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Review

HSC Aging and Senescent Immune Remodeling

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Aging-associated changes in the function of the immune system are referred to as senescent immune remodeling (SIR). Here we review the current understanding on the cellular and molecular mechanisms underlying SIR. We focus on aging-associated changes in T and B cells, and discuss recent evidence supporting the notion that aging of the hematopoietic stem cell (HSC) compartment directly contributes to SIR due to aging-associated alterations in stem cell differentiation. We conclude by outlining strategies to attenuate SIR, including approaches to rejuvenate HSCs, which may open new avenues for targeting SIR in the clinic.

Introduction

Adults 65 years and older present with increased incidence of respiratory, urogenital, and gastrointestinal infections, higher susceptibility to autoimmune disease, and mortality resulting from these challenges [1,2]. The incidence of *Clostridium difficile* associated diarrhea in older adults is almost 10-fold higher than in younger individuals, and mortality during an outbreak can be as high as 17%, depending on the vulnerability of the patient and virulence of the individual strain [3,4]. Similarly, the incidence of community-acquired pneumonia as a consequence of viral infections is highest among the very elderly (over 80 years of age) [5] with mortality rates as high as 75% [6]. Mortality rates in the very elderly to rhinovirus, influenza, and streptococcus pneumonia are 20-fold higher as compared to younger adults (45–64 years of age) [7,8]. Contributing to higher mortality rates in infectious disease are impaired cell-mediated immunity, which also contributes, in the case of influenza, to poor responses to vaccination [9]. Trivalent influenza vaccines have been shown to convey protection in approximately 50% of the elderly population (age 65+), compared to 70% in adults younger than 65 years [10,11].

These aging-associated changes in the immune system have been referred to as immunosenescence [12] or, perhaps more accurately (because immune function is not only impaired but changed), as SIR [13,14]. SIR impacts on both innate and adaptive immunity. Elderly patients present have altered ratios of $CD4^+$:$CD8^+$ T cells in peripheral blood [15]. This ratio is termed the immune risk profile (IRP), and a higher IRP has been shown to correlate with clinically defined frailty and disease, but not with healthy aging; centenarian survivors have an IRP similar to younger adults [16]. Age-related alterations in innate immunity are often associated with high levels of inflammation, often referred to as inflammaging. Clinically, inflammaging can be a major contributor to aging-associated disease in non-hematopoietic tissues [17,18].

We review here current understanding of the cellular and molecular mechanisms that underlie SIR. In particular, we examine the contribution of aging of HSCs to SIR, and discuss rejuvenation of aged HSCs as a potential approach towards the amelioration of SIR.

Trends

Thymic involution in SIR does not preclude maintenance of a naïve T cell pool upon aging. This implies that attenuation of SIR and restoration of a young naïve T cell pool do not require attenuation of thymic aging.

Large epidemiological studies on the oldest adults demonstrate a strong correlation between markers of inflammaging and frailty, but not for example between chronic CMV infection and frailty, indicating a crucial role for inflammaging in the clinical manifestations of SIR.

SIR is already initiated by aging of HSCs, the stem cells that form most immune cells. Aged HSCs display, among others, enhanced differentiation into myeloid cells at the cost of differentiation into functional lymphoid cells.

Aging of HSCs is reversible, thus rejuvenation of HSCs to attenuate or even revert SIR is a novel therapeutic option for further investigation.

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Senescent Immune Remodeling: Cellular Mechanisms

A hallmark of aging-associated SIR is a reduction in the number of naïve T cells [19] and overall changes in the numbers of lymphocyte populations (remodeling), including a reduction in the number of both helper/inducer ($CD4^+$) and suppressor/cytotoxic ($CD8^+$), as well as $CD19^+$ B cells [19]. An inversion of the ratio of $CD4^+$ to $CD8^+$ T cells in peripheral blood [15] is observed in some elderly individuals, as well as an increase of activated T cells ($CD3^+$ $HLA\text{-}DR^+$) and T lymphocytes expressing natural killer cell (NK) markers [20]. Aging-associated changes in T and B cells and potential clinical implications of these are summarized in Table 1.

