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Advanced Age Alters Monocyte and Macrophage Responses

Joslyn M. Albright,[1,2] Robert C. Dunn,[2,3] Jill A. Shults,[1,2] Devin M. Boe,[4] Majid Afshar,[2,3,5,6] and Elizabeth J. Kovacs[4]

Abstract

Significance: With the growing population of baby boomers, there is a great need to determine the effects of advanced age on the function of the immune system. ***Recent Advances:*** It is universally accepted that advanced age is associated with a chronic low-grade inflammatory state that is referred to as inflamm-aging, which alters the function of both immune and nonimmune cells. Mononuclear phagocytes play a central role in both the initiation and resolution of inflammation in multiple organ systems and exhibit marked changes in phenotype and function in response to environmental cues, including the low levels of pro-inflammatory mediators seen in the aged. ***Critical Issues:*** Although we know a great deal about the function of immune cells in young adults and there is a growing body of literature focusing on aging of the adaptive immune system, much less is known about the impact of age on innate immunity and the critical role of the mononuclear phagocytes in this process. ***Future Directions:*** In this article, there is a focus on the tissue-specific monocyte and macrophage subsets and how they are altered in the aged milieu, with the hope that this compilation of observations will spark an expansion of research in the field. *Antioxid. Redox Signal*. 25, 805–815.

Keywords: aging, clinical, immunology, infection, inflammation

Introduction

THE ELDERLY POPULATION is the fastest-growing segment of the U.S. population, with more than 20% of the country projected to be age 65 or older by 2030, compared with 13% in 2010 (70). As the population ages, research examining specific changes in the immune system is relevant not only by sheer number of people affected but also by increasing expense. At age 65, the first year of Medicare eligibility, average annual healthcare charges are less than $5000 per person per year, a figure that more than doubles by age 80 (8). To get an impression of the magnitude of that expense at a national level, the United States treasury department has reported that national healthcare costs due to aging will grow at a rate of ~2% per year and that government expenditure on healthcare will increase to more than 6% of the potential gross domestic product (GDP) over the next 10 years (88). Government spending on Medicare and Medicaid was 4.8% of the potential GDP in 2010. Not only do aged individuals have worse outcomes after illness or injury, but they also contract infectious diseases such as flu and pneumonia at much higher rates than their younger counterparts (21). For example, during most flu seasons in the United States, an estimated 90% of flu-related deaths and 50% to 60% of flu-related illness occur in people aged 65 or older.

Advanced age is associated with a chronic low-grade inflammatory state that is referred to as inflamm-aging (32). This process is perceived to be responsible for the impaired innate and adaptive immune responses seen in the elderly (34, 80). Although the factors responsible for initiating inflamm-aging have not been fully defined, there are several theories

[1]Department of Surgery, Loyola University Chicago Health Sciences Campus, Maywood, Illinois.
[2]Burn and Shock Trauma Research Institute, Loyola University Chicago Health Sciences Campus, Maywood, Illinois.
[3]Stritch School of Medicine, Loyola University Chicago Health Sciences Campus, Maywood, Illinois.
[4]Department of Surgery, University of Colorado Denver Anschutz Medical Campus, Aurora, Colorado.
[5]Department of Medicine, Loyola University Chicago Health Sciences Campus, Maywood, Illinois.
[6]Department of Public Health Sciences, Loyola University Chicago Health Sciences Campus, Maywood, Illinois.

that involve both intrinsic and extrinsic effects on leukocytes and the environment in which they mature and reside. Hallmarks of inflamm-aging include basal levels of pro-inflammatory cytokines interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNFα), which are elevated even in healthy aged individuals (32).

Prevailing theories by which inflamm-aging is initiated are numerous and include chronological age-dependent alterations in the following: (i) post-translationally modified macromolecules, including DNA and proteins that stimulate leukocytes and other cells to secrete pro-inflammatory cytokines; (ii) senescence of immune and nonimmune cells, leading to an increased release of inflammatory mediators *via* a senescence-associated secretory phenotype; and (iii) increased intestinal permeability, allowing bacteria and bacterial products (*e.g.,* endotoxin) to enter the circulation and change in the bacterial communities or microbiome of the gastrointestinal tract (50, 85). These theories are detailed elsewhere (9, 33) and are beyond the scope of this article. Additionally, the association of aging and inflammation with human diseases, such as atherosclerosis, metabolic syndrome, and osteoporosis, is a well-documented pairing that is also beyond the range of this article but has been recently reviewed (38, 43). The focus of this article is on monocytes and macrophages, key cells in the inflammatory cascade and its regulation (Fig. 1).

