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Innate immunity and aging

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Abstract

Advanced age is associated with defects in all of the cells of the innate immune system, including numbers, function, their, and early stages of activation. In this review, the current state of the field on the impact of age on the innate immune system is presented. The analysis of the literature suggests that a dysfunctional innate immune system is a contributing factor to aberrant outcomes after injury or infection and to the development of many of the diseases observed in the elderly. Gaining an understanding of the nature of the defects in innate immune cells may allow the development of therapeutic strategies aimed at restoring innate immune function in aged individuals.

Keywords

Neutrophils; macrophages; NK; NK-T; mast cells; eosinophils; age

1. Introduction

Aging is associated with a decline in health, partially attributed to defects in immunity [reviewed in (Katz et al., 2004)]. The complex process of immunosenescence affects both the innate and the adaptive arms of the immune system (Allman and Miller, 2005b; Gomez et al.,

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2005; Weng, 2006). Commonly observed in the elderly are decreased T cell memory, exhaustion of the naïve T cell population with involution of the thymus (Weng, 2006), decline in B cell production reflected in defective humoral immunity (Allman and Miller, 2005a; Riley et al., 2005), and a chronic inflammatory state that has come to be called, “inflamm-aging” (Franceschi et al., 2000). As a result, older subjects are less able to mount an immune response following an infectious challenge than are young adults (Linton and Dorshkind, 2004) and more susceptible than young to viral and bacterial infections, opportunistic infections, reactivation of latent viruses, autoimmune diseases, and neoplasias (Effros, 2003; Murasko and Jiang, 2005; Pawelec et al., 2005; Prelog, 2006).

Cumulative evidence indicates that aging exerts significant effects on all cells of the innate immune system (Agrawal et al., 2008; Gomez et al., 2005; Plackett et al., 2004; Plowden et al., 2004a; Sebastian et al., 2005; Solana et al., 2006). Impairment of multiple neutrophil functions, such as phagocytic capacity, synthesis of reactive oxygen intermediates, and intracellular killing efficiency is observed in the elderly (Fulop et al., 2004; Tortorella et al., 2007). Advanced age also affects macrophage functions, including phagocytic activity, cytokine and chemokine secretion, antibacterial defenses, infiltration and wound repair, and antigen presentation (Sebastian et al., 2005). There is contradictory evidence regarding the effects of aging on natural killer (NK) and natural killer T (NKT) cells numbers and functional properties (Mocchegiani et al., 2003; Peralbo et al., 2007). Although studies are limited, mast cell (Hart et al., 1999; Montagna and Carlisle, 1990; Nguyen et al., 2005) and eosinophil (Kasper et al., 1999; Leng et al., 2005; Yagi et al., 1997) numbers and functional properties have also shown age-related alterations. These latter findings are particularly interesting in the context of the age-associated increase in morbidity and mortality with asthma, autoimmune disorders and atherosclerosis. Thus, differences in both the number and function of multiple cell type, contribute to the defective innate immunity with advanced age. Moreover, new evidence has pointed to intracellular molecular pathways that lead to impaired activation of immune cells. We next review, in detail, the age-associated changes in number, function and cell signaling in cells of the innate immune system.

2. Neutrophils

Neutrophils are the predominant phagocytes of circulating blood. Typically, they are recruited to the site of infection by chemokines and products released from microorganisms (Chilvers et al., 2000; Davis et al., 1987; Lehrer et al., 1988). Chemotaxis towards the infection, results in adherence of neutrophils to endothelial cells through cell adhesion molecules and ultimately in migration through endothelial walls. The ingestion of pathogens by neutrophils occurs by means of phagocytosis and killing through the generation of reactive oxygen species (ROS) and the release of toxic granular enzymes (Lehrer et al., 1988). Neutrophils are short-lived cells, with a half-life of 8–12 hours and are removed by apoptosis, leading to their recognition and phagocytosis by macrophages (Savill et al., 1989). However, in response to priming agents, such as endotoxin, granulocyte macrophage colony stimulating factor (GM-CSF), platelet activating factor (PAF) and pro-inflammatory cytokines, including tumor necrosis factor- α (TNF α), their life-span is increased, resulting in elevated bactericidal capacity (Chilvers et al., 2000). In addition, this prolongation of the neutrophil life-span can contribute to excessive local tissue damage through the release of proteolytic enzymes, elastase and collagenase, (Sibille and Marchandise, 1993; Sibille and Reynolds, 1990).

The effects of aging on the generation and function of neutrophils is shown in Figure 1. For example, there is consistency in the literature in that advanced age does not modify the total number of circulating neutrophils (Albright and Albright, 2003; Chatta and Dale, 1996). In addition, bone marrow cells from young and aged subjects have similar responses to stem cell stimulants such as granulocyte macrophage colony stimulating factor (GM-CSF) and

interleukin (IL)-3 (Chatta and Dale, 1996). Chemotaxis of neutrophils primed with GM-CSF towards the formylated tripeptide N-formyl-methionyl-leucyl-phenylalanine (fMLP) and GM-CSF is reduced in cells from healthy aged volunteers, relative to their younger counterparts (Fortin et al., 2006; Fulop et al., 2004). As a consequence of the defects in chemotactic activity of neutrophils from the elderly, decreased infiltration at sites of injury should be anticipated (Lord et al., 2001). However, *in vivo* studies reveal a different picture. In a dermal excisional injury model, for example, comparable neutrophil myeloperoxidase (MPO) activity levels were found in the wound bed and in wound homogenates from young and aged mice (Swift et al., 2001). Moreover, pulmonary inflammation was greater in aged mice at 24 hours after receiving an intraperitoneal injection of lipopolysaccharide (LPS), when compared to young animals (Gomez et al., 2007a). This response was accompanied by higher levels of the neutrophil chemotactic cytokines, macrophage inflammatory protein (MIP-2)/CXCL2 and KC/CXCL1, and elevated levels of interleukin (IL)-1 β , in the lungs of aged mice receiving LPS, relative to young LPS treated mice (Gomez et al., 2007a). Finally, 24 hours after receiving a 15% total body surface area (TBSA) burn injury, the lungs of aged mice showed a marked increase in neutrophils, and KC, but no changes in MIP-2 and IL-1 β , compared to young, burn-injured animals (Nomellini et al., 2008a). Thus, *in vivo* versus *in vitro* models produce different outcomes. In addition, comparing the type of insult, the small excisional wound model, which generates only a local response, will likely yield a different magnitude of response than one involving a systemic response like LPS or burn injury.

