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Review

Leukocyte function in the aging immune system

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ABSTRACT

Aging is associated with a progressive dysregulation of immune responses. Whether these changes are solely responsible for the observed increased mortality and morbidity amongst the elderly is uncertain. Recent advances have highlighted the age-associated changes that occur beyond T and B lymphocytes. Additionally, multiple human and animal studies have identified a relationship between chronic low-grade inflammation and geriatric syndromes, such as frailty, suggesting that the phenomenon of "inflamm-aging" may provide a rationale for the increased vulnerability to chronic inflammatory diseases in older adults. In the present review, we broadly summarize our current understanding of age-dependent changes in leukocyte function and their contribution to aging-related disease processes. ***J. Leukoc. Biol.*** **87: 1001–1009; 2010.**

Introduction

The immune system undergoes characteristic and multifaceted changes with aging. These changes occur in all leukocytes and accordingly, affect innate and adaptive immune functions. Although certain specific immune responses are diminished with aging, others are unaffected or even exacerbated. Paradoxically, a state of low-grade chronic inflammation is also commonly observed in the elderly. This chronic inflammatory state, referred to as "inflamm-aging", was first described when it was shown that PBMCs from aged people are able to produce higher amounts of proinflammatory cytokines than those from young subjects [1], an observation that on the surface, appears to defy the dominant hypothesis at the time—that elderly individuals are immunodepressed. Inflamm-aging results from exposure to continuous antigenic stress, ultimately leading to the up-regulation of cellular and molecular processes [2] and favoring susceptibility to age-related diseases. In the following sections, we will highlight the changes in leukocyte function during aging and their implications for the pathogenesis of age-associated diseases. It should be emphasized that in many instances, the reports in the literature are inconsistent and even contradictory. This may be attributed to the varying sources of the cells (such as humans or mice), culture conditions, and methodological approaches used in the studies. In the case of leukocytes that migrate to peripheral tissues, the effect of the local environment studied in vitro and the effect of the aged microenvironment in vivo may also account for discrepancies in the results. Similarly, comparing results from different human studies is difficult because of varying criteria for the selection of subjects, their comorbidities, and medication use.

Abbreviations: BM=bone marrow, cDC=conventional dendritic cell, DC=dendritic cell, LC=Langerhans cell, MO-DC=monocyte-derived dendritic cell, PAMP=pathogen-associated molecular pattern, pDC=plasmacytoid dendritic cell, RA=rheumatoid arthritis, ROS=reactive oxygen species, SDF-1=stromal-derived factor-1, Treg=regulatory T cell

GRANULOCYTES

Neutrophils

Neutrophils are key components of the inflammatory response that shape immune responses and participate in the breakdown and repair of tissue [3]. A growing body of data has implicated neutrophils in immunosurveillance and metastasis, but how aging-dependent changes in neutrophil function contribute to diseases in the elderly is incompletely understood. A recent review by Fortin et al. [4] highlights the role of neutrophils in age-related pathologies, such as Alzheimer's disease, atherosclerosis, cancer, and autoimmune diseases. Although no change in the number of mature, circulating neutrophils has been noted with aging, numerous functional changes have been described, but in almost all instances, these findings have been inconsistent (reviewed in ref. [5]). Although some investigators have observed impaired chemotaxis in human neutrophils, others failed to uncover any differences [6–10]. This discrepancy may be attributable to different chemotactic stimuli being used and whether the neutrophils were primed. For example, Fulop et al. [10] detected an age-dependent decrease in the chemotaxis of freshly isolated human neutrophils toward fMLP and GM-CSF but not LPS. Interestingly, priming

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with GM-CSF was able to enhance chemotaxis of young neutrophils significantly (compared with basal status without GM-CSF exposure) but unable to exert the same effect in old neutrophils [10]. This impairment in priming with GM-CSF has also been linked to defects in the respiratory burst of neutrophils [11]. Age-related changes in GM-CSF-induced signaling have been highlighted in a recent review [12]. Whether the production of ROS is altered in aged neutrophils remains a controversial issue [13, 14]. Ogawa et al. [15] recently detected an increase in spontaneous ROS production by aged human neutrophils but no difference in fMLP or LPS-induced ROS production. Studies by Fulop et al. [10] indicate that the kinetics of ROS production may be altered in aging, as superoxide anion levels from aged human neutrophils are lower than those from young neutrophils at early time-points (2 min and 24 h) but higher at later time-points (48 h). In a small study conducted in Spain involving young and middle-aged individuals as well as centenarians, superoxide anion concentrations in neutrophils were found to increase with age [16]. On the other hand, the neutrophils of centenarians contained superoxide anion levels that were comparable with those of young adults. In support of data that link activity of the antioxidant enzyme catalase to longevity in mice [17], the neutrophils of centenarians also displayed the highest catalase activity when compared with young and middle-aged subjects [16]. A limited number of studies have addressed age-dependent changes in murine neutrophil function. A report published by Nishio et al. [18] suggests that greater numbers of neutrophils are required for wound repair in old than in young mice, perhaps as a result of reduced neutrophil function. Although neutrophils have been implicated in the pathogenesis of atherosclerosis since the late 1980s, surprisingly, their presence in atherosclerotic lesions was only demonstrated recently in two widely used mouse models of atherosclerosis, the apolipoprotein E-deficient mouse and the low-density lipoprotein receptor-deficient mouse [19, 20]. Zernecke et al. [20] observed that expression of the chemokine receptor CXCR4 is up-regulated in murine neutrophils that have been "aged" in vitro [21–23], and its ligand CXCL12 (SDF-1) plays a pivotal role in the recruitment of neutrophils to atherosclerotic plaques. However, although up-regulation of CXCR4 expression has been established in human neutrophils that have been aged in culture [23], there is no published work that describes the expression of CXCR4 on neutrophils from aged individuals. Clearly, as atherosclerosis is considered primarily to be a disease associated with aging, further investigations aimed at unraveling how age-dependent changes in neutrophil function contribute to the pathogenesis of atherosclerosis demand our prompt attention.

Eosinophils and basophils

Eosinophils and basophils are effector cells of the innate immune system that play a central role in the pathogenesis of allergic inflammation and protective immunity in response to parasitic infections with helminths. Aged mice displayed greater airway eosinophilia in response to OVA challenge than young mice [24]. Conversely, airway hyper-responsiveness was reduced in aged mice, suggesting that eosinophil effector function declines with aging [24]. In contrast, Yagi et al. [25] found that aged rats failed to accumulate eosinophils in allergic inflammation of the airway. The only human study in asthma patients by Mathur et al. [26] indicates that airway eosinophilia is comparable in younger and older subjects. Degranulation of peripheral blood eosinophils in response to IL-5 (but not fMLP) was suppressed in elderly asthmatics, whereas adhesion and chemotaxis were unchanged. In addition, no significant difference in superoxide anion production was found between eosinophils from younger and older subjects following stimulation with PMA. However, age-dependent changes in functional characteristics of airway eosinophils (which are known to be distinct from peripheral blood eosinophils) were not examined in this study [26].

At present, our understanding of age-dependent effects on basophil development and in vivo function is incomplete [27]. In humans, IgE-mediated releasability of histamine and basophil sensitivity to anti-IgE increase with age [28]. Emerging findings that basophils can support humoral memory responses, drive IgE-dependent dermatitis, and shift an immune response toward Th2-dominated inflammation have resulted in a resurgence in interest in these granulocytes [29]. Furthermore, it was demonstrated recently that basophils readily generate large quantities of Th2 cytokines such as IL-4 and IL-13 in humans and mice [30]. These cytokines are central regulators in conditioning Th2-type immune responses. Basophil-derived IL-4 has been shown to drive the differentiation of naïve murine CD4 T cells to Th2 cells [31]. Basophils also stimulate B cells to synthesize IgE in vitro in an IL-4- and CD40 ligand-dependent manner. These newly identified functions of basophils clearly suggest that their role in aging-dependent immune responses and pathological processes necessitates further investigation.

