regulate expression of granzyme B after co-stimulation with concanavalin A and IL-2 (Grossman et al., 2004).

Recent studies defined T cell markers in immunosenescence of vaccinated elderly participants. Among these markers, telomerase expression levels, T cell receptor excision circle (TREC) frequency, $CD4^+/CD8^+$ ratio, and CD28 expression of $CD4^+$ and $CD8^+$ T cells in correlation to gene expression or regulation were tested. Although TRECs are solely generated as byproducts in the process of T cell receptor maturation, they can serve as well-accepted tools for clinical and research application. By measuring TREC contents, the thymic activity can be analysed, and conditions such as T cell immunodeficiencies, HIV infection, autoimmune diseases, reconstitution after bone marrow transplantation, and aging processes can be diagnosed and monitored (reviewed in Somech (2011)). TREC amounts also correlate with various signalling pathways, such as T cell receptor and p53 signalling. Moreover, miRNAs, which are associated with telomerase expression, were shown to be involved in controlling pathways responsible for immune functions, e.g., mTOR, p53, and MAPK signalling (Kennedy et al., 2016).

It is well known that Treg cells are important in maintaining tolerance and regulating the immune system after cessation of the immune response. However, the effect of aging and Treg cells is controversial. Development of Treg cells takes place in the thymus and declines during aging, suggesting that the Treg cell function is decreased in the elderly. In this context, Tsaknaridis et al. (2003) provided evidence that activity of human Treg cells during aging had an effect on the Treg cell function. Zhao et al. (2007) confirmed these results four years later in mice. In contrast, Garg et al. (2014) have demonstrated an increased Treg cell function in aged mice, and high amounts of Treg cells can be found in aged mice as well as in humans. This partially explains a higher risk of tumorigenesis in aged individuals, since cancer cells can use the suppressive environment to evade the immune system (Herrero et al., 2001; Gregg et al., 2005; Sharma et al., 2006). In summary, the balance between regulatory and effector T cells is crucial for a successful immune response and the ratio of regulatory/effector T cells can be used as a T cell aging marker due to their increased ratio during aging. The alterations in T cell population ratios are directly linked to pathological processes that underlie diseases and lead to lower humoral responses to infections, cancer cells, and vaccination (van der Geest et al., 2014).

3. B cells

Elderly people often show lower humoral responses to infections and vaccination. B cells derive from haematopoietic stem cells (HSCs) in the bone marrow (BM); however, HSCs functionally decline with age, the precursor B cell pool decreases, and the development of potential differentiating B cells is impaired (Miller and Allman, 2003; Tsuboi et al., 2004).

The microenvironment affects B cell activation and maturation. In senescence-accelerated mice, reduced production of TGF-β was observed in marrow stromal cells. Co-culture of stromal cells from young mice with pro-B cells from old mice led to the formation of lower numbers of mature B cells. Additionally, more pre-B cells were recovered in co-culture with stromal cells from young mice. Furthermore, neutralization of TGF-β was directed to higher proliferation of pro- and pre-B cells (Tsuboi et al., 2004). IL-7 belongs to B cell regulatory factors and is responsible for pro- and pre-B cell proliferation. BM stroma cells in aged mice produce less IL-7, as shown in non-senescence-accelerated mice (Updyke et al., 1993). It was reported that the response of B cell lineage precursors to IL-7 is limited because of defects in IL-7R signalling (Miller and Allman, 2003). IL-7 also inhibits expression of TGF-β, resulting in a reduced control of pre-B cell proliferation and differentiation. Interestingly, the numbers of precursor B cells were shown to decrease, while the amount of mature B cells was preserved during aging. Such findings support the suggestion that mature B cells live longer in aged mice (Tsuboi et al., 2004). An additional reason for weaker humoral responses in the elderly is a decreased interaction of T helper cells with B cells due to lower expression of CD154 (a ligand for CD40, CD40L), which explains a decrease in antibody diversity and function (Cancro et al., 2009). The interaction of CD40L on $CD4^+$ T helper cells with the CD40 surface molecule on B cells is an important mediator during B-lymphocyte differentiation. Impairment of CD40/CD40L activation can lead to modulation of humoral and cellular immunity (Lederman et al., 1994). Changes in the CD40/CD40L interaction are also linked to various diseases associated with the aging process, such as Alzheimer's disease (Giunta et al., 2010). Allmann and Miller (2005) concluded that the diversity of B cell receptors (BCRs) of pro-B cells decreases in older mice due to an impaired recombination potential of V, D, and J genes.