Neutrophils have a short life-span, consequently, a defect in apoptotic cell death may be a contributor to aberrant function of these cells with advanced age. While spontaneous apoptotic death is not affected by advanced age, the ability of priming agents—such as LPS, granulocyte-colony stimulating factor (G-CSF), GM-CSF, IL-6 and steroids—to delay neutrophil apoptosis is significantly impaired in elderly persons (Fortin et al., 2007a; Fulop et al., 1997a; Tortorella et al., 2001; Tortorella et al., 2006). In addition, changes in the ratio of pro- and anti-apoptotic members of the bcl-2 family (Fulop et al., 2002), and defective activation of the Janus kinase (Jak)/signal transducer and activator of transcription (STAT) signaling pathway (Fortin et al., 2007a) have been found in the neutrophils of elderly individuals compared to young subjects. Altogether, these findings suggest that advanced age affects the activation of signaling pathways involved in the rescue of neutrophils from spontaneous apoptosis. The defect in signal transduction pathways coupled to some receptors, which should result in an increased susceptibility to neutrophil apoptosis in the elderly, may be an important contributor of aberrant inflammatory responses during senescence.

Neutrophils from elderly humans are less phagocytic than those from younger adults (Butcher et al., 2001; Lord et al., 2001; Wenzel et al., 2000). Moreover, the respiratory burst has been shown to be altered in neutrophils from aged volunteers, although reports vary depending on experimental conditions (Butcher et al., 2001; Fulop et al., 2004). For example, superoxide (O_2^-) and hydrogen peroxide (H_2O_2) production *in vitro* by neutrophils from aged humans is decreased (Di Lorenzo et al., 1999; Fulop et al., 1985; Nagel et al., 1982) when compared to cells from young. In contrast, others have reported normal to slightly raised O_2^- or H_2O_2 production in by neutrophils from healthy aged humans stimulated *in vitro* (Ito et al., 1998; Lord et al., 2001). These apparently contradictory data may be explained by the type of stimuli used, including fMLP along with gram positive and gram negative bacteria (Fulop et al., 2004; Lord et al., 2001). In addition, the timing of the analysis may be a contributing factor. For example, the production of O_2^- was lower after 24 hours of culture in neutrophils from aged volunteers relative to young subjects, but higher after 48 hours (Fulop et al., 2004). Taken together, these results indicate that age affects the microbicidal capacity of neutrophils.

In relation to the molecular mechanisms involved in the age-related alterations in neutrophil function, impaired intracellular signaling has been implicated as pivotal contributor. Decreased

intracellular Ca^{2+} after fMLP stimulation may help to explain reduced phagocytic ability (Fulop et al., 1997b) decreased bactericidal activity (Wenisch et al., 2000) and eventually the declined chemotaxis and bactericidal capacity of neutrophils from the aged. Similarly, actin polymerization is markedly reduced after stimulation of neutrophils from aged subjects with fMLP or phorbol myristate acetate (PMA)—an activator of protein kinase C (PKC)—relative to young (Rao, 1986). The defective actin depolymerization found in neutrophils from aged volunteers has been associated with impaired O_2^- production (Piazzolla et al., 1998).

Intracellular signaling following receptor ligation may be responsible for many of the observed changes in neutrophils from aged individuals. First, phosphorylation of extracellular receptor-activated kinase (ERK) mitogen-activated protein (MAP) kinase was lowered in neutrophils obtained from healthy aged volunteers following stimulation with GM-CSF (Larbi et al., 2005). Similar results were found for both MAP kinases ERK and p38 in response to fMLP (Fulop et al., 2004). In addition, the generation of IP_3 , and diacylglycerol (Lipschitz et al., 1991), as well as the activation of cAMP pathways (Chaves et al., 2007), were decreased in neutrophils from the elderly. GM-CSF–modulated granulopoiesis does not appear to be impaired in the aged, however, a reduction in the ability of neutrophils from the elderly to be primed by GM-CSF or to activate the respiratory burst has been extensively documented [reviewed in (Tortorella et al., 2007)]. Evidence suggests that, at least in elderly humans, decreased signal tyrosine phosphatase-1 (SHP-1) and suppressor of cytokine signaling (SOCS) (Tortorella et al., 2006) are involved in the age-related failure of GM-CSF to stimulate neutrophil function via inhibition of phosphoinositide 3-kinase (PI3-K)/ERK and signal transducer and activator of transcription (STAT) pathways (Tortorella et al., 2006). Triggering receptor expressed on myeloid cell-1 (TREM-1) is a receptor with important roles in diverse neutrophil properties, such as phagocytosis, respiratory burst and degranulation (Radsak et al., 2004), as has been demonstrated in neutrophils obtained from aged humans. Following TREM-1 engagement, neutrophils from aged donors had diminished respiratory burst relative to that of neutrophils from younger donors (Fortin et al., 2007b). In addition, the phosphorylation of TREM-1 effectors, such as AKT and phospholipase C- γ , was also altered with aging (Fortin et al., 2007b). In summary, alteration in intracellular signaling following receptor ligation is a candidate to explain decreased functionality in neutrophils from the elderly.

