

## Invited Mini Review

## Impact of mesenchymal stem cell senescence on inflammaging

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Life expectancy has dramatically increased around the world over the last few decades, and staying healthier longer, without chronic disease, has become an important issue. Although understanding aging is a grand challenge, our understanding of the mechanisms underlying the degeneration of cell and tissue functions with age and its contribution to chronic disease has greatly advanced during the past decade. As our immune system alters with aging, abnormal activation of immune cells leads to imbalance of innate and adaptive immunity and develops a persistent and mild systemic inflammation, inflammaging. With their unique therapeutic properties, such as immunomodulation and tissue regeneration, mesenchymal stem cells (MSCs) have been considered to be a promising source for treating autoimmune disease or as anti-aging therapy. Although direct evidence of the role of MSCs in inflammaging has not been thoroughly studied, features reported in senescent MSCs or the aging process of MSCs are associated with inflammaging; MSC niche-driven skewing of hematopoiesis toward the myeloid lineage or oncogenesis, production of pro-inflammatory cytokines, and weakening their modulative property on macrophage polarization, which plays a central role on inflammaging development. This review explores the role of senescent MSCs as an important regulator for onset and progression of inflammaging and as an effective target for anti-aging strategies. [BMB Reports 2020; 53(2): 65-73]

## INTRODUCTION

Life expectancy has steadily increased for nearly 200 years, mainly because of the reduction in early and mid-life mortality. Furthermore, the decline in late-life mortality in recent years has resulted in the continuing increase in life

expectancy. In fact, worldwide life expectancy will increase with a probability of at least 65% for women and 85% for men by 2030, it will reach over 90 years particularly in Korea, with a 57% probability (for women) (1). As society gets aged, social burdens and costs for supporting individual longevity, such as health and medical care, are increased. To deal with public-health expenditure and social requirements more efficiently and economically, comprehensive and in-depth understandings of the aging process and physiology in the old are strongly needed.

As the immune system possibly records all the immunological experiences and stimuli it was exposed to, our immune system shows prominent changes during the aging process (2). An 'aging immune system' is often termed 'Immunosenescence', and it refers to both innate and adaptive immune changes. Immunosenescence is considered to have a clinical significance, because it might be the origin of diseases of the elderly, such as infections, cancer, autoimmune disorders, and chronic inflammatory diseases (3). Aging has been associated with changes in the hematopoietic system, including diminished long-term repopulation and lineage-biased hematopoiesis of immune cells (4, 5), leading to a subsequent impaired immune defense against various infections (3) and spontaneous proinflammatory activation (6). Several studies have reported that an age-associated chronic proinflammatory state would be responsible for detrimental degenerative diseases, such as rheumatoid arthritis (7), atherosclerosis (8), and neurodegenerative disease (9). Franceschi and colleagues termed the persistence of low-grade chronic inflammatory status as 'inflammaging' and have intensively investigated this pleiotropic phenomenon since 2000 (10). For two decades, the study of inflammaging has been widely developed by the efforts of researchers in the various fields, including microbiology and endocrinology, and has emerged as an important concept to provide a dynamic reassessment of immune changes with aging (11, 12).

Among the emerging cell therapy or anti-aging remedies, mesenchymal stem/stromal cell (MSC) therapy has attracted attention because of the cells' unique properties, stemness, and immunomodulatory ability. MSCs are considered as a 'safer source' for cell therapy with minimal risk of transplanted stem cells forming tumors and becoming cancerous, however, it has a limited self-renewal ability similar to other adult stem

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cells. The aging of MSCs leads to an age-associated decline in their number and functions including multilineage differentiation, homing, immune modulation, and wound healing (13). To accomplish a successful anti-aging therapy, it is important to figure out whether the therapeutic efficacy of MSCs could be affected by dysfunctions associated with the aging process. Our group and others showed relatively impaired therapeutic ability of aged MSCs in animal models such as colitis or chronic wounds (14, 15). However, present studies about aging and stem cell therapy have focused on *in vitro* stem cell aging itself, so called 'replicative senescence' (16). It has been highlighted for recent years that aging of MSC niche causes spontaneously inflammatory responses and interferes the effect of MSC therapy (17). Furthermore, although studies of inflammaging mainly target the prediction for disease susceptibility and successful anti-aging therapy, and uncovering the secret of the aging process and its related dysfunction, less is known about how MSCs are associated with the features of inflammaging. In this review, we investigated how aging of MSCs residing in the body and related microenvironment could exacerbate inflammaging and hamper the therapeutic potentials of emerging therapies including allo- and auto-transplantation of MSCs. Therefore, we sought to summarize previous results and propose possible hypotheses about the effect of MSCs on inflammaging.

