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| Feature Article |

Aging-Associated Decline in Innate Immunity and Therapeutic Strategies to Counteract It

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Abstract : Accumulated cellular defects over time and a decline in immune function are hallmarks of aging. Since the principal role of the immune system is to protect the host from infections and illness, defects in immune functions increase the susceptibility to and severity of infection or cancer, decreasing quality of life. Compared to the extensive studies of age-associated changes to the adaptive immune system, alterations to the innate immune system with advanced age are not well documented. Aging affects every aspect of innate immunity, including alterations in cellular composition, cell number, phenotype and function, and these alterations may significantly contribute to impairments in the adaptive immune system. Understanding the underlying mechanism of immune aging will allow the development of preventive or therapeutic strategies to restore age-associated immune defects that are beneficial for the elderly and that may dramatically enhance their qualities of life. Recent advances in potential therapeutic options for immunity restoration in the aging innate immune system are discussed.

Key words: *aging, innate immunity, hematopoiesis, cell therapy, RAGE*

1. Introduction

Improved sanitation, development of preventive and therapeutic vaccines, development of effective antibiotics, and advances in medical technologies greatly increase human life expectancy and contribute to the increased aged population. This, however, became a significant concern for the global public healthcare system, as a doubling of the aged population (age over 65 years) is predicted in the next 20 years (Fig 1). In 2004, the aged population accounted for 12% of the total US population but consumed more than 30% of the healthcare expenditures.[1] This rapid and inevitable population aging has serious impacts on both the economy and the healthcare system.

Aging is accompanied by a decline in immune functions, referred to as immune aging or immune senescence. In addition, life-long exposure to environmental factors and countless interactions with infectious agents lead to chronic inflammatory states in older individuals, known as inflammaging, which is characterized by increased proinflammatory mediators in the serum.[2] Due to a decline in immune function, the elderly suffer more frequently and more severely from infectious agents than do younger people.[3] In addition, the elderly respond poorly to the same preventive vaccines to infectious agents compared to the responses in young people. Cancer incidence and autoimmune disorders are also known to be increased with age.[4] Although the causes for these events are complex, it is evident that immunity plays a key role in the health of the elderly, as disease susceptibility significantly depends on immune function. Aging is clearly associated with defects in both the innate and adaptive immune systems. While immune aging in the adaptive immune system is relatively well-documented,[5,6] the innate immune system is less well understood. This study attempts to address the current knowledge on age-associated changes in innate immunity and potential interventions that may restore or rejuvenate an aged immune system.

2. Aging and Hematopoiesis

Cells of the immune system have limited life spans and a small reserve of hematopoietic stem cells (HSCs) in the bone marrow to continuously replenish them throughout a lifetime.[7] Bone marrow contains HSCs and stromal non-hematopoietic

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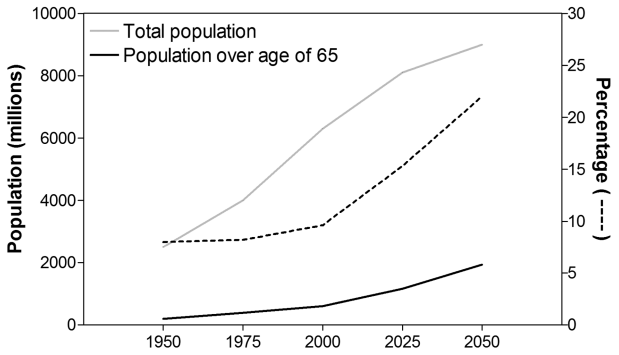

Figure 1. The total world population, the number of people aged 65 or greater and the proportion of aged population. The figure is based on statistics from the World Health Organization website.

stem cells that provide a microenvironment (niche) for hematopoiesis. Although HSC compartments are not exempt from aging, little is known about the effects of aging on HSC compartments or the effects of aged HSC on the downstream function of the immune system.

