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REVIEWS

Cell Therapy Strategies to Combat Immunosenescence

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ABSTRACT. Declining function of the immune system, termed "immunosenescence," leads to a higher incidence of infection, cancer, and autoimmune disease related mortalities in the elderly population.[1] Increasing interest in the field of immunosenescence is well-timed, as 20% of the United States population is expected to surpass the age of 65 by the year 2030.[2] Our current understanding of immunosenescence involves a shift in function of both adaptive and innate immune cells, leading to a reduced capacity to recognize new antigens and widespread chronic inflammation. The present review focuses on changes that occur in haematopoietic stem cells, macrophages, and T-cells using knowledge gained from both rodent and human studies. The review will discuss emerging strategies to combat immunosenescence, focusing on cellular and genetic therapies, including bone marrow transplantation and genetic reprogramming. A better understanding of the mechanisms and implications of immunosenescence will be necessary to combat age-related mortalities in the future.

KEYWORDS. aging, bone marrow, cell therapy, gene therapy, haematopoietic stem cell, immunosenescence, macrophage, T-cell, thymus

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INTRODUCTION TO IMMUNOSENESCENCE

Human life expectancy has increased from 40 to 80 years of age just over the past 2 centuries largely due to medical advances.[3] However, it is likely that the human immune system did not evolve to protect the host over such an extended lifespan. Immunosenescence is a term that describes the changes in the immune system that are seen in the aging population. The hallmarks of immunosenescence include a reduced capability to respond to new antigens, increased memory responses, and a lingering level of low-grade inflammation that has been termed "inflamm-aging."[4] Decline of the immune system is associated with increased incidence of infection, immune disease, and cancer in the elderly.[5] While immunosenescence is often described as a decline in the number and function of immune cells, myeloid cells have been shown to increase in the aged population and some secreted peptides are also expressed in greater amounts. Therefore, it is important to keep in mind that immunosenescence is more appropriately conceptualized as a change in the actions of the immune system, rather than an overall decline of all functions and constituents.

The term "immunosenescence" does not accurately describe the cellular mechanisms responsible for the age-related changes of the immune system. Immunosenescence can only be explained in part by cellular senescence, and may also be influenced by cumulative history of antigen exposure, environmental stressors, and dealings with acute or latent pathogens. Mechanisms of cellular senescence have been rigorously described in some cell types, namely fibroblasts, but there are likely differences in the characteristics of cellular senescence observed between organisms, tissues, and even cell microenvironments. In general, senescence occurs when a cell permanently or irreversibly exits the cell cycle.[6] Hayflick and colleagues first observed that human fibroblasts have a limited capacity to proliferate in culture, a principle known as the Hayflick limit. After several population doublings, the cultured fibroblasts lost the ability to divide, although sufficient space and nutrients were provided. Interestingly, the cells remained viable in the non-dividing state for many weeks and were thus termed senescent. These findings suggested that senescent cells might represent a physiological response to prevent cellular immortality and growth, as is common in cancer, and could help to explain the decline of tissue regeneration and repair that is observed with aging. More recent findings have shown cellular senescence can be triggered by many stimuli, including telomere shortening (also known as replicative senescence), DNA damage, chromatin perturbation, oncogene expression, and stress.

While senescent cells are not actively growing, they are distinct from quiescent or dormant cells. Senescent cells undergo extreme changes in gene expression and become resistant to apoptosis. Cell cycle inhibitors including cyclin-dependent kinase inhibitors (CDKIs), such as p21 and p16, are often expressed by senescent cells. Senescent cells secrete many factors, including extracellular matrix remodeling proteins and inflammatory cytokines, which lead to changes in the microenvironment. These inflammatory cytokines may serve to recruit cells from the immune system that will subsequently remove the senescent cells from the surrounding tissue.[7] However in the aging population, it has been suggested that the declining immune system cannot keep up with the need to eliminate senescent cells.[8] The increase in senescent cells then secrete more inflammatory cytokines and reactive oxygen species, which may drive neighboring cells into senescence and contribute to the cycle of inflamm-aging.[9] Over time, the accumulation of non-functional senescent cells may lead to organ failure and death of the host.[10] Further studies are needed to better understand cellular senescence and how it relates to the functional decline of the immune system and the chronic, low-grade inflammation that is linked to many diseases in the elderly population.