## INFLAMMAGING

Inflammaging refers to a persistent low-grade systemic pro-inflammatory status that appears in the normal aging process of mammals. Inflammation is a defense mechanism against life-threatening invasion by harmful agents and maintains homeostasis in child- and adulthood, but chronic inflammation can have a deleterious effect on the body and might be a significant risk factor increasing morbidity and mortality for most if not all degenerative diseases and geriatric syndromes in elderly people (18).

The etiology of inflammaging would be divided into endo- and exo-genous reasons. An important feature of inflammaging is an accumulation of damaged macromolecules and cellular debris because of its increased production and chronically inhibited damage surveillance and repair functions in multiple tissues (19). Aging on the cellular level may be responsible for inflammaging. Secretion of proinflammatory cytokines from senescent cells accumulated in tissues with age, the so-called 'senescence-associated secretory phenotype' (SASP), contributes to the onset of inflammaging (20). Age-associated changes in the immune system (Immunosenescence), which refers to impaired adaptive immunity and compensatory activation of the innate immune system, are also included in the sources of inflammaging (21). In addition to these microbial products leaking into surrounding tissues, dysregulated activation of the coagulation system and inadequate regulation of the complement pathway would be another cause for inflammaging

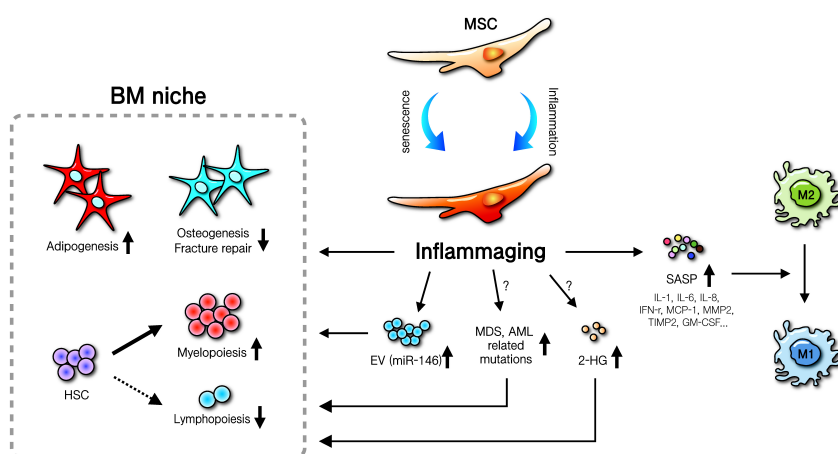
(19). Among exogenous reasons, viral and bacterial infections and the subsequent exposure to nucleic acids of the microorganisms, which facilitate activation of innate immune receptors such as Toll-like receptors (TLR) and NOD-like receptors (NLR) as Pathogen-Associated Molecular Patterns (PAMPs) were categorized as exogenous reasons (22). In addition to this, infection-mediated pro-inflammatory cytokines (23) and disorder in phytochemical consumption (24-26) also deteriorates modulation of oxidative stress and inflammatory signaling.