MONOCYTES/MACROPHAGES

After leaving the BM and entering the blood, monocytes differentiate continuously into macrophages en route to peripheral tissues. Aging-associated changes in monocyte and macrophage function have been recognized as major contributors to dysregulation of the immune system in a variety of disease processes. Recently, the hypothesis that macrophage populations are distinctly heterogenous, and age-dependent changes in macrophage function occur in a tissue-specific manner has gained widespread acceptance [32, 33]. Macrophages are highly plastic, and the tissue microenvironment in which they reside as well as the aging process can modify their function continuously. Dace and Apte [32] observed in a murine model of age-related macular degeneration that macrophages shift from an antiangiogenic, proinflammatory phenotype dominant in young animals to a proangiogenic, anti-inflammatory phenotype present in old mice. IL-10 was identified as a key mediator of this switch, and concentrations of this cytokine are elevated in

macrophages from aged rodents and humans [32]. Kelly et al. [34] reported that in mice, aging is associated with enhanced ocular angiogenesis following laser injury, which is linked to increased macrophage IL-10 production.

TLRs are expressed on a variety of different cell types including classic APCs, such as DCs, monocytes, and macrophages, as well as on B, epithelial, and endothelial cells [35]. They are activated by recognizing phylogenetically well-preserved PAMPs [35]. Recent studies indicate that signaling via TLR1/2 heterodimers, as well as TLR-induced costimulatory expression in monocytes, is reduced significantly in the elderly [35]. These defects may contribute to the impaired phagocytosis of infectious agents and diminished response to vaccinations that are associated with aging. Investigations of age-dependent changes in macrophage function have yielded inconsistent findings regarding production of the cytokines TNF-α and IL-6, expression of cyclooxygenase-2, phagocytosis, and chemotaxis [5]. When comparing macrophages derived from the BM of young and old mice, Sebastián et al. [36] found recently that telomeres shorten with age. Telomeres are tandem hexanucleotide repeats capped by telomere DNA-binding proteins at the ends of eukaryotic chromosomes. With each duplication of the chromosome, a small segment of the telomere is lost, and when the telomeres reach a critically short length, cells lose the ability to divide, thus entering a state of replicative senescence, a phase in which their functions and activities change. Hence, telomere shortening during cell division represents a molecular clock that triggers the entry of cells into senescence [37]. In macrophages, telomere shortening leads to decreased proliferation in response to GM-CSF but not M-CSF as a result of decreased phosporylation of the transcription factor STAT5a. Macrophages from old mice were more vulnerable to oxidants and had higher concentrations of intracellular ROS than those from their young counterparts [36]. Oxidation of STAT5a is required for its phosphorylation and was reduced in macrophages of aged mice. Interestingly, macrophages from telomerase knockout mice displayed the same phenotype as aged macrophages, suggesting that telomere shortening may cause enhanced oxidative stress, reduced STAT5a oxidation, and phosphorylation, which ultimately, result in the impaired GM-CSF-dependent macrophage proliferation [36]. For a comprehensive review of aging-related phenotypic and functional changes of circulating monocytes, the reader is referred to a recent book chapter by Ginaldi and De Martinis [38] and the references cited therein. Age-related effects on macrophage polarization and their implications for angiogenesis have been reviewed by Dace and Apte [32].

DCs

DCs are a sparsely distributed, heterogeneous, BM-derived population of potent APCs that can be divided into two major categories: those present in peripheral blood (including cDCs and pDCs) and those present in tissues/organs including LCs in the skin, interstitial DCs in tissues, and interdigitating DCs in the thymus and other lymphoid organs [39].

More than three decades after their discovery, DCs have now emerged as key modulators of a repertoire of immune processes, including immune surveillance, induction of immunological tolerance, regulation of T cell immune responses, autoimmunity, and antimicrobial immunity [40, 41]. The diverse functions of DCs depend on the heterogeneity of DC subsets and their functional plasticity, as well as their location and activation or maturation status [42–44].

In contrast to the rapidly increasing amount of knowledge pertaining to DC physiology in young individuals, relatively little is known about DCs in aged humans and animals. The following sections summarize the currently available studies. It is important to note that the results are often contradictory and difficult to compare as the origin of the cells, their culture conditions, and maturation protocols vary greatly. Standardization of such culture procedures and more studies in frail and healthy elderly are necessary to achieve a consensus regarding the effect of aging on DC-mediated immune responses.

Number

A decreased density of LCs has been described consistently in the aged murine and human skin (recently reviewed in refs. [45, 46]). Several studies were performed to address the question of whether the number of human pDCs and cDCs was reduced in old age. Using DC subset-specific cell-surface markers and flow cytometry analysis, two recent reports showed a significant age-related numerical decline of pDCs but unchanged cDC numbers in peripheral blood from healthy young and elderly donors [47, 48]. Interestingly, Jing et al. [47] found that the preserved cDC frequency with age among healthy subjects can be impacted profoundly and negatively by declining health status in the elderly. The reduced pDC and unaltered cDC numbers in aged donors have been reported in at least three other studies for each subset (refs. [49–51] and [52–54], respectively), whereas two other groups have reported no difference in the number of pDCs with aging [55, 56]. The use of Ficoll-enriched cells versus whole blood, as well as differences in sample population, sample size, and number of events acquired during the flow cytometry analysis may contribute to the inconsistent findings with age in those studies.

With respect to ex vivo-derived DCs, we [57] and others [52, 54, 58, 59] have found no age-associated changes in the number and morphology of cDCs generated from human blood MO-DCs or murine BM-DCs in the presence of GM-CSF and IL-4.

Migration

The migration of mature DCs that have encountered antigen in peripheral tissues to the secondary lymphoid organs, where they can induce an immune response, is dependent on chemokine signaling between lymphoid homing receptor CCR7 expressed by DCs and its ligands expressed by the microenvironment. Findings from our laboratory [60] show that despite similar levels of expression of CCR7, aged murine BM-DCs have an impaired capacity, not only to migrate in vitro in response to the CCR7 ligand secondary lymphoid

tissue chemokine but also to be recruited in vivo to draining lymph nodes. This was associated with a defect in CCR7 signal transduction. Agrawal et al. [55] reported similar defective in vitro chemokine-dependent migration of human MO-DCs using MIP-3β, another CCR7 ligand, and attributed their results to defects in the downstream signaling pathway, possibly in the PI3K pathway. However, in another study, Pietschmann et al. [53] found that the in vitro migration of human MO-DCs, through an endothelial monolayer, was unimpaired. Whether the migration of LCs is affected by the aging process also remains controversial. Although some reports indicate reduced LC migration in response to allergen in aged mice [61] and TNF-α in the elderly [62], others demonstrate that the capacity of LCs to migrate from the skin to the draining lymph nodes was not impaired in aged mice [63]. This suggests a selective effect of aging on DC responsiveness to the signals involved in the migratory process.

Phagocytosis

Although a reduction in the phagocytic capacity of DCs with age would not only result in reduced clearance of infections but would also contribute to age-associated loss of peripheral self-tolerance and chronic inflammation, data about the effect of aging on this DC function are limited. The only human study by Agrawal et al. [55] described decreased potential for micropinocytosis of Lucifer Yellow dye, endocytosis of dextran beads, and phagocytosis of apoptotic Jurkat T cells in MO-DCs. The authors proposed that the observed, reduced phagocytosis in aged DCs is a result of an impaired PI3K signaling pathway via decreased phosphorylation of AKT. Paula et al. [64] reported that phagocytosis of necrotic B16F10 melanoma tumor cells was unaffected by age in BM-DCs from C57BL/6 mice. Using the same strain of mice, we found [60] no significant changes in the uptake of whole OVA protein or OVA peptides by BM-DCs. Notably, decreased expression of C-type lectin receptors, CD205 and DC-specific/intracellular adhesion molecule type 3-grabbing nonintegrin, in murine aged DCs has been reported by our group [57, 60] and others [65]. However, the consequences of this altered expression for DC-mediated phagocytosis remain to be ascertained.