The impact of advanced age on macrophage activation and signaling has been examined by several groups, mainly in rodent models of aging [reviewed in Gomez *et al.* (40)]. Published projects working with human macrophages are rare. Circulating monocytes are the most accessible mononuclear phagocytes, making them and monocyte-derived macrophages the most frequently inspected in human studies, but even those results are sparse and conflicting (90). The bulk of the material presented here will be from animal work, and human data will be highlighted where appropriate.

Regulating Inflammation

Mononuclear phagocytes play a central role in both the initiation and resolution of inflammation. Secreted cytokines are key mediators of these processes (7, 95). In the tissues, macrophages produce various pro-inflammatory cytokines in response to infectious stimuli, including TNFα, IL-1β, IL-6, nitric oxide (NO), reactive oxygen species, and neutrophil chemokines CXCL2 and CXCL8 (human homologs to murine macrophage inflammatory protein 2 (MIP-2), CXCL1, and KC) (20, 45, 63). Prolonged pro-inflammatory activation of macrophages leads to unregulated collateral tissue damage

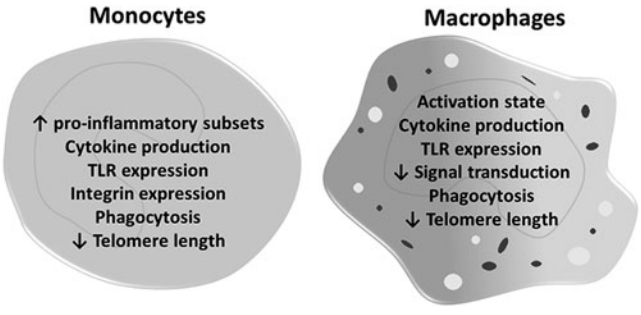

FIG. 1. Macrophage characteristics altered in aging.

(7, 15, 94). Therefore, by necessity, tissue inflammation is a highly regulated process and mononuclear phagocytes are integral to this process.

Resolution of inflammation is achieved by several mechanisms that incorporate resident tissue macrophages: (i) removal of pathogens by neutrophils and macrophages; (ii) downregulation of neutrophil chemokines; and (iii) removal of apoptotic neutrophils (4). Efferocytosis is the phagocytosis of dying cells, including neutrophils and bacteria, by professional phagocytes and other cells, a process that downregulates IL-12, TNFα, and NO secretion and upregulates anti-inflammatory IL-10 and transforming growth factor beta (TGFβ) (5, 35, 51, 71, 93). For example, in the lung, macrophage efferocytosis helps facilitate restoration of tissue integrity *via* macrophage release of epithelial growth factors, platelet-derived growth factor, vascular endothelial growth factor, and hepatocyte growth factor (42, 64). In addition, they release prostaglandin E_2 to stimulate endothelial cell migration and promote angiogenesis (17). Migration of macrophages to nearby lymph nodes and the apoptosis of macrophages themselves also help to resolve inflammation (49, 52). Overall, both macrophage efferocytosis and apoptosis are important in the restoration and remodeling of injured tissue; however, dysregulation of anti-inflammatory signals also has consequences. Animal experiments have shown an inadequate pro-inflammatory response and insufficient pathogen clearance after excessive efferocytosis (61). Taken together, the initiation and resolution of inflammation is a narrowly orchestrated process that is dependent on macrophages for coordinating both pro- and anti-inflammatory responses.

Recent research into the resolution of inflammation has uncovered an important role for monocyte- and macrophage-produced lipid signaling molecules that are known as specialized pro-resolving mediators (SPMs). Apart from the prostaglandins and leukotrienes classically characterized as pro-inflammatory lipid mediators, SPMs are a group of ω-3 polyunsaturated fatty acid-derived molecules encompassing families such as lipoxins, protectins, maresins, and the D- or E-series resolvins. Mainly metabolized from docosahexanoic acid and eicosapentaenoic acid, SPMs contribute to the resolution of inflammation by regulating the production of cytokines and chemokines, limiting neutrophil influx, and enhancing the pro-resolving actions of macrophages such as efferocytosis of apoptotic cells and clearance of bacteria and debris [reviewed in Serhan *et al.* (76)]. Maresins, for example, promote a transition from pro-inflammatory to anti-inflammatory macrophages in an autocrine manner, encouraging resolution of tissue inflammation and wound healing (77, 78). The pro-resolution activities of SPMs do not seem to act in an immunosuppressive manner (69, 74, 83). Animal experiments offer evidence that SPMs may also improve microbial removal and play a protective role against infections, lowering the antibiotic requirements to effectively clear bacterial infections (26, 74). In a murine model of age-associated adipose inflammation (14), low doses (1 n*M*) of lipoxin-A4, a potent SPM, reduced production of IL-6 and increased IL-10 levels in adipose tissue explants, as well as attenuated *in vitro* secretion of TNFα and monocyte chemotactic protein 1 (MCP-1) by lipopolysaccharide (LPS)-treated J774 macrophages. Furthermore, studies by Arnardottir *et al.* (6) demonstrated that aged mice