Inflammaging is a situation in which immunity exerts antagonistic pleiotropy programmed during evolution, and it has several distinctive features. The most prominent feature is chronic activation of innate immunity via dysregulated stimulation of pattern-recognition receptors by non-degraded waste in the body (Damage-Associated Molecular Pattern; DAMP), in which macrophages play a pivotal role. Innate immune cells subsequently secrete a robust amount of pro-inflammatory cytokines/chemokines, including interleukin (IL)-6 (22). Accordingly, cell-surface receptors are stimulated by secreted pro-inflammatory cytokines, and intracellular signaling cascades are initiated to activate transcription factors, including NF- $\kappa$ B (nuclear factor kappa-light-chain enhancer of activated B cells) and STAT (signal transducer and activator of transcription) (27, 28). Furthermore, NF- $\kappa$ B-mediated activation of NLRP3 inflammasomes, with the release of pro-inflammatory cytokines such as IL-1 $\beta$  and IL-18, facilitates the activation of inflammaging (29). To avoid an inadequate accumulation of bodily waste, rescue machinery termed 'autophagy/mitophagy' exists in vertebrates, and the function becomes defective with the condition of inflammaging (30). Dysregulation of the ubiquitin-proteasome system, activation of the DNA damage response, and dysbiosis are also included in the category of features of inflammaging. In the following part of this review, we will discuss a possible role of aged MSCs residing in the body and exogenously introduced in the development or progression of inflammaging (Fig. 1).

## HOW DO SENESCENT MSCs EXACERBATE INFLAMMAGING?

### Senescent MSCs: Another source for DAMP

Both innate and adaptive immune systems have continuously evolved to protect our bodies from harmful agents. Those life-threatening stressors can be divided into two different categories by their origins; external and internal. Microbes, including bacteria, viruses, fungi, and parasites, and even ingredients we've taken for energy, belong to external stressors. On the other hand, all types of materials created by tissues and cells during cell turnover and metabolism in living organisms, including cellular debris, metabolites, products of incomplete degradation or non-enzymatic processing, termed 'molecular garbage', are internal factors (2, 18). To deal with molecular garbage and maintain homeostasis in the body, several adaptive strategies, such as the recognition of PAMP or



**Fig. 1.** MSCs as a key modulator of inflammaging. The diagram illustrates how senescence and inflammation regulate MSC fate and lead to inflammaging. Cellular senescence facilitates MSCs to differentiate toward adipogenesis, and biased adipogenesis in the BM niche skews hematopoietic reconstitution, inhibits lymphopoiesis but increased myelopoiesis. Aged MSCs show increased secretion of extracellular vesicles (miR-146a) and SASP that stimulates innate immune cell receptors or macrophage polarization from M2 to M1. Age-related alterations may contribute to increased MDS or AML-related mutations or 2-HG production that possibly further exacerbated hematopoietic niche function or the pathogenesis of myeloid malignancies. BM, bone marrow; EV, extracellular vesicle; MDS, myelodysplastic syndrome; AML, Acute myeloid leukemia; 2-HG, 2-hydroxyglutarate; SASP, Senescence-associated secretory phenotype.

MAMP (Microbial-Associated Molecular Pattern), which can directly activate pattern-recognition receptors (PRRs) and downstream inflammatory cascades, have been developed. As individuals age, the physiological machinery becomes decrepit and functionally impaired; elderly people subsequently become vulnerable to infections like cytomegalovirus (CMV) or human immunodeficiency virus (HIV) (23, 31). In contrast, an age-mediated increase in the production of DAMPs or alarmins can reportedly fuel the progression of inflammaging.

MSC treatment has emerged as an anti-aging therapy because of its unique immunomodulatory function and tissue regenerative capacity (32, 33). Especially, paracrine secretion of MSCs plays a crucial role in clinical feasibility, including anti-aging therapy, and with these secretory factors, the conditioned medium (CM) of MSCs can be administered to various diseases. MSC CM contains not only anti-inflammatory cytokines but also encapsulated vesicles of nucleic acids and proteins, called 'exosomes', which exert a therapeutic effect on various diseases, including cancer (34) and myocardial infarction (35). Furthermore, recent studies have demonstrated that debris from MSCs is one of the effectors to activate immunomodulation through the activation of an alternative type of macrophages (36). Nonetheless, various abilities of MSCs gradually diminish during cellular senescence. As MSCs become aged, the cells become enlarged and heterogeneous in shape, granules and cell inclusions are accumulated in the cytoplasm, and debris is formed in the culture medium (37). Moreover, aging exerts telomere shortening, impaired functions in DNA machineries, such as methylation or histone acetylation, and an increase in levels of ROS and nitric oxide

(NO) (38). As described above, antigenic load, such as materials related to DAMP, is a major contributor to inflammaging. MSCs residing all over in our body presumably spout out much cell debris similar to that from other usual aged cells, and products derived from MSCs activate innate immune response through the activation of innate immune-cell receptors, such as TLRs, NLRs, and receptors of advanced glycation end-products (RAGE) (39-41). Accordingly, aged MSCs would exacerbate the initiation and progression of inflammaging by producing excessive cellular wastes throughout the body.