When considering the mechanisms of immunosenescence, it is important to remember that changes in the function of immune

cells can be cell-intrinsic or cell-extrinsic. Cell-intrinsic changes in the genome might arise as a result of epigenetics or DNA damage in the form of oxidative damage, telomere shortening, or impairment of DNA repair responses.[11] Cell-extrinsic changes, such as the increased inflammatory milieu, may also play a role in immunosenescence.[12] It is likely that both cell intrinsic and extrinsic changes are involved in immunosenescence, but an understanding of the exact mechanisms affecting certain cell types will allow scientists to appropriately design targeted therapies that can counteract immunosenescence.

Mouse models have predominantly been used in the study of immunosenescence because of their close evolutionary relationships to humans and the availability of several immunologically relevant genetic and infectious disease models. In addition to using naturally aged models to study immunosenescence, immune diseases and infections such as rheumatoid arthritis and HIV have also shed light into mechanisms of immunosenescence.[13,14] There are many important differences in aging between humans and mice that must be addressed. First, 24 month-old mice may not be physiologically equivalent to elderly 80-year old human beings, which is particularly relevant as cellular replication is a important mechanism of aging and senescence.[4] In contrast to most human cells, murine cells contain telomeres that are 10 times longer.[15] Despite this, murine cells senesce after only a few doublings in culture due to supraphysiological oxygen concentrations, while human cells are not as sensitive. Finally, immunological aging is shaped by infection and antigenic load, and it is important to keep in mind the difference in pathological history, especially viral infection, of mice and men.

HSCS AND AGING

The immune system is generated and maintained by asymmetric division of multipotent haematopoietic stem cells (HSCs) in the bone marrow.[16] The immune system has 2 arms, the innate and the adaptive systems, which work together to eliminate pathogens and neoplastic cells, respond to vaccination, and regulate processes such as tissue turn over and wound healing.[16] The cells of the innate immune system include monocytes/macrophages, dendritic cells, natural killer cells, and polymorphonuclear leukocytes. The cells of the adaptive immune system include antigen-specific lymphocytes, T-cells and B-cells.[16] This review will focus on changes in HSCs, macrophages, and T-cell lineages, as these have been the most widely studied.

Increasing evidence shows that HSCs themselves undergo age-related changes and have a limited replicative lifespan. HSC aging was demonstrated by serial transplantation of whole bone marrow, which only supported 4–6 rounds of transplantation, suggesting the possibility of stem cell exhaustion or replicative senescence.[17] Interestingly, HSCs from long-lived C57BL/6 mice have relatively slow replication, whereas short-lived DBA/2 mice exhibit faster replication, associating lifespan with replicative senescence of HSCs.[18] In addition, accumulation of DNA damage has a profound impact on HSCs, leading to loss of proliferation, diminished self-renewal, increased apoptosis, and subsequent exhaustion.[19] Differentiation of the HSCs is also affected by aging, where HSCs committed to the myeloid lineage outnumber lymphoid cells in both mice and men.[20]

MACROPHAGES AND AGING

The increased incidence of infections in the elderly suggests defects in the ability of innate immunity, particularly macrophages, to function as effective barrier cells. Classically activated macrophages recognize pathogens, perform phagocytosis, and produce cytotoxic bursts of nitric oxide and superoxide. These classically activated macrophages have been termed “M1,” for the production of Th1 cytokines. Alternatively, macrophages can express Th2 cytokines, and are termed “M2,” and can also play roles in tissue surveillance, wound healing, and tissue regeneration.[21] While macrophages are commonly classified as being in an “M1 or M2 activation state,” in vivo data

suggests that macrophages are heterogeneous and may have characteristics of both M1 and M2 activation states at any given time. Therefore macrophage activation may exist along a spectrum rather than an "all-or-nothing" response, largely controlled by the microenvironment.[22]