Toll-like receptors (TLR) 2 and 4 can recognize components of the bacterial cell wall such as peptidoglycans and lipopeptides (TLR2) and LPS (TLR4). The levels of expression of TLR2 and TLR4 on neutrophils are not affected by age (Fulop et al., 2004). However, when the distribution of TLR4 between lipid raft and non-raft fractions at the basal levels and after LPS stimulation was studied, neutrophils from aged donors showed differential recruitment of the receptor to lipid rafts when compared to cells from young donors (Fulop et al., 2004). Furthermore, a recent publication has demonstrated that, following TREM-1 activation, neutrophils from the aged failed to recruit TREM-1 to lipid rafts (Fortin et al., 2007b). Overall, these data suggests that changes in membrane fluidity seen with age could provide a general mechanism for many of the dysfunctional signaling pathways seen in neutrophils from the elderly (Larbi et al., 2008).

3. Macrophages

Monocytes originate from hematopoietic cells in the bone marrow in response to growth factors, such as macrophage-colony stimulating factor (M-CSF), GM-CSF and IL-3 (Barreda et al., 2004). After entering the blood from the bone marrow, monocytes continue to differentiate into macrophages as they migrate into tissues. Macrophages have specific functions depending on the tissue in which they reside: macrophages in secondary lymphoid organs (spleen, lymph nodes, etc.) phagocytose effete red blood cells (spleen) or lymph antigen

and debris, alveolar macrophages phagocytose dust/bacteria and surfactant, Kupffer cells reside in liver sinusoids and clear particulate matter, microglial cells in the brain clear debris in the central nervous system, osteoclasts in bones participate in bone resorption and remodeling (Albright and Albright, 2003). In general, macrophage functions involve phagocytosis of antigens, microorganisms and cellular debris, and killing of invading microorganisms and tumors through oxygen-dependent and -independent mechanisms (Underhill and Ozinsky, 2002). In addition, macrophages secrete cytokines, which, in turn, play a role in regulation of multiple immune functions, especially inflammatory responses (Albright and Albright, 2003). Each of these functions is enhanced when macrophages are activated from their resting state by a variety of stimuli, including exposure to microorganisms and cytokines (Martinez et al., 2008). In conclusion, macrophages are highly versatile cells with a broad range of functional capabilities that may be affected by senescence.

Macrophages show effects of advanced age in many of their biological properties (Figure 2). While some reports have shown decreased chemotaxis and phagocytosis in macrophages from aged humans and mice (De La Fuente, 1985; Fietta et al., 1993), other studies using aged rats have found completely opposite results (Corsini et al., 2005; Hilmer et al., 2007) or even no age-related effects (Miller et al., 2007). These differences may reflect the activation state of the macrophages, their source, or particular experimental conditions (Plowden et al., 2004a). Additionally, relative to macrophages from young mice, those from aged mice have decreased capacity for antigen presentation (Plowden et al., 2004b; Solana et al., 2006). These results have been associated with decreased expression of MHC class II molecules (Herrero et al., 2002). This compromise in chemotaxis with advanced age is also observed in other professional antigen presenting cells, such as dendritic cells (Plackett et al., 2004).

With advanced age skin is affected, and these changes have implications for its functions as a protective barrier, including its ability to heal wounds (Gosain and DiPietro, 2004). Macrophages play an important role during the inflammatory phase of wound healing, as they keep the wound bed free from infection and promote angiogenesis (Barbul and Regan, 1995). Studies performed in mice have demonstrated defective wound healing (Danon et al., 1989; Swift et al., 2001) associated with decreased percentage of phagocytic macrophages (Swift et al., 2001), as well as impaired macrophage function (Danon et al., 1989; Swift et al., 2001). Following a laser injury to the retina, ocular macrophages from aged mice, relative to those from young mice, exhibited an impairment of their anti-angiogenic responses, as evidenced by their incapability to inhibit choroidal neovascularization (Kelly et al., 2007). Compared to young animals, macrophage-rich retinal lesions in aged mice had significant downregulation of TNF α , IL-6, IL-12, IL-23, and FAS mRNA, as well as upregulation of IL-10 mRNA (Kelly et al., 2007). The results from this study suggest inability of ocular macrophages from aged mice to regulate angiogenesis. The lack of production of anti-angiogenic surveillance activity by macrophages may help to explain the higher susceptibility of aged individuals to age-related macular degeneration and certain cancers (Kelly et al., 2007).

There is not clear evidence with regard to age-dependent effects on the generation of macrophages from their monocyte precursors (Sebastian et al., 2005). While the number of blood monocytes does not appear to be changed with age, a reduction in cellularity, increased apoptosis, and decreased percentage of macrophages was reported in the bone marrow from aged volunteers (Ogawa et al., 2000). In addition, evidence from studies in bone marrow stromal cells obtained from aged mice support an age-associated impairment in insulin growth factor-1 (IGF-1) signaling, normally involved in recruitment of osteoclasts to the bone marrow surface (Cao et al., 2007). However, contradictory results have been found in other studies. Stromal/osteoblastic cells regulate the number and activity of osteoclasts through expression of growth factors, such as receptor activator of nuclear factor-kappa B (NF κ B) ligand (RANKL), M-CSF, and osteoprotegerin (OPG) (Teitelbaum and Ross, 2003). In one study,

Cao and collaborators found that OPG expression gradually decreased with advanced age in stromal/osteoblastic cells from aged mice, however, RANKL and M-CSF expression increased (Cao et al., 2005). When cultured *ex vivo*, stromal/osteoblastic cells from aged animals had a greater ability to recruit and stimulate the growth of osteoclasts and exhibited a higher expression of M-CSF mRNA, relative to young animals (Cao et al., 2005). The results from this study suggest that there are increased macrophage progenitors in the bone marrow of aged mice relative to young animals. Further studies are needed to establish the effects of aging on proliferation and differentiation of macrophages' precursors, as well as the involvement of stromal cells on age-associated pathology, such as osteoporosis.