MSCs are known to generate higher amounts of extracellular vesicles (EVs), including microvesicles (MVs; 0.1-1 μm in diameter) and exosomes (40-100 nm in diameter), which are important mediators of intercellular communications, than do other cell types (42). MVs derived from cell surfaces reflect the properties of their parental cells and take part in cellular physiologies with neighboring cells and their own parental cells by diffusing around the adjacent microenvironment. MVs shed from MSCs express markers CD29, CD44, CD73, CD90 and CD105, as expressed on the MSC cell surface (43) and contribute to tissue regeneration, immunomodulation, and hematopoietic support, which are known to be unique biological functions of MSCs. Importantly, as shown in MSC aging, MVs shed from senescent MSCs undergo several phenotypical changes, such as that the number of secreted MVs from aged MSCs considerably increased while the average size decreased (44). Furthermore, the release of MVs or exosomes increased in senescent human fibroblasts, tumor cells (45) and endothelial cells (46). Cellular stress such as telomere attrition or DNA damage induced by aging or disease

may induce a p53-dependent increase in the biogenesis of MVs (45). Increase of MVs in senescent endothelial cells appears to be mediated Rho kinase activity as treatment with fasudil (Rho kinase inhibitor) blocked MV formation (46). Similar to the cellular products as described above, secretion of MVs in aged MSCs seems to facilitate more frequent stimulation of innate immune-cell receptors. According to the paper using multi-omics analyses, several molecules mediated DAMP reaction such as S100A6, S100A11, and HSPs were significantly increased in the aged MSCs (47). Moreover, miRNAs can be crucial factors mediated DAMP response (48). Expression of miR-146a (49), known to have an important role for induction of age-associated pro-inflammatory response, significantly increases in senescent MVs. Lastly, the treatment with old MVs to normal MSCs causes a decrease in osteogenic genes, including ALP, RUNX2, and OCN, and pro-osteogenesis abilities, and consequently leads to impoverished bone formation in bone marrow (BM) niche (44). The situation may be akin to hematopoietic stem cells (HSCs) from old individuals, in which a high number of HSCs is found in aged human or mouse than young counterpart, but showed impaired functionality (50, 51).

#### **Aged MSCs secrete a robust amount of pro-inflammatory cytokines**

Inflammation plays an important role in protecting our bodies from various infections for a lifetime, but it may become detrimental in the late period of life. It recruits large quantities of pro-inflammatory cytokines in the inflammaging process (52). Senescent cells generally present a distinctive property to secrete enormous amounts of cytokines and chemokines called SASP, which corrupts indispensable cellular processes, such as proliferation, migration, differentiation, and tissue remodeling, and consequently triggers the onset of fatal degenerative diseases, including cancers (53). In a similar context, senescent MSCs excessively release secretome, including IL-6, IL-8, interferon-gamma (IFN- $\gamma$ ), monocyte chemoattractant protein (MCP)-1, and matrix metalloproteinases (MMP2, TIMP2). A systemic inflammatory response caused by these secretory molecules reduces the immunomodulatory function of MSCs and promotes cancer progression (54, 55). As we have already discussed, secreted subcellular organelles, such as exosomes or MVs, play a crucial role in secretion of SASP in MSCs (43). During aging, MSCs show a persistent increase in the activation of TLR signaling, which mediates excessive production of pro-inflammatory cytokines (56). Furthermore, an age-dependent increase of adipogenesis might be a possible contributor for SASP through activations of peroxisome proliferator-activated receptor gamma 2 and CCAAT/enhancer binding protein, and adipose tissue acts as a reservoir for cytokines (57, 58).

Among cytokines, a pleiotropic cytokine, IL-6, has been at the center of the stage of inflammaging research field from the very beginning. Although the IL-6 level in the plasma of young

individuals remains low or undetectable, it prominently increases as individuals age. Even in the serum of healthy centenarians, IL-6 interestingly shows a higher level than in young people. Augmented IL-6 level in the serum of the elderly is associated with an impediment of physical ability, cognitive dysfunction, onset of cancers, and disease progression of general degenerative disorders (52). A growing body of evidence has proven that MSCs aggressively secrete IL-6 with advancing age, which is the result of accumulated genetic damage and activation by the other cytokines, such as TNF- $\alpha$  and IFN- $\gamma$  (59). Upregulated IL-6 secretion has been reported to promote proliferation and migration of cancer cells (60), and disrupt homeostasis of HSCs and hematopoietic progenitor cells (HPCs) (61). Moreover, pro-inflammatory cytokines accelerate the secretions of other cytokines by MSCs. It is well known that priming with cytokines, representatively IFN- $\gamma$  and TNF- $\alpha$ , improves the production of nitric oxide synthase (NOS) or PGE<sub>2</sub> in MSCs (62). Furthermore, G-CSF downregulates the secretion of CXCL12 from BM-MSCs and releases HSCs to populate (63).

#### **Aged MSCs facilitate a shift in macrophage polarization**

Inflammaging is a macrophage-centered phenomenon (18). The cells residing in the tissue and circulation are responsible for chronic inflammation in the elderly. Moreover, SASP factors modulate M1/M2 fate decision by shifting M2 cells toward M1 (64). Conversely, MSCs can exert beneficial effects on the macrophage polarization from M1 to M2, so that the cells could be employed to treat various immune-related disorders (65). Given that senescent MSCs augments myeloid cell generation and innate immune activation, aging of MSCs also affects macrophage polarization (66). Interestingly, macrophages co-cultured with young MSCs expressed M2 markers, Arg1 and IL-10, whereas the cells with aged MSCs increased M1-related TNF- $\alpha$ . In addition to this, macrophages co-cultured with aged MSCs increased the migratory ability, which is a typical property of classically activated M1 macrophages (67). As described above, aged MSCs altered their features to produce essential inducers for M1 differentiation, including IFN- $\gamma$ , IL-1 and DAMP, therefore activating the NF- $\kappa$ B signal (54). Consequently, MSCs in the aged micro-environment lost their unique competence of M1 suppression.

#### **Inflammaging of BM niche accelerates skewed blood-cell differentiation**

Hematopoiesis is an essential and life-long process, which unceasingly produces and eliminates mature blood cells for the constitution of circulating and residing cells in the body throughout an individual's lifetime. Blood cells, including red blood cells (RBCs), platelets, myeloid cells, and lymphoid cells, arise from a unique and small cellular population termed HSCs and HPCs in the BM. Cell fate decisions, such as self-renewal, differentiation, and cell death, are securely regulated by both cell-intrinsic and extrinsic factors (68). Interestingly,

recent studies have revealed, using single-cell transplantation and lineage-tracing technology, that hematopoiesis of defined HSCs and HPCs proceeded heterogeneously, which indicates that cells have subtypes and preferences to differentiate into a particular type of blood cell; lineage priming (69, 70).

Functionally impaired hematopoiesis is a hallmark of the aging process, as is a remarkably decreased self-renewal capacity of HSCs (71). Of note, hematopoietic changes with advancing age are intimately related to inflammaging, which includes depression in both the adaptive and the innate immune system, vulnerability to infections, spontaneous development of autoimmune diseases, and hematopoietic malignancies (72). In addition, the pattern of hematopoiesis and subsequent composition of mature blood cells in systemic circulation are considerably affected by age-associated changes. Most of all, mounting evidence has supported that myeloid cells are increased in number, but their actual functionality is impeded (5). For example, phagocytic activity of macrophages reduces even in the presence of stimuli, and a dysregulated skewing of a pro-inflammatory (classical) type of macrophage is frequently detected (73, 74). In a similar context, the transplantation of aged HSCs showed biased clonal expansion toward myeloid lineage, indicating an age-associated autonomous HSC differentiation pattern (75). The number of naïve B cells is decreased and the diversity of the B cell repertoire is also reduced, accompanying impeded antibody affinity and even autoantibody production (76). In addition, the ratio of CD4 and 8 T cells is gradually increased with aging (77).

The HSC niche, including MSCs and endothelial cells (ECs), plays a crucial role in homeostasis of HSCs by maintaining the microenvironment in the BM (78). As the aging process progresses, MSCs cannot maintain their functional and regenerative capacities and sometimes promote inflammation and cancer progression. Recent studies uncovered that the ineffective hematopoiesis caused by aberrant crosstalk between HSCs and MSCs caused by the primary alteration of MSCs and it might lead to myelodysplastic syndrome (MDS) (79), which supports niche-driven oncogenesis in the hematopoietic system. It is obvious that cellular senescence helps MSCs to differentiate eccentrically toward adipocytes (80, 81). It is suggested that biased adipogenesis in aged BM aggravates hematopoietic reconstitution, extracellular matrix composition, bone formation, and subsequent fracture repair (82, 83). As differentiated adipocytes reportedly interrupt lymphopoiesis (84), age-associated adipogenesis might exert an increased propensity to differentiate toward the myeloid rather than the lymphoid lineage, which could be another reason for hematopoietic malignancies including MDS. This hypothesis could be further supported by the results from previous studies that young HSCs transplanted toward aged BM showed myeloid-biased long-term repopulation (85).

### Age-associated mutations impede MSC niche function

Skewed differentiation of HSCs toward the myeloid lineage in elderly individuals coincides with several recurrent somatic mutations, especially *TET2* (Ten-Eleven Translocation 2) and it is associated alteration in DNA methylation (86). Notably, *TET2* mutation is frequently detected in patients with myeloid leukemia, myelomonocytic leukemia (CMML) (~50%), myeloid proliferative neoplasm (MPN) (~13%), MDS (~25%), and acute myeloid leukemia (AML) (~23%) (87). It is reported that 2-hydroxyglutarate (2-HG), an oncometabolite produced by mutated isocitrate dehydrogenase (IDH) 1 and 2, catalytically inactivates the *TET2* enzyme, and the mutation is also found in AML (~20%), gliomas (60-80%), and cholangiocarcinomas (7-28%) (88). With advancing aging, a general decrease in O<sub>2</sub> supply and impaired O<sub>2</sub> diffusion to target tissues, caused by age-associated poor vascularization, leads to tissue hypoxia (89). Interestingly, a hypoxic environment helps mammalian cells increase the production of oncometabolite, 2-HG (88, 90). Although hypoxic preconditioning has frequently been employed as a strategy for improving the immunomodulation or angiogenesis ability of MSCs (32), a hypoxic environment conversely leads to decline in osteogenic differentiation, related to functional impairment in hematopoiesis (91). Oncometabolite 2-HG-induced mutation of *IDH1* and 2 impairs osteoblast differentiation, presumably suggesting a mechanism underlying hypoxia-mediated reduced osteogenic differentiation of MSCs (90). Moreover, systemic chronic hypoxia facilitates premature senescence of MSCs in BM by altering the diversity of gut microbial communities, particularly a decrease in *Lactobacilli* colonies and subsequent accumulation of D-galactose, a well-known agent for senescence induction (92).

Somatic genetic mutations in the gene *additional sex comb-like 1* (*ASXL1*) are the other risk factors promoting dysregulated clonal myelopoiesis, which is frequently mutated in patients with MDS (15-25%) and AML (10-15%) (93). *ASXL1* proteins control transcriptional repression by recruiting polycomb repressive complex 2 (PRC2) to promoters, with a subsequent increase in H3K27 methylation for gene inactivation (94). In fact, *Asxl1* mutant mice showed spontaneous development of peripheral leukopenia and dysplastic myeloid cells in BM, and *ex vivo* analyses revealed various pathogenic features of MDS, including aberrant myeloid differentiation with a significant increase of granulocytic and monocytic cell fraction, and considerable growth arrest of HSC pool mediated by apoptosis and cell-cycle arrest. In addition to hematopoiesis, the mice showed impaired development in body size and weight (95). It is well defined that deletion of the epigenetic modulator gene *ASXL1* in myeloid cells results in upregulated osteoclastogenesis in association with reduction of H3K27me3 through inhibition, suggesting diminished bone mass and subsequently impaired BM niche function (94). Moreover, mutations in *ASXL1* expression in the BM-MSCs could exert reduced hematopoietic supportive ability and biased myelopoiesis, which is proved by using genetically

modified animals (96). Given the possible role of frequent age-related mutations in BM niche function, MSC aging may facilitate inflammaging through exacerbated hematopoietic supportive function and biased hematopoiesis in the elderly, which could lead to the pathogenesis of myeloid malignancies.

## CONCLUDING REMARKS AND FUTURE PERSPECTIVES

Finding the determinants of the aging process that help us understand age-related diseases has long been a quest by gerontologists and practitioners in the geriatric field for decades. With emerging interests in microbiology and immunology, researchers have tried to connect and expand their knowledge to find the clues to the secrets of aging and its related dysfunction. Recently, a new paradigm of aging-related concepts termed 'immunosenescence' and 'inflammaging' emerged, which explains the aging phenomena in terms of immunology (6, 10, 11). Aging-dependent alterations of immune cells and the BM microenvironment lead to subclinical inflammatory states, and subsequent chronically sustained inflammatory stimuli contribute to the functional decline of the immune system and even increased risks for leukemogenesis, supporting the inflammaging concept (68). As a BM niche factor, MSCs play a key role in modulating the immune system in the body; however, their role in the aging-immune-inflammation axis remains to be elucidated. Thus, herein, we discussed the previous reports related to the senescent MSCs and their behaviors, and propose their potential role in the progression of inflammaging.

One crucial factor for induction and progression of inflammaging is sustained stimulation of DAMP originating from senescent MSCs as their secretion of damaged macromolecules and self-debris increased with age and could lead to a subsequent spontaneous innate immune response. Age-biased bone marrow adipogenic differentiation, causes not only weakened BM niche function and bone homeostasis but also dysregulates hematopoiesis, resulting in biased myelopoiesis. Inflammaging of MSCs might be involved in genomic instability of the hematopoietic system by accumulation of recurrent *ASXL1* mutations in a niche microenvironment or through mutagenic enzyme production. Probably the most prominent contribution of MSCs to inflammaging is mass production of pro-inflammatory cytokines during the aging process, which then further promote M1 polarization of macrophages.

Despite present achievements in investigating the effect of MSCs on inflammaging progression, there is still plenty of unexplored territory remaining to be elucidated, which includes other mechanisms that might contribute to inflammaging progression. For example, MSCs can suppress the inflammasome, which is important to their therapeutic potential against immune-related disorders (65, 97), but the effect of senescence on the regulation of inflammasomes is less investigated.

Studies on centenarians show very different inflammaging phenotypes, such as pro-inflammatory cytokine levels in plasma, even in the individuals without chronic pathologies (18); so future study should be conducted to define features of individuals and cases (such as threshold on the stress or genotoxins), or how the inflammaging-related cytokines act in specific tissue-residing MSCs which could regulate the onset of specific chronic disease.

It also could be envisioned how inflammaging contributes to changing the therapeutic efficacy of transplanted MSCs. Although some studies have reported that *in vitro* senescent MSCs present reduced therapeutic potentials, including immunomodulation and engraftment (98), their specific mode of action in the elderly and influences by inflammaging are less investigated, regardless of whether the transplanted cells are early- or late-passaged MSCs. Inflammation is essential for survival and has beneficial effects on harmful signal neutralization in young individuals; however, inflammation in the elderly is detrimental, and this environment possibly stimulates transplanted MSCs for further hyperactive production of SASP, followed by inhibition of regenerative or immunomodulatory ability (18). Therefore, we also need to consider the antagonistic pleiotropy of pro-inflammatory cytokines on the therapeutic mechanism of transplanted MSCs. Lastly, most of the inflammaging studies have focused on which stimuli, such as molecules and cytokines, are important for induction and progression, but, to define the relation between inflammaging and MSCs, a systemic approach to understanding the interaction of MSCs with each niche- or tissue-specific signaling cascade of receptors is needed. Our effort to understand the complex interplay between MSCs and the aged environment and to integrate MSC therapy in the concept of inflammaging will contribute to improving preventive and personalized medicine for elderly individuals.

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## CONFLICTS OF INTEREST

The authors have no conflicting interests.

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