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Aging of the Innate Immune System

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Summary

The innate immune system is composed of a network of cells including neutrophils, NK and NKT cells, monocytes/macrophages, and dendritic cells that mediate the earliest interactions with pathogens. Age-associated defects are observed in the activation of all of these cell types, linked to compromised signal transduction pathways including the Toll-like Receptors. However, aging is also characterized by a constitutive pro-inflammatory environment (Inflamm-aging) with persistent low-grade innate immune activation that may augment tissue damage caused by infections in elderly individuals. Thus, immunosenescence in the innate immune system appears to reflect dysregulation, rather than exclusively impaired function.

Introduction

Immunosenescence in the innate immune system has considerable complexity. For example, in a mouse model of influenza infection slower recovery, prolonged morbidity, and longer duration of viremia were observed in older C57BL/6 mice, but were associated with a wide range of responses ranging from a delay in granulocyte and conventional DCs (cDCs) infiltration to the lungs, to increased macrophage persistence in the lung. Production of pro-inflammatory compounds such as IL-12 and MCP-1 in the lung were diminished in old mice, but for others such as IL-6 a delayed peak was observed, while TNF- α and IL-1 α levels were higher [1]. Thus, while many responses in the innate immune system are diminished with aging, there is substantial evidence for age-associated hyper-reactivity of innate immunity as well. The effects of aging on the innate immune system in humans and mice have been reviewed recently [2–4]; here, we provide an overview emphasizing recent developments in the field.

Neutrophils

Neutrophils constitute the primary immune defense against rapidly dividing bacteria, yeast and fungal infections, deploying microbicidal mechanisms including generation of reactive oxygen and nitrogen species, release of proteolytic enzymes and microbicidal peptides from

cytoplasmic granules. Neutrophils can also engage and kill microbes extracellularly through the extrusion of Neutrophil Extracellular Traps (NETs) [5]. To date there are no reports concerning NET production by neutrophils from older adults, though NET generation is reduced in neonates compared to adults, indicating that this aspect of neutrophil function is modified through development [6]. Cells of the immune system are derived from haematopoietic stem cells (HSCs) which proliferate and commit to either lymphoid or myeloid lineages. With increasing age, there is a skewing towards myeloid progenitors at the expense of lymphoid progenitors. Beerman *et al* have shown recently that this is the result of the clonal expansion of myeloid biased HSCs [7•]. There is thus no reduction in neutrophil numbers with age and no loss of ability to generate a robust neutrophilia in response to infection. In contrast, human neutrophils show compromised activity with aging, with most aspects of neutrophil microbicidal function affected. The reduced chemotactic ability seen in neutrophils from older donors [8] would affect the time taken for neutrophils to reach the site of infection allowing rapidly dividing bacteria to establish a strong core of infection. Inefficient chemotaxis would also increase bystander tissue damage as neutrophils secrete proteases such as elastase to aid their migration through tissues. The latter would be expected to extend inflammation and impair resolution of inflammation in older adults. Nomellini *et al* assessed neutrophil accumulation in the lungs of young and old mice following burn injury. They showed the rate of neutrophil accumulation did not differ with age, but the time taken to resolve the inflammation was significantly longer in the old mice suggestive of greater tissue damage [9•]. Chemotaxis is also the mechanism by which effete neutrophils return to the bone marrow at the end of their short lifespan following upregulation of CXCR4 [10]. Whether the return of effete neutrophils to the bone marrow is also affected by aging is not known, but we suggest that this is a possibility. Neutrophils aged *in vitro* gradually senesce and show reduced phagocytosis and superoxide generation prior to entry into apoptosis. If the removal of older effete neutrophils from the circulation is impeded they would remain in the blood for longer thus reducing average neutrophil function (Figure 1).

For some aspects of age-related neutrophil decline the mechanisms are partially understood, for example reduced phagocytosis of opsonised *E. Coli* is associated with reduced surface expression of the Fc γ receptor CD16 [11]. However for most other aspects of neutrophil senescence our understanding is incomplete. Aging affects a broad range of cellular processes in the neutrophil, mediated by ligation of receptors with distinct signaling mechanisms. With the exception of CD16, the literature shows that expression of receptors mediating neutrophil activation is unaltered with age. As a broad range of downstream signaling events employed by these receptors are affected by aging, including altered calcium, Phosphoinositide-3 kinase (PI-3K), MAP kinase, Protein Kinase B, Jak-STAT and SHP-1 signaling (reviewed in [12]), this suggests that changes to generic signaling processes are most likely to underlie these observations.

Lipid rafts are crucial for the efficient functioning of many membrane bound receptors, facilitating formation of multimeric signaling complexes. Changes to the composition of membrane lipids crucial for lipid raft structure, for example by depletion of cholesterol, compromises receptor mediated signaling and could explain the multiple age-related

changes in neutrophil receptor signaling. A single report examining rat peritoneal neutrophils revealed an age-dependent increase in membrane fluidity associated with a decline in the cholesterol/phospholipid ratio and a rise in total polyunsaturated fatty acids [13]. Crucially, neutrophils from 24 month old rats had a dramatically reduced superoxide burst in response to fMLP that was overcome when cells were treated with phorbol myristate (PMA), supporting our proposal that the age-related loss of neutrophil function is due at least in part to compromised proximal signaling events. Further support for this proposal is provided by Fortin *et al* who reported that recruitment of Triggering Receptor Expressed on Myeloid cells-1 (TREM-1) to lipid rafts was reduced in neutrophils from elderly donors, with concomitant reduction in downstream signaling events [14]. As TREM-1 regulates TLR signaling, this observation may also help to explain data showing reduced levels of TLR4 in lipid raft fractions in response to lipopolysaccharide in neutrophils from elderly donors [15].

NK and NKT cells

NK cells mediate MHC-independent cytotoxicity in the innate immune defense against viral infections and some malignancies. Absolute numbers of NK cells increase with age, reflecting an increase in CD56dim cells, but NK cell cytotoxicity on a per cell basis is decreased and levels of cytokines and chemokines such as RANTES, MIP1 α , and IL-8 produced upon NK cell activation are also reduced (reviewed in [16]). These age-associated alterations in NK function may result in part from changes in zinc homeostasis in older individuals, and there is evidence that NK cell function can be improved with zinc supplementation [17]. A recent report postulated a more direct link between NK cells and reduced adaptive immunity. In aged Balb/c mice, an increased proportion of NK cells were found in an early B cell bone marrow developmental population (Hardy Fraction A); depletion experiments revealed that this NK population may directly inhibit surrogate light chain expression in developing B cells, thereby potentially contributing to B cell immunosenescence [18•].

Classical NKT cells express a T cell receptor (V α 14/V β 8.2 in mice and V α 24/V β 11 in humans) that is CD1d-restricted and represent a rare class of “innate immune lymphocyte”. Studies in mice have demonstrated an age-associated increase in NKT cell numbers with augmented function [19–21]. A role for NKT cells in modulating the inflammatory response to viral infection has emerged from a study by Stout-Delgado *et al* which employed a Herpes Simplex Virus-2 (HSV-2) murine infection system. Such infection resulted in markedly elevated levels of IL-17A in older, compared to young mice, and was associated with increased neutrophil recruitment to the liver and chemokine production. Notably, NKT cells from aged mice were identified as greater producers of elevated IL-17A levels compared to young NKT cells, and adoptive transfer of aged NKT cells into young mice resulted in hepatic injury [22••]. This concept of an enhanced inflammatory environment with aging has also been associated with augmented IL-17-dependent T cell allogeneic responses [23], and with an increase in IL23/p19 gene expression in aged murine bone marrow-derived dendritic cells [24•]; such hyper-responsive inflammatory responses could well contribute to adverse outcomes associated with infection or sepsis in older individuals.

Monocytes and Macrophages

Monocytes represent a highly mobile component of the innate immune system located in the spleen and blood which respond to inflammation by differentiating into antigen presenting cells such as macrophages and dendritic cells. Monocyte absolute numbers increase with age [25], a change independently associated with clinical frailty [25a]. This increase in monocyte numbers is contrasted with an age-associated decrease in macrophage function, particularly in the context of Toll-like Receptor (TLR) activation. Age-associated decreases in LPS-induced (activating TLR4) IL-6, TNF- α and IL-1 β have been reported in both C57BL/6 and Balb/c mice [26–28], and a more generalized decrease in cytokine production in response to activation of TLR2/6, 3, 4, 5, and 9 was observed in splenic and peritoneal macrophages from C57BL/6 mice [29] that was associated with decreased TLR gene expression. However, in aged Balb/c mice, decreased TLR signal transduction for example via MAP and JNK kinase pathways, rather than decreased TLR expression, was reported [26,27,30]. Similar defects in signal transduction and increased oxidative stress were observed in macrophages from both telomerase-deficient and aged wild-type mice [31].

Human studies of TLR function in monocytes have also revealed age-associated declines in cytokine production. A study of 79 young (age 21–30) and 80 older (age 65) subjects revealed an age-associated decrease in TLR1/2-induced IL-6 and TNF- α production in monocytes [32]. Impairment in TLR1/2 function was associated with decreased monocyte surface expression of TLR1 but not TLR2; notably, intracellular TLR1 expression was unchanged, suggesting a post-translational alteration in surface TLR1 expression in monocytes from older individuals. A generalized age-associated defect in the upregulation of the co-stimulatory protein CD80 on monocytes was observed for all TLR ligands tested (TLR1/2, TLR2/6, TLR4, TLR5, and TLR8) [33]. Crucially, the ability to increase CD80 expression upon TLR engagement was strongly associated with the generation of a protective antibody response to influenza vaccination, confirming consequences for adaptive immunity.

Defects in macrophage function in aged humans have also been described in a recent study evaluating delayed type hypersensitivity (DTH) responses to *Candida* antigens, which are known to diminish with age [34••]. Using skin biopsy specimens from young (<40 years) and older (>70) individuals, substantial decreases in TNF- α , IL-6 and IFN- γ levels were found in skin suction blister fluid samples from old, compared to young individuals. Decreased intracellular production of TNF- α was observed in dermal macrophages, potentially contributing to decreased migration of T cells in response to antigen challenge in the skin. Since dermal macrophages from older individuals could be induced to produce TNF- α *ex vivo*, macrophage dysfunction in the skin of older individuals may reflect alterations in the skin microenvironment. These findings have implications for cutaneous innate immunity, as well as for the use of DTH in clinical contexts, such as tuberculin skin testing.

By contrast, other studies suggest that aging is associated with a pro-inflammatory environment—so-called “inflamm-aging” (reviewed elsewhere in this issue)—characterized by constitutively elevated levels of cytokines such as IL-6 and TNF- α as well as acute phase

reactants [35]. Dysregulation of TLR responses, as has been reported for the increased TLR3 expression observed following infection of aged human macrophages with West Nile Virus, may contribute to morbidity from viral infections in elderly individuals [36•]. In murine models of sepsis using either LPS intraperitoneal injection (Balb/c) or cecal ligation and puncture (FVB/N), aged mice were found to have an enhanced inflammatory response, as measured by cytokine and chemokine production or neutrophil infiltration, compared to young mice [37–39]. Notably, mice deficient in macrophage migration inhibitory factor (MIF), a cytokine with protean influences on inflammation, insulin signaling and cell cycle regulation, were recently found to have increased lifespan [40••]. Thus, it is conceivable that age-associated ex vivo defects in macrophage cytokine production can be reconciled with a pro-inflammatory milieu that may result in enhanced basal levels of cytokines and chemokines.

Dendritic cells

In general, aging results in decreased DC function, but some parameters may be unchanged and some examples of enhanced function have been reported. In murine systems, an age-associated decrease in DC-SIGN expression was observed on immature bone marrow derived DCs [41]; but, myeloid splenic and bone marrow-derived DCs have preserved function in T cell priming and TLR function in aged mice [42]. Some defects in mDCs from aged mice have been observed in the context of a murine tumor antigen model, characterized by defective DC trafficking and impaired CCR7 signal transduction [43]. The function of pDCs isolated from bone marrow appeared compromised in aged C57BL/6 mice using an HSV-2 infection model dependent on TLR9 activation [44•]. Decreased production of IFN- α upon HSV-2 infection was observed in pDCs from old mice, with defective induction of IRF-7 expression upon TLR9 activation. These findings potentially provide an additional basis for the impaired control of viral infections observed in aging individuals. An additional area for investigation is the follicular dendritic cell (FDC), a reticular cell present in primary and secondary follicles that is particularly adept at presenting immune complexes to facilitate B cell adaptive responses [45]. FDC networks in aged mice are markedly disrupted, and likely contribute to B cell immunosenescence, but FDC contribution to human immunosenescence remains poorly understood [46,47].

In humans, a recent study of 18 young and 19 older individuals elucidated an age-associated defect in TLR-7 and TLR-9-induced IFN- α production in pDCs, as assessed via intracellular cytokine staining [48]; no age-associated alterations in mDC cytokine production were observed, but mDC TLR function in this case was interrogated via cytokine ELISA of pooled young or older PBMCs. Panda *et al* carried out a study of TLR-induced cytokine production in 52 young (21–30) and 52 older (> 65) individuals, using intracellular staining to quantify cytokine levels in primary mDCs and pDCs [49•]. An age-associated decrease in TLR7 and TLR9-induced IFN- α production was observed in pDCs and a generalized age-associated defect in the intracellular production of TNF- α , IL-6 and the p40 subunit shared by IL-12 and IL-23 was observed for virtually all TLRs evaluated (TLR1/2, TLR2/6, TLR3, TLR4, TLR5, and TLR8). The extent of TLR-induced cytokine production was strongly associated with influenza vaccine antibody response. Notably, elevated basal levels of intracellular cytokine production were observed in older, but not young mDCs and pDCs.

Thus, it is attractive to speculate that the observed TLR functional defects reflect a constitutive level of cytokine production that may contribute to an age-related pro-inflammatory environment, but cannot be substantially increased with additional TLR engagement.

In this context, monocyte derived DCs from older, compared to young individuals were found to have increases in LPS and single-stranded RNA-induced TNF- α and IL-6 production, as well as increased self-DNA-induced IL-6 and IFN- α production [50,51•]. These functional increases in cytokine output were accompanied by impairments in phagocytic function and migration in vitro, and were hypothesized to be attributable to diminished PI-3K activity, which has been implicated in both DC migration and as a possible negative regulator of TLR signaling. It remains possible that the growth factor treatment used to derive DCs from monocytes could attenuate age-associated functional differences; alternatively, this class of DC could model inflammatory DCs derived in vivo at the sites of infection—potentially another contributing factor to inflamm-aging.

Extrinsic moderation of innate immune function

When considering the immune system and the impact of aging upon its function, it is important to remember that it does not operate in isolation. It is now accepted that immunity is modulated by a plethora of hormones, including adrenal steroids, sex hormones and the expanding family of adipokines. As aging is associated with dramatic changes to the hormonal environment, this in turn will contribute to individual variability in immune decline.

Aging is accompanied by profound changes to the hypothalamic-pituitary-adrenal axis, termed the adrenopause. From the age of approximately 30 years the serum level of dehydroepiandrosterone sulfate (DHEAS) declines steadily until by the age of 70 only 10–20% of maximal levels remain. In contrast cortisol levels are maintained throughout life. DHEAS primarily functions as a precursor to the androgenic hormone DHEA, but there is evidence that DHEA and DHEAS also have immune enhancing functions. Although this area is still quite controversial, the reported effects of these steroids include *in vitro* potentiation of monocyte, neutrophil and NK cell function and increased protection from bacterial infection in mice [52]. Interestingly, Radford et al have shown recently that neutrophils appear to be unique in their ability to respond to DHEAS as they were the only leukocytes to express a membrane transporter (OATP-D) for this sulfated steroid [53]. There are thus potential consequences for immunity in old age at times of stress as the increased production of the immune suppressive cortisol will not be counterbalanced by DHEA/DHEAS. An increased cortisol:DHEAS ratio is seen after physical trauma (hip fracture) in older adults and is associated with reduced neutrophil superoxide generation and increased risk of infection [54]. Investigation into these and other extrinsic factors such as host functional status, together with the evaluation of age effects on other innate pattern recognition receptors such as the NOD-like and RIG-I-like receptors will lend further insights to future studies of age-associated changes in innate immunity.

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Figure 1. Consequences of altered membrane lipid content for neutrophil function

Studies of peritoneal neutrophils from aged rats [13] have shown reduced levels of cholesterol and increased membrane fluidity, associated with reduced superoxide generating ability. Other studies have shown dysregulation of receptor recruitment to lipid rafts, which will have very broad effects in compromising receptor signaling functions and downstream biological effects. Abbreviations: PKB, protein kinase B; PI3K, phosphoinositide-3-kinase; JAK, JAK, JAK-STAT signaling (JAK, Janus kinase; STAT Signal Transducers and Activators of Transcription).