Overall, the B cell number does not decline with aging, and B cells are still able to produce antibodies. However, a shift occurs between $CD5^+$ (T cell-independ-

ent antibody release), $CD27^+$ (memory B cells), and $CD40^+$ (T cell-dependent antibody production) B cells toward reduced $CD5^+$ B cells and increased $CD27^+$ B cells (Colonna-Romano et al., 2003). This might be another reason why the elderly have a diminished response toward vaccination. Although antibody development and the BCR repertoire are altered, B cells from the elderly produce more IgM, lower their affinity for antigens, and redirect antibody specificity from foreign antigens to self-antigens ("auto-antibodies"). The latter process can lead to severe autoimmune diseases (Rowley et al., 1968; Dailey et al., 2001; Han et al., 2003). The changes described above can be related to an impaired class switch recombination (CSR).

Cepeda et al. (2018) recently reported an age-associated increase in the frequency of thymic B cells and significant changes in thymic B cell phenotype, which are caused both by intrinsic changes and changes in the thymic microenvironment. These changes are associated with significantly decreased induction of autoimmune regulator Aire, as well as reduced induction of Aire-dependent self-antigens.

During aging, the response of B cells to antigenic stimulation is reduced, and B cells produce fewer antibodies (Aydar et al., 2002). CD40 molecules interact with CD40L (CD154) present on T cells and are responsible for T cell-dependent antibody production. T cells of the elderly are deficient in CD40L as well as CD28, resulting in the lack of cooperation with B cells (Weyand et al., 1998). Despite the partial loss of $CD5^+$ B cells (Colonna-Romano et al., 2003), the prevalence of T-independent vs. T-dependent response is typical and important for maintaining immunity in senescence. Although B cells from aged mice have a decreased ability for antibody class switch (Frasca et al., 2007), this function is still preserved and independent of CD40L signalling. Collectively, the humoral arm of the immune system of the elderly is able to develop a T cell-independent antibody response to possibly compensate for T cell-dependent immunity.

4. Natural killer cells and natural killer T cells

Natural killer (NK) cells derive from $CD34^+$ common lymphoid progenitors (CLPs) in the BM. CLPs differentiate into pre-NK cell precursors (pre-NKP) and further to $CD56^{bright}$ immature (iNK) and $CD56^{dim}CD57^+$ mature NK (mNK) cells (Geiger and Sun, 2016). During the aging process, the ratio of iNK/mNK is shifted towards a mature phenotype. CD57 is hereby used as a marker of differentiation, which is increased in the elderly. The explosion of CD57 expression on the cell surface of NK cells is interpreted as a remodelling process of the NK population towards a differentiated state with reduced proliferation (Przemska-Kosicka et al., 2018).

Investigations of telomere length in human NK cells revealed that their average telomere length decreases during aging (Ouyang et al., 2007). Moreover, the telomere length is significantly higher in $CD56^{bright}CD16^-$ NK cells compared to $CD56^{dim}CD16^+$ NK cells, the latter representing a more differentiated state (Lu and Finkel, 2008).

Cytotoxic T lymphocytes (CTLs) eliminate virus-infected cells by recognizing viral-derived antigens presented by MHC I molecules. This is in contrast to NK cells, which eliminate virus-infected cells when MHC molecules are down-regulated (Karre, 2008). Natural killer T (NKT) cells, which share properties of both T cells and NK cells, have a restricted TCR diversity and recognize microbial as well as self-derived glycolipids presented by CD1d molecules. In comparison to a specific T cell response, which in general takes several days to develop, activated NKT cells react after minutes or hours, respectively, by secreting T cell cytokines addressed to Th1 (IFN-γ), Th2 (IL-4), or Th17 (IL-17) cytokines (Balato et al., 2009; Mallevaey and Selvanantham, 2012). Viral infection, e.g. by the herpesviridae family, activates NKT cells, which are critical for an antiviral immune response (Grubor-Bauk et al., 2003; Chung et al., 2015). NK cells in aged individuals are impaired in their function (Solana et al., 1999), leading to a higher risk of infection. NKT cells, on the other hand, probably compensate for the deregulation of NK cells in a very old population, indicating that NKTs might play an important role in the aged-related, impaired immune response against viral infections (Mocchegiani and Malavolta, 2004).