The effects of age on other tissue macrophage populations has also have been studied. For example, advanced age resulted in an increased frequency of Kupffer cells in rat livers (Hilmer et al., 2007). There is controversy regarding whether age alters phagocytic activity by those cells (Hilmer et al., 2007; Videla et al., 2001; Vomel et al., 1981; Yamano et al., 2000). The observed differences may be derived from the use of various methods, e.g. colloidal carbon vs. polystyrene microspheres, to assess the uptake of particles by sinusoidal cells. While no age-related differences were reported in microglia in specific regions of hippocampus of brains from male mice (Long et al., 1998), greater numbers of microglia were found in aged females animals compared to young females (Mouton et al., 2002). Moreover, female mice had 25–40% more microglia in dentate gyrus and field CA1 of Ammon's horn (CA1) regions than age-matched male mice (Mouton et al., 2002). These age and sex-specific defects could be originated as a consequence of the effects of sex hormones and reproductive aging (Gomez et al., 2008b). Despite of these divergent observations, the age-associated changes in number and function of resident macrophages may contribute to increased morbidity and mortality associated with pathogens, as well as to the development of age-associated pathology.

For the most part, aging has been generally associated with increased intracellular levels and activity of the enzyme, cyclooxygenase-2 (COX-2) (Wu et al., 2001), however, there are contradictory data. Following inhalation of air borne particulate matter and inhaled gases, greater lung and systemic effects, such as spontaneous cardiac arrhythmias, are observed in aged rodents, relative to their younger counterparts (Elder et al., 2000; Nadziejko et al., 2004; Sunil et al., 2007). In one study, after inhalation of an aerosol containing ozone reaction products (present in cleansers and air fresheners), the levels of COX-2 protein were reduced in alveolar macrophages obtained from aged rats (Sunil et al., 2007). This diminution may be a potential contributor to the increased susceptibility in the elderly to air pollutants. The reported elevation in COX-2 levels has been linked to an increase in one of its products, prostaglandin E₂ (PGE₂) (Wu et al., 2001). In aged mice, overproduction of PGE₂ by NP(366–374) peptide (ASNENMDAM)-pulsed, thioglycollate-elicited peritoneal macrophages leads to inhibition of MHC class II expression and IL-12 synthesis in CD8⁺ T cells (Plowden et al., 2004b). In general, prostaglandins are described as immunosuppressive because they attenuate lymphocyte proliferation and cytokine production by T cells and macrophages (Hilkens et al., 1996). Thus, the increase in the release of PGE₂ by macrophages from aged individuals could play a role in the observed suppression of T cell function.

The effects of aging on cytokine production by macrophages have showed conflicting results with stimulation of purified macrophage populations *in vitro* versus systemic exposure *in vivo*. For example, performing *in vitro* studies, we and others reported that macrophages from aged mice produce less TNF α and IL-6 after exposure with LPS than comparably stimulated cells from young mice (Boehmer et al., 2004; Boehmer et al., 2005; Chelvarajan et al., 2005; Chelvarajan et al., 2006; Renshaw et al., 2002). This observation is consistent regardless of the source of macrophages, either thioglycollate-elicited peritoneal exudates cells (Boehmer et al., 2004; Renshaw et al., 2002) or splenic macrophages (Boehmer et al., 2005; Chelvarajan et al., 2005; Chelvarajan et al., 2006).

Despite the general agreement about decreased cytokine production by macrophages from aged rodents, studies carried out in monocytes obtained from humans have shown discordant results. The levels of the pro-inflammatory cytokines, TNF α and IL-6 (Delpedro et al., 1998), and chemokines, IL-8 (Clark and Peterson, 1994), RANTES and MIP-1 α (Mariani et al., 2002) were elevated in peripheral blood monocytes from the elderly after *in vitro* stimulation with LPS. However, evaluation of intracellular cytokines in peripheral blood monocytes (van Duin et al., 2007) revealed defective TLR1/2-induced TNF- α and IL-6 production with advanced age (van Duin et al., 2007). In spite of this, cytokine production following ligation of TLR2/6, TLR4, and TLR5 remained largely intact (van Duin et al., 2007). These differences were maintained even after correcting for variables including gender, race, medications, and comorbidities (van Duin et al., 2007). Inconsistencies between these results and those in aged animals could be related to the species employed in the studies, the maturity of the cells (fully differentiated tissue macrophages versus blood monocytes), or the methodological approaches used for the assessment of cytokine production. To complicate this further, *in vivo* evidence documents the presence of “inflamm-aging” in healthy aged subjects (Franceschi et al., 2000; Johansen et al., 2008; Salvioli et al., 2006), and elevated inflammatory response after an infectious challenge or injury in both humans and animal models (Gomez et al., 2006b; Gomez et al., 2007a; Krabbe et al., 2004; Martin et al., 2006; Nomellini et al., 2008a; Saito et al., 2000; Saito et al., 2003; Tateda et al., 1996). Since macrophages may be a source of pro-inflammatory cytokines *in vivo*, many possible explanations for this inconsistency have been proposed (Gomez et al., 2005). Among the multiple possibilities, the effect of the local environment *in vitro* versus the effect of the aged microenvironment *in vivo* should be considered.

Macrophages are exposed to a variety of agents (including hormones, cytokines, chemokines, adrenergic and cholinergic agonists, fatty acids, hormones and immunoglobulins), which can affect their functional and phenotypic characteristics (Stout et al., 2005; Stout and Suttles, 2005). As many of these agents change in quantity with advanced age, the *in vivo* function of macrophages can profoundly be affected. Thus, the age-specific microenvironment could play a key role in defining the functionality and activation properties of macrophages. We reported that serum from aged rats increased the levels of IL-6 by thioglycollate-elicited peritoneal macrophages obtained from young animals, cultured *in vitro* without stimulants (Gomez et al., 2006a). These observations suggest that the elevated production of inflammatory mediators in the aged results, in part, from the interaction of macrophages with their external milieu.

The effect of the aged-specific microenvironment has also been analyzed. In a recent study Wu et al. analyzed the levels of inflammatory mediators in the adipose tissue from aged mice (Wu et al., 2007). Higher levels of expression of the mRNA for pro-inflammatory cytokines IL-1 β , IL-6 and TNF α , and lower expression of the anti-inflammatory, peroxisome proliferator-activated receptor-gamma (PPAR- γ) were found in adipose tissue from aged mice, relative to those of young mice (Wu et al., 2007). The numbers of macrophages in adipose tissue were comparable between young and aged mice, as well as their ability to produce cytokines *in vitro* (Wu et al., 2007). However, adipocytes from aged mice produced more of these inflammatory mediators than those from young mice (Tchkonina et al., 2007; Wu et al., 2007). Moreover, conditioned medium from adipocytes of aged mice increased cytokine production by peritoneal macrophages from aged mice significantly more than that from adipocytes from young mice (Wu et al., 2007). Altogether, these data suggest that the inflammatory state developed in adipose tissue obtained from aged mice is regulated primarily by adipocytes. In addition, the contribution of macrophages to the inflammatory state may be a product of their interaction with cellular components of the aged microenvironment.

The microenvironment in aged subjects can also contribute to other facets of macrophages phenotype. The heterogeneous population of macrophages can develop into two subsets:

classically (M1) or alternatively (M2) activated macrophages (Mantovani et al., 2002). The development into one or another phenotype depends on the presence of factors, such as cytokines, specific pathogen-associated molecular patterns, endogenous danger signals, immune complexes, apoptotic cells and hormones (Martinez et al., 2008). Age may impact the polarization of macrophages towards either the M1 or M2 phenotype (Dace and Apte, 2008). For instance, the decrease in production of TNF- α , IL-1 β , IL-6 and IL-12 (Boehmer et al., 2004; Boehmer et al., 2005; Chelvarajan et al., 2005; Chelvarajan et al., 2006; Renshaw et al., 2002) observed in macrophages from aged mice stimulated *in vitro* with LPS, contrasted with an elevated IL-10 production (Boehmer et al., 2005; Spencer et al., 1996). This secretory profile, characteristic of a M2 phenotype (Martinez et al., 2008), can lead to an anti-inflammatory phenotype. Interestingly, when we studied *in vitro* cytokine production by splenic macrophages obtained from wild type (WT) vs. IL-6 knockout (KO) mice in response to LPS, the cells from aged IL-6 KO mice had a cytokine production that was similar to that of young WT animals (Gomez, C.R. and Kovacs E.J., unpublished observations). These results suggest that IL-6, an important component of the age-associated microenvironment and may play a role in regulating the age-dependent defects in macrophages.

The classical way of activating macrophages involves stimulation with LPS, or IFN- γ (Celada and Nathan, 1994). As with neutrophils, defects in various signaling pathways can be found in macrophages from aged individuals. The impact of aging on macrophage signaling through the IFN- γ receptor-mediated signal transduction pathway has been examined. No age-related differences in surface expression of IFN- γ receptor in caseinate-elicited peritoneal macrophages from mice has been reported (Ding et al., 1994). However, a marked reduction in total active STAT-1 α protein and a complete inhibition of STAT-1 gene expression in response to IFN- γ was reported in peritoneal macrophages from aged compared to young mice (Yoon et al., 2004). The incapacity of macrophages from aged mice to express and activate STAT-1 α could affect the ability to initiate the transcription of specific targets of IFN- γ stimulation (Yoon et al., 2004). However, we have failed to correlate this observation using the production of cytokines as parameters of activation after IFN- γ stimulation in splenic macrophages from aged mice (E.D., Boehmer, and E.J., Kovacs, unpublished observations). Thus, more studies are needed to confirm the validity of the initial observation.

In addition to its function as the receptor for LPS, TLR4 also binds endogenous ligands, such as oxidized low-density lipoproteins and A β peptide (Fassbender et al., 2004; Mullick et al., 2006). While the expression of TLR4 on macrophages from aged mice does not appear to change with age (Boehmer et al., 2004; Boehmer et al., 2005; Chelvarajan et al., 2005; Chelvarajan et al., 2006), surface levels of CD14, a co-receptor for TLR4 (Ingalls et al., 1999) were reduced in macrophages from aged animals when compared to those from young mice (Chelvarajan et al., 2005; Vega et al., 2004). Recently, the effect of advanced age on the surface expression of TLRs 1, 2, and 4 was evaluated in peripheral blood monocytes from human volunteers (van Duin et al., 2007). The study excluded immuno-compromised individuals and those who reported symptoms of recent infection. In addition, a repeated measures mixed effects statistical model allowed the evaluation of the effect of age, while accounting for heterogeneous baseline individual differences (van Duin et al., 2007). Relative to those of young adults, TLR1 surface expression levels were lower on monocytes of older subjects, TLR2 surface expression was not affected by age, and only a small decrease in expression was observed for TLR4 in the older group (van Duin et al., 2007). Overall, these observations suggest that proximal components of macrophage signaling cascades are affected by advanced age.

Studies aimed at analyzing the effects of age on the activation of intracellular signaling pathways have documented additional defects in macrophages from aged relative to young mice. For example, a reduction in the levels of the MAP kinases ERK, p38 and JNK was

reported in macrophages from aged mice relative to young before and after LPS stimulation (Boehmer et al., 2004; Boehmer et al., 2005; Chelvarajan et al., 2006). Alveolar macrophages obtained from young rats, expressed the p56 and p54 isoforms of JNK, whereas those from older rats expressed the p54 and p46 isoforms (Sunil et al., 2007). In this same study, following inhalation of limonene ozone reaction products, alveolar macrophages from young rats increased their ERK levels, whereas ERK expression was decreased in cells from aged animals (Sunil et al., 2007). From these report, it can be concluded that levels and the isoform expression for MAP kinases are affected in macrophages from aged animals.

Activation of macrophages triggers additional signal pathways that have been found to be altered in cells from aged individuals. Examples of this include the decreased plasma membrane translocation of PKC- α , PKC- β I, and PKC- β II in monocytes obtained from aged humans (Delpedro et al., 1998), as well as the increased cytoplasmic degradation of inhibitory κ B (I κ B) and increased nuclear translocation of NF- κ B in thioglycollate elicited macrophages from aged mice (Wu et al., 2003). Decreased expression (Chelvarajan et al., 2006) or activation (Sunil et al., 2007) of members of the NF- κ B pathway has also been found in cells from older rodents. Microarray analysis performed on RNA from resting and LPS-stimulated splenic macrophages from young and aged mice showed down-regulated expression of signal transduction genes involved in the TLR-signaling pathway leading to NF- κ B activation (Chelvarajan et al., 2006). These signaling components include the adaptor molecule, MyD88, and several members of the NF- κ B pathway (Rel-a, Rel-b, NF- κ B p50 and p52, and TRAF6) (Chelvarajan et al., 2006). Moreover, an increase in the expression of IL-1 receptor-associated kinase 3 (IRAK3), a negative regulator of this pathway was found in splenic macrophages from aged animals relative to young (Chelvarajan et al., 2006). These new data add to the story demonstrating age-related defects in cell signaling, and suggest that the TLR-dependent pathways are working at reduced efficiency in macrophages from the aged. Those alterations represent mechanisms that could be responsible for the age-associated defects in macrophage function, such as cytokine dysregulation, altered wound healing or decreased cellular response to bacterial infections.

4. NK and NKT cells

Within the immune system, NK cells play a significant role in defense against a broad variety of infections and in the inhibition of tumor growth and metastases. Although a number of studies have investigated the effects of age on NK cell number and function in both rodents and humans, there remains considerable controversy about whether advanced age adversely affects NK cell function (Table 1). Earlier studies by Albright and Albright demonstrated that the NK cell activity demonstrable in rodents at 8 weeks of age was nearly absent in mice that were 25 months of age (Albright and Albright, 1983). In humans, however, it has been shown that NK cell cytotoxicity is well preserved in centenarians and that an increase in the actual number of NK cells can be observed in healthy aging (Franceschi et al., 1996; Franceschi et al., 1995; Sansoni et al., 1993). In disease states, however, age-associated alterations in NK cell kinetics and function have been reported and include diminished proliferation rates (Zhang et al., 2007), association with higher incidences of infection (Ogata et al., 1997), onset of atherosclerosis (Bruunsgaard et al., 2001), and increased susceptibility to nutritional deficiencies (Ravaglia et al., 2000).

The discrepancy over the effects of age on NK cell number and function has arisen due to a combination of reports that either do or do not adhere to the SENIUR protocol and other age and disease parameters (Mocchegiani and Malavolta, 2004). When a more restrictive selection criteria is followed, such as the guidelines followed by the SENIUR protocol, it appears that there is an age-related increase in the overall numbers of circulating NK cells in humans (Mariani et al., 2001; Ogata et al., 1997; Solana and Mariani, 2000). However, despite the

increased number, as the age of the subject being considered advances, there appears to be a modest decline in cytotoxic ability of NK cells toward NK-sensitive targets *in vitro* (Mysliwska et al., 1992a; Mysliwska et al., 1992b; Ogata et al., 1997), while NK cell antibody-dependent cell-mediated cytotoxicity appears intact (Fernandes and Gupta, 1981). Additional reports have indicated that NK cells from aged subjects exhibit a modest impairment in their ability to produce IFN γ and proliferate in response to IL-2 (Borrego et al., 1999). Further studies are needed to further clarify age-related alterations on NK cell function *in vivo*.

Unlike NK cells, NKT cells are innate lymphocytes that express an $\alpha\beta$ T cell receptor that recognizes glycolipid antigens presented in the context of CD1d molecules that are expressed on professional antigen presenting cells (Bendelac et al., 2007; Joyce, 2001). NKT cells are found throughout the lymphoid compartment, in the circulation, and comprise the majority of hepatic lymphocytes. They are a rare cell, only constituting about 1% or less of the total lymphocyte pool. Classified as innate lymphocytes (because they express a highly invariant TCR that recognizes lipids instead of peptides), NKT cells are known for their critical importance in the clearance of viral and bacterial infections as well as facilitating anti-tumor immunity and regulating immune tolerance and autoimmunity (Bendelac et al., 2007; Joyce, 2001). Only a limited number of studies have examined the effects of aging on NKT cell number and function (Table 1), although studies do exist both in mice and humans. In general, it is accepted that as age advances, the absolute number of NKT cells within the lymphoid compartment increases (Dubey et al., 2000; Faunce et al., 2005; Ishimoto et al., 2004; Poynter et al., 1997). It is not clear however, whether the increase results from a longer life-span of NKT cells (vs. conventional lymphocytes) (Berzins et al., 2006), active expansion of the population within the aged immune microenvironment, or perhaps an age-related alteration in recruitment from the peripheral circulation to the lymphoid compartment. Interestingly, several reports have shown an age-related decrease of CD1d-restricted NKT cells in the peripheral circulation as well as decreased proliferative capacity (DelaRosa et al., 2002; Peralbo et al., 2007; Peralbo et al., 2006), which might support the notion that aging causes differential trafficking of the NKT cell population. Although they should not be confused with canonical invariant TCR-expressing NKT cells (i.e., V α 24-J α Q TCR), it has been reported that NKT-like cells (non-CD1d-restricted, but still expressing CD56 and other NK-associated molecules) are in fact, elevated in the peripheral circulation (Peralbo et al., 2007).

Although NKT cells are known for their capacity to influence both antigen presenting cell and T cell function, only a few studies have examined the contribution of NKT cells to immunosenescence. Systemic inhibition of NKT cell activation significantly prevented the age-associated decline of both *in vitro* T cell proliferative responses and *in vivo* delayed type hypersensitivity responses (Faunce et al., 2005). In these studies, it was further reported that NKT cells contributed to the age-associated increase of the immunosuppressive cytokine, IL-10 (Faunce et al., 2005). Additional reports demonstrated that NKT cells contribute to the age-associated increase in IL-4, a cytokine that is known to directly inhibit various aspects of T cell immunity and antigen presenting cell function (Faunce and Palmer, 2008; Poynter et al., 1997), while other work demonstrates an age-related decrease in IFN γ (Mocchegiani and Malavolta, 2004). Lastly, it has also been shown that age negatively affects NKT cell-mediated cytotoxic activity in mice (Mocchegiani et al., 2004; Tsukahara et al., 1997). Although NKT cells have been shown to play a significant role in shaping the overall immune response and advanced age appears to alter NKT cell number and function, more research is required in both experimental animal models and humans to fully appreciate the impact of aging on NKT cell biology and further understand the mechanisms by which NKT cells might contribute to immunosenescence.

5. Mast cells

Mast cells have wide tissue distribution including epithelia, blood vessels, nerves, the airways and gastrointestinal tract, smooth muscle cells and mucus-producing glands (Galli et al., 2005). In some species, including mice, mast cells also reside within mesothelium-lined cavities, such as the peritoneal cavity (Finlay-Jones et al., 1999). Upon activation—for example via aggregation of the FcεRI—mast cells can produce a vast array of mediators, chemokines, and cytokines with pro-inflammatory, anti-inflammatory, and/or immunomodulatory effects (Galli et al., 2005).

The effect of advanced age on the number of mast cells has been studied (Table 1). A decrease in mast cells was reported in the skin of aged humans (Montagna and Carlisle, 1990). Meanwhile, an age-associated increase in the numbers of dermal mast cells was found in BALB/c (Hart et al., 1999), but not C57BL/6 (Hart et al., 1999; Nguyen et al., 2005) mice. Interestingly, no changes were reported in the number of mast cells in lamina propria cells in the jejunum biopsy specimens collected from elderly patients with gastrointestinal symptoms, but no evidence of immunological, infectious, neoplastic, or allergic disease (Arranz et al., 1992). Thus, the number of mast cells in aged individuals depends on the tissue source and the species under analysis.

In addition to their roles in the initiation of adaptive immune responses (McLachlan et al., 2003), bacterial (Di Nardo et al., 2003) and antiparasitic clearance (Furuta et al., 2006; McDermott et al., 2003), and the promotion of inflammatory responses (Nigrovic et al., 2007), mast cells are also involved in numerous inflammatory diseases, including autoimmune disorders and atherosclerosis (Metz and Maurer, 2007; Nigrovic et al., 2007). As many of these disorders are more frequent in the elderly, it is intriguing to speculate how the dysregulations in mast cell function may contribute to the development of these pathologies. As an example, an increase in mast cell number and degranulation was reported with aging in patients subjected to aortocoronary bypass and undergoing varicose vein surgery (Pascual et al., 2007). In addition, enhanced mature transforming growth factor beta -1 (TGF-β1) correlated with age and with the varicose condition (Pascual et al., 2007). The authors of this publication have speculated that mast cells, as sources of proteases (Galli et al., 2005), could be major contributing factors to the cleavage or maturation of TGF-β1. As increased mast cells and TGF-β1 contribute to failure of the vein wall, this study suggests they participate in the induction of fibrosis that represents the final stages of venous insufficiency.

6. Eosinophils

The primary role of eosinophils is in host defense against parasites by releasing cytotoxic cellular contents, including pro-inflammatory cytokines, chemokines, and lipid mediators (Kariyawasam and Robinson, 2006). In addition, eosinophils act as antigen-presenting cells and serve as inducers of tissue damage by releasing proteins and lipid mediators (Rothenberg and Hogan, 2006). Regarding the effects of aging on eosinophil numbers, a positive correlation between increased peripheral eosinophils and serum IL-6 levels was described after analyzing data from a cross-sectional analysis, including aged women from the Women's Health and Aging Study I (Leng et al., 2005). While the mechanisms underlying the origin of the association between the elevation of eosinophils and IL-6 and described in this study need further investigation, these findings suggest a contribution of eosinophils to the age-related elevation in circulating IL-6 levels.

Eosinophils are involved in the pathogenesis of asthma, and their levels in the airway correlate with disease severity (Trivedi and Lloyd, 2007). While advanced age is associated with an increase in morbidity and mortality with asthma (Braman, 2003), no differences were found in the levels of eosinophils in the sputum of young and aged patients (Vignola et al., 2003).

(Table 1). These results suggest that aging per se may not lead to increased severity of the disease, but rather, the duration of the disease may be a defining factor for the complication observed in aged patients (Vignola et al., 2003). In a recent study, eosinophil function was analyzed in young and aged asthma patients (Mathur et al., 2008). Relative to young patients, degranulation of eosinophil-derived neurotoxin (EDN) in response to IL-5 *in vitro* was decreased in aged asthma patients relative to young (Mathur et al., 2008). In addition, a tendency for reduced fMLP-stimulated EDN degranulation and production of O₂⁻ in response to PMA was found in the older group (Mathur et al., 2008). Other eosinophil functions, such as adhesion, chemotaxis towards eotaxin, as well as parameters of lung function and the percentage of sputum eosinophils were similar between young and aged patients (Mathur et al., 2008). These data confirm previous literature showing comparable airway eosinophilia in young and aged asthma patients (Vignola et al., 2003). While these findings do not represent age-related changes in the function of airway, they suggest that advanced age impairs eosinophil effector functions. Thus, differences in the response to allergens in the elderly due to age-related changes eosinophil function may help to explain the increased severity of asthma in aged subjects.

