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Immunosenescence in Monocytes, Macrophages, and Dendritic Cells: Lessons Learned from the Lung and Heart

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Abstract

In the absence of an immune challenge, healthy, aged individuals have a significantly higher basal inflammatory state where circulating levels of cytokines, including IL-6, TNF-α and IL-1β, are elevated[1]. This progressive pro-inflammatory state, termed "inflamm-aging," affects the phenotype/function of cells present in the aged as well as renders the older individuals more susceptible to a poor prognosis after systemic insults. Although it is important to understand the mechanisms that underlie the progression of disease, most preclinical analyses of disease therapies are performed in young adult mice that have an intact, functional immune system. Oftentimes, this is not necessarily representative of the immune disposition in the aged, let alone diseased, aged. Herein, two distinct responses that are not only commonly associated with aging but that also have dendritic cells and/or monocytes and macrophages as key players are discussed: pulmonary infection and myocardial infarction. Although studies of pulmonary infection in the aged have progressed significantly, studies of monocytes and macrophages in inflammation and cardiac injury following ischemia in the aged have not been as forthcoming. Nonetheless, several elegant studies have established the dynamic role of monocytes and macrophages post infarction. These will be discussed in light of what is known with aging.

Keywords

aging; monocytes; macrophage; dendritic cell; lung; heart

1. Introduction

Macrophages and dendritic cells (DCs)[1] play pivotal roles in modulating immune function following infection and tissue injury [2-4]. As professional antigen presenting cells, these

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[1]Abbreviations: angiotensin II – AngII; conventional dendritic cells – cDC; damage associated molecular protein – DAMP; Found In Inflammatory Zone 1 – FIZZ1; glycogen synthase kinase-3 – GSK-3; inducible nitric oxide synthase – iNOS; microRNA – miRNA; pathogen associated molecular protein – PAMP; reactive oxygen species – ROS; senescence-associated secretory phenotype – SASP

cells help shape the innate and adaptive immune responses. In response to insult, DCs are activated to induce adaptive immunity, while monocytes and macrophages initiate an inflammatory response. Monocytes and macrophages respond through the production of pro-inflammatory cytokines and anti-microbial mediators. Macrophages are not only key players in the initiation of inflammation, but also orchestrate its resolution, and in the case of sterile injury, wound healing. Plasticity is characteristic of macrophages, whose phenotype and function evolves in response to changing conditions within their microenvironment.

Macrophages can be broadly categorized into two subsets. The classically activated or M1 macrophages are induced by the bacterial cell wall component lipopolysaccharide (LPS) or a combination of Th1 cytokines, IFN-γ and TNF-α. The alternatively activated or M2 macrophages are induced by Th2 cytokines such as IL-4 and IL-13[5]. M1 macrophages are characterized by the production of reactive oxygen species (ROS), reactive nitrogen intermediates, IL-1, IL-12, and TNF-α[3,6]. In addition, M1 macrophages drive Th1 responses and kill intracellular foreign pathogens[4]. Anti-inflammatory, i.e., M2 macrophages, express arginase-1, scavenger and mannose receptors, and the intracellular proteins Found In Inflammatory Zone 1 (FIZZ1) and Ym1[7,8]. M2 macrophages have elevated levels of IL-10 and predominately function in immunoregulation, neovascularization and tissue remodeling[4,9].

Aging is characterized by the elevation in baseline inflammation (called “inflamm-aging”) with a refractory response to immune challenge (known as “immunosenescence”). Importantly, the chronic exposure to systemic low levels of pro-inflammatory cytokines modulates phagocytic mononuclear cell activity, and skews the M1/M2 populations present in the aged independent of injury or infection[10-13]. Superimposed on these changes in the ‘milieu’ and steady state populations, is the aged response. Whether responding to tissue injury or infection, a state of diminished and prolonged inflammation ensues. In addition to affecting pathogen clearance, age-related changes in nitric oxide production and phagocytic activity lead to impaired removal of damaged tissue and promote adverse remodeling following injury. Thus, a disruption in the balance between inflammation and immune activation may contribute to a variety of co-morbidities and increased mortalities following local and systemic insults[14-19].