5. Professional antigen-presenting cells (APCs)

Invading pathogens are recognized by immune cells by identification of the pathogen-associated molecular patterns (PAMPs). Pathogens are internalized by phagocytosis and digested in a stepwise fashion. Antigen-presenting cells are then able to present fragments of a digested pathogen as foreign antigens on their surface by the antigen presenting receptor MHC. Generally, antigens are processed and presented via the MHC class I (MHC I) or the MHC II pathway. In the MHC I pathway, cytosolic antigens are shredded by several proteases from cytosolic sources and loaded to the MHC I molecule. The MHC I-peptide complex then traffics to the cell surface for inspection by CTLs (Rammensee, 2006). In the MHC II pathway, the antigen-processing event of exogenous antigens is performed by several classes of proteases belonging to cysteine (CatB, C, H, S, L, X, and AEP), aspartic (CatD and CatE), and serine proteases (CatA and CatG). T helper cells are activated via antigenic peptides loaded onto MHC II molecules. Furthermore, cytosolic antigens can be digested by cathepsins and loaded to MHC II during the process of autophagy, and there is a crosstalk between exogenous antigens and presentation to CTLs via MHC I in macrophages, DCs, and B cells (Muller et al., 2012).

During the process of infection, dendritic cells (DCs) play a key role in the activation of innate and adaptive

immunity, which is a prerequisite to eliminate infections. After cessation of an immune response, DCs prevent effector T cells from an uncontrolled activation status, which otherwise can cause harm. Additionally, DCs are important for both, self-tolerance and maintaining immune homeostasis (Banchereau and Steinman, 1998). Migration as well as phagocytosis, pinocytosis, and antigen capture in a receptor-dependent and -independent manner is diminished in aged monocyte-derived DCs (Agrawal et al., 2007b). Another set of observations revealed that lipopolysaccharide (LPS)-stimulated monocyte-derived DCs from elderly donors secrete higher amounts of TNF-α and IL-6, which accounts for impaired PI3 kinase (PI3K) signalling, initiating decreased migration and phagocytosis (Agrawal et al., 2007a). Plasmacytoid DCs (pDCs) from elderly people significantly reduce response to viruses and vaccines with decreased release of IFN-α, and are responsible for higher susceptibility to influenza infection (Jing et al., 2009).

Various studies demonstrated that the number of DCs did not change with advancing age and that the elderly harboured a similar surface marker profile compared to young individuals (Steger et al., 1996; Lung et al., 2000). This is in accordance with studies in mice, where no differences in the phenotype and amount of DCs were observed (Grolleau-Julius et al., 2006; Tesar et al., 2006). However, the levels of pDCs are reduced with age (Jing et al., 2009), indicating an additional reason for a declined immune response to viruses during aging. Although the phenotypes of DCs from young and aged individuals are comparable, DCs exhibit functional differences in the elderly, for instance, impaired phagocytic capacity. Moreover, compromised PI3K signalling possibly plays an important role in the uptake of antigens by phagocytosis (Clague et al., 1995). This decrease in the antigen uptake capacity of DCs can probably be linked to reduced T cell priming as well as impaired clearance of transformed, infected, or apoptotic cells.

As a result, the compromised clearance of apoptotic cells might contribute to inflammation and is associated with aging. Additionally, it was shown that DCs of aged subjects secrete more pro-inflammatory cytokines (IL-6 and TNF-α), which seem to contribute to chronic inflammation (Agrawal et al., 2007b). While migration is critical for the induction of an effective immune response, DCs in aged subjects showed a decreased migration ability (Gunn, 2003; Agrawal et al., 2007b). These findings are in contradiction with previously reported results, where the authors did not find any changes in the migration ability of DCs from the elderly compared to control DCs (Pietschmann et al., 2000). It is most likely that the described data was influenced by contamination of the DC population with other cells. Although differences in phagocytosis and cytokine secretion were detected, the DC migration capability in the elderly compared to young individuals is still a controversial matter.

6. Macrophages

Most of the tissue resident macrophages emerge from the embryonic yolk sac, whilst the rest of them develop by differentiation from monocytes, which are circulating innate immune cells. Their age-related dysfunction affects the pathology of a variety of chronic and infectious diseases. Recently, this dysfunction has been linked to down-regulation of the gene expression patterns related to cellular respiration (Metcalf et al., 2017). The actual impairment in the monocyte mitochondrial function is most significant during maximal respiration and highest oxidative energy demand, and it can be speculated that mitochondrial dysfunction leads to impairment of immune responses to distinct pathogens (Pence and Yarbro, 2018).