exhibit delayed resolution of acute inflammation in parallel with altered lipid signaling dynamics, finding that aged mice produced lower levels of SPMs and increased quantities of pro-inflammatory prostaglandins and thromboxanes relative to young controls. Remarkably, levels of pro-inflammatory lipid mediators were higher in the aged mice even at baseline, indicating that dysregulated SPM signaling is an inherent factor in age-related immune dysfunction. The authors also employed resolvins D1 and D3 to enhance efferocytosis by macrophages from aged mice and to reduce prolonged inflammation in aged mice, partially compensating for deregulated SPM production. The pro-resolving activities of SPMs, as well as their potent ability to help macrophages control inflammation, make SPMs and their synthetic analogs attractive candidates for immune-modulating therapeutics, with a wide range of applications in experimental models and human disease (28, 75).

Macrophage Activation Nomenclature

Macrophage transition from equilibrium to inflammation and back again is made possible by the remarkable plasticity of macrophages (36, 59). Reversible activation into a pro-inflammatory state depends on factors present within the microenvironment. Classification guidelines of macrophage activation profiles have recently changed. Leading experts in macrophage research provided a consensus classification terminology in 2014 (65). Three recommendations were put forth to describe the activation state of macrophages. First, identify the model (*in vitro vs. in vivo*) and method of isolation. Second, identify the mediators used to stimulate macrophage activation. Lastly, describe the up- or down-regulation of cell-surface or intracellular markers that are associated with mediator-specific induction.

Before these guidelines, most reports generalized macrophages into either a classical, pro-inflammatory M1-activated macrophage or an alternative, anti-inflammatory M2-activated macrophage (this included all postulated subsets of M2: M2a, M2b, and M2c). Figure 2 outlines a generalized classification of M1 and M2 macrophages. M1/pro-inflammatory macrophages are characterized by mediators of activation, including interferon gamma (IFNγ), TNFα, and LPS. These factors activate signal transducer and activator of transcription (STAT) 1 signaling and upregulate a scavenger receptor macrophage receptor with collagenous structure, inducible nitric oxide (iNOS, Nos2), and IL-6 and TNFα gene expression. In contrast, M2/anti-inflammatory macrophages are activated by IL-4, IL-10, IL-13, and TGFβ. These mediators stimulate the STAT3 or STAT6 signaling cascade and upregulate gene expression of mannose receptor CD206, IL-4 receptor, IL-10, TGFβ, arginase 1 (ARG1), resistin-like α (Retnla, Fizz1), and chitinase 3-like 3 (Chi3l3, Ym1) (81). Publications are now starting to utilize this environmental nomenclature to define macrophage populations (29, 89). Thus, the studies reviewed here will attempt to use the terms pro-inflammatory and anti-inflammatory as well as these markers for the general classification of macrophages and to avoid terms such as M1, M2, classical, regulatory, *etc.*

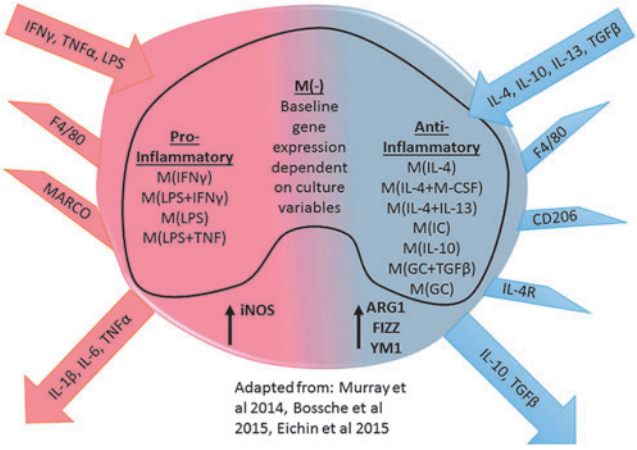

FIG. 2. Macrophage mediator-based nomenclature. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

Advanced Age and Mononuclear Cells

No differences have been found in the number of peripheral blood monocytes in the circulation of elderly subjects compared with their younger counterparts. However, there is an age-dependent shift in the proportion of monocyte subsets and subsequent inflammatory profiles (60, 86). Human monocytes from elderly participants have increased levels of $CD14^{++(high)}CD16^{+}$ and $CD14^{+(low)}CD16^{+}$ pro-inflammatory/nonclassical monocytes and decreased levels of $CD14^{+}CD16^{-}$ monocytes (44, 67, 73). However, these monocytes have a markedly attenuated inflammatory response (decreased levels of both IL-6 and TNFα) after TLR1/2 stimulation compared with the younger age group alongside a decreased TLR1 expression (67).

In elderly participants compared with young subjects, Hearps *et al.* (44) showed that CD11b, an integrin involved in transendothelial migration and important in plaque formation (82), had greater expression on circulating monocytes (91). L-selectin is responsible for leukocyte rolling and adhesion to endothelial cells and is downregulated on monocytes from elderly individuals (27). The altered expression of CD11b and L-selectin may affect monocyte migration and function in elderly individuals. Additionally, Hearps and coworkers demonstrated that TLR4-stimulated monocytes from elderly participants had impaired phagocytosis, shorter telomeres, and elevated intracellular TNF; these results suggest a dysregulation of monocyte function in the elderly. Clinically, the pro-inflammatory nature of elderly monocytes may prove to be a beneficial target to help maintain healthy aging. Recent evidence from a study of Japanese centenarians shows that a pro-inflammatory state, marked by elevated serum C-reactive protein, IL-6, and TNFα, is a significant predictor of longevity over telomere length (3).

Renshaw *et al.* revealed that defects in the production of cytokines by monocytes and macrophages from aged mice in response to *in vitro* LPS stimulation stemmed from aberrant expression of Toll-like receptor (TLR) mRNAs (72). We and others have corroborated the observed diminished cytokine release by macrophages from aged mice (11–13, 16, 24, 47, 56, 58). The mechanism responsible for this defect has not been confirmed. However, it seems that chronic exposure to low-level elevations of IL-6, a factor frequently implicated in inflamm-aging, is associated with age-related dampening of the macrophage pro-inflammatory response. In 2010, Gomez

et al. published their observations on the role of IL-6 and the behavior of macrophages from aged mice (39). Using young and aged IL-6 knockout mice, *in vitro* cytokine production by splenic macrophages was tested after LPS stimulation. When IL-6 was present in aged mice, an impaired inflammatory response was seen in these macrophages (lower TNFα, IL-1β, IL-6, and IL-12). However, in the aged IL-6 deficient mouse model by the same group, the aged mice had a stronger inflammatory response compared with young mice. The presence of elevated levels of IL-6 in aged mice did not seem to play a role in the number of macrophages in the splenocyte population or the surface expression of TLR4. Two independent studies from separate groups also failed to show age-dependent differences in cell-surface expression of TLR4 in macrophages from aged mice (11, 23). Human monocyte studies also fail to show a clear age-related difference in TLR expression; factors such as exercise, sex, and comorbidities seem to play a role as well [reviewed in van Duin and Shaw (90)]. Possible explanations for the discrepancy in TLR4 expression could be related to cell populations and purity (resident macrophages *vs.* peripheral blood mononuclear cells) or methods of assessment (PCR *vs.* flow cytometry). It should also be noted that although pro-inflammatory stimuli, such as LPS and IFNγ, are the more commonly used *in vitro* mediators, aged macrophage response to anti-inflammatory activation, for example, IL-4, is also altered (31, 48, 58).

Boehmer *et al.* demonstrated the relationship between aging and defects in mitogen-activated protein kinase (MAPK) signaling, leading to decreased cytokine production (11–13). A subsequent publication identified that the activation/signaling deficiencies in macrophages from aged mice were limited to the TLR2 and TLR4 signaling pathways. Parallel studies, using alternate means of activating macrophages with IFNγ, failed to show an age-dependent reduction in cytokine production (12). Relative to macrophages from young mice, cells from aged animals also have decreased levels of cytoplasmic p38 and c-Jun N-terminal kinase (JNK) MAPKs (12, 13), which may help explain the inability of macrophages in aged mice to be appropriately activated. A simplified signal transduction model illustrating these findings is included in Figure 3. Work from another group, also using interferon activation, revealed that macrophages from aged mice had a reduction in the activation of STAT1, relative to cells from younger mice (96). In both sets of studies, the changes in the activation of signaling molecules parallels a reduction in the total protein in cells from aged mice. In the setting of infection, a recent study examining human monocyte-derived macrophage showed altered responses in PI3K-AKT signaling in cells from elderly subjects. Before infection, there were no differences in protein kinase B (AKT) phosphorylation but the baseline AKT phosphorylation in cells from the elderly varied widely, perhaps again pointing to environmental factors such as diet and exercise that can modulate the basal inflammatory state of an elderly individual. Then after exposure to heat-killed bacteria, there was greater activation of AKT in macrophages derived from monocytes obtained from younger volunteers (92). This observation is not consistent with murine splenocyte studies in which an increase in PI3K-AKT activation was seen in splenic macrophages from aged mice. In the latter study, activation with bacterial ligands yielded a decreased cytokine production (30). These divergent observations may reflect

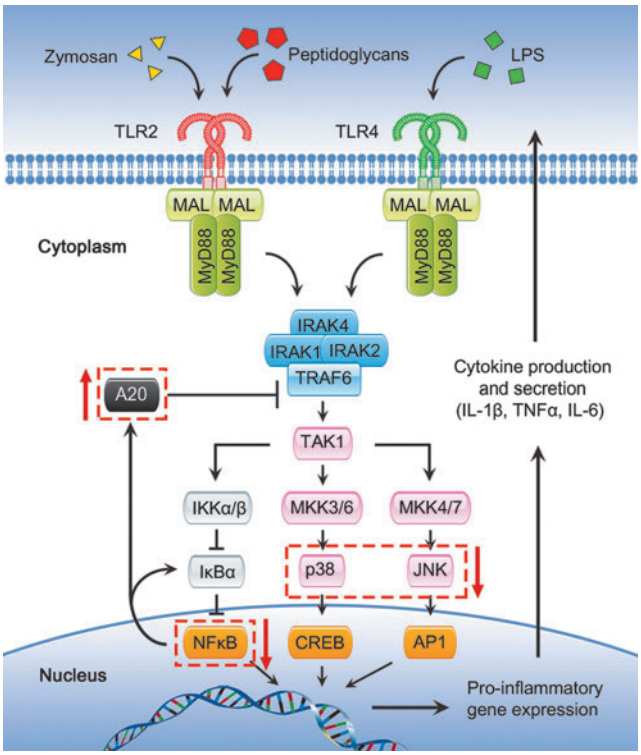

FIG. 3. TLR2 and TLR4 signaling pathways and aging. This figure illustrates intracellular TLR signaling pathways as discussed in the text of this article. *Red arrows* and *dashed boxes* signify components of signal transduction pathways that are found to be involved in age-related monocyte/macrophage dysfunction (11–13, 16, 46). To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

species-specific differences, variations in activation parameters or culture conditions. PI3K-AKT signaling is involved in bacterial killing, NO production, and cytokine secretion.

Recently, Hinojosa *et al.* (46) focused on the cytosolic suppressor of nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) and MAPK known as A20. The A20 molecule has been shown to deubiquitinate and inhibit TNF receptor-associated factor 6 (TRAF6), which activates the NFκB and MAPK signaling cascades *via* interactions with TGFβ-activated kinase 1 (TAK1) (Fig. 3). It was found that A20 is elevated in some of the tissues and macrophages of aged mice, specifically in the lungs and alveolar macrophages. The overexpression of A20 was found to dampen the cytokine response to bacteria but had no effect on the ability of macrophages to phagocytize. This study also describes a potential mechanism by which TNFα contributes to inflamm-aging, since *in vitro* stimulation of alveolar macrophages with TNFα induced a rise in A20 levels. The authors discussed that the elevated TNFα in this model could be secreted from senescent lung cells, further supporting the thought that multiple factors play a role in regulating cytokine production by macrophages.

When examining phagocytosis in macrophages from aged mice, differences in age and tissue type again play an important role. The ability of peritoneal macrophages from aged mice to phagocytose fluorescent particles was reduced relative to young mice, but bone marrow monocytes and bone marrow-derived macrophages from aged mice had phagocytic

ability similar to their younger counterparts. No intrinsic defect in macrophages from aged mice could be found, but it seems that the microenvironment in the peritoneum likely caused the impairment of macrophage function in this model (57). The aged mice had a decreased proportion of macrophages in the resident peritoneal population, as well as increased peritoneal levels of B cell-derived IL-10, leading to compromised macrophage phagocytosis. Takahashi *et al.* (87) recently showed that *in vivo* phagocytosis of necrotic cells was attenuated in aged peritoneal macrophages. In this study, the decreased necrotic cell clearance led to prolonged peritoneal inflammation, which may contribute to deleterious clinical outcomes.

Further investigation revealed that the altered behavior of macrophages from aged subjects is not necessarily due to intrinsic aged macrophage defects but the tissue-specific inflamm-aging microenvironment (48, 54, 58). These studies set out to characterize the aged phenotype and, though they are conflicting at times, there are a few common elements. Some inconsistencies can be explained by differences in experimental technique and design. Nevertheless, bone marrow-derived cells seem to be the least affected by the aging microenvironment (Table 1). Mononuclear cells separated directly from aged bone marrow as well as those cultured from harvested bone marrow did not tend to show differences in cytokine production or phagocytosis when

Table 1. Unique Age-Dependent Findings in Select Recent Publications

Species, strain, and age	*Major macrophage findings*	*Additional comments*	*Reference*
Mouse C57BL/6 20–24 months	In aged mice: ↑ percentage of circulating monocytes and macrophages in the spleen ↓ percentage of macrophages in the peritoneum ↑ number of mitochondria and mitochondrial reactive oxygen species after LPS stimulation ↓ autophagy	Showed that autophagy deficient macrophages (Atg7 knockout) have similar phenotype to aged macs in their studies	(85)
Mouse C57BL/6 18–24 months	↓ percentage of peritoneal macrophages in aged animals with ↓ phagocytosis of necrotic cells.	Decreased necrotic cell clearance *in vivo* (peritoneum) lead to elevated peritoneal MIP-2 Prolonged inflammation in aged mice	(87)
Mouse C57BL/6 21 months	↑ A20 expression in alveolar macrophages from healthy aged mice ↓ NFκB and MAPK signaling A20 can be induced by TNFα but not IL-6.	Dietary fish oil lowers A20 levels and protects aged mice from *Streptococcus pneumoniae* infection	(46)
Mouse Swiss Albino 12 and 16 months	↓TLR2 and TLR4 expression in resident peritoneal macrophages from aged mice		(79)
Mouse BALB/c 19–21 months	Response to infection by alveolar macrophages from aged mice: ↓ TNFα and IL-6 production ↓ NFκB, JNK, and p38 activation ↑ ERK activation	Aged lung lower levels of IL-6 and IL-1β after infectious challenge	(16)
Mouse BALB/c 17–18 months	↓ number of marginal zone macrophages in the spleen of aged mice No age-dependent difference in phagocytosis	Anatomical breakdown of the marginal zone with age.	(10)
Mouse BALB/c 18–20 months	↓ splenic macrophages, pro-inflammatory response after LPS stimulation and other pro-inflammatory stimuli ↓ anti-inflammatory response after incubation with IL-4 No difference in pro-and anti-inflammatory phenotype markers between bone marrow-derived macrophages from young and aged mice	From primary macrophages: global suppression of macrophage function. From bone marrow-derived macrophages: age-dependent differences in macrophage phenotype lost after prolonged cell culture	(58)
Mouse/C57BL/6 and B6.SJL-Ptprc[a] Pepc[b]/BoyJ 15–20 months	↓ phagocytosis by peritoneal macrophages from aged mice both *in vivo* and *in vitro*. No difference in phagocytosis by bone marrow-derived macrophages or bone marrow monocytes		(57)

IL, interleukin; JNK, c-Jun N-terminal kinase; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MIP-2, macrophage inflammatory protein 2; NFκB, nuclear factor kappa-light-chain-enhancer of activated B cells; TLR, Toll-like receptor; TNFα, tumor necrosis factor alpha.

compared with young cells (57, 58). This could suggest that, though there is evidence of age-related changes (48, 68), the bone marrow is not as affected by inflamm-aging (58). On the other hand, peritoneal macrophages appear to be the most impaired of tissue-specific types but this has not been well delineated. Differences between publications could be attributed to inconsistencies between resident cells and thioglycolate-elicited macrophages and/or the increasing numbers of peritoneal lymphocytes and their secretions as animals age (22, 25, 54, 57).

Additionally, the aged phenotype can be returned to a young phenotype with *ex vivo* cellular interventions or by *in vivo* treatments in an animal model. For example, aged macrophage response to stimuli is similar to young macrophage response after removal from the tissue and *in vitro* treatment with cytokines such as IFNγ. This demonstrates that macrophages from aged mice do not lose their functional plasticity/adaptivity, and it further reveals that altered responses by macrophages from aged mice are due to microenvironmental effects (58, 84). Structural differences in macrophages from aged mice include reduced receptor expression and telomere length, but these changes do not seem to have as dramatic an effect as the cytokine milieu. Finally, and perhaps most clinically relevant, several investigators have found that some of the variances in macrophages from an aged milieu can be abrogated with diet and exercise (2, 37, 46, 54, 62). Exercise-trained older mice had greater resistance to viral infection than the nonexercise group, and their cytokine production also normalized to younger mice levels after LPS stimulation; whereas nonexercising older mice did not show the same change. Exercise has also been shown to enhance TNFα release and antiviral resistance in macrophages from aged mice (53). Hinojosa *et al.* found that the age-associated elevations in A20 were nullified by supplementing the mouse diet with anti-inflammatory n-3 polyunsaturated fatty acids from fish oil (46).

Responses to systemic stressors such as viral infections and neoplasia are also different in aging. Elsewhere in this Forum, tumor-associated macrophages will be reviewed (Submitted by Jo Van Ginderachten), but it is worth pointing out in this article that there is evidence that the systemic innate immune changes related to neoplasia are different in aged mice compared with young mice (84). For example, Jackaman *et al.* found that both young and aged mice macrophages were polarized to an anti-inflammatory response when challenged with a tumor. However, aged mouse macrophages made large amounts of IL-4 in response to a neoplastic environment whereas young mice did not, leading to an immunosuppressive tumor microenvironment in the aged (48). After stimulation with herpes simplex virus 1 (HSV-1), peritoneal macrophages from old and middle-aged mice showed better intrinsic viral resistance compared with younger mice. However, alveolar macrophages from middle-aged mice were more effective than those in both young and aged mice. Resident peritoneal macrophages from aged mice also produced less TNFα on HSV-1 stimulation, and alveolar

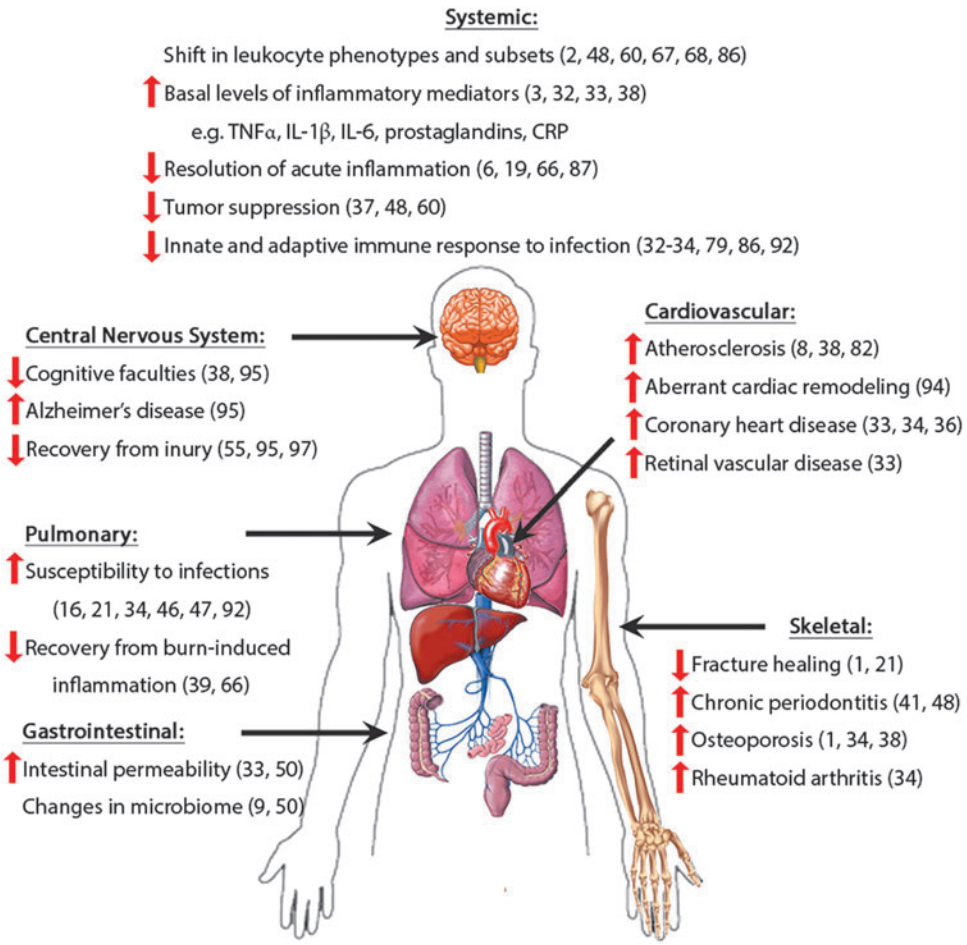

FIG. 4. The physiological effects of inflamm-aging. Examples of organ dysfunction and pathologies related to immunosenescence and innate immune dysregulation in the elderly. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

macrophages secreted less IL-12 compared with the younger group, showing both age- and location-related changes in macrophage response. Viral infections are common in the elderly population and typically lead to worse outcomes. Both alveolar and peritoneal resident macrophages seem to have impaired extracellular combat of viral infections and may prove to be important cellular targets for improving clinical outcomes.

Models of aging that involve an *in vivo* inflammatory challenge (*i.e.,* injury or systemic infection) show that tissue-specific macrophages from aged mice have a heightened inflammatory response and an impaired anti-inflammatory response (31, 55). There are, however, conflicting results from human studies of infection. For example, Verschoor *et al.* reported that monocyte-derived macrophages from elderly subjects produced less TNFα and IL-6 after exposure to *Streptococcus pneumoniae in vitro*. This decrease in cytokine production paralleled a decrease in bacterial killing compared with cells derived from young subjects, but there was no difference in phagocytosis (92). Pro-inflammatory responses such as IL-1β, TNFα, and iNOS have all been shown to be upregulated more in aged animals compared with the response seen in younger mice after injury (19, 66). IL-4 receptor expression and cellular anti-inflammatory response to IL-4 treatment are also reduced after exposure to *in vivo* inflammation.

Yabluchanskiy *et al.* showed that cardiac outcomes were worse in aged mice but improved if anti-inflammatory macrophages were present (94). Aged animals had elevated plasma levels of matrix metalloproteinase-9 (MMP-9), corresponding to increased left ventricular dilation and worse left ventricular ejection fraction. In a model with gene deletion of MMP-9 expression, mice had improved cardiac function and survival. Importantly, the macrophages isolated from the cardiac scar in the null animals had significantly higher expression of the following tissue-repairing, anti-inflammatory markers: CD163, mannose receptor, TGFβ, and Ym1.

Models that represent diseases commonly found in the elderly have also shown age-related differences in macrophage activation. Using naturally occurring periodontitis in aged Rhesus monkeys, Gonzalez *et al.* found that macrophages from healthy aged gingival tissue had increased expression of pro-inflammatory genes compared with young monkeys (41). These genes were then further elevated in the setting of aging and periodontitis. Studies such as these suggest that in certain anatomic locations, healthy aged tissues host a macrophage phenotype that promotes increased inflammation and tissue destruction that is made worse in a diseased state (1, 97).

Conclusions, Opportunities, and Challenges

Research in aging macrophages presents unique challenges. First, defining tissue-specific basal aged phenotypes remains elusive. In areas of the body with constant inflammatory stimulus and exposure to environmental pathogens, the system seems to be heightened with an elevated resting inflammatory state and a subsequent excessive reaction. However, in other areas with limited environmental exposure, the macrophages tend to present an anti-inflammatory phenotype with a subdued reaction to stimulation compared to responses in younger individuals.

Second, it seems as if the ''aged phenotype'' is reversible. Once removed from the inflamm-aging microenvironment, the macrophage response can be restored to generate a response similar to the young macrophage activation profiles. This presents therapeutic opportunities that may involve a patient's own macrophages to not only treat acquired diseases but also affect many pathologies and physiological dysfunctions associated with the aging process (Fig. 4) (18).

Acknowledgments

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Address correspondence to:
Dr. Elizabeth J. Kovacs
Department of Surgery
University of Colorado Denver Anschutz Medical Campus
12700 East 19th Avenue
RC2, 6012, Mail Stop #8620
Aurora, CO 80045

E-mail: elizabeth.kovacs@UCDenver.edu

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Abbreviations Used

AKT = protein kinase B (PKB)
AP1 = activator protein 1
ARG1 = arginase 1
Chi3l3 = chitinase 3-like 3 (Ym1)
CREB = cAMP response element binding protein
CRP = C-reactive protein
CXCL1 = KC
CXCL2 = macrophage inflammatory protein 2 (MIP-2)
CXCL8 = interleukin-8 (IL-8)
HSV-1 = herpes simplex virus 1
IFNγ = interferon gamma
IKK = IκB kinase
IL-10 = interleukin-10
IL-1β = interleukin-1 beta
IL-4 = interleukin-4
IL-6 = interleukin-6
iNOS = inducible nitric oxide (Nos2)
JNK = c-Jun N-terminal kinase
LPS = lipopolysaccharide

Abbreviations Used (Cont.)

MAL = MyD88 adaptor-like protein
MAPK = mitogen-activated protein kinase
MARCO = macrophage receptor with collagenous structure
MCP-1 = monocyte chemotactic protein 1 (CCL-2)
MKK = MAPK kinase
MMP-9 = matrix metalloproteinase-9
MyD88 = myeloid differentiation primary response gene 88
NFκB = nuclear factor kappa-light-chain-enhancer of activated B cells
NO = nitric oxide
PI3K = phosphatidylinositide 3 kinase
Retnla = resistin-like α (Fizz1)
SPMs = specialized pro-resolving mediators
STAT1 = signal transducer and activator of transcription 1
TAK1 = transforming growth factor beta-activated kinase 1
TGFβ = transforming growth factor beta
TLR = Toll-like receptor
TNFα = tumor necrosis factor alpha
TRAF6 = TNF receptor-associated factor 6