Maturation

DC maturation is dependent on the binding of PAMPs derived from bacteria, fungi, parasites, and viruses to TLRs [66]. Although TLR expression by murine BM-DCs or splenic DCs seems to remain unaltered with age [67], there is some controversy regarding the responsiveness of these cells to maturation stimuli in old age, as measured in terms of phenotypic modification and cytokine production (reviewed in ref. [60]). Similarly, no consensus has been reached yet concerning DCs from elderly individuals [45, 46]. Two recent studies have focused on the impact of aging on the ability of human pDCs to secrete type I IFN in response to viruses. Both revealed decreased IFN-α production in response to influenza virus [47] and HSV-2 stimulation [68], resulting in defective host responses to viral infections. This was associated with increased oxidative stress and decreased TLR-7 and TLR-9 expression/signaling pathways. Because of the importance of pDCs in antiviral responses, a functional decline of pDCs is likely to contribute to the age-related decrease in the Th1 response to influenza and the impaired immune response to viral infections in the elderly.

T cell stimulatory properties and consequences for DC-based immunotherapies

No consensus has been reached yet regarding the impact of aging on DC capacity to induce T cell responses. Whereas some studies [64, 69], including ours [57, 60], have reported a decline in class I and/or class II antigen presentation capacity, others [52, 54, 58, 63] have described no change or even an increase. DCs represent useful tools for the development of novel immunotherapies, in particular, as carriers of tumor vaccines or other immunization regimes. Our group and others (recently reviewed in ref. [60]) have investigated whether aging affects DC anti-tumor activity and found that DC-based vaccination was undermined in aging as a result of poor priming of tumor antigen-specific T cells as well as other DC age-related changes. Importantly, Sharma et al. [70] showed that protective anti-tumor immune responses in mice could be restored with appropriate costimulation. An original study by Moretto et al. [71] also demonstrated that aging affects the ability of mucosal murine DCs to mount a robust T cell response against *Encephalitozoon cuniculi*, an intracellular parasite. The authors observed that old murine DCs have a defect in IL-15 induction and that treatment of the cells with this cytokine has the potential to restore their function. These studies indicate that aged DCs can be exploited for the induction of optimal immunity and consequently, lead to more effective therapies in the elderly.

T CELLS

Increase in memory T cells and decrease in naïve T cells

Memory and naïve human T cells are often distinguished based on their expression of members of the CD45 family of surface antigens. Thus, CD45RA antigen is expressed primarily on naïve T lymphocytes, and CD45RO is present on the cell surface of a primed population of memory T lymphocytes. With normal aging, the slow turnover and long lifespan of naïve T cells are preserved [72], but thymus output diminishes gradually and ultimately, becomes insufficient to replace naïve T cells lost from the periphery and to maintain the breadth of the T cell repertoire [73, 74]. Conversely, cumulative exposure to foreign pathogens and environmental antigens promotes the accumulation of memory T cells with age [75]. Thus, elderly individuals have more $CD29^+$ and $CD45RO^+$ and less $CD45RA^+$ peripheral blood CD4 and CD8 cells. The natural decline in the number of naïve T cells coupled with the narrowing of the T cell repertoire has profound consequences for immune function, rendering the elderly less responsive to immune stimulation and vaccination, as well as predisposing them to cancer [76]. For example, a higher ratio of

memory T cells and Treg lymphocytes versus naïve T cells has been observed in aged SJL/J mice, which are prone to Hodgkin's-like lymphoma [77]. The resulting "shrunken", naïve T cell pool leads to an age-associated imbalance, which may contribute to the occurrence of lymphoma in this mouse model. A recent review by Nikolich-Zugich [78] summarizes the state-of-the-art knowledge concerning aging-dependent changes in T cell subsets and their relationship to infections with latent, persistent pathogens. In addition to the accumulation of memory T cells with age, selected changes in lymphocyte turnover have been reported in animal and human aging. For example, there is a marked reduction in turnover of memory CD8 in mice [79]. This may be explained partially by the antiproliferative effect of increased Bcl-2 expression and high levels of type-1 IFNs in aging. Contraction of the naïve T cell repertoire was shown to result in impaired CD8 T cell responses in a mouse infection model [80]. In humans, there is evidence of significant contraction of the naïve human CD4 T cell repertoire in the seventh and eighth decade of life [74]. By examining the uptake of deuterated glucose into cells, Wallace et al. [72] showed that surprisingly, naïve and memory T cell and Treg subsets have similar turnover in young and old people. The investigators also found that CD8 memory cells have a much longer half-life than other T cell subsets in the elderly. Furthermore, these persistent cells include large clonal populations.

Impaired IL-2 production and proliferation

T cell proliferation and growth are induced by IL-2, but as T cells age, they lose their capacity to produce and respond to IL-2. When exposed to antigen, memory T cells will divide and proliferate rapidly to elaborate more T cell clones but only proliferate upon stimulation with IL-2. If insufficient concentrations of IL-2 are produced or if T cells cannot respond effectively to IL-2, T cell function is greatly impaired. Age-related impairments in the activation of transcription factors AP-1 and NF-AT have been closely associated with decreased expression of IL-2 by human T cells [81–83]. Additional to the aberrant cytokine response, naïve T cells in aged humans and rodents display multiple defects in early signaling events and impaired calcium influx compared with their younger cohorts [84].

T cell migration

In RA, migration of T cells to the synovium is facilitated by a number of chemokines, including MIP-1α and SDF-1. In addition to promoting angiogenesis and degradation of cartilage matrix by stimulating release of matrix metalloprotease-13 from human chondrocytes [85], SDF-1 inhibits activation-induced apoptosis of T cells [86]. MIP-1α, present at elevated concentrations in synovial fluid when compared with serum of patients with RA, has been implicated in macrophage recruitment to the rheumatoid synovium. Recent work from our laboratory [87] indicates that expression of selected proinflammatory chemokines and chemokine receptors is increased in aged human and murine T cells. The enhanced proinflammatory T cell chemotactic responses may play a role in the pathogenesis of inflammatory diseases in aging, including cardiovascular diseases and autoimmunity. Conversely, the decrease in T cell CCR7 expression and function, in part related to the age-related decline in naïve T cell populations, may explain the observed defective T cell homing to secondary lymphoid organs in aging.

Tregs and Th17 cells

Whether the number of $CD4^+CD25^{hi}$ Tregs changes during aging is uncertain. Although most studies report no correlation between circulating Treg numbers and aging, a select few have demonstrated a positive association [88, 89]. It has been suggested that aged Tregs inhibit CD8 cell cytotoxicity via reduced production of IFN and perforin. An increase in Treg numbers or function may therefore provide a potential mechanism for the impaired immune response seen in older adults. Although the effect of aging on human Th17 cells remains uncertain, studies in rats have revealed that protein expression of IL-17 is elevated in coronary arteries of old animals compared with those of young animals [90]. Moreover, elevated concentrations of IL-17 and TNF-α have been detected in the serum and synovial fluid of patients with RA and osteoarthritis compared with healthy controls [91].