Macrophages directly eliminate pathogens and tumour cells and release cytokines to recruit other cells of the immune system. In addition, macrophages present antigens on MHC I and II molecules, which leads to activation of an adaptive immune response. The damaged tissue as well as apoptotic cells can be removed, or the damaged tissue can be renewed by regeneration processes induced by macrophages, implying that macrophages play an additional role in tissue homeostasis (Dalli and Serhan, 2017). Generally, macrophages of the M1 type infiltrate to the site of inflammation and can subsequently differentiate into two different cell types, M1 and M2 macrophages, which trigger a Th1 or a Th2 response, respectively. M1 macrophages are activated by IFN-γ and LPS, producing pro-inflammatory cytokines that trigger, for instance, a Th1 type immune response. On the other hand, M2 macrophages are generated when immature macrophages are stimulated. They are more active in the late stage of inflammation, remove the debris, control wound healing, are associated with a Th2 type response, and attract Th2 as well as Treg cells. Both M1 and M2 macrophages are not stably differentiated since they are able to change their phenotype depending on the microenvironment (Lloberas and Celada, 2002; Linehan and Fitzgerald, 2015; Isobe et al., 2017). Even though this review uses the two polarization predicaments M1 and M2 as definite classification states, it has to be noted that this terminology is fluid and the polarization must be seen as a continuum (Martinez and Gordon, 2014).

Importantly, the aging process polarizes macrophages towards the M2 phenotype (Linehan and Fitzgerald, 2015) and rises the number of this type of macrophages in the spleen, lymph nodes, and bone marrow of old mice (Jackaman et al., 2013). Macrophages express opsonin-dependent and opsonin-independent receptors, which bind various foreign molecular structures and are subsequently internalized in the so-called phagosome. The phagosome fuses with a lysosome and the content is proteolytically degraded, which leads to an activation cascade triggering different responses (Linehan and Fitzgerald, 2015). It is plausible that the number of macrophages, which play a pivotal role in the innate im-

mune response, is declined during immune aging. Indeed, macrophages from older mice exhibit a decreased ability to kill tumour cells, have a lower opsonization function, and showed reduced expression of toll-like receptors (TLRs) (Stahl and Brown, 2015).

Antigen presentation is one of the major roles of macrophages and is diminished in aged mice. After stimulation with IFN-γ, macrophages from aged mice display up to 50 % lower MHC II expression compared to macrophages from young mice (Linehan and Fitzgerald, 2015). This low expression of MHC II is not caused by mRNA degradation, but rather by low mRNA synthesis. Moreover, the level of prostaglandin E2 (PGE2) changes during aging and affects antigen presentation. Hayek et al. (1997) revealed that aged mice express more PGE2 than young mice. PGE2 can inhibit the IFN-γ activation pathway, thereby steadily decreasing antigen presentation. Moreover, it was illustrated that the amount of transcription factors, bound to the promoter of the MHC II gene, was decreased in macrophages from old, in contrast to young mice. Consequently, MHC II-mediated antigen presentation is impaired in macrophages from aged mice (Herrero et al., 2001).

7. Neutrophils

Neutrophils are the cells belonging to the innate immune system and initially participate in an immune response. Since immunity is impaired in the elderly, one might assume that either the functional efficiency declines or/and the number of neutrophils drops during aging. A reduction of the neutrophil count was actually postulated after analysing blood samples from young and elderly donors (De Martinis et al., 2004). However, these findings are in disagreement with published results indicating no alteration in total numbers of neutrophils during aging in the blood (Born et al., 1995) and neutrophil precursors in the bone marrow (Chatta et al., 1993). The observed discrepancies might account for different age categories used in the assay, as convincingly summarized by Domingues-Faria et al. (2016). In a more recent study, an increased neutrophil proportion was detected in lymphoid organs of healthy elderly mice, exhibiting several phenotypic differences compared to neutrophils from their younger counterparts, such as extended lifespan of neutrophils from elderly mice (Tomay et al., 2018).