With a focus on monocytes, macrophages and DCs, this review will cover age-associated changes in the development and function of these cells in the context of two distinct responses, a respiratory infection and a sterile insult, i.e., myocardial infarction. These responses were chosen due to their high incidence of morbidity and mortality in the aged population. Recent studies have elucidated the controversy regarding the lineage of monocytes and macrophages with an emerging picture that some macrophages derive from a hematopoietic stem cell lineage that involves specific progenitor intermediates and monocytes[20], whereas other macrophages derive from primitive macrophages that have colonized tissue prior to hematopoiesis[21-24]. The observations that macrophages can polarize to various functional states[25,26] as well as the emerging identification of distinct monocyte subsets[27] has fostered the idea that macrophages are fated for specific functions, suggesting that harmful subsets can be therapeutically targeted while those that are beneficial can be spared. Although in some cases the findings are so new that potential

changes with age have yet to be determined, this review will cover macrophage lineage and heterogeneity in the young noting areas of potential differences in the aged.

2. Pulmonary Response

2.1. Mononuclear phagocytes in the lung

Constant exposure to airborne pollutants and microbial challenges renders the respiratory tract especially vulnerable to infection and damage. Highlighting this state of permanent alert, the lung, comprised of parenchyma and alveolar space, contains numerous mononuclear phagocytes, including DCs and macrophages. In the steady state, macrophages are the major cell component of the alveolar space and are key for removal of pathogens and debris[28]. Like in other epithelia and skin, throughout the lung airways and interstitium is an elaborate network of DCs that performs unique sentinel function for pulmonary immune responses. At least five phenotypically distinct DC subsets have been identified, a minority population of the plasmacytoid type, and several distinct myeloid “conventional” subsets[29]. The definition of tissue DCs and macrophages has relied upon phenotypic determinations, which are not definitive, leading to some confusion surrounding the exact contribution of DCs *vs*. macrophages to tissue immunity. More exacting analyses, such as transcriptional profiling should shed more light on this issue[30].

Alveolar macrophages and interstitial macrophages are distinct phenotypically and functionally[31,32]. Alveolar macrophages are unique amongst tissue-resident macrophages in both phenotype and function. Their phenotype is similar to DCs, e.g. high levels of CD11c and CD205 expression. Their ability to cross-present antigen is higher than other macrophage populations[31] and they are able to clear particulates and pathogens without the induction of inflammation or recruitment of neutrophils or monocytes[33-35]. The lung microenvironment that is particularly higher in oxygen tension and levels of GM-CSF and M-CSF, are critical for the induction and maintenance of the alveolar macrophage population[31,36]. Alveolar macrophages differ from the pulmonary interstitial macrophages in origin and lifespan as well. They populate the lung during embryogenesis and renew largely from tissue resident populations, with little contribution from the bone marrow[24,37]. Blood-borne precursors are thought to undergo an obligate intermediate differentiation step within the parenchyma transitioning to macrophages that subsequently migrate into the alveolar space[28]. Under steady-state, alveolar macrophages turn over very slowly with a half-life of 30-60 days[38] and a population half-life of 40%/year[39]. In contrast, pulmonary interstitial macrophages originate from bone marrow-derived monocytes and have a shorter lifespan[28,40]. Upon activation they actively recruit other inflammatory cells[33,35,41,42]. Viral and bacterial infection results in the appearance of other monocytic and macrophage-like cells within the lungs. Some of these cells likely differentiate into DCs[43,44] and participate in pathogen clearance; however, an overabundance of these cells may result in pathologic changes in the lung.

DCs are classified into conventional and plasmacytoid DCs (PDC) subsets that differ in phenotype and ontogeny. PDC have a limited ability to populate non-lymphoid tissues in steady state or to phagocytose and present antigen. Within the lung, in the absence of inflammation, two populations of conventional DCs can be identified, the $CD103^+$ and the

CD11b$^+$ subsets. The CD103$^+$ population forms a network within the epithelial layer of the airways and extends protrusions between the basolateral spaces. The CD11b$^+$ DC subsets reside underneath the basement membrane within the lamina propria[43]. Pulmonary DCs turnover rapidly, with a half-life of 1.5-2 days in the airway and 3-4 days in the parenchyma[45]. The majority of DCs in the lung are derived from the circulating blood monocyte pool, while the PDC population arises from Flt3$^+$ conventional DC (cDC)[29]. The pulmonary CD103$^+$CD11b$^-$ DCs are potent at production of IL-12, cross-presentation of antigens to CD8 T cells, and induction of CD8 T cell differentiation[46-49].

DCs sample their environment through a collection of receptors that bind various pathogens or damage associated molecular patterns (PAMPS and DAMPS)[29,45]. Reduced expression of these receptors or alterations in their signaling pathways can increase susceptibility to infection[50-52]. Upon ingestion of antigen, DCs become activated, up-regulating surface expression of activation markers, mobilizing to local lymph nodes and secreting cytokines. The production of Type I and III interferons (IFNs) by the PDC subset is critical for anti-viral responses, while the profile of cytokine production by the cDC subsets directs Th cell polarization. Similarly, lung resident macrophages recognize PAMPS and DAMPS by expressing anti-microbial reactive oxygen and nitrogen species; thus, shaping the innate and adaptive immune responses through the release of a variety of pro-inflammatory mediators at early time points post injury or infection. These cells also dampen the inflammatory response through phagocytosis of apoptotic cells and secretion of soluble factors, e.g., IL-10, and can participate in tissue remodeling and repair[4,9].

Advancing age affects the respiratory system in a variety of ways including alterations in macrophage and DC function that impact innate and adaptive immunity. Among the earlier observed alterations in DCs were changes in the overall numbers and subset distribution present in various tissues, activation-induced expression of co-stimulatory molecules, cytokine synthesis, migration, antigen-presentation and T cell activation (reviewed in[53-56]. Variable results and conflicting conclusions are abundant, likely reflecting differing methods used in the identification of cell populations, different cell sources, reagents, and health status, but consensus is emerging. The preponderance of evidence indicates that aging does not result in a change in the number of DCs in the lungs, or most other lymphoid tissues, although there may be changes in subset frequency[56]. The basal expression levels of most markers on DCs, including Toll-like receptor (TLR) are also unchanged across the lifespan. However, the TLR expression profile in macrophages may change with age. It has been reported that all TLRs are decreased in peritoneal and splenic macrophages of aged C57BL/6 mice[57,58] whereas no differences were reported in TLR2 and TLR4 in aged BALB/c mice[59-61]. Basal levels of pro-inflammatory cytokine production is elevated in aged DCs and macrophages derived from several tissue sources[62-65]. Indeed, elevated inflammatory cytokine levels in the lung and other evidence of chronic inflammation are commonly identified in aged humans[63,66,67] and mice[68]. However, TLR-mediated pro-inflammatory cytokine production by aged DCs and macrophages is often reduced relative to young cells[18,55,62-64,69]. *In vivo* stimulation with live or attenuated virus or bacteria bolster this conclusion, indicating that the capacity of aged DCs and macrophages for cytokine production is both diminished and

delayed[63,70-77]. TLR ligand stimulus or viral infection-induced Type I and III IFN synthesis is universally found to be lower in aged DCs[53,56,78]. Similarly, age-associated defects in the signaling of other pattern recognition receptors, i.e., RIG-1 and NLRP-3, have led to impaired IFN or IL-1β production after stimulation with West Nile virus and influenza virus, respectively[74,79].

2.2. Molecular Mechanisms: GSK-3, miRNA, histone modifications, and oxidative stress

At the center of the signal transduction network regulating inflammatory cytokine gene expression is the glycogen synthase kinase-3 (GSK-3) family of serine/threonine kinases[80-83]. GSK-3 is a constitutively active protein kinase that has broad regulatory influence due to the multiplicity of substrates that it can phosphorylate. These substrates include metabolic enzymes, signaling molecules, structural proteins and transcription factors, typically involved in regulating cell proliferation and differentiation, cellular metabolism, cell survival and cell cycle regulation[82,84]. Evidence suggests that GSK-3 is a novel regulator of aging that retards age-related pathologies in a wide variety of tissues[85]. De-regulation of GSK-3 on the other hand, has been associated with the initiation or progression of many diseases[86-88] and the induction of cellular senescence[89].

GSK-3 plays a pivotal role in regulating the production of pro- and anti-inflammatory cytokines in DCs and macrophages. This enzyme is comprised of two isoforms both of which are constitutively active under basal conditions and whose activity can be differentially regulated by phosphorylation[83], intracellular localization, and protein complex formation[86]. In general, GSK inactivation by N-terminal serine phosphorylation in DCs and macrophages augments anti-inflammatory cytokine production while concurrently suppressing the production of pro-inflammatory cytokines[82], although the outcome of GSK-3 inactivation is complex and context specific.

Recent findings implicate the de-regulation of GSK-3 activity in age-related changes in pro-inflammatory cytokine production, particularly by means of altered phosphoinositide-3 kinase (PI3K) activity in aged macrophages and DCs. PI3Ks are a family of lipid kinases that phosphorylate the hydroxyl group of the inositol ring of phosphoinositides. The resulting phosphorylated products regulate a multitude of cellular events including cytokine production[90]. PI3K recruits and activates the serine-threonine kinase Akt that subsequently phospho-inactivates GSK-3, thus shifting the balance of pro-and anti-inflammatory cytokine production[91]. Fallah, *et al.*[71] reported that in aged murine splenic macrophages gene expression for both the catalytic and regulatory subunits of the Class IA PI3K was higher than in young macrophages. This increased mRNA for PI3K was associated with heightened phosphorylation of Akt and GSK-3 in the aged cells. Interestingly, the age-associated decline in pro-inflammatory cytokine production by aged macrophages to *Streptococcus pneumoniae* activation was corrected by inhibition of PI3K. Also implicating GSK-3 associated signaling pathway alterations mediating aging changes were the results of Boyd *et al.*[70]. This group reported alveolar macrophages taken from aged mice displayed lower phosphorylation of p65 NF-κB, JNK and p38 MAPK and an increase in ERK phosphorylation, consistent with an up-stream inhibition through GSK.

These data suggest that alterations in signal transduction pathway activity participate in the reduced innate immune inflammatory responses of the elderly, but clearly other mechanisms are active as well.

Xu, *et al.*[92] report that Langerhans cells in the skin of aged mice display a unique microRNA (miRNA) pattern. MiRNAs are small, non-coding RNA molecules that are key regulators of gene expression by means of their capacity to limit translation of specific RNAs. Although a comparative analysis of miRNA expression in young and aged whole lungs did not reveal age-associated changes[93], Jiang, et al.[94] found high levels of miR-146a in murine macrophages in aged animals. miR-146a negatively regulates the expression of IL-1β and IL-6. These authors further found that histone modifications play a role regulating miR-146a expression, as suppression of histone deacetylase activity improved the inflammatory response of aged macrophages. Histone modification, such as by methylation or acetylation can have a dramatic impact on chromatin structure and thereby play a critical role in gene activation and induction of expression through controlling DNA accessibility to polymerases and transcription factors. An analysis of chromatin structure of human monocyte-derived DCs revealed that the promoter regions for genes encoding IL-29 and IFN-A2 are more highly associated with the repressor histone in aged populations than in young[78]. This was accompanied by a decreased association of these promoters with the activator histone after activation.

ROS are physiologically produced by all cells and mostly derived from leakage of the electron transport chain in mitochondria[95,96]. In inflammatory cells a second important source of ROS production is the “oxidative burst”, where the NADPH oxidase complex catalyzes the formation of hypochlorite, hypochlorous, and hypobromous acid to eradicate pathogens[97,98]. In aging cells an imbalance due to increased ROS production and a decrease in the levels of activity of the ROS-converting enzymes, leads to the non-enzymatic oxidation of proteins, carbohydrates, lipids and nucleic acids, a process generally known as oxidative stress[99-105]. Mildly oxidized cytosolic proteins are degraded by the proteasome system or by chaperone-mediated autophagy[106-108]. Extensively oxidized proteins become irreversibly aggregated and are degraded through aggrephagy, a selective form of macroautophagy. In aged cells of the immune system, there is an increased level of free radicals[109], a decreased level/function of enzymes involved in clearing free radicals[110] which participate in compromising phagocytosis, proteasomal activity and TLR signaling[19,69,111]. Recently, Cannizo *et al.*[112] showed that DCs isolated from the spleen and lymph nodes as well as the $CD34^+$ bone marrow precursors of old mice accumulate oxidatively modified proteins with side chain carbonylation, advanced glycation end products and lipid peroxidation, and that endosomal accumulation of oxidatively modified proteins interferes with the efficient processing of exogenous antigens and degradation of macroautophagy delivered proteins. Further, this group [112] demonstrated that *in vivo* treatment with antioxidant improves aged DC antigen processing and presentation. It is likely pulmonary DCs also accumulate oxidatively modified proteins which negatively impacts their activity, contributing to immunosenescence in this tissue.

2.3. Senescence associated secretory profile (SASP) and the aged microenvironment

Macrophage phenotypic and functional profiles are highly plastic and dependent on external cues, and DC function is also strongly influenced by the microenvironmental milieu, as previously mentioned. It has recently become apparent that the development of age-associated chronic inflammation has significant impacts on these cell types, modifying innate immune reactions in the aged. The source of the inflammatory cytokines and the mechanisms that maintain the chronic inflammatory state are somewhat enigmatic, but it has been hypothesized that cellular senescence underlies the phenomenon. Cellular senescence is the state of permanent inhibition of cell division. It is associated with a unique secretory phenotype, termed the senescence-associated secretory phenotype (SASP), characterized by production of pro-inflammatory factors[113-115]. It is likely the SASP phenomenon explains the paradoxical situation of increased basal inflammation accompanied by reduced pathogen-induced inflammatory responses. The consequences of SASP on disease susceptibility have been clearly demonstrated, affecting both the incidence and severity of inflammatory diseases such as COPD and IDF [116] as well as infectious disease.

The aging lung displays elevated basal inflammation[63,66-68,117] that contributes to increased susceptibility to infectious disease in a number of ways. The pulmonary SASP includes increased level of the markers, keratin 10, laminin receptor and platelet activation factor receptor, all of which act as adhesion receptors for bacteria, including *S. pneumoniae* [117]. Elevated circulating levels of TNF-α, another pro-inflammatory factor produced by senescent cells, strongly affects the function of aged pulmonary macrophages, as demonstrated in an elegant series of papers by the Orihuela group[70,118-120]. These investigators identified age-dependent changes in the activity of alveolar macrophages that included reduced phagocytosis and induced cytokine production, a complex of changes that they termed age-dependent macrophage dysfunction (ADMD). They demonstrated that after infection with *S. pneumoniae*, the reduction in pro-inflammatory cytokine production by aged alveolar macrophages was not due to lowered expression levels of TLR, but to reduced NF-κB activation. This signaling defect could be replicated in young alveolar macrophages by pre-treatment of the cells with physiologic levels of TNF-α, linking the response profile of the aged macrophages to the changes in the milieu that occurs as the epithelial cells undergo cellular senescence and express the SASP phenotype. Further work dissecting the molecular signaling pathways indicated that reduced NF-κB activation was in part due to increased expression in the aged cells of A20 whose activity reduces TRAF6 polyubiquitination and thus lowers NF-κB activation, and consequently the expression of pro-inflammatory cytokines. Enhanced A20 expression is also linked to exposure to TNF-α, whose basal levels is elevated as a sequela of epithelial cell senescence.

Respiratory DC migration upon viral infection also is reduced in aged mice due to elevated levels of prostaglandin D2 expression in the lungs[121], another characteristic of senescent cells. Taken together these data suggest that senescence-associated changes in the lung parenchymal cells, particularly the epithelial compartment, create a microenvironment that depresses normal, immunoprotective activities of the resident macrophages and DCs[6]. Of relevance to improving immune function in the elderly, alveolar macrophage and DC activity can be improved both by dietary manipulations[119] as well as administration of

mTOR inhibitory drugs, reversing the negative effects of pulmonary cellular senescence[122].

3. Cardiac Ischemia

3.1. Role of monocytes/macrophages in myocardial ischemia

Atherosclerotic lesions that rupture in coronary arteries cause myocardial infarction. This ischemic event kills cardiomyocytes and triggers the influx of myeloid cells (see Table I). Neutrophils are the first leukocyte population to infiltrate the ischemic site[123]. Shortly thereafter, monocytes and macrophages can be found in the infarct at numbers up to a million cells. Cells with an inflammatory phenotype (Ly-6C^{high} monocytes/M1 type macrophages) dominate initially, followed by cells with a lesser inflammatory phenotype promoting tissue repair (Ly-6C$^{low/int}$ monocytes/M2 type macrophages). Other leukocytes invading the infarct in lower cell numbers include DCs[124], lymphocytes[125,126] and mast cells[127]. During the wound healing process, a deficiency in the influx of CD4 T cells delays the transition from an inflammatory to reparatory phase and DC depletion affects the resolution of inflammation[124]; however the mechanism underlying these changes are unclear. When inflammation resolves, non-leukocyte cells join the rebuilding activities in the infarct. Angiogenic factors induce the growth of numerous vessels into newly forming granulation tissue. Myofibroblasts produce collagen that strengthens the emerging infarct scar.

Monocytes accumulating in the infarcted myocardium arrive from the bone marrow and spleen[128] in two sequential phases (i.e., inflammatory and reparative): Ly-6C^{high} monocytes arrive during the first days post myocardial infarction in response to MCP-1, whereas days later Ly-6C$^{low/int}$ monocytes arrive second in response to fractalkine (CX3CL1)[123]. This time course corresponds to expression of M1-type markers in tissue early after injury and M2-type macrophage markers later[129]. In the infarct, myeloid cells turnover very rapidly, and monocytes are recruited at a high rate[130]. Thereafter, most cells become apoptotic, and a fraction of the accumulated CD11b^{+} population exits the infarct within 24hrs and accumulates in the liver, lymph node and spleen[130]. During days 1 to 4 after ischemia, the milieu is highly inflammatory with Ly-6C^{high} cells secreting TNF-α and proteases[123]. Dead cells, extracellular matrix and debris surrounding the infarct are cleared by phagocytosis.

At certain levels, inflammation may promote adverse effects, e.g., the tissue-destabilizing function of proteases may lead to infarct expansion, rupture, and dilation of the left ventricle[131]. However, inflammation is necessary to clear dead cardiac tissue and begin the active process of resolving inflammation, as well as to promote scar formation. Marginated leukocytes clear the dying and necrotic cardiomyocytes and support fibrogenic and angiogenic responses[131,132]. Thus, modulation of the inflammatory response post myocardial infarction contributes to the quality of heart repair[123,133,134]. Harel-Adar *et al*.[135] showed that modulation of cardiac macrophages to a reparative state at a predetermined time after myocardial infarction promoted angiogenesis, the preservation of small scars, and prevented ventricular dilatation and remodeling.

During this rebuilding phase, the inflammatory activity resolves and gives way to Ly-6C$^{low/int}$ monocytes/macrophages. These cells release vascular endothelial growth factor (VEGF) and TGFβ, supporting angiogenesis and collagen production. In the aged (Table I), studies have reported a prolonged course of wound repair, associated with a prolonged inflammatory state, and delayed neovascularization and restoration of the extracellular matrix[16,136]. Specifically, in older mice post myocardial infarct, there is impaired inflammation with decreased and delayed neutrophil and macrophage infiltration, reduced cytokine and chemokine expression and impaired phagocytosis of dead cardiomyocytes[16,136]. The impaired inflammation and suboptimal clearance of dead cardiomyocytes that may lead to maladaptive vascular remodeling and tissue repair in the healing heart and therefore accelerate transition into heart failure.

3.2. Monocyte/macrophage lineage and heterogeneity at steady state and in the infarct

3.2.1. Steady State—Although evidence for lineage relationship in the infarcted myocardium is rather sparse, the developmental relationship between monocyte subsets and macrophages is emerging. Using complementary *in vivo* cell tracking, parabiosis, bone marrow transplants and fate-mapping studies, Epelman *et al*.[21] found the majority of cardiac macrophages were established during embryogenesis and persist into adulthood in substantial numbers. Unlike the lung, there are very few macrophages present in the uninjured heart at steady state. The heart contains two separate and discrete cardiac macrophage pools[21]. The first pool (CCR2^{-}, CD11c^{low}) includes the majority of MHC-II^{high}, MHC-II^{low}, and Ly-6C^{+} macrophages. These macrophage subsets are separate from the blood monocyte pool and represent an embryonically established lineage made up of progeny from yolk sac macrophages and fetal monocytes. The second pool of macrophages is smaller in number and is derived from blood CCR2^{+} Ly-6C^{high} monocytes. Both embryonic and adult derived macrophages are maintained through local proliferation and replacement by blood monocytes, respectively.

In the mouse, circulating monocytes are phenotypically and functionally heterogeneous and can be separated based on Ly-6C expression[123,137]. In the steady state 50-60% of monocytes belong to the Ly-6C^{high} CCR2^{high} CX3CR1^{low} CD62L^{+} subset. These inflammatory or classical monocytes have a relatively short circulating life span and accumulate preferentially in inflammatory sites where they give rise to macrophages. The remaining Ly-6C^{low} CCR2^{low} CX3CR1^{high} CD62L^{-} subset, referred to as nonclassical, patrols the vasculature and accumulates at low numbers in the steady state[138,139]. In the steady state, monocyte conversion from Ly-6C^{high} to Ly-6C^{low} may occur in blood and bone marrow[24].

3.2.2. Post Infarct—Soon after coronary artery ligation[123] or after angiotensin II (AngII) infusion[21], Ly-6C^{high} monocytes infiltrate the heart in large numbers [The AngII signaling cascade is induced in virtually all forms of cardiovascular disease[140] and has been shown to mobilize splenic monocytes to the infarcted myocardium[128]]. Many of these monocytes may not differentiate to macrophages but either exit or die in the tissue. Those that differentiate acquire M1-like properties, continue to express Ly-6C, CCR2, MHC-II^{high} and contribute to inflammation[21,130]. Over time, as inflammation gives way

to resolution, a second Ly-6C$^{low/int}$ population emerges. Whether macrophages dominating this regenerative wound-healing phase arise via the differentiation of Ly-6C^{high} monocytes and less via differentiation of Ly-6C^{low} monocytes or local M1 to M2 macrophage conversion needs to be elucidated. As inflammation completely subsides, a population of F4/80^{high} macrophages resembling steady state macrophages returns. Studies by Epelman *et al*.[21] suggest that despite the autonomy between resident and monocyte-derived macrophages, after depletion and in a setting of competitive resident macrophage proliferation, blood monocyte-derived macrophages have the ability to take up residence within tissue and become the dominant macrophage population. After repopulation is complete, tissue macrophage autonomy is restored, albeit with a large complement of adult monocyte-derived macrophages as the new resident macrophage population.

Studies in ischemic myocardium indicated that recruitment, rather than local proliferation, is the primary mechanism regulating monocyte and macrophage numbers[130]. Within the first 24hrs after coronary artery ligation in mice, approximately half of all monocytes recruited to the heart are derived from the spleen[128]. These monocytes reside in the subcapsular red pulp of the spleen and resemble their circulating blood counterparts. The bone marrow and blood also substantially contribute to the monocytes recruited to the infarct. Because the residence time of monocytes and macrophages is short, and because large numbers of cells are continuously recruited to the heart post infarction, the splenic monocyte pool is exhausted in a short time. However, upon myocardial infarction the bone marrow outsources the production of monocytes to extramedullary sites through the export and relocation of hematopoietic stem cells and progenitor cells to the spleen[141,142]. In mice, the splenic reservoir of monocytes is replenished by day 6 after coronary artery ligation and continues to supply the infarct with a significant number of cells[130].

The findings of Epelman[21] in which various macrophage subsets may have different functional roles might explain why, in models of cardiac injury, blocking monocyte influx is protective, whereas broad macrophage depletion strategies that also target resident cardiac macrophages abolish protection[132,143]. These data suggest that preserving resident cardiac macrophage expansion via proliferation, while targeting peripheral monocyte recruitment, might lead to improved myocardial recovery after injury. Moreover, the data underscore the importance in dissecting the roles of various monocyte/macrophage populations during the course of an ischemic response. Given these findings in young mice and the known findings of prolonged inflammation and delayed recovery in the aged, raises many questions. How does “inflamm-aging” affect resident macrophage populations and the Ly-6C^{high} monocyte population from the spleen, blood and bone marrow? Is there skewing in the representation of the various monocyte/macrophage populations with age? Do cells in each of the subsets respond as well as their young counterpart, and to what extent are differences driven by the aged microenvironment or by intrinsic changes (e.g., oxidative stress)? Can we restore responsiveness in the aged by targeting specific monocyte/macrophage populations?

3.3. Clinical Relevance

A number of clinical studies support concepts of cardiac monocyte and macrophage heterogeneity and dynamics that have been recently described in mice. A bi-phasic monocyte response has been observed in the blood of patients after myocardial infarction[144]. A number of clinical studies confirm the correlation of blood leukocyte levels in heart failure with prognosis[145,146]. Other observations in patients after myocardial infarction include an increased level of hematopoietic progenitors in the blood[147], increased metabolic activity of the bone marrow[141], and evidence for an increased progenitor activity in the spleen[142]. Clinical signs of inflammation in heart failure patients support an active role of macrophages in heart disease.

Still lacking is a clear understanding of the potentially dynamic changes in monocyte/macrophage heterogeneity that occur over a lifespan, clarification of alterations in functional monocyte/macrophage responses due to microenvironmental *vs*. intrinsic influences, and a determination of these changes in steady state *vs*. post infarction. Understanding these changes in the aged will aid in the design of efficacious interventions that may ameliorate maladaptive remodeling and improve outcome post myocardial infarction. This becomes especially significant given that the majority of cardiovascular deaths occur in the elderly despite improved therapies[148]. Although several factors may contribute to this, it is clear that more studies are needed using aged animals.

4.Conclusion/Overview

Phagocytic leukocytes, macrophages and DCs, are amongst the initial responders to tissue injury and infection. Their response may include the synthesis of microbicidal mediators, chemokines and cytokines, up-regulation of the expression of cell surface activation markers and mobilization. Unlike the lung where sufficient cell numbers are present to confront pathogens, the number of phagocytic leukocytes present at steady state in the heart is low and upon injury, a large number of monocytes/macrophages are mobilized to the infarcted area. In both models, an efficacious outcome requires initiation of an adequate inflammatory response with a timely resolution. Both macrophages and DCs are diverse populations and the macrophage lineage in particular displays extreme functional and phenotypic heterogeneity. Distinct subpopulations can be found in circulation and resident in various tissues. Tissue distribution of these subpopulations frequently changes over the course of response to injury or pathogenic challenge, in answer to microenvironmental cues.

Advancing age erodes the capacity for appropriate disease and injury management and is characterized by an increased level of chronic inflammation, but diminished and protracted acute inflammatory responsiveness upon challenge. Although several factors may contribute to inflamm-aging and immunosenescence, it seems increasingly likely that the development of the senescence associated secretory phenotype creates a unique “senescent” tissue microenvironment, dramatically altering macrophage and DC function. The chronic presence of pro-inflammatory mediators alters the molecular processes and signal transduction pathways triggered in response to tissue damage or infectious challenge. An increased understanding of these changes will provide unique opportunities for therapeutic intervention to restore appropriate innate immune activity in the elderly.

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Highlights

- Age erodes the capacity for appropriate disease and injury management
- Age is associated with elevated chronic inflammation and diminished acute inflammatory response
- Distribution of populations change over the course of response in answer to microenvironment cues
- Inflamm-aging alters molecular processes triggered in response to tissue damage or infection

Table I

Dynamic Changes in Myocardium Post Infarction[16,136,149]

	INFLAMMATORY PHASE		**REPARATIVE PHASE**
	Neutrophil influx -phagocytose dead cells/debris -release inflammatory mediators	*Influx of Ly6-C^{hi} mono to elevated MCP-1* -phagocytosis -produce pro-inflammatory mediators and proteases --tissue destabilization	*Increase Ly6-C$^{lo/int}$ mono to elevated CX$_3$CL1* -phagocytosis -produce VEGF, TGFβ --support angiogenesis and collagen synthesis
AGED RESPONSE	Reduced neutrophil influx; Timely neutrophil clearance	Fewer early macrophages; Reduced levels of MCP-1, IL-1β, TNF-α, IL-6, M-CSF; Increased levels of IL-10; Persistence of dead cardiomyocytes in infarcted area	Macrophages peak later; Defective scar formation (reduced myofibroblast density and decreased collagen deposition in infarcted area); Comparable TGFβ levels