Inflammatory macrophages are derived from circulating blood monocytes which enter the tissue due to chemoattractant signals during injury or infection.[16] Monocytes themselves are formed from HSCs in the bone marrow along the common myeloid progenitor cell pathway.[16] While monocyte-derived macrophages are short-lived and are not believed to proliferate in sites of infection, tissue resident macrophages, which develop from the embryonic yolk sac or fetal liver, can survive for at least 6 weeks and maintain a presence in the tissue through homeostatic proliferation.[23] Tissue-resident macrophages are present in many tissues with slightly different phenotypes including liver (Kupffer cells), brain (microglia), lungs (alveolar macrophages), bone (osteoclasts), skin (Langerhans's cells), Peyer's patches in the gut, red and white pulp in the spleen, the peritoneal cavity, and the interstitium of organs such as kidney, heart, and pancreas.[23] Because of their widespread presence throughout the body and their heterogeneous functions, it is not surprising that macrophages are a key component of the innate immune system. The increase in infection in the aging population may be a result of decreased macrophage number and function.[23]

Despite the increased output of myeloid progenitor cells in the elderly, the number of macrophage precursors in the blood is reduced compared to the younger population.[24] In addition, the number and function of Langerhans's cells in the epidermal tissue, has been reported to decline with age.[25] Alternatively activated M2 macrophages have been reported to increase in aged skeletal muscle and are correlated with the detrimental replacement of functional muscle with fibrotic tissue.[26] Bone marrow transplantation of old mice with donor cells from young mice reduced the number of M2 cells in skeletal muscle and subsequently reduced fibrosis, suggesting a cell-intrinsic mechanism of the aged bone marrow or M2 macrophages.[26] However, these mice were not tracked over the long term, so reemergence of fibrosis from cell-extrinsic factors cannot be ruled out. From these studies, it is likely that the number and function of macrophages in an aged organism will differ based on the tissue and activation state of interest, further complicating our understanding of immunosenescence.

Classically activated macrophages are also affected in aging. Like dendritic cells, macrophages are professional antigen-presenting cells (APCs) that can stimulate T-cell activation.[16] Aged macrophages have reduced MHC class II molecules, reported in both humans and mice, likely contributing to the known decline in T-cell response in the elderly.[27,28] Some of the most classic features of the macrophage are phagocytosis and respiratory burst. Phagocytosis, the process of engulfing a pathogen or cellular debris for subsequent destruction in the phagosome, was found to be decreased in aged murine peritoneal macrophages.[29] Receptors that mediate phagocytosis may have changes in expression or function with aging, however this has not yet been examined. The decline of phagocytic ability has also been correlated to a decline in macrophage-derived chemokines such as macrophage inflammatory protein (MIP) and eotaxin.[30] In addition, decreases in nitrous oxide and superoxide have been reported in aged rats.[31] Decreases in adherence, opsonization, and tumor cell killing by aged macrophages in aged mice were also observed.[32-34]

As for additional cytokines and chemokines secreted by macrophages during aging, the literature has shown mixed results. Some studies find that aged macrophages secrete more pro-inflammatory cytokines such as IL-1 and IL-6,[35,36] other studies determine aged macrophages secrete less,[37,38] while others find no change.[39] These discrepancies are likely the result of different tissue sources and activation states of the macrophages tested, as well as differences in the health of the test population. It is, however, accepted that aged macrophages secrete more prostaglandin E2 than younger counterparts.[40] Prostaglandin E2 may be responsible for the decrease in MHC class II

molecules on macrophages, as well as increased production of IL-10 which suppresses T-cell activation, thereby weakening the response to vaccination and the elimination of pathogens.[41]

Toll-like receptors (TLRs), which play an important role in pathogen detection, have decreased surface expression on peritoneal and splenic macrophages in aging mice.[42] This decrease in TLR expression may be responsible, in part, for the susceptibility of the elderly to bacterial, mycotic, and viral infections. Loss of the TLR signaling pathway may also reduce pro-inflammatory cytokine secretion that is crucial for mounting an attack against a pathogen by recruiting other cellular and humoral components of the immune system.