7. Perspectives

In the previous sections, we have addressed the contribution of cells of innate immune system to immunosenescence. While important in the context of aging, one must also consider that the aging phenotype is a consequence of the combined effects of dysfunctional immune, endocrine and nervous systems. In addition, the genetic makeup, in combination with environmental factors, such as oxidative damage, ultraviolet light and other types of radiation, individual lifestyles and geography all play a major role in determining the phenotype of the aged (Straub et al., 2001). The result of the interaction between all of the variables must be considered when the range of outcomes of the aged phenotype is observed. Of note, genetics is a main determinant of individual aging (Candore et al., 2006; Cournil and Kirkwood, 2001). In recent years, population studies have suggested that certain polymorphisms, including those involving innate immune responses, are linked to lifespan, responsiveness to stressors and pathology in the elderly (Franceschi et al., 2000; Salvioli et al., 2006). That is to assume that those genetic variants will be beneficial for some individuals to resist challenges and insults, such as infections, trauma or injury as they age.

While the genetic makeup can not be modified, interventions may help to reduce the aging phenotype and/or act as therapeutic agents (Nomellini et al., 2008b). In particular, we would like to briefly discuss the potential of those aimed at changing the components of the aged microenvironment towards a more youthful milieu. As a result of the aging process, dramatic changes occur in the endocrine system, particularly in the sex hormones (Gomez et al., 2008b). Thus, it is possible that the sex hormone environment may play a role in the effects of aging on the immune system, as well as in immune responses to injury and infection. When given at low doses, estrogen has a potent anti-inflammatory effect (Flake et al., 2006; Kovacs et al., 2002). In aged humans and rodents, estrogen administration prior to insult can accelerate dermal wound healing (Ashcroft et al., 1999), protects from cardiovascular disease (Moorthy et al., 2004), and improves systemic and cellular inflammatory responses to traumatic injury (Gomez et al., 2007b; Kovacs et al., 2004a; Kovacs et al., 2004b). However, as estrogen supplementation in postmenopausal women has been restricted due to increased cardiovascular disease and cancer (Kim and Morley, 2005; NAMS, 2007), precaution should be exerted in the design of therapeutic strategies involving this hormone.

In aged individuals without underlying disease, “inflamm-aging” (Franceschi et al., 2000), is characterized in part by circulating levels of IL-6 (Ershler, 1993; Ershler et al., 1994; Ershler et al., 1993). “Inflamm-aging” has been associated with mortality and disability among the

elderly (Ferrucci et al., 1999). In an attempt to better define the role of this cytokine in the systemic inflammatory response in the aged, we challenged young and aged WT and IL-6 KO mice with LPS. Our results comparing LPS exposure in aged IL-6 KO mice with the same treatment in aged WT mice revealed improved survival (Gomez et al., 2006b), decreased acute phase response (Gomez et al., 2006b), and reduced hepatic injury (Gomez et al., 2008a). These observations suggest that blocking this cytokine's production and/or action on target cells may diminish aberrant innate immune responses and organ damage associated with hyper-inflammatory responses in aged individuals. Determining the contribution of the diverse elements that define the aging phenotype to aberrant functioning of the innate immune system in the elderly will help to elucidate potential strategies for age-associated pathology, response to insults and extended longevity in the elderly.

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Figure 1.

Abbreviations: fMLP: N-formyl-methionyl-leucyl-phenylalanine; GM-CSF: granulocyte monocyte colony stimulating factor; TLR4: toll-like receptor 4; PMA: phorbol myristate acetate; ERK: extracellular signal regulated kinase; IP₃: inositol triphosphate; DAG: diacyl glycerol; cAMP: cyclic adenosine monophosphate; pTREM-1: triggering receptor expressed on myeloid cell-1.

Figure 2.

Abbreviations: MHCII: major histocompatibility complex II; CD14: cluster of differentiation 14; TLR: toll-like receptor; COX-2: cyclooxygenase-2; STAT-1 α : signal transducer and activator of transcription-1 alpha; MAPK: mitogen activated protein kinase; PKC: protein kinase C; NF- κ B: nuclear factor-kappaB.

Table 1

Effects of aging on NK, NKT, mast cells and eosinophils

Cell type	Change with advanced age	References
NK cells	↑ numbers in circulation	(Mariani et al., 2001; Ogata et al., 1997; Solana and Mariani, 2000)
	↓ activity	(Albright and Albright, 1983)
	↓ cytotoxicity	(Mysliwska et al., 1992a; Mysliwska et al., 1992b; Ogata et al., 1997)
	Intact ADCC	(Fernandes and Gupta, 1981)
	↓ proliferation and IFN- γ production in response to IL-2	(Borrego et al., 1999)
NKT cells	↑ numbers in lymphoid organs and blood	(Dubey et al., 2000; Faunce et al., 2005; Ishimoto et al., 2004; Poynter et al., 1997)
	Contribute to age-associated:	
	↓ in vitro T cell proliferation	(Faunce et al., 2005)
	↓ in vivo DTH	(Faunce et al., 2005)
	↑ IL-10 and IL-4	(Faunce et al., 2005; Poynter et al., 1997)
Mast cells	↓ cytotoxicity	(Mocchegiani et al., 2004; Tsukahara et al., 1997)
	↓ numbers in the skin of humans	(Montagna and Carlisle, 1990)
	↑ numbers in the skin of BALB/c mice; no change in C57BL/6 mice	(Hart et al., 1999; Nguven et al., 2005)
Eosinophils	↑ degranulation in varicose disease	(Pascual et al., 2007)
	No change in cell number in sputum	(Vignola et al., 2003)
	↓ release of EDN in response to IL-5 in asthma patients	(Mathur et al., 2008)
	↓ release of EDN in response to fMLP	(Mathur et al., 2008)
	↓ production of O ₂ ⁻ in response to PMA	(Mathur et al., 2008)
	No change in adhesion or chemotaxis in asthma patients	(Mathur et al., 2008)

Abbreviations: NK: natural killer; ADCC: antibody-dependent cell mediated cytotoxicity; IFN: interferon; IL: interleukin; NKT: natural killer T; DTH: delayed-type hypersensitivity; EDN: eosinophil-derived neurotoxin; fMLP: formyl-methionyl-leucyl-phenylalanine; O₂⁻: superoxide; PMA: phorbol myristate acetate.