Decline of Th1 and enhanced Th2 response

A number of studies suggest an imbalance between Th1 and Th2 responses in aging. However, the nature of this imbalance remains an issue of significant controversy. Some, but not all, published data support the notion that human aging is associated with a decline of Th1 and enhancement of Th2 immune responses [92–94]. In contrast, studies in mice have generally provided evidence for enhanced Th1 and impaired Th2 immune responses [95, 96]. Most recently, Uciechowski et al. [97] reported a decreased Th2/Th1 ratio in old compared with young individuals. These researchers also concluded that zinc deficiency is common in the elderly and that moderate zinc supplementation is linked to an increase in Th2 cells.

APOPTOSIS

Aging affects all three major signaling pathways of apoptosis in T cells (recently reviewed by Gupta [98]). The effect of aging on the lymphocyte CD95 (APO/Fas) apoptotic signaling pathway was studied by Potestio et al. [99]. This group of researchers found increased CD95 expression in humans up to the age of 75 years, followed by a decline in CD95 expression in CD4 but not CD8 T cells from the very old [99]. The increase in CD95 expression may be, in part, secondary to the more activated state of old T cells and correlates to enhanced apoptosis [99, 100]. The functional consequences of these T cell changes are unclear, as CD8 cells from aged individuals have increased perforin expression that would suggest that they may also have preserved cytotoxic function [101]. Current data by Gupta and Gollapudi [102] indicate that aging-associated deficiencies in naïve and central memory subsets of $CD4^+$ and $CD8^+$ T cells may be linked to their increased sensitivity to CD95-mediated apoptosis.

T cell immune replicative senescence

T lymphocytes, like all human somatic cells, have a finite, proliferative lifespan that is determined by telomere length. Age-dependent decreases in telomere length have been reported in B cells and T cells [103–107]. At birth, virtually all human T cells express CD28, a costimulatory receptor that plays a pivotal role in their antigen-mediated activation, proliferation, and survival [108]. It has been observed that human aging is associated with a decreased ratio of $CD28^+/CD28^-$ T cells, particularly in the CD8 subset. The loss of CD28 expression is a marker of T cell replicative senescence [109–111], although not all $CD28^-$ cells are senescent cells. In addition to loss of CD28 gene expression and shortened telomeres, cultured CD 8 T cells that are subjected to repeated cycles of antigen-driven proliferation also eventually display irreversible cell-cycle arrest, apoptosis resistance, as well as reduced expression of major stress proteins in response to heat shock (reviewed in ref. [112]). Interestingly, compared with the "young-old" population (65 years and above), successfully aged nonagenarians have a similar number of $CD28^+$ and $CD28^-$ cells but have better preservation of CD8 T cell functions, including activation-induced cell death [113]. A recent review by Weng et al. [108] highlights advances in our understanding of $CD28^-$ T cells and their role in the age-associated decline of immune function. Short telomeres in peripheral blood T lymphocytes as well as granulocytes have been reported in RA [114], supporting the contention that RA is associated with intrinsic abnormalities in telomere length and may represent a form of premature aging. Recent work using HIV-specific CD8 cells has suggested that telomerase-based therapeutic approaches may prevent or at least retard replicative senescence [115].

NKT cells

NKT cell cytotoxic function declines in humans and rodents with age [116–119], despite the fact that T cells acquire many markers of NK cells (including CD16, CD56, CD57, and CD94) over one's lifetime. Thus, it was shown that T cells from older human adults with these NK cell markers are hyporesponsive to antigen stimulation [117]. These changes may contribute to the age-dependent, increased susceptibility to parasitic and viral infections [118, 119]. For a detailed analysis of age-related changes in NKT cell function, the interested reviewer is referred to several recent reviews by Panda et al. [120], Mocchegiani et al. [121, 122], and Peralbo et al. [123].

B CELLS

Memory B lymphocytes express the surface marker CD27. Colonna-Romano et al. [124] observed that the percentage of $CD27^+$ cells was slightly elevated in the elderly and that serum concentrations of IgD correlate negatively with $CD27^+$ B cells. Naïve B cell (IgD^+CD27^-) numbers, on the other hand, were reduced significantly in the elderly. In contrast to this report, Shi et al. [125] found that memory B cells decline with age, whereas Chong et al. [126] reported that numbers of memory B cells decrease, and those of naïve B cells increase with age. Although the overall number of B cells is relatively stable across a human lifespan, there are clear changes in B cell generation and repertoire. In general, this results in a shift in the antibody specificity away from foreign to autologous antigens. Furthermore, there is an associated narrowing of the diversity of the B cell antibody response resulting in the impaired ability of the aging immune system to generate high-affinity antibodies, the appearance of mAb, and clonal B cell expansion. Clinical consequences of these changes include impaired responses to infection, cancer cells, vaccination, and the potential for late-life B cell lymphomas. Using DNA samples from peripheral blood, work by Gibson et al. [127] revealed that the dramatic collapse of B cell repertoire diversity in the elderly correlated strongly with general health status and was a marker of frailty. Recent reviews by Cancro et al. [128], Siegrist and Aspinall [129], and Caruso et al. [130] focus on aging-mediated changes in B cells and their responses to vaccination, respectively.

CONCLUSION

Understanding immune system changes in aging has important implications as humans pursue longevity and at the same time, aim to reduce the burden of disease that comes with aging. Recent advances in the field should allow researchers to devise novel strategies to prevent and treat ailments that preferentially afflict the elderly, such as infectious diseases and cancer. The realization that old age is associated with a state of low-grade inflammation that provides the background for susceptibility to diverse diseases also presents a new avenue for research into reversing these changes and to improve the outcome of chronic inflammatory diseases in the elderly.

REFERENCES

1. Fagiolo, U., Cossarizza, A., Scala, E., Fanales-Belasio, E., Ortolani, C., Cozzi, E., Monti, D., Franceschi, C., Paganelli, R. (1993) Increased cytokine production in mononuclear cells of healthy elderly people. *Eur. J. Immunol.* **23,** 2375–2378.
2. Franceschi, C. (2007) Inflammaging as a major characteristic of old people: can it be prevented or cured? *Nutr. Rev.* **65,** S173–S176.
3. Nathan, C. (2006) Neutrophils and immunity: challenges and opportunities. *Nat. Rev. Immunol.* **6,** 173–182.
4. Fortin, C. F., McDonald, P. P., Lesur, O., Fulop Jr., T. (2008) Aging and neutrophils: there is still much to do. *Rejuvenation Res.* **11,** 873–882.
5. Gomez, C. R., Nomellini, V., Faunce, D. E., Kovacs, E. J. (2008) Innate immunity and aging. *Exp. Gerontol.* **43,** 718–728.
6. Niwa, Y., Kasama, T., Miyachi, Y., Kanoh, T. (1989) Neutrophil chemotaxis, phagocytosis and parameters of reactive oxygen species in human aging: cross-sectional and longitudinal studies. *Life Sci.* **44,** 1655–1664.
7. Wenisch, C., Patruta, S., Daxbock, F., Krause, R., Horl, W. (2000) Effect of age on human neutrophil function. *J. Leukoc. Biol.* **67,** 40–45.
8. Biasi, D., Carletto, A., Dell'Agnola, C., Caramaschi, P., Montesanti, F., Zavateri, G., Zeminian, S., Bellavite, P., Bambara, L. M. (1996) Neutrophil migration, oxidative metabolism, and adhesion in elderly and young subjects. *Inflammation* **20,** 673–681.
9. Esparza, B., Sanchez, H., Ruiz, M., Barranquero, M., Sabino, E., Merino, F. (1996) Neutrophil function in elderly persons assessed by flow cytometry. *Immunol. Invest.* **25,** 185–190.
10. Fulop, T., Larbi, A., Douziech, N., Fortin, C., Guerard, K. P., Lesur, O., Khalil, A., Dupuis, G. (2004) Signal transduction and functional changes in neutrophils with aging. *Aging Cell* **3,** 217–226.
11. Tortorella, C., Polignano, A., Piazzolla, G., Serrone, M., Jirillo, E., Antonaci, S. (1996) Lipopolysaccharide-, granulocyte-monocyte colony stimulating factor and pentoxifylline-mediated effects on formyl-methio-