Aging-associated changes in the naïve T cell population – both in terms of total numbers and antigen receptor repertoire – has been thought to be primarily a consequence of thymic involution, but recent studies have questioned this notion (reviewed in [21]). The amount of T cell receptor excision circles (TREC) in peripheral blood, a surrogate measurement of thymic function, was found to exponentially decline with age [22]. However, mathematical models have suggested that a decrease in thymic production cannot solely account for the reduction in TREC with age [23]. In line with these findings, Braber *et al.* could show that, in humans, the naïve T population is largely maintained via cell division of naïve T cells in the periphery upon aging [24]. High initial diversity in the T cell pool in young animals, in combination with little impact of thymic output on the peripheral naïve T cell pool [25], support a model in which proliferation of the T cell pool in humans in the periphery is crucial, but only starts to contribute to this peripheral pool in older adults [26]. This proliferation in the periphery is probably driven by the age-constant levels of interleukin (IL)-7, tonic T cell receptor (TCR) signals, and other hemostatic cytokines [26]. In addition, recent studies based on TCR sequencing and TREC analyses indicate that the extent of the contraction of the TCR repertoire upon aging might have been overestimated [27]. Finally, the peripheral repertoire in the elderly is also affected by selection of highly-reactive clones in the periphery. For example, highly-responsive naïve $CD8^+$ cells in the periphery of older adults might competitively override any new selected naïve cells emigrating from the thymus, irrespective of its functional decline [28], which will further contribute to the reduced repertoire in older adults. Thus, the impact of thymic involution on the maintenance of a diverse and reactive naïve T cell pool is not fully clear, and neither is the extent to which naïve T cell numbers and repertoire diversity are diminished with aging. A more precise understanding of the contribution of thymic involution to the peripheral T cell pool in healthy adults will be a prerequisite to evaluating its contribution to SIR. In examining the contribution of thymic involution to the peripheral T cell

Table 1. Age-Related Cellular Changes in the Human Adaptive Immune System

Compartment	Age-Associated Changes	Clinical Implications (Selected)	Refs
T cells	Decrease in naïve T cells narrowing the T cell receptor repertoire TRECs in peripheral blood decline throughout adulthood Imbalance between Th1 and Th2 responses Increased sensitivity to CD95-mediated apoptosis Decreased ratio of $CD8^+$ $CD28^+/CD28^-$ T cells, T cell replicative senescence? Increase in Tregs with age	Less responsive to immune stimulation/infection and vaccination Predisposition to cancer Reactivation of chronic infections	[22,31,38, 41,82–84]
B cells	No change in the total number of B cells Changes in B cell generation and repertoire Decline in antibody diversity Reduced number of specific antibodies, increased number of non-specific antibodies B cell clonal expansions, appearance of monoclonal antibodies	Autoimmunity Impaired responses to infection, cancer cells, and vaccination Potential for late-life B cell lymphomas	[43–45,83]

repertoire, it will be important to take into account the notion that aging may be associated with increased sensitivity of naïve T cells to apoptosis (reviewed in [29]).

Altered expression of co-stimulatory receptors on $CD8^+$ T cells is another hallmark of an aging immune system, notably the reduced expression of CD28 [30]. Elderly individuals have higher numbers of $CD8^+$ $CD28^-$ T cells in peripheral blood, and it is thought that these cells are senescent, given the roles of CD28 in proliferation and protection from apoptosis. However, $CD8^+$ $CD28^-$ cells from older adults proliferate upon mitogen treatment *in vitro* [31]. The functional capabilities of these cells *in vivo*, and the impact of their increased frequency in the immune competence of the elderly, remain unclear.