The hematopoietic compartment of bone marrow is known to decrease with age, accompanied by replacement with adipose tissue.[8] Disturbances in the cytokine and hormonal milieu (systemic environment) substantially affect both HSCs and non-hematopoietic stroma and contribute to the age-dependent changes in bone marrow that lead to alterations in hematopoiesis. In line with this, treatment of aged mice with recombinant growth hormones significantly enhanced the cellularity with decreased adiposity of bone marrow,[9] implying that (1) HSCs retain much of their intrinsic regenerative potential with age and (2) the systemic environment and the niche in which the HSCs reside limit their full potential with advancing age. Serial murine bone marrow transplantation experiments demonstrated that HSCs from young and old mice are indistinguishable in their progenitor activities,[10] implying that stem cell exhaustion does not accompany normal aging. However, another study showed that there are quantitative differences in HSC activity with aging.[11] Limiting dilution assays of HSCs from old and young mice revealed that their progenitor activities *in vivo* were indistinguishable; HSCs contents were five times higher in the bone marrow of old mice, with significantly reduced bone marrow homing and repopulation efficiency. Furthermore, HSCs of aged mice exhibited an increased propensity toward myeloid lineage differentiation compared to that of lymphoid lineage differentiation, possibly contributing to decline in lymphopoiesis with aging.[12,13] This is in part due to the decrease in IL-7 production by bone marrow stromal cells[14], an essential cytokine for lymphopoiesis, further supporting the notion that systemic and local environments limit HSC potential. These data suggest that the abilities of HSCs and bone marrow stroma to nurture hematopoiesis are substantially altered during aging. In addition, genetic studies in murine models[15,16] showed that aged HSCs differ from their younger counterparts. The global gene expression profile revealed coordinated changes in lineage-specification genes and chromatin remodeling genes. Since all cells of the immune system are derived from HSCs, age-associated changes in HSC compartments greatly affect the innate and adaptive arms of the immune system. An understanding of the overall impacts of aging on hematopoiesis and its microenvironment is of clinical relevance, particularly in cell therapy settings, including bone marrow or HSC transplantation.

3. Age-Associated Alterations in Innate Immune Cells

Clinical studies have shown that the ability of the elderly to respond to infection is greatly diminished compared to that in younger populations. A decline in function of the innate immune system with advanced age[17] is implicated as the primary cause of this problem (Fig 2).

3.1 Polymorphonuclear Cells

Polymorphonuclear cells (PMNs) are one of the key effector cells in the innate immune response against microbial infection and injury and are thus indispensible for host defense. They are quickly recruited to the sites of infection in response to tissue damage, complement activation or microbial products and participate in the removal of pathogens via phagocytosis of opsonized particles or production of bacteriostatic and bacteriocidal products. In addition, these cells influence adaptive immunity through the production of chemotactic factors and cytokines. Their capabilities, including chemotactic migration, adhesion, and phagocytosis, are critical for proper defense against microbial invasion and injury. Although the number of circulating neutrophils does not appear to be significantly affected by aging, there are conflicting results regarding the functional defects of PMNs in the aged population both *in vitro* and *in vivo*. Wenisch et al.[18] reported a significant reduction of PMN function with increasing age. In contrast, Niwa et al.[19] showed that the function of PMNs in aged individuals was diminished and there was no significant difference between people older than 80 years old and the young volunteers. However, this observation may reflect the results of selective pressure because only the healthiest people survived into the aged group. Human PMNs from young and