nyl-leucine-phenylalanine-stimulated neutrophil respiratory burst in the elderly. *Microbios* **85,** 189–198.
12. Tortorella, C., Simone, O., Piazzolla, G., Stella, I., Antonaci, S. (2007) Age-related impairment of GM-CSF-induced signaling in neutrophils: role of SHP-1 and SOCS proteins. *Ageing Res. Rev.* **6,** 81–93.
13. Fulop Jr., T., Foris, G., Worum, I., Paragh, G., Leovey, A. (1985) Age related variations of some polymorphonuclear leukocyte functions. *Mech. Ageing Dev.* **29,** 1–8.
14. Lord, J. M., Butcher, S., Killampali, V., Lascelles, D., Salmon, M. (2001) Neutrophil aging and immunesenescence. *Mech. Ageing Dev.* **122,** 1521–1535.
15. Ogawa, K., Suzuki, K., Okutsu, M., Yamazaki, K., Shinkai, S. (2008) The association of elevated reactive oxygen species levels from neutrophils with low-grade inflammation in the elderly. *Immun. Ageing* **5,** 13.
16. Alonso-Fernandez, P., Puerto, M., Mate, I., Ribera, J. M., de la Fuente, M. (2008) Neutrophils of centenarians show function levels similar to those of young adults. *J. Am. Geriatr. Soc.* **56,** 2244–2251.
17. Brown-Borg, H. M., Rakoczy, S. G. (2000) Catalase expression in delayed and premature aging mouse models. *Exp. Gerontol.* **35,** 199–212.
18. Nishio, N., Okawa, Y., Sakurai, H., Isobe, K. (2008) Neutrophil depletion delays wound repair in aged mice. *Age (Dordr.)* **30,** 11–19.
19. Van Leeuwen, M., Gijbels, M. J., Duijvestijn, A., Smook, M., van de Gaar, M. J., Heeringa, P., de Winther, M. P., Tervaert, J. W. (2008) Accumulation of myeloperoxidase-positive neutrophils in atherosclerotic lesions in LDLR−/− mice. *Arterioscler. Thromb. Vasc. Biol.* **28,** 84–89.
20. Zernecke, A., Bot, I., Djalali-Talab, Y., Shagdarsuren, E., Bidzhekov, K., Meiler, S., Krohn, R., Schober, A., Sperandio, M., Soehnlein, O., Bornemann, J., Tacke, F., Biessen, E. A., Weber, C. (2008) Protective role of CXC receptor 4/CXC ligand 12 unveils the importance of neutrophils in atherosclerosis. *Circ. Res.* **102,** 209–217.
21. Martin, C., Burdon, P. C., Bridger, G., Gutierrez-Ramos, J. C., Williams, T. J., Rankin, S. M. (2003) Chemokines acting via CXCR2 and CXCR4 control the release of neutrophils from the bone marrow and their return following senescence. *Immunity* **19,** 583–593.
22. Sainz, J., Sata, M. (2008) Open sesame! CXCR4 blockade recruits neutrophils into the plaque. *Circ. Res.* **102,** 154–156.
23. Weisel, K. C., Bautz, F., Seitz, G., Yildirim, S., Kanz, L., Mohle, R. (2009) Modulation of CXC chemokine receptor expression and function in human neutrophils during aging in vitro suggests a role in their clearance from circulation. *Mediators Inflamm.* **2009,** 790174.
24. Busse, P. J., Zhang, T. F., Srivastava, K., Schofield, B., Li, X. M. (2007) Effect of aging on pulmonary inflammation, airway hyperresponsiveness and T and B cell responses in antigen-sensitized and -challenged mice. *Clin. Exp. Allergy* **37,** 1392–1403.
25. Yagi, T., Sato, A., Hayakawa, H., Ide, K. (1997) Failure of aged rats to accumulate eosinophils in allergic inflammation of the airway. *J. Allergy Clin. Immunol.* **99,** 38–47.
26. Mathur, S. K., Schwantes, E. A., Jarjour, N. N., Busse, W. W. (2008) Age-related changes in eosinophil function in human subjects. *Chest* **133,** 412–419.
27. Ohnmacht, C., Voehringer, D. (2009) Basophil effector function and homeostasis during helminth infection. *Blood* **113,** 2816–2825.
28. Marone, G., Giugliano, R., Lembo, G., Ayala, F. (1986) Human basophil releasability. II. Changes in basophil releasability in patients with atopic dermatitis. *J. Invest. Dermatol.* **87,** 19–23.
29. Gurish, M. F. (2009) The expanding universe of the basophil. *Blood* **113,** 2616.
30. Moore, M. L., Newcomb, D. C., Parekh, V. V., Van Kaer, L., Collins, R. D., Zhou, W., Goleniewska, K., Chi, M. H., Mitchell, D., Boyce, J. A., Durbin, J. E., Sturkie, C., Peebles Jr., R. S. (2009) STAT1 negatively regulates lung basophil IL-4 expression induced by respiratory syncytial virus infection. *J. Immunol.* **183,** 2016–2026.
31. Sokol, C. L., Barton, G. M., Farr, A. G., Medzhitov, R. (2008) A mechanism for the initiation of allergen-induced T helper type 2 responses. *Nat. Immunol.* **9,** 310–318.
32. Dace, D. S., Apte, R. S. (2008) Effect of senescence on macrophage polarization and angiogenesis. *Rejuvenation Res.* **11,** 177–185.
33. Stout, R. D., Suttles, J. (2005) Immunosenescence and macrophage functional plasticity: dysregulation of macrophage function by age-associated microenvironmental changes. *Immunol. Rev.* **205,** 60–71.
34. Kelly, J., Ali Khan, A., Yin, J., Ferguson, T. A., Apte, R. S. (2007) Senescence regulates macrophage activation and angiogenic fate at sites of tissue injury in mice. *J. Clin. Invest.* **117,** 3421–3426.
35. Van Duin, D., Shaw, A. C. (2007) Toll-like receptors in older adults. *J. Am. Geriatr. Soc.* **55,** 1438–1444.
36. Sebastián, C., Herrero, C., Serra, M., Lloberas, J., Blasco, M. A., Celada, A. (2009) Telomere shortening and oxidative stress in aged macrophages results in impaired STAT5a phosphorylation. *J. Immunol.* **183,** 2356–2364.
37. Iancu, E. M., Speiser, D. E., Rufer, N. (2008) Assessing aging of individual T lymphocytes: mission impossible? *Mech. Ageing Dev.* **129,** 67–78.
38. Ginaldi, L., De Martinis, M. (2009) Phenotypic and functional changes of circulating monocytes in elderly. In *Handbook on Immunosenescence* (T. Fulop, C. Franceschi, K. Hirokawa, G. Pawelec, eds.), New York, NY, USA, Springer Science, 511–528.
39. Takeuchi, S., Furue, M., Takeuchi, S., Furue, M. (2007) Dendritic cells: ontogeny. *Allergol. Int.* **56,** 215–223.
40. Steinman, R. M., Banchereau, J. (2007) Taking dendritic cells into medicine. *Nature* **449,** 419–426.
41. Banchereau, J., Steinman, R. M. (1998) Dendritic cells and the control of immunity. *Nature* **392,** 245–252.
42. Ueno, H., Klechevsky, E., Morita, R., Aspord, C., Cao, T., Matsui, T., Di Pucchio, T., Connolly, J., Fay, J. W., Pascual, V., Palucka, A. K., Banchereau, J. (2007) Dendritic cell subsets in health and disease. *Immunol. Rev.* **219,** 118–142.
43. Klechevsky, E., Liu, M., Morita, R., Banchereau, R., Thompson-Snipes, L., Palucka, A. K., Ueno, H., Banchereau, J. (2009) Understanding human myeloid dendritic cell subsets for the rational design of novel vaccines. *Hum. Immunol.* **70,** 281–288.
44. Ueno, H., Klechevsky, E., Morita, R., Aspord, C., Cao, T., Matsui, T., Di Pucchio, T., Connolly, J., Fay, J. W., Pascual, V., Banchereau, J. (2007) Dendritic cell subsets in health and disease. *Immunol. Rev.* **219,** 118–142.
45. Agrawal, A., Agrawal, S., Tay, J., Gupta, S. (2008) Biology of dendritic cells in aging. *J. Clin. Immunol.* **28,** 14–20.
46. Shurin, M. R., Shurin, G. V., Chatta, G. S. (2007) Aging and the dendritic cell system: implications for cancer. *Crit. Rev. Oncol. Hematol.* **64,** 90–105.
47. Jing, Y., Shaheen, E., Drake, R. R., Chen, N., Gravenstein, S., Deng, Y. (2009) Aging is associated with a numerical and functional decline in plasmacytoid dendritic cells, whereas myeloid dendritic cells are relatively unaltered in human peripheral blood. *Hum. Immunol.* **70,** 777–784.
48. Pérez-Cabezas, B., Naranjo-Gómez, M., Fernández, M. A., Grífols, J. R., Pujol-Borrell, R., Borràs, F. E. (2007) Reduced numbers of plasmacytoid dendritic cells in aged blood donors. *Exp. Gerontol.* **42,** 1033–1038.
49. Narbutt, J., Lesiak, A., Zak-Prelich, M., Wozniacka, A., Sysa-Jedrzejowska, A., Tybura, M., Robak, T., Smolewski, P. (2004) The distribution of peripheral blood dendritic cells assayed by a new panel of anti-BDCA monoclonal antibodies in healthy representatives of the polish population. *Cell. Mol. Biol. Lett.* **9,** 497–509.
50. Shodell, M., Siegal, F. P. (2002) Circulating, interferon-producing plasmacytoid dendritic cells decline during human aging. *Scand. J. Immunol.* **56,** 518–521.
51. Vuckovic, S., Gardiner, D., Field, K., Chapman, G. V., Khalil, D., Gill, D., Marlton, P., Taylor, K., Wright, S., Pinzon-Charry, A., Pyke, C. M., Rodwell, R., Hockey, R. L., Gleeson, M., Tepes, S., True, D., Cotterill, A., Hart, D. N. (2004) Monitoring dendritic cells in clinical practice using a new whole blood single-platform TruCOUNT assay. *J. Immunol. Methods* **284,** 73–87.
52. Lung, T. L., Saurwein-Teissl, M., Parson, W., Schonitzer, D., Grubeck-Loebenstein, B. (2000) Unimpaired dendritic cells can be derived from monocytes in old age and can mobilize residual function in senescent T cells. *Vaccine* **18,** 1606–1612.
53. Pietschmann, P., Hahn, P., Kudlacek, S., Thomas, R., Peterlik, M. (2000) Surface markers and transendothelial migration of dendritic cells from elderly subjects. *Exp. Gerontol.* **35,** 213–224.
54. Steger, M. M., Maczek, C., Grubeck-Loebenstein, B. (1996) Morphologically and functionally intact dendritic cells can be derived from the peripheral blood of aged individuals. *Clin. Exp. Immunol.* **105,** 544–550.
55. Agrawal, A., Agrawal, S., Cao, J. N., Su, H., Osann, K., Gupta, S. (2007) Altered innate immune functioning of dendritic cells in elderly humans: a role of phosphoinositide 3-kinase-signaling pathway. *J. Immunol.* **178,** 6912–6922.
56. Della Bella, S., Bierti, L., Presicce, P., Arienti, R., Valenti, M., Saresella, M., Vergani, C., Villa, M. L. (2007) Peripheral blood dendritic cells and monocytes are differently regulated in the elderly. *Clin. Immunol.* **122,** 220–228.
57. Grolleau-Julius, A., Garg, M. R., Mo, R., Stoolman, L. L., Yung, R. L. (2006) Effect of aging on bone marrow-derived murine CD11c+CD4–CD8α– dendritic cell function. *J. Gerontol. A Biol. Sci. Med. Sci.* **61,** 1039–1047.
58. Komatsubara, S., Cinader, B., Muramatsu, S. (1986) Functional competence of dendritic cells of aging C57BL/6 mice. *Scand. J. Immunol.* **24,** 517–525.
59. Saurwein-Teissl, M., Schonitzer, D., Grubeck-Loebenstein, B. (1998) Dendritic cell responsiveness to stimulation with influenza vaccine is unimpaired in old age. *Exp. Gerontol.* **33,** 625–631.
60. Grolleau-Julius, A., Abernathy, L., Harning, E., Yung, R. L. (2009) Mechanisms of murine dendritic cell antitumor dysfunction in aging. *Cancer Immunol. Immunother.* **58,** 1935–1939.
61. Cumberbatch, M., Dearman, R. J., Kimber, I. (2002) Influence of aging on Langerhans cell migration in mice: identification of a putative deficiency of epidermal interleukin-1β. *Immunology* **105,** 466–477.
62. Bhushan, M., Cumberbatch, M., Dearman, R. J., Andrew, S. M., Kimber, I., Griffiths, C. E. (2002) Tumor necrosis factor-α-induced migration of human Langerhans cells: the influence of aging. *Br. J. Dermatol.* **146,** 32–40.
63. Choi, K. L., Sauder, D. N. (1987) Epidermal Langerhans cell density and contact sensitivity in young and aged BALB/c mice. *Mech. Ageing Dev.* **39,** 69–79.

64. Paula, C., Motta, A., Schmitz, C., Nunes, C. P., Souza, A. P., Bonorino, C. (2009) Alterations in dendritic cell function in aged mice: potential implications for immunotherapy design. *Biogerontology* **10,** 13–25.
65. Linton, P. J., Li, S. P., Zhang, Y., Bautista, B., Huynh, Q., Trinh, T. (2005) Intrinsic versus environmental influences on T-cell responses in aging. *Immunol. Rev.* **205,** 207–219.
66. Mazzoni, A., Segal, D. M. (2004) Controlling the Toll road to dendritic cell polarization. *J. Leukoc. Biol.* **75,** 721–730.
67. Tesar, B. M., Walker, W. E., Unternaehrer, J., Joshi, N. S., Chandele, A., Haynes, L., Kaech, S., Goldstein, D. R. (2006) Murine [corrected] myeloid dendritic cell-dependent Toll-like receptor immunity is preserved with aging. *Aging Cell* **5,** 473–486.
68. Stout-Delgado, H. W., Yang, X., Walker, W. E., Tesar, B. M., Goldstein, D. R. (2008) Aging impairs IFN regulatory factor 7 up-regulation in plasmacytoid dendritic cells during TLR9 activation. *J. Immunol.* **181,** 6747–6756.
69. Sprecher, E., Becker, Y., Kraal, G., Hall, E., Harrison, D., Shultz, L. D. (1990) Effect of aging on epidermal dendritic cell populations in C57BL/6J mice. *J. Invest. Dermatol.* **94,** 247–253.
70. Sharma, S., Dominguez, A. L., Lustgarten, J. (2006) Aging affect the anti-tumor potential of dendritic cell vaccination, but it can be overcome by co-stimulation with anti-OX40 or anti-4-1BB. *Exp. Gerontol.* **41,** 78–84.
71. Moretto, M. M., Lawlor, E. M., Khan, I. A. (2008) Aging mice exhibit a functional defect in mucosal dendritic cell response against an intracellular pathogen. *J. Immunol.* **181,** 7977–7984.
72. Wallace, D. L., Zhang, Y., Ghattas, H., Worth, A., Irvine, A., Bennett, A. R., Griffin, G. E., Beverley, P. C., Tough, D. F., Macallan, D. C. (2004) Direct measurement of T cell subset kinetics in vivo in elderly men and women. *J. Immunol.* **173,** 1787–1794.
73. Kohler, S., Wagner, U., Pierer, M., Kimmig, S., Oppmann, B., Mowes, B., Julke, K., Romagnani, C., Thiel, A. (2005) Post-thymic in vivo proliferation of naive CD4+ T cells constrains the TCR repertoire in healthy human adults. *Eur. J. Immunol.* **35,** 1987–1994.
74. Naylor, K., Li, G., Vallejo, A. N., Lee, W. W., Koetz, K., Bryl, E., Witkowski, J., Fulbright, J., Weyand, C. M., Goronzy, J. J. (2005) The influence of age on T cell generation and TCR diversity. *J. Immunol.* **174,** 7446–7452.
75. Saule, P., Trauet, J., Dutriez, V., Lekeux, V., Dessaint, J-P., Labalette, M. (2006) Accumulation of memory T cells from childhood to old age: central and effector memory cells in CD4+ versus effector memory and terminally differentiated memory cells in CD8+ compartment. *Mech. Ageing Dev.* **127,** 274–281.
76. Hakim, F. T., Gress, R. E. (2007) Immunosenescence: deficits in adaptive immunity in the elderly. *Tissue Antigens* **70,** 179–189.
77. Han, G. M., Zhao, B., Jeyaseelan, S., Feng, J. M. (2009) Age-associated parallel increase of Foxp3(+)CD4(+) regulatory and CD44(+)CD4(+) memory T cells in SJL/J mice. *Cell. Immunol.* **258,** 188–196.
78. Nikolich-Zugich, J. (2008) Aging and life-long maintenance of T-cell subsets in the face of latent persistent infections. *Nat. Rev. Immunol.* **8,** 512–522.
79. Zhang, X., Fujii, H., Kishimoto, H., LeRoy, E., Surh, C. D., Sprent, J. (2002) Aging leads to disturbed homeostasis of memory phenotype CD8(+) cells. *J. Exp. Med.* **195,** 283–293.
80. Yager, E. J., Ahmed, M., Lanzer, K., Randall, T. D., Woodland, D. L., Blackman, M. A. (2008) Age-associated decline in T cell repertoire diversity leads to holes in the repertoire and impaired immunity to influenza virus. *J. Exp. Med.* **205,** 711–723.
81. Haynes, L., Linton, P-J., Eaton, S. M., Tonkonogy, S. L., Swain, S. L. (1999) Interleukin 2, but not other common {γ} chain-binding cytokines, can reverse the defect in generation of CD4 effector T cells from naive T cells of aged mice. *J. Exp. Med.* **190,** 1013–1024.
82. Nagel, J. E., Chopra, R. K., Chrest, F. J., McCoy, M. T., Schneider, E. L., Holbrook, N. J., Adler, W. H. (1988) Decreased proliferation, interleukin 2 synthesis, and interleukin 2 receptor expression are accompanied by decreased mRNA expression in phytohemagglutinin-stimulated cells from elderly donors. *J. Clin. Invest.* **81,** 1096–1102.
83. Whisler, R. L., Beiqing, L., Chen, M. (1996) Age-related decreases in IL-2 production by human T cells are associated with impaired activation of nuclear transcriptional factors AP-1 and NF-AT. *Cell. Immunol.* **169,** 185–195.
84. Sadighi Akha, A. A., Miller, R. A. (2005) Signal transduction in the aging immune system. *Curr. Opin. Immunol.* **17,** 486–491.
85. Chiu, Y-C., Yang, R-S., Hsieh, K-H., Fong, Y-C., Way, T-D., Lee, T-S., Wu, H-C., Fu, W-M., Tang, C-H. (2007) Stromal cell-derived factor-1 induces matrix metalloprotease-13 expression in human chondrocytes. *Mol. Pharmacol.* **72,** 695–703.
86. Nanki, T., Hayashida, K., El-Gabalawy, H. S., Suson, S., Shi, K., Girschick, H. J., Yavuz, S., Lipsky, P. E. (2000) Stromal cell-derived factor-1-CXC chemokine receptor 4 interactions play a central role in CD4+ T cell accumulation in rheumatoid arthritis synovium. *J. Immunol.* **165,** 6590–6598.
87. Mo, R., Chen, J., Han, Y., Bueno-Cannizares, C., Misek, D. E., Lescure, P. A., Hanash, S., Yung, R. L. (2003) T cell chemokine receptor expression in aging. *J. Immunol.* **170,** 895–904.
88. Dejaco, C., Duftner, C., Schirmer, M. (2006) Are regulatory T-cells linked with aging? *Exp. Gerontol.* **41,** 339–345.
89. Gregg, R., Smith, C. M., Clark, F. J., Dunnion, D., Khan, N., Chakraverty, R., Nayak, L., Moss, P. A. (2005) The number of human peripheral blood CD4+ CD25high regulatory T cells increases with age. *Clin. Exp. Immunol.* **140,** 540–546.
90. Csiszar, A., Ungvari, Z., Koller, A., Edwards, J. G., Kaley, G. (2003) Aging-induced proinflammatory shift in cytokine expression profile in coronary arteries. *FASEB J.* **17,** 1183–1185.
91. Hussein, M. R., Fathi, N. A., El-Din, A. M., Hassan, H. I., Abdullah, F., Al-Hakeem, E., Backer, E. A. (2008) Alterations of the CD4(+), CD8(+) T cell subsets, interleukins-1β, IL-10, IL-17, tumor necrosis factor-α and soluble intercellular adhesion molecule-1 in rheumatoid arthritis and osteoarthritis: preliminary observations. *Pathol. Oncol. Res.* **14,** 321–328.
92. Ginaldi, L., De Martinis, M., D'Ostilio, A., Marini, L., Loreto, M. F., Martorelli, V., Quaglino, D. (1999) The immune system in the elderly: II. Specific cellular immunity. *Immunol. Res.* **20,** 109–115.
93. Ginaldi, L., De Martinis, M., D'Ostilio, A., Marini, L., Loreto, M. F., Corsi, M. P., Quaglino, D. (1999) The immune system in the elderly: I. Specific humoral immunity. *Immunol. Res.* **20,** 101–108.
94. Shearer, G. M. (1997) Th1/Th2 changes in aging. *Mech. Ageing Dev.* **94,** 1–5.
95. Li, S. P., Miller, R. A. (1993) Age-associated decline in IL-4 production by murine T lymphocytes in extended culture. *Cell. Immunol.* **151,** 187–195.
96. Kovacs, E. J., Duffner, L. A., Plackett, T. P. (2004) Immunosuppression after injury in aged mice is associated with a TH1-TH2 shift, which can be restored by estrogen treatment. *Mech. Ageing Dev.* **125,** 121–123.
97. Uciechowski, P., Kahmann, L., Plümäkers, B., Malavolta, M., Mocchegiani, E., Dedoussis, G., Herbein, G., Jajte, J., Fulop, T., Rink, L. (2008) TH1 and TH2 cell polarization increases with aging and is modulated by zinc supplementation. *Exp. Gerontol.* **43,** 493–498.
98. Gupta, S. (2005) Molecular mechanisms of apoptosis in the cells of the immune system in human aging. *Immunol. Rev.* **205,** 114–129.
99. Potestio, M., Pawelec, G., Di Lorenzo, G., Candore, G., D'Anna, C., Gervasi, F., Lio, D., Tranchida, G., Caruso, C., Romano, G. C. (1999) Age-related changes in the expression of CD95 (APO1/FAS) on blood lymphocytes. *Exp. Gerontol.* **34,** 659–673.
100. Aggarwal, S., Gupta, S. (1998) Increased apoptosis of T cell subsets in aging humans: altered expression of Fas (CD95), Fas ligand, Bcl-2, and Bax. *J. Immunol.* **160,** 1627–1637.
101. Chamberlain, W. D., Falta, M. T., Kotzin, B. L. (2000) Functional subsets within clonally expanded CD8(+) memory T cells in elderly humans. *Clin. Immunol.* **94,** 160–172.
102. Gupta, S., Gollapudi, S. (2008) CD95-mediated apoptosis in naive, central and effector memory subsets of CD4+ and CD8+ T cells in aged humans. *Exp. Gerontol.* **43,** 266–274.
103. Weng, N. P., Granger, L., Hodes, R. J. (1997) Telomere lengthening and telomerase activation during human B cell differentiation. *Proc. Natl. Acad. Sci. USA* **94,** 10827–10832.
104. Weng, N. P., Hathcock, K. S., Hodes, R. J. (1998) Regulation of telomere length and telomerase in T and B cells: a mechanism for maintaining replicative potential. *Immunity* **9,** 151–157.
105. Kaszubowska, L. (2008) Telomere shortening and aging of the immune system. *J. Physiol. Pharmacol.* **59** (Suppl. 9), 169–186.
106. Goronzy, J. J., Fujii, H., Weyand, C. M. (2006) Telomeres, immune aging and autoimmunity. *Exp. Gerontol.* **41,** 246–251.
107. Effros, R. B., Dagarag, M., Spaulding, C., Man, J. (2005) The role of CD8+ T-cell replicative senescence in human aging. *Immunol. Rev.* **205,** 147–157.
108. Weng, N. P., Akbar, A. N., Goronzy, J. (2009) CD28(–) T cells: their role in the age-associated decline of immune function. *Trends Immunol.* **30,** 306–312.
109. Brzezinska, A., Magalska, A., Szybinska, A., Sikora, E. (2004) Proliferation and apoptosis of human CD8(+)CD28(+) and CD8(+)CD28(–) lymphocytes during aging. *Exp. Gerontol.* **39,** 539–544.
110. Effros, R. B., Boucher, N., Porter, V., Zhu, X., Spaulding, C., Walford, R. L., Kronenberg, M., Cohen, D., Schachter, F. (1994) Decline in CD28+ T cells in centenarians and in long-term T cell cultures: a possible cause for both in vivo and in vitro immunosenescence. *Exp. Gerontol.* **29,** 601–609.
111. Weyand, C. M., Brandes, J. C., Schmidt, D., Fulbright, J. W., Goronzy, J. J. (1998) Functional properties of CD4+ CD28– T cells in the aging immune system. *Mech. Ageing Dev.* **102,** 131–147.
112. Effros, R. B. (2004) Replicative senescence of CD8 T cells: effect on human aging. *Exp. Gerontol.* **39,** 517–524.
113. Hsu, H. C., Scott, D. K., Zhang, P., Zhou, J., Yang, P., Wu, Q., Schroeder Jr., H. W., Gerald, L. B., Ravussin, E., Jazwinski, S. M., Mountz, J. D. (2006) CD8 T-cell immune phenotype of successful aging. *Mech. Ageing Dev.* **127,** 231–239.
114. Akbar, A. N., Beverley, P. C., Salmon, M. (2004) Will telomere erosion lead to a loss of T-cell memory? *Nat. Rev. Immunol.* **4,** 737–743.

115. Effros, R. B. (2009) Kleemeier Award Lecture 2008—the canary in the coal mine: telomeres and human healthspan. *J. Gerontol. A Biol. Sci. Med. Sci.* **64,** 511–515.
116. De la Fuente, M., Baeza, I., Guayerbas, N., Puerto, M., Castillo, C., Salazar, V., Ariznavarreta, C., F-Tresguerres, J. A. (2004) Changes with aging in several leukocyte functions of male and female rats. *Biogerontology* **5,** 389–400.
117. Tarazona, R., DelaRosa, O., Alonso, C., Ostos, B., Espejo, J., Pena, J., Solana, R. (2000) Increased expression of NK cell markers on T lymphocytes in aging and chronic activation of the immune system reflects the accumulation of effector/senescent T cells. *Mech. Ageing Dev.* **121,** 77–88.
118. Speziali, E., Bethony, J., Martins-Filho, O., Fraga, L. A., Lemos, D. S., Souza, L. J., Correa-Oliveira, R., Faria, A. M. (2004) Production of interferon-γ by natural killer cells and aging in chronic human schistosomiasis. *Mediators Inflamm.* **13,** 327–333.
119. Ogata, K., An, E., Shioi, Y., Nakamura, K., Luo, S., Yokose, N., Minami, S., Dan, K. (2001) Association between natural killer cell activity and infection in immunologically normal elderly people. *Clin. Exp. Immunol.* **124,** 392–397.
120. Panda, A., Arjona, A., Sapey, E., Bai, F., Fikrig, E., Montgomery, R. R., Lord, J. M., Shaw, A. C. (2009) Human innate immunosenescence: causes and consequences for immunity in old age. *Trends Immunol.* **30,** 325–333.
121. Mocchegiani, E., Malavolta, M. (2004) NK and NKT cell functions in immunosenescence. *Aging Cell* **3,** 177–184.
122. Mocchegiani, E., Giacconi, R., Cipriano, C., Malavolta, M. (2009) NK and NKT cells in aging and longevity: role of zinc and metallothioneins. *J. Clin. Immunol.* **29,** 416–425.
123. Peralbo, E., Alonso, C., Solana, R. (2007) Invariant NKT and NKT-like lymphocytes: two different T cell subsets that are differentially affected by aging. *Exp. Gerontol.* **42,** 703–708.
124. Colonna-Romano, G., Bulati, M., Aquino, A., Scialabba, G., Candore, G., Lio, D., Motta, M., Malaguarnera, M., Caruso, C. (2003) B cells in the aged: CD27, CD5, and CD40 expression. *Mech. Ageing Dev.* **124,** 389–393.
125. Shi, Y., Yamazaki, T., Okubo, Y., Uehara, Y., Sugane, K., Agematsu, K. (2005) Regulation of aged humoral immune defense against pneumococcal bacteria by IgM memory B cell. *J. Immunol.* **175,** 3262–3267.
126. Chong, Y., Ikematsu, H., Yamaji, K., Nishimura, M., Nabeshima, S., Kashiwagi, S., Hayashi, J. (2005) CD27(+) (memory) B cell decrease and apoptosis-resistant CD27(−) (naive) B cell increase in aged humans: implications for age-related peripheral B cell developmental disturbances. *Int. Immunol.* **17,** 383–390.
127. Gibson, K. L., Wu, Y. C., Barnett, Y., Duggan, O., Vaughan, R., Kondeatis, E., Nilsson, B. O., Wikby, A., Kipling, D., Dunn-Walters, D. K. (2009) B-cell diversity decreases in old age and is correlated with poor health status. *Aging Cell* **8,** 18–25.
128. Cancro, M. P., Hao, Y., Scholz, J. L., Riley, R. L., Frasca, D., Dunn-Walters, D. K., Blomberg, B. B. (2009) B cells and aging: molecules and mechanisms. *Trends Immunol.* **30,** 313–318.
129. Siegrist, C. A., Aspinall, R. (2009) B-cell responses to vaccination at the extremes of age. *Nat. Rev. Immunol.* **9,** 185–194.
130. Caruso, C., Buffa, S., Candore, G., Colonna-Romano, G., Dunn-Walters, D., Kipling, D., Pawelec, G. (2009) Mechanisms of immunosenescence. *Immun. Ageing* **6,** 10.

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