In general, neutrophils circulate along blood vessels until they are recruited by chemoattractants, which are released during the invasion of pathogens. Subsequently, chemoattractans bind to the chemotactic receptor of neutrophils, N-formyl-methionyl-leucyl-phenylalanine (fMLP) receptor. The interaction triggers a conformational change, leading to activation of phospholipase C and PI3K and changing the actin cytoskeleton. Consequently, neutrophils adhere to endothelial cells and migrate to the site of invading pathogens (Cicchetti et al., 2002). Although chemotaxis seems to be impaired, neutrophils do not lose the adhesion and migration abilities during aging, suggesting that the reason of impaired chemotaxis may result from fMLP pathway alterations (Biasi et al., 1996; Esparza et al., 1996; Butcher et al., 2001).

The phagocytic ability of neutrophils is significantly reduced in older individuals, in contrast to younger counterparts, as implicated in several studies. Attempting to explain this phenomenon, Mege et al. (1988) suggested alterations in the receptor function. Two main receptors on neutrophils are responsible for phagocytosis: Fc receptors and complement receptors (CRs). The ligands for these receptors are opsonins, which mark invading pathogens and trigger phagocytosis (Mege et al., 1988). CD16 is a receptor for the Fc fragment of immunoglobulins. During aging, the density of CD16 on the cell membrane is decreased, indicating that diminished CD16 expression might be responsible for impaired phagocytosis (Butcher et al., 2001). On the other hand, the densities of Fc receptors and CRs do not change during aging and phagocytosis is not impaired. Instead, decreased killing of pathogens by neutrophils is attributed to an altered oxidative metabolism in these cells (Fülöp et al., 1985). Neutrophils can kill microorganisms through production of reactive oxygen species (ROS). The first correlation between neutrophils and oxygen was discovered in 1932, when neutrophils were shown to exhibit an increased uptake of oxygen when exposed to bacteria (Baldridge and Gerard, 1932). At that time, the authors did not have an explanation for these findings. Approximately 25 years later, Iyer et al. (1961) solved the mystery as they proved that stimulated neutrophils produced hydrogen peroxide, mainly generated by NADPH oxidase, for killing invading bacteria. NADPH oxidase can be activated by the fMLP pathway and is connected to lipid rafts. It was observed that the response of neutrophils from aged subjects to ligands of the fMLP receptor was diminished. Therefore, the production of ROS is impaired in elderly individuals, which might contribute to the decreased clearance of pathogens by neutrophils (Biasi et al., 1996). Oxidative stress caused by the production of ROS can also damage tissues of the host.

Furthermore, lower production of superoxide in the elderly can be beneficial bearing in mind that chemotaxis is gradually slowed down. In contrast, there is an association between the levels of free radicals and aging, because the release of oxygen metabolites leads to damage to cell molecules and boosts an age-related phenotype (Harman, 1972). This theory was strengthened by the suggestion that the ROS levels are increased in the elderly (Lu and Finkel, 2008). It is known that ROS can induce the nuclear factor κ B (NF-κB) pathway (Pantano et al., 2006). NF-κB regulates expression of various genes encoding mediators of the immune response, such as cytokines and chemokines involved in inflammatory and stress responses, cellular growth, antigen presentation, and additional biological processes, such as apoptosis (Yamamoto and Gaynor, 2001). As a result, down-regulation of ROS and NF-κB affects anti-

gen presentation, whereas up-regulation of ROS and NF-κB leads to production of high amounts of cytokines.

The decreased function during immunosenescence in neutrophils has several consequences. As their chemotactic ability is reduced, they move slowly to the site of infection, which impairs an effective immune response. Since neutrophils release proteases degrading tissue structures to support their migration, neutrophils can cause damage in the course of their movement (Barna and Kew, 1995). As a consequence, pathogen elimination is impaired by a declined phagocytic ability, ROS production, and antigen presentation, resulting in a less efficient function of the adaptive immune system.

In response to infections, the formation of neutrophil extracellular traps (NETs) represents an important strategy. NETs are highly decondensed DNA structures, also containing histones, proteases, and antimicrobial peptides. NETs are formed in the context of dying neutrophils, representing a new form of cell death called NETosis, a process which is still effective in elimination of bacteria. In old mice, reduced NET release was detected resulting from an age-related defect of Atg5 and subsequently impaired autophagy. While NETosis was decreased, an increase in apoptosis among neutrophils was ascertained (Xu et al., 2017).

A recent metabolomic study identified alterations in the neutrophil metabolism when comparing untreated neutrophils from young healthy donors with neutrophils from aged individuals. Among the investigated metabolites, a significant increase in the levels of NAD^+ (4- to 9-fold) and decreased levels of ATP (0.3-fold) and hypotaurin (0.8-fold) were detected. Taurin and hypotaurin are both important for neutrophil function by acting as antioxidants to prevent damage at the site of inflammation. NAD^+ is well known for its function in energy metabolism, but it is also required as a co-substrate for the sirtuin class of histone deacetylases. Therefore, an increased amount of NAD^+ could influence gene expression in the aged neutrophils. Low levels of ATP and high levels of NAD^+ might also indicate an increased energy demand or impaired transport of energy within neutrophils from elderly individuals (Richer et al., 2018).

8. Diet

Because many factors driving the aging processes are related to our modern lifestyle, changing our way of life can have beneficial effects. Well-balanced nutrition might have the property to modulate the immune cell function and ability to interfere with immunosenescence by restoring an aging immune system. By these measures, called nutritional immunology, an imbalance of mediators in age-related diseases in general can be corrected. A simple strategy to reverse immunosenescence and extend lifespan is caloric restriction, which is an inexpensive and non-invasive method without side-effects and was demonstrated in rodents as well as non-human primates. Caloric restriction reduces T cell senescence (Messaoudi et al., 2008), age-related involution of the thymus (Yang et al., 2009), and HSCs of caloric restriction mice showed significantly higher repopulation potential, higher self-renewal capacity, and the potential to generate cells of the lymphoid lineage (Tang et al., 2016). One additional possibility to interfere with aging of the immune system is the intake of vitamins. As an example, vitamin C can function as an anti-oxidant and might ameliorate the damage to proteins, DNA, and lipids, which is predominantly caused by ROS and naturally accumulates with age (Lee et al., 1998). Uchio et al. (2015) found that high dosages of vitamin C could also counteract thymic involution in the aged individuals, leading to an increase of naïve and memory T cell numbers. Furthermore, vitamin E holds anti-oxidant properties similarly as vitamin C (Burton and Traber, 1990). Previous research also indicated that vitamin E can be used to regulate functions of DCs, for example by up-regulation of anti-aging protein klotho, an important inhibitor of age-related phenotype changes (Kuro-o et al., 1997; Kurosu et al., 2005).

Conclusion

During its lifetime, a human organism is exposed to numerous harmful environmental factors, including reactive oxygen species, body injuries, pathogen-induced diseases, stressful lifestyle, or environmental contamination. The adverse impact of such factors gradually accumulates, also affecting immune cells and finally resulting in the process of immunosenescence. It is also obvious that immunosenescence is a physiologically normal process basically also affecting healthy individuals. Collectively, immunosenescence may be defined by changes in the phenotype and repertoire, as well as by deregulation in the function and metabolism of immunocompetent cells, causing a modified response to external and internal stimuli. As a consequence, the majority of elderly people are more susceptible to autoimmunity, infections, and cancer, resulting in higher mortality (Fig. 1). Along with aging, the mechanisms of protease activity, cytokine secretion, and inflammation are progressively deregulated.

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Authors' contributions

J. B., F. G., K. Z., R. K., C. S., E. H., T. B., U.K., and M. Z. wrote the paper. All the authors edited the paper and approved its final version.

Ethics approval and consent to participate

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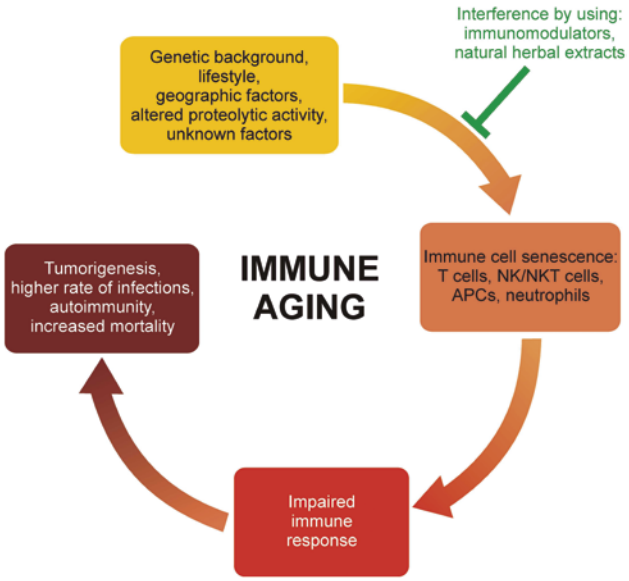

Fig. 1. The complexity and consequences of immune aging and possible preventive interventions

Competing interests

The authors declare that they have no competing interests.

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