As alluded to above, macrophages play important roles in wound healing in both the M1 and M2 activation states. Studies in humans and rodents have revealed poor cutaneous wound healing, characterized by enhanced platelet aggregation, delayed re-epithelialization, delayed angiogenesis, delayed collagen deposition, and decreased wound strength. The decline in wound healing is positive correlated with a delayed infiltration of macrophages.[43] Ashcroft et al. collected punch biopsies of the wounds of healthy human subjects, aged 19-96, at various time points up to 3-months post-wounding.[44] The authors found that macrophage and lymphocyte infiltration was delayed in the older populations, with cell numbers peaking at Day 84 as opposed to Day 7 in young patients for macrophage/monocytes.[44] Another study showed that the rate of wound repair in aged mice could be partially restored by transplanting peritoneal macrophages from young mice, demonstrating the importance of macrophages in the wound healing process that becomes unsynchronized in aging.[45] Decreases in angiogenesis of the wounds may be a result of decreased TLR expression on macrophages, which are also involved in VEGF signaling.[46] In addition, a decrease in adhesion molecules VLA-4 on monocytes or the receptor VCAM-1 on endothelial cells may explain the delayed rate of macrophage infiltration to the wound.[44] In the future, it will be important to unite the field of immunosenescence by characterizing differences in aged macrophages from different tissue sources using the same functional assays and conditions to reduce some of the contradictories currently present in the literature. It is clear, however, that delayed infiltration of macrophages and decreased functions such as phagocytosis, respiratory burst, and antigen-presentation are likely responsible, at least in part, for the increase in infection-related deaths in the elderly population.

T-CELLS AND AGING

Lymphoid progenitor cells that will differentiate into T-cells are formed in the bone marrow from HSCs then migrate to the thymus for maturation.[16] The developing T-cells in the thymus, known as thymocytes, undergo a series of processes to become fully mature, antigen-recognizing cells. Developing thymocytes, which lack both CD4 and CD8, are known as double negative cells. Double negative cells that lack expression of CD44, but do express CD25, undergo β-selection, which is the formation of antigen-specific T-cell receptors (TCRs). Gene rearrangement leads to the production of a repertoire of over 10^8 TCRs, which is sufficient to protect against the range of pathogens likely encountered throughout life.[16] Formation of TCR with CD3 molecules leads to survival, proliferation, and differentiation of thymocytes into double positive cells that express both CD4 and CD8. Cells that do no undergo β-selection are eliminated by apoptosis. Double positive cells undergo positive selection for their antigen by interacting with cortical epithelial cells. Thymocytes that engage in antigen/MHC with appropriate affinity survive, whereas others that bind too weakly are eliminated. Then thymocytes migrate into the medulla of the thymus where they are presented self-antigens by APCs, including macrophages and dendritic cells. Cells that interact too strongly with APCs undergo apoptosis, the process of negative selection, to prevent potential autoimmune reactions. Following these maturation steps, mature T-cells become singly positive for either CD4 or CD8, and exit the thymus for circulation in the blood stream.

CD8 cells are known as effector T-cells, which are cytotoxic killers, and recognize antigens by MHC Class I restricted molecules. CD4 cells are known as helper cells, which are professional cytokine factories that can enhance or subdue other functions of the immune system, and recognize antigens by MHC Class II restricted molecules.[16] T-cells are major contributors to immunosenescence related complications, especially infection and cancer. Aging results in low numbers of CD8 naïve cells, persistent memory cells, and reduced diversity of the TCR repertoire.[1] Several studies have reported that naive T-cells are increasingly dysfunctional with aging, whereas the functions of memory cell populations are preserved.[47]

The genesis of naïve T-cells in adults is entirely dependent on ongoing but vastly diminished thymic function.[48] In C57/BL6 mice, the thymus begins to involute at puberty, where thymic cellularity decreases drastically from 1–3 months of age followed by less extreme involution from 3–7 months of age.[49] In humans, function of the thymus decreases beginning as early as the first year of life at a rate of 3% per year, then slows to a rate of 1% per year around middle age until death.[50] Involution of the thymus is likely responsible for loss of naïve T-cells and reduced T-cell receptor diversity observed with aging.[51] Defects in T-cell synapses with antigen-presenting cells are also reported. The defects are likely a result of glycosylation of cell-surface molecules such as CD28 or changes in cell membrane lipid properties.[52,53] Reduced production of IL-2 after T-cell activation may be the single most important consequence of the synapse defect in mice, since addition of exogenous IL-2 can rescue many of the age-related deficits of the T-cell activation.[54] In humans, diminished signaling through Erk as a result of the phosphatase DUSP6 has been identified as a mechanistic explanation to decline in T-cell activation.[55]

Recently some studies have challenged the importance of thymic involution to immunosenescence. An in silico study found that even complete loss of thymic function at the age of 20 may not influence TCR repertoire diversity.[56] According to the model, multiple changes in growth behavior are required for TCR contraction. Restoration of thymic output cannot prevent or rescues shrinkage of the TCR repertoire. While this may be the case, a large proportion of the elderly have undergone depletion of peripheral T-cells due to chemotherapy or antiretroviral therapy, and therefore reintroduction of a thymus would still prove useful to combat age related mortalities.[4]

Lower abundance of TCR diversity, and diminished ability to clonally expand and produce cytotoxic mediators in CD8 T-cells may be responsible for increased mortality from bacterial and viral infections in the elderly. Old mice succumb more readily to infection with West Nile virus or Listeria than younger mice.[57] The occurrence of intrinsic defects in naïve cells, versus memory cells, is counterintuitive since naïve T-cells have lower turnover and are therefore less likely to develop replicative senescence. However, studies in mice have reported increased longevity of naïve CD4 T-cells, correlating with a decrease in proapoptotic Bim expression, and suggests that this longer cell lifespan may lead to accumulation of defects.[58]

In Sweden, 2 longitudinal studies have been carried out to study the very elderly (>85 year old) and develop an immune risk profile (IRP) that can predict mortality.[59,60] The first study, known as OCTO, followed people in very good health over the age of 85. The OCTO study discovered an immune risk profile predictive of mortality that included high levels of CD8 T-cells, low levels of CD4 T-cells, and poor proliferative response to concanavalin A. The second study, termed NONA, selected a more representative population, with only 10% in of enrolled participants in perfect health. Interestingly a similar IRP was discovered. The NONA study found that increased amounts of memory and effector CD8-positive, CD28-negative T-cells and depleted quantities of naïve T-cells able to recognize new antigens are associated with mortality. The small number of subjects who reached the ages of 90 or even 100 years old never entered the IRP group during the 2-6 years of follow ups. One unexpected result was correlation of the IRP with persistent cytomegalovirus infection (CMV). CMV is generally considered a harmless

infection, although 50-80% of adults over the age of 40 are likely to have CMV infection in the United States.[61] The accumulation of CMV specific CD8 T-cells and large clonal expansions associated with CMV antigens provide additional support for the hypothesis that CMV contributes markedly to immune dysfunction with aging. It is currently unclear if the findings of the OCTO and NONA study will apply universally to younger populations or elderly populations outside of Sweden. Changes in HSCs, macrophages, and T-cells are summarized in **Figure 1**.

TARGETED THERAPIES FOR AGED IMMUNE CELLS

Currently, there are no clinically used therapies aimed at rejuvenating aged HSCs, macrophages, or T-cells to prevent or treat age-related disease. Emerging cellular and genetic therapies that could be applied to immunosenescence are being explored using animal models with some success.

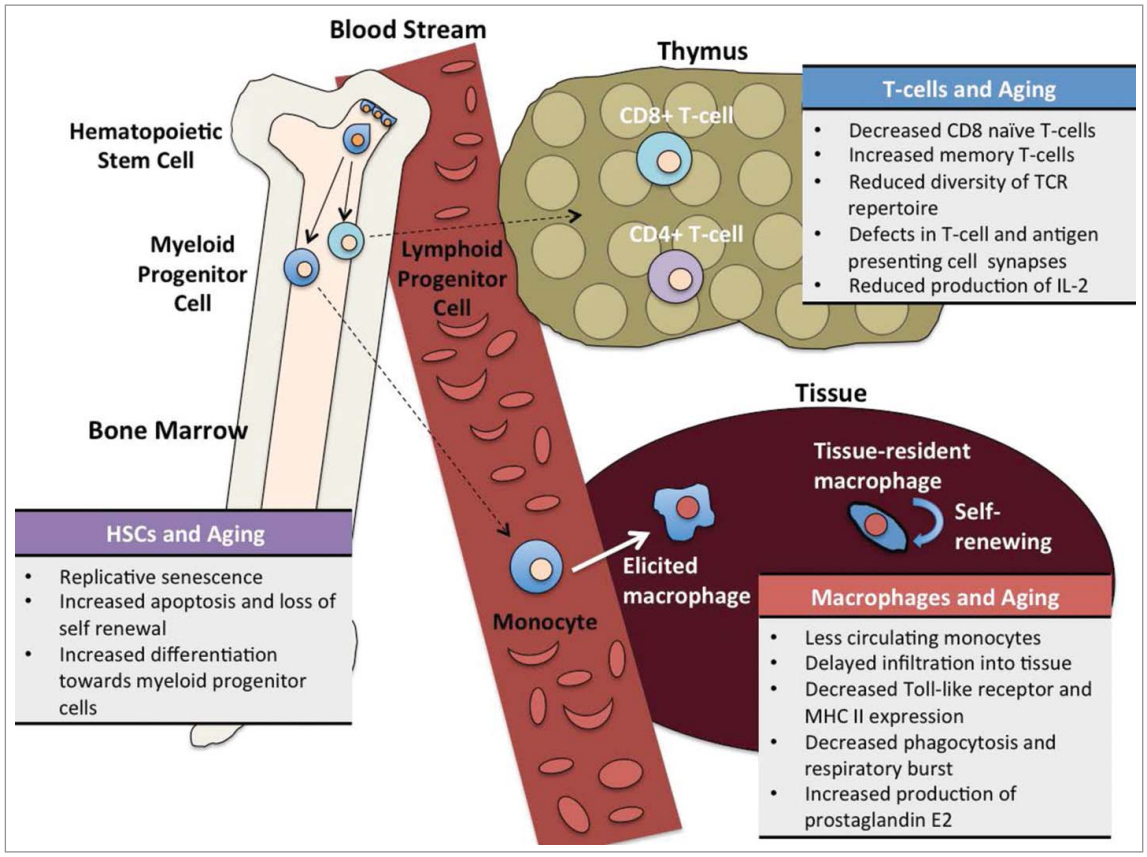
Rejuvenating Aged Immune Cells

Many age-associated defects have been observed in HSCs, which give rise to all downstream effectors cells of the immune system, including macrophages and T-cells. Therefore, rejuvenating the HSCs might improve some of the dysfunction of both macrophages and T-cells, as well as many other cell types, observed in aging. Bone marrow transplantation from a young donor to an elderly patient could be used to rejuvenate the exhausted, aged progenitor pool. However, imperfect tissue matches often lead to rejection and even graft-vs.-host disease, a major hurdle to overcome in many fields of study.

Induced pluripotent stem cells (iPSCs) could theoretically be used to generate HSCs from a patient's own cells, thereby eliminating donor-recipient mismatch. Interestingly, reprogramming terminally differentiated cells into iPS cells induces telomerase reverse transcriptase gene (TERT), leading to telomere elongation and maintenance, in a process that can be described as "reverse aging." A recent study showed that human senescent cells can successfully undergo genetic reprogramming and become functional once again.[62] IPSCs created from senescent and centenarian cells had reset telomere length, gene expression profiles, mitochondrial metabolism, and oxidative stress patterns that were indistinguishable from human embryonic stem cells. In addition, the iPSCs were able to redifferentiate into fully rejuvenated cells.[62] Therefore, iPS technology may be the key to the fountain of youth, where any senescent cell could in theory be reborn. Techniques to differentiate HSCs from iPS cells exist, but efficiency and safety are major hurdles that this technology must yet overcome.[63] In addition, genetic reprogramming will likely need to take place ex vivo to prevent collapse of organ function in the intermediate, undifferentiated cell state, so repopulation of tissue resident macrophages and lymphocytes will take several weeks or months from a single bone marrow transplantation. Also, effectiveness of rejuvenated HSCs would be limited by thymic output for T-cells and would likely not replace tissue resident macrophages, which are self-sustaining. However, repopulating the bone marrow with autologous IPSC-derived HSCs is a promising approach to rejuvenating the majority of immune system, especially the innate effector response.

Inducing autophagy in aged cells and organisms by caloric restriction has been shown to reduce age-related damage,[64] suggesting that decline in cell function may be prevented by maintaining autophagy levels, or potentially even rescued by the induction of autophagy in aged cells. A recent study showed that macrophages from aged mice exhibit reduced autophagic flux compared to young mice, therefore chemically increasing autophagy might be one strategy to reduce aged macrophage dysfunction.[65] In addition to caloric restriction, exercise is a known factor that can boost lifespan and health span. One study found that long-term moderate exercise in male BALB/c mice tended to reverse age-associated changes in

FIGURE 1. Summary of Age-Related Changes in the Immune System. Steady-state turnover of the immune system begins in the bone marrow with haematopoietic stem cells (HSCs), which give rise to both myeloid and lymphoid lineages. Along the lymphoid lineage, T-cells mature in the thymus and become positive for CD4 or CD8. Monocytes, derived from myeloid progenitor cells, travel through the blood steam to sites of recruitment and enter tissues as elicited macrophages. HSCs, T-cells, and macrophages all experience cellular changes with aging.



macrophage function using cells isolated from the lungs and peritoneal cavity.[66]

Another method to boost macrophage function, especially recognition of foreign pathogens, is to treat with cytokine or growth factor cocktails. Treatment of aged macrophages with IFN-γ [67] or with insulin-like growth factor (IGF) [68] significantly improved both inflammatory and effector responses to lipopolysaccharide (LPS) stimulation. One challenge to this technique will be in vivo delivery of cytokines and growth factors to relevant anatomical locations and in relevant doses. Alternatively, macrophages could be isolated, stimulated ex vivo, and reinfused to the patient as a living adjuvant to reduce infection.

As mentioned previously, macrophages are heterogeneous cells with behaviors that are heavily influenced by the microenvironment. One group recently demonstrated that effector functions can be restored in macrophages from aged mice by simply removing them from the aged environment.[69] Therefore, therapies aimed at altering the environment, rather than the cells themselves, could be an alternative approach to treating aging.

Finally, strategies aimed at macrophage rejuvenation may also influence the T-cell

compartment. One study found that IL-2-/CD40-activated macrophages rescue the production of IFN-γ by T-cells in geriatric mice. Therefore, targeting macrophages with specific antibodies may improve both innate and adaptive functions in aging hosts.[70] In addition, vitamin E improves T cell responsiveness in old mice mostly by reducing macrophage prostaglandin E2 production.[71]

Rejuvenating the Thymus

The thymus begins to significantly deteriorate around 10 years of age in humans, and likely plays a role in the decline of the immune system, especially the diversity of the T-cell repertoire, during aging. Rejuvenating or somehow regulating thymic output is an intriguing approach to combat age-related decline of T-cells. Sex hormones have been shown to modulate thymus size and function. In fact castration of 9-month-old mice enhances the number of early thymic progenitors.[72] While this technique may have limited application in humans, male patients undergoing sex steroid ablation therapy for prostate cancer have increased circulating naïve T-cells, demonstrating the link between sex hormones and thymic health in humans.[73] Bolotin et al. reported an enhancement of thympoiesis after bone marrow transplant with administration of IL-7 in mice.[74] Indeed, IL-7 therapy alone has been shown to rejuvenate the thymus in aged mice, although with size and output slightly less than observed in the young mice.[75] IL-7 therapy has yet to be tested in humans, and has been shown to have species-specific functions, possibly limiting its efficacy.

Other approaches to replacing or regenerating the thymus include tissue and cell transplantation. Transplantation of cultured thymic tissue from human cadavers into the kidney capsule of patients with DiGeorge syndrome successfully restored immune function for up to 10 years.[76] However there are limitations to this approach for treating the aging population due to lack of donated tissue, invasive surgery, and tissue rejection.

Regenerative medicine, including tissue engineering and cell and gene therapy, offer alternative approaches to replacing the thymus. Many groups have identified murine multipotent progenitors, termed thymic epithelial cells (TEC), that can grow into a 3-dimensional thymus and support normal T-cell development when transplanted into the kidney capsule.[77] Human TECs have yet to be isolated in sufficient numbers, however protocols to push human embryonic stem cells toward TEC lineage are becoming consistently more efficient.[78]

In addition to the kidney capsule, lymph nodes have emerged as an intriguing site for ectopic organ formation. Recently, Lagasse's group showed that minced thymuses from newborn mice can be injected into the jejunal lymph nodes of athymic mice and reconstitute functional lymphocytes.[79] The ectopic thymuses were organized into epithelial thymus structures with areas corresponding to both the thymic medullary and cortical epithelia and contained maturing CD4/CD8+ double positive T-cells. The transplanted mice rejected both a skin allograft and injected tumor cells, suggesting the newly developed T-cells were functional.

Even more recently, the thymus was the first organ ever to be successfully regenerated in a living organism. Diminished expression of the TEC specific transcription factor, Forkhead box N1 (FOXN1), is implicated in the mechanism of thymus involution. Blackburn's group found that forcing the expression of FOXN1 in the involuted thymus of aged mice caused complete thymus regeneration, characterized by increased thymopoiesis and naïve T-cell output, as a result of robust TEC progenitor responses.[80] The study showed that regeneration of an organ in an aged animal can be controlled by manipulation of a single transcription factor, perhaps ushering in a new era of regenerative medicine. The authors do not comment on how this therapy could one day be applied to human patients.

CONCLUSION

Immunosenescence is a complex problem arising from age-associated changes to the cells of the immune system, a process that may increase susceptibility to cancer, infection, autoimmune disease, or simply organ failure as

a result of chronic inflammation and build up of non-functional senescent cells. Changes in the function of haematopoietic stem cells, macrophages, and T-cells just scratch the surface of the complexities of immune system decline with age. Cellular and genetic therapies are beginning to make headway in rejuvenating the immune system, which could ultimately improve lifespan and/or health span of the human race. Genetically reprogramming cells into induced pluripotent stem cells can rejuvenate any cell type through telomere elongation, overcoming hurdles of replicative senescence. Activating transcription factors such as FOXN1 may completely regenerate the thymus and reestablish functional naïve T-cells in the elderly. Finally, altering the systemic inflammatory environment through caloric restriction to increase autophagy, exercise, and vitamin supplementation are practical ways to combat immunosenescence right now. Increasing our understanding of immunosenescence using standard cell isolation and culture methods, as well as the increasing the feasibility of cellular and genetic techniques are crucial to push the field forward. Currently genetic reprogramming generally has low efficiency and raises concerns about ethics and safety. It is also unclear how genes can be altered in vivo without detrimental effects. In addition, rejection of cell and tissue transplantation is a major hurdle in many fields, including immunosenescence, which scientists are continuously working to solve. Significant progress in the field of immunosenescence has been made over the last 40 years; it will be remarkable to see what the next few years bring.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

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