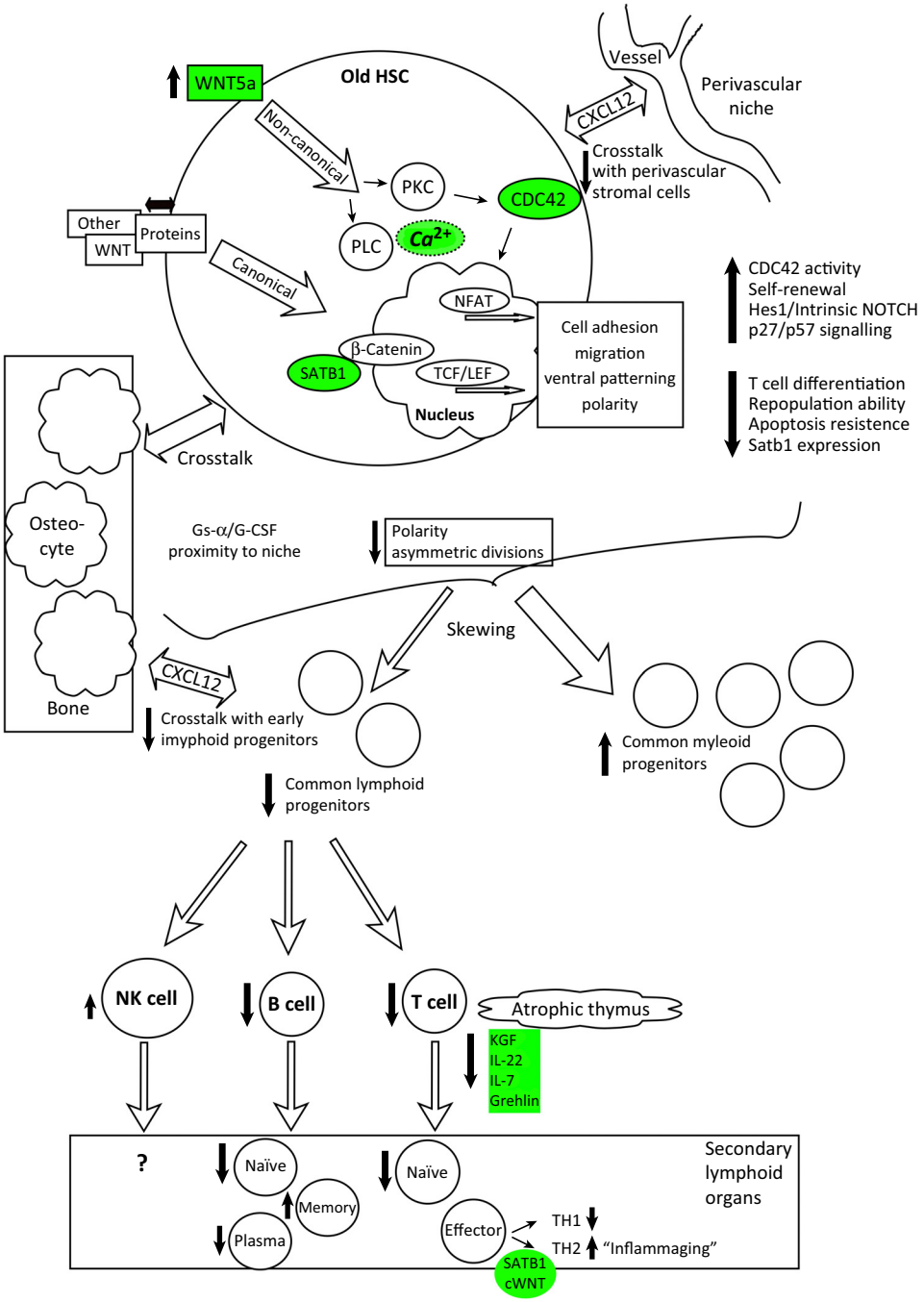
Cytomegalovirus (CMV) infection is considered an environmental contribution to SIR because latent CMV infection in the very elderly (86–94 years of age) has been associated with a non-favorable IRP [32], and the frequency of CMV-specific CD8 T cells is highest in older adults [33]. It has been speculated that the filling of the 'immunological space' with CMV-specific T cells may constrict the T cell repertoire and strongly impact on the memory compartment, and that these effects may be relevant to SIR [34]. In support of this notion, latent CMV infection in mice indeed resulted in pronounced changes in the T cell compartment consistent with impaired naïve T cell function [35]; however, these studies were not performed in aged mice. The peripheral naïve T cell population in humans not infected with CMV exhibited a higher number of naïve T cells and a lower CD4/CD8 ratio [36]. However, CMV seropositivity and pathophysiology associated with SIR, such as inflammation, are not consistently linked [37], and CMV seropositivity does not always result in reduced immune competence in older adults [36]. Thus, the impact of CMV infection on SIR remains to be further defined.