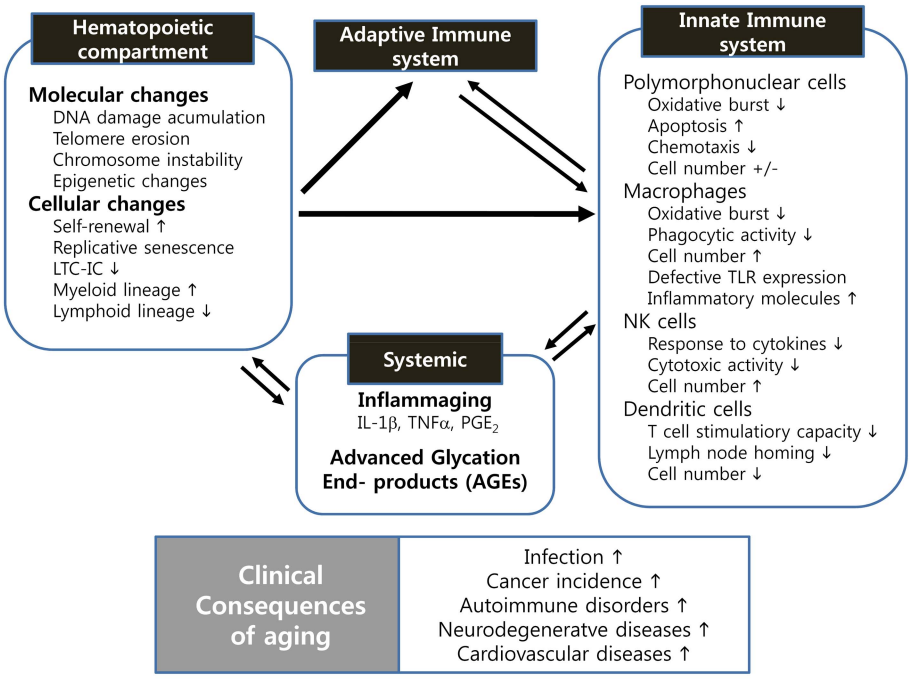

Figure 2. A model of the contributions of cellular and molecular changes accompanying different components of the immune systems due to aging to clinical consequences in the aged population. Hematopoietic stem cells give rise to all the cell types of the immune system under signals from the systemic and local microenvironments. Age-associated alterations in the functional parameters of innate immunity, as well as plausible interactions between components of the immune systems are summarized. LTC-IC, long-term culture initiating cells.

aged populations have displayed compatible capacity for adhesion and phagocytosis.[20] However, a significant decrease in the bactericidal activity through a reduction in the reactive oxygen species (ROS) producing capacity[21] was noted in the elderly, suggesting that longer durations are inevitable to resolve infection in elderly individuals. In addition, aged PMNs express more proapoptotic proteins and are more prone to undergo programmed cell death due to their inability to neutralize reactive oxygen species.[20] The lifespan of a PMN is relatively short (5.4 days in circulation),[22] and the enhanced apoptotic cell death of aged PMNs further decrease their life spans. This alteration may provide insufficient protection against pathogens, leading to an increased risk of infection. Thus, the functions of PMNs are compromised in the aged population. Although these age-related alterations to PMN functions can be overcome through compensatory adaptive immune responses, the changes in the adaptive immune system in aged individuals further weaken the innate arm, leading to an increased susceptibility to microbial infections. Thus, restoration of senescent immunity in aged individuals requires strategies which repair both the innate and adaptive immune systems.

3.2 Monocytes/Macrophages

Monocytes originate from myeloid precursors in the bone marrow and are located both in circulation and within tissues. They respond to infection and inflammation by differentiating into antigen presenting cells such as macrophages or dendritic cells (DCs).[23] Thus, monocytes serve as a bridge between adaptive and innate immunity. They are able to directly remove pathogens via phagocytosis or through the production of bacteriostatic and bacteriocidal products which are induced by components of microorganisms or by cytokines from T cells, natural killer (NK) cells and DCs. Macrophages also participate in wound healing by preventing infection and promoting angiogenesis.[24] Although the number of monocytes increases with age, there are age-dependent decreases in their function.[25] Associations with either decreased pathogen receptor (toll-like receptor, TLR) expression[26] or defective TLR-derived signal transduction[27] have been reported in murine models. Studies of human monocytes have also revealed age-associated declines in cytokine responses to TLR.[28] Macrophages from aged mice produced less ROS upon stimulation,[29] which leads to reduced bactericidal activity. As a consequence, elderly individuals tend to require a longer duration to recover from infections and

injuries. In addition, defects in CD80 up-regulation are observed in monocytes upon TLR engagement[30] and have a significant impact on adaptive immunity, as this molecule is associated with humoral immunity to viruses. This implies that defects in monocyte/macrophage function can also contribute to the alterations seen in other components of the immune system through soluble factors and cytokines. Observations of increased proinflammatory molecules in the sera of aged individuals added an additional layer of complexity to the interpretation of functional defects in monocyte/macrophage as these may be associated with inflammaging.[31] Macrophages are considered to be the primary producer of pro-inflammatory cytokines and may actively contribute to immune aging via secretion. For example, macrophages of aged mice produce higher levels of prostaglandin E_2 (PGE_2), which directly inhibits T cells and dendritic cell function, compared to those in their young counterparts.[32]

3.3 NK Cells

These large granular lymphocytes participate in early defense against certain malignancies and viral infections either via direct contact-dependent lysis or indirect antibody dependent cell-mediated cytotoxicity (ADCC). Several age-related alterations in NK cells have been described.[33] Compared to young healthy subjects, NK cells from the elderly had lower proliferation and production rates, which may be associated with telomere shortening in HSCs. The absolute number of NK cells increases with age, accompanied by an increase in the $CD56^{dim}$ mature cytotoxic subpopulation of NK cells.[34] While there was previous controversy over NK cell activity in peripheral blood lymphocytes, studies using purified NK cells clarified that cytolytic activity decreases with age.[35] Thus, the diminished NK cell activity during aging may be compensated for by the increase in NK cell number. In addition to cytolytic functions in malignant or virus-infected cells, NK cells modulate cells of the innate and adaptive immune systems through the production of cytokines. However, defects in cytokine production by NK cells may compromise the immunity against viral infections and malignancies. Additionally, there is a strong correlation between infection risk and mortality in aged individuals with reduced NK cell activity.[36]

3.4 Dendritic Cells

DCs are a heterogeneous population of specialized antigen presenting cells that constantly sample the surroundings for pathogens such as viruses and bacteria through pattern recognizing receptors such as toll-like receptors (TLRs). Their strategic localization at various portals of pathogen entry and capacities for uptake, processing, and presentation of antigens to T cells impart DCs with unique roles in bridging the innate and adaptive immune systems.[37] While various DC subsets share key functions in the processing and presentation of antigens to naïve T cells for the induction of adaptive immune responses, there are considerable differences among the subsets with regard to phenotype, migratory pattern, tissue localization and cytokine production. At present, the effects of age on DCs are still not fully understood.

Upon contact with pathogens through TLRs, dendritic cells undergo a maturation process and migrate to the lymph nodes, where they present processed antigens to naïve T lymphocytes, initiating an adaptive immune response.[38] The number of myeloid DCs in circulation progressively declines with age and is accompanied by a decrease in circulating CD34+ HSCs and an increase in monocytes,[39] implying that the generation of DCs is partially dysregulated in aged individuals. In addition, the capacities of DCs to uptake and migrate to secondary lymph nodes are impaired with age.[40]

The reduction of antigen uptake can also affect the subsequent function of dendritic cells in antigen processing and presentation, ultimately leading to changes in T cell-mediated immune responses. Inflammaging in elderly individual can also influence the activation and function of DCs, thereby affecting both the innate and adaptive arms of the immune system. DCs from elderly individuals also displayed a more mature phenotype with reduced capacity to induce MHC class II molecules and altered capacity to produce Th1 cytokines, such as IL-12.[40] Since proper functioning of DCs is essential for optimal immune responses to infection and cancer, impairments in DC function in aged individuals may compromise the immune system's ability to protect the host.

4. Systemic Mediators of Immune Aging

4.1 Inflammaging

Inflammatory responses are an essential component for host defense against infectious pathogens. On the other hand, a strong correlation of chronic inflammation with the development or progression of long-term tissue damage, cancer, autoimmune disorders, cardiovascular diseases, atherosclerosis, neurodegeneration and higher mortality has been documented.[41] Cytokines, chemokines, growth factors and lipid metabolites are the key regulators of immune cell function and differentiation, and thus dysregulation of these regulators is believed to be associated with various human diseases. Age-associated systemic elevations in TNF-α, IL-6,

PGE_2 and IL-1β have been described as a subclinical inflammatory status defined as inflammaging[42] and are attributed to lifelong exposure of the immune system to the environment. Inflammaging in the elderly can contribute to defects in the host immune system through the augmentation of infection-related tissue damage. The increased levels of systemic inflammatory cytokines might be caused by pre-existing disease conditions in the elderly or by age-related defects in the innate immune system.

Recent studies of gene expression profiles from different age groups showed that age is accompanied by a decline in first-line defense mechanisms, with augmentation of the inflammation process,[36] supporting the concept of inflammaging. The clinical significance of inflammaging has been demonstrated in a recent study that reduction of the inflammatory milieu increased lifespan in mice.[43] Thus, the detrimental effects of chronic inflammation on cardiovascular disease, age-associated muscle loss, metabolic syndrome and other health issues of the elderly are being realized.[44] In line with this, enhanced anti-inflammatory cytokines in the circulation of the elderly, such as IL-10, or inflammatory modulating drugs, such as statins and nonsteroidal anti-inflammatory drugs (NSAIDs), may be beneficial for preventing these conditions.[45] Although these approaches cannot completely restore the altered immunity, they are useful in attenuating some of the inflammaging-associated disease progression. While heightened basal levels of inflammation activity in elderly individuals might be a survival disadvantage for infection, cancer or autoimmune diseases, active biomedical or pharmacological modulation of inflammaging may promote healthy aging.

4.2 Advanced Glycation Endproducts (AGEs), TLRs and Inflammation

Proper activation of innate immunity depends on non-specific recognition of various pathogens by innate immunity receptors such as TLRs and receptors for advanced glycation endproduct (RAGE). TLRs are a set of germline-encoded pathogen-associated molecular pattern (PAMP)-recognizing receptors that mainly recognize conserved molecular patterns of exogenous signals from microorganisms, whereas RAGE recognizes ligands, such as HMGB1, which are danger signals that emanate from endogenous tissue damage. Because TLR and RAGE recognize both exogenous and endogenous danger signals and alert the body to potential danger, they are collectively referred to as alarmins.[46] TLRs with broad ligand specificity trigger innate immune responses by recognizing conserved elements of molecular patterns in pathogens and activate antigen presenting cells such as dendritic cells and macrophages, playing a crucial role in the innate immune response.[47]

At present, there are 11 known human TLRs and 13 murine TLRs. Of these, 9 are well defined with analogous ligands in humans and mice. TLRs exist as heterodimers or homodimers and bind to pathogen-associated molecular patterns such as di/triacylated lipopeptides, lipopolysaccharide (LPS) and flagellin.[47] The di/triacylated lipopeptides mainly interact with TLR 1/2, whereas LPS and flagellin interact mainly with TLR 4/4 and TLR 5/5, respectively.[47] Activation of TLRs on inflammatory cells results in the production of various pro-inflammatory cytokines such as TNF-α, IL-6, macrophage inflammatory protein 1-α (MIP-1α), and RANTES.[47] TLRs are important for activation of antigen presenting cells and therefore play an important role in linking the innate immune response with activation of the adaptive immune response.[48,49]

Aging is associated with a decrease in TLR activity, which may contribute to impaired innate immunity in the elderly. In a study comparing an elderly population older than 65 years (N=80) with young adults between 21-30 years of age (N=80), there was a significant decrease in the response of monocytes to TLR 1/2 agonist (Pam 3CSK4) in the elderly.[28] Compared to young individuals, there was a 50% decrease in the productions of TNF-α and IL-6 in response to a TLR 1/2 agonist, which was in part due to a 36% lower expression of TLR 1/2 on the surface of monocytes in the elderly. Aging is associated with an accumulation of injuries attributed to increased formation of ROS, such as superoxide anions, hydroxyl radicals and hydrogen peroxide. ROS-induced DNA damage results in activations of poly(ADP-ribosyl) polymerase and poly(ADP-ribosyl)ation of glyceraldehydes-3-phosphate dehydrogenase (GA3PDH), resulting in nuclear translocation of GA3PDH and ultimately its inhibition. The resulting accumulation of upstream intermediates of the glycolytic pathway and dihydroxyacetone phosphate (DHAP) results in increased formation of advanced glycation endproducts such as methylglyoxal, which act to promote formation of advanced glycation endproducts (AGEs).[50]

Administration of D-galactose to 5-month-old mice resulted in modified AGEs and induced changes resembling accelerated aging including immune aging and neurological impairment. These changes were prevented by administration of an AGE formation inhibitor (aminoguanindine).[51,52] Thus, this model suggests that AGE, at least partially, accounts for the aging in the immune system. Interaction of AGEs with RAGE leads to perturbation in diverse states of diseases, such as diabetes, inflammation, autoimmune disease, cancer, cardiovascular disease and neurodegenerative disease.[53] RAGE is a type 1 membrane protein in the immunoglobulin superfamily and is a

pattern recognizing receptor against diverse pathogens, playing important roles in innate immunity but also interacting with diverse endogenous ligands such as HMGB1, S100A12 as well as AGE, resulting in chronic inflammation. This protein is expressed abundantly in normal lung tissue and in macrophages and at low levels in non-activated T cells, podocytes, Müller cells of the retina neurons, glial cells vascular smooth muscle cells and endothelial cells. However, in pathologic states, expression in vascular smooth muscle cells and endothelial cells is up-regulated.[53] RAGE ligation is known to triggers a series of diverse cellular signaling events, including activation and nuclear translocation of NFκB, leading to up-regulation of myriads of cytokines, cell adhesion molecules and ROS generation, all of which promote inflammation and recruitment of proinflammatory cells.[53-55] Thus, increased accumulation of AGEs with aging and subsequent increases in AGE-RAGE interactions may be one of the mechanisms for increased age-associated inflammation (inflammaging). In a study by Hallam et al.[56] which compared the vascular functions of elderly Fisher rats (24-36 months) compared to those of young rats (4 months), there was a significant accumulation of methylglyoxal and increased expression of RAGE in the aorta of elderly rats. Administration of soluble RAGE resulted in significant improvement of endothelial function of the aorta in the elderly rats, demonstrating that age-associated vascular dysfunction may be mediated in part by increased AGE-RAGE interaction.[56]

Decreased expression and function of TLRs, along with increased AGE-RAGE interactions in aging, may partly explain inflammaging, the paradoxical finding of increased inflammation and simultaneous immunocompromise.

5. Immunorestorative Interventions in the Aged Population: Perspectives

There is accumulating experimental evidence suggesting that innate and adaptive immunities are weakened with age, leading to increased incidence and susceptibility to diseases (infection, cancer, autoimmune diseases, cardiovascular and neurodegenerative diseases). A number of different strategies can be used to counteract the decline in immune function in the elderly (Table 1).[57,58] While there are a number of means to intervene in immune aging including balanced nutrition and exercise, there remains a strong need for specific, effective and safe immune-restoring therapies for elderly individuals with reduced immunity.

Since immune aging is clearly associated with hematopoiesis, it can, in theory, be reversed by improving the qualities of HSC compartments. Administration of hematopoietic growth factors or cytokines can modulate hematopoiesis to compensate for the functional and numerical deficits of innate immunity. Indeed, administration of M-CSF to aged mice partially restored immunity against opportunistic infection.[59] Animal models of heterochronic parabiotic pairs of aged and young mice demonstrated that young systemic factors rejuvenated old hematopoietic compartments.[60] Identification of factors that have such critical influences on hematopoiesis and innate immunity has great clinical implication. In a clinical setting, this can be partly fulfilled through the collection of serum and HSCs from young patients and the transplantation of long-term banked

Table 1. Strategies to restore immunosenescence in aged individuals.

Preventive or curative strategies to restore altered innate immunity	Examples; Mode of action
Hematopoietic growth factors or cytokines (systemic or local administration)	M-CSF; stimulate and correct hematopoiesis to compensate for the functional deficits of the cells of innate immunity, enhance the function of neutrophils and NK cells
Modulation of inflammaging through pharmacological or antibodies	Anti-TNF-α; reduce basal level of systemic inflammation
Modulation of AGE formation or inhibition of AGE-RAGE interaction	Prevent AGE-mediated accelerated aging processes, including immune aging
Hormonal or nutritional intervention	DHEA, zinc supplements, vitamins and melatonin; enhance the functions of neutrophils and NK cells.
Local IGF-1 neutralization	Anti-IGF-1; enhancement of hematopoietic activity in aged bone marrow
Autologous blood/plasma transfusion	Autologous blood at a young age; replenish functionally competent immune cells of the innate and adaptive arms of the immune system
Hematopoietic stem cell therapy	Autologous or allogeneic HSC transplantation to restore hematopoietic compartments and the immune system

autologous HSCs and serum for restoration of immunity at an advanced age.

Correction of dysfunctional microenvironments or systemic inflammaging via pharmacological means or through the use of antibodies to inflammatory mediators may also contribute to rejuvenation of the innate immune system. The finding that blocking antibodies and soluble receptors for pro-inflammatory cytokines, such as TNF-α, have notable therapeutic effects on several autoimmune diseases[61] supports the potential benefit of this approach in age-associated chronic inflammation. Inhibitors of AGE formation[51] or AGE-RAGE interaction[56] can be administered to improve age-associated subclinical inflammation. Hormonal/cytokine therapies may be useful for the treatment of the age-associated decline. For example, dehydroepiandrosterone (DHEA) supplements are known to enhance the functions of neutrophils and NK cells.[62] IGF-1 has controversial effects on immune aging.[63] Although a low level of IGF-1 utilization is known to be associated with longevity in a centenarian population,[64] this cytokine increases the functions of NK cells and macrophages.[65] Furthermore, enhancement of hematopoietic activity in aged bone marrow due to IGF-1 neutralization[60] underlines the importance of this cytokine in the regulation of stem cells. Although this cytokine approach is attractive for immune rejuvenation, known toxic side effects may limit their clinical application.

Autologous blood cell collection and cryopreservation at a young age may be a unique and ideal source for restoring immunity in the elderly,[66] as it contains functionally competent immune cells of the innate as well as adaptive arms of the immune system. These cell sources have obvious advantages over allogeneic sources because they are fully compatible to the recipients with no concerns of rejection, disease transmission or other transfusion-related adverse effects. Furthermore, techniques for blood/bone marrow collection, *in vitro* cell manipulation, long-term cryopreservation and transfusion have greatly improved and have become routine procedures. Although current data suggest that alterations in hematopoietic stem cell compartments in the aged correlate with immune aging, it is not clear whether replacing aged HSCs with young ones will restore innate immunity, and more studies are still needed.

The ideal therapeutics for restoration or rejuvenation of defective immunity in the elderly should be easy to administer, easy to produce or synthesize, and safe. Although rejuvenation of an aging immune system to the levels of those in young individuals may not be achievable, a modest enhancement in immune function may be of clinical benefit. A better understanding of immune aging is essential for the development of new therapeutic strategies that ultimately lead to improvements in the quality of life in the elderly population.

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