With regards to the regulatory T cell (Treg) compartment, multiple studies provide evidence for the accumulation of $FOXP3^+$ (forkhead box P3) Treg cells with age ([38]). However, the functional relevance of this increased representation in peripheral blood to immune competence in the elderly and its impact on SIR are not clear. Recent epidemiological studies reported a positive correlation between long-term (8 year) survival of the very elderly and a higher frequency of $CCR4^+$ Treg cells [38]. A recently described population of Treg cells [$CD25^+$ $CCR7^+$ $CD62L^+$ CTLA-4^+ $FOXP3^+$) that exhibits high proliferative potential *in vitro* decreased with age, in contrast to the general accumulation of Treg cells with aging [39]. Whether this population of Treg cells exerts a significant impact on features of SIR, such as altered $CD4^+$:$CD8^+$ ratios and increased inflammation, remains unclear.

Aging also results in alterations in the balance between type 1 T helper cell (Th1) and Th2 immune responses, as determine by measurement of cytokine profiles of peripheral blood mononuclear cells (PBMCs), with the very elderly presenting a skewing towards a Th2 profile, although both

Table 2. Aging-Related Changes of HSCs that Are Likely To Influence SIR

Feature	Change in Function
Myeloid/lymphoid potential of HSCs	Aged stem cells are prone to produce more myeloid cells in transplantation assays, and neglect differentiation into lymphoid progenitor cells. The question is still whether individual stem cells are prone to this or whether the increase in the number of myeloid-biased stem cells upon aging is responsible for the shift [53,54,85]
Stem cell self-renewal potential	Both aged murine and human HSCs display reduced serial transplantation ability compared to young HSCs, implying reduced stem cell self-renewal potential [86]
Polarity	Aged murine HSCs present with apolarity [87]. Interestingly, polarity was recently also described as a hallmark of functional memory T cells [88]



Trends in Immunology

Figure 1. Senescent Immune Remodeling (SIR) Starts with Aging of HSCs. WNT signaling in old hematopoietic stem cells (HSCs) switches from a canonical β-catenin-dependent pathway to a non-canonical pathway associated mainly to WNT5a [69]. This again increases the activity of the small RhoGTPase CDC42 and results in an increase in Ca^{2+} levels (or induction of JNK pathways) to regulate transcription factors such as NFAT. There seems to be substantial WNT/NOTCH crosstalk resulting in upregulated intrinsic NOTCH signaling and less polarity. Apolarity might be linked to the mode of the cell division and thus fate of the daughter cells. These changes in polarity could also be influenced by downregulation of the global chromatin regulator SAT1B with aging [89] and by changes of cytokines in the niche (i.e., CXCL12) [90]. Potential modification targets that have been identified so far are CDC42 inhibitor (CASIN) [54], SAT1B [89], WNT pathways [67,69,91], and calcium signaling (e.g., via physical activity [91]). With respect to the later stages of T cell development,

interferon (IFN)-γ and IL-4 were expressed at higher amounts in $CD4^+$ and $CD8^+$ T cells of aged as compared to young individuals after *in vitro* stimulation [40,41]. Interestingly, with respect to possible clinical interventions, there seems to be a correlation between the magnitude of this shift and zinc deficiency. Zinc deficiency is common in the elderly and is known to result in decreased production of Th1 cytokines, and zinc supplementation might thus alleviate some of the underlying shift towards Th1 responses upon aging [42].

Aging appears to result in a reduced diversity of naïve B cells (IgD^+ $CD27^-$), but no significant changes in the number of peripheral B cells, suggesting that the impact of aging on the B cell compartment may be primarily qualitative (reviewed in [43]). Spectratyping analyses reveal a constriction of the B cell repertoire with aging, which correlates with a narrowing of the spectrum of antibody responses and correlated with frailty and susceptibility to infection [44]. A correlation between Epstein–Barr virus (EBV) seropositivity and B cell clonal expansion in the very elderly (80 years and older) has also been reported, although this is not linked to persistent CMV infection [45]. Most humoral immune responses require cognate T cell help and, as noted above, SIR is associated with alterations in the $CD4^+$ compartment; however, how SIR-associated alterations in $CD4^+$ cells relate to changes in B cell responses in the elderly has not been directly examined.

Inflammaging: A Systemic Issue

Older adults frequently present with a systemic chronic low-grade inflammation that has been termed 'inflammaging' [46]. Inflammaging is characterized by increased levels of proinflammatory cytokines [IL-1, IL-6, IL-8, tumor necrosis factor (TNF)-α and C-reactive protein (CRP)] and is associated with an increased risk of morbidity, mortality, sarcopenia, and frailty. Epidemiological studies have provided the strongest evidence for inflammaging, notably the Newcastle study that examined a large cohort (n = 845) of adults aged 85 years or more; interestingly, this study revealed no correlation between frailty and CMV serum-positivity or IRP [47–49]. Proinflammatory cytokines associated with inflammaging are thought to be involved in the pathophysiology of cardiovascular and neurodegenerative diseases [50]. However, the cellular sources of these cytokines are not known. The alterations in lymphocyte compartments associated with SIR may play a role, but this has not been demonstrated. Senescent fibroblasts have been shown to produce inflammatory cytokines in some contexts, and approaches towards reducing the senescence associated secretory phenotype (SASP) of these cells, such as manipulation of the NLRP3 (NLR family, pyrin domain containing 3) inflammasome-dependent proinflammatory cascade [51] and inducible deletion of $p16^+$ (cyclin-dependent kinase inhibitor 2A/CDKN2A) senescent cells [52], impact on systemic inflammation associated with aging in mice. How the production of inflammatory cytokines by senescent cells relates to SIR and alterations in lymphocyte compartments remains to be examined.

HSC Aging: The Beginning of SIR?

In the young, hematopoietic stem cells (HSCs) provide a balanced output of myeloid and lymphoid progenitor cells, which in turn give rise to the innate and adaptive immune compartments. Aging in both humans and mice results in a shift from lymphoid to myeloid differentiation, with a bias of aged HSC towards differentiation into common myeloid progenitor cells (CMPs)

particular cytokines such as IL-7, IL-12, IL-15, and IL-22, as well as growth factors such as KGF (important for the stability of the thymic microenvironment), have been identified as potential targets for restoring T cell function, immunity, and response to vaccination [14]. Notably, in these stages SAT1B also seems to be crucial for Th2-type T cell development in a (canonical) WNT-dependent manner [92]. Potential novel and currently used targets for attenuation of SIR are marked in green. Abbreviations: CDC42, cell division cycle 42; CXCL12, chemokine CXC motif ligand 12; IL, interleukin; JNK, c-Jun N-terminal kinase/mitogen-activated protein kinase 8 (MAPK8); KGF, keratinocyte growth factor/fibroblast growth factor 7 (FGF7); LEF, lymphoid enhancer-binding factor 1; NFAT, nuclear factor of activated T cells, cytoplasmic, calcineurin-dependent; SAT1B, spermidine/spermine *N1*-acetyltransferase 1B; TCF1, transcription factor 1; Th2, type 2 T helper cell; WNT, wingless/int.

and a concomitant reduction in the frequency of common lymphoid progenitor cells (CLPs); this ultimately results in decreased B and T cell lymphopoiesis upon aging [53,54] (Table 2 and Figure 1). Clonal expansion of individual HSCs within the HSC pool in the bone marrow as an individual ages has also been reported [55], and it is tempting to speculate that the clonality that arises in mature immune effector cells might be at least in part a consequence of the clonality seen in the HSC compartment in aging. It is currently unclear whether the aging-associated myeloid bias of hematopoiesis is a consequence of such a clonal shift, or whether aging directly impacts on the differentiation potential of HSCs themselves [53]. Further downstream, impaired differentiation in the lymphoid lineage upon aging is linked to impaired IL-7 stimulation of CLPs owing to lower levels of both IL-7, reduced expression on IL-7 receptor (IL7-R) on T cell progenitors [56] and reduced expression of differentiation regulators such as NOTCH 1 and GATA3 (GATA binding protein 3) in HPSCs [57]. Aging is associated with reduced differentiation of CLPs into the B cell lineage and reduced expression of the B lineage-specifying factors (early B cell factor, EBF; and paired box 5, Pax5); transduction of CLP from older mice with a constitutively active form of STAT5 (signal transducer and activator of transcription 5) restored both EBF and PAX5 expression and increased B cell potential [58].

Aging has also been shown to impact on T cell function. The expression of miR-181a in naïve $CD4^+$ T cells in older adults is reduced as compared to younger individuals, and this reduction is associated with decreased TCR sensitivity. miR-181a regulates the expression of DUSP6 (dual specificity phosphatase 6), a cytoplasmic phosphatase that targets phospho-ERK (extracellular signal-regulated kinase) downstream from the TCR, and increasing the expression of miR181a in $CD4^+$ T cells from older adults restored TCR signaling [59]. miRNAs exert multiple regulatory roles in HSC self-renewal and differentiation [60], and thus it will be of interest to determine whether aging-associated changes in miRNAs contribute to SIR.

HSCs express cell surface receptors associated with inflammation such as Toll-like receptors (TLRs) and purinergic receptors [61]. Mice treated with low doses of lipopolysaccharide (LPS) exhibited myeloid skewing and impaired serial transplantation, suggesting that this treatment resulted in premature HSC aging [62]. The functional significance of this is not clear; it could be speculated that, upon pathogen challenge, the rapid production of myeloid cells may present an advantage to the organism. Cytokines associated with inflammaging such as TNFα, IFN-α, IFN-γ, and IL-6 have been shown to reduce HSC self-renewal potential and result in myeloid skewing [63,64]. For example, repeated treatment of mice with poly-IC, which induces expression of IFN-α and IFN-γ in the bone marrow, resulted in HSC exhaustion and bone marrow failure [65]. Recently, a contribution of cytokines associated with cellular senescence (SASP) to the aging of HSCs has been reported [66]. Thus, SIR may result from a cycle that involves inflammation and HSC aging, but the relative contributions of each of these factors and the mechanisms involved remain to be determined.

There is some insight into molecular pathways that may have relevance to aging HSCs and their subsequent impact on SIR. Age-associated changes in WNT (wingless/int) signaling appear directly related to T cell lineage differentiation. Human HSCs exhibit reduced levels of β-catenin upon 'aging' in *in vitro* cultures, and this correlated with impaired or delayed differentiation of an early T progenitor cell subset [67]. The NOTCH pathway, a potential negative regulator of stem cell aging [68], is modulated by non-canonical Wnt signaling; aged murine HSCs express high amounts of NOTCH1 and the NOTCH target gene *Hes1* (Hes family BHLH transcription factor 1), and this NOTCH signature can be induced by expression of WNT5a in young HSCs [69]. The WNT and NOTCH pathways may also play a role in aging-associated bias towards NK cell differentiation in both humans and mice [70]. The small RhoGTPase CDC42 (cell division control 42) has been identified as a key regulator downstream of the non-canonical Wnt pathway. CDC42 activity increases in the bone marrow and other tissues with age, and this increase has

been causally linked to HSC polarity, differentiation, engraftment, and aging [54]. The elevated activity of CDC42 in aged HSCs seems to be a direct consequence of increased stem cell-intrinsic expression of WNT5A and, thereby, a shift from canonical to non-canonical Wnt signaling [69]. The mechanism by which WNT5A expression in aged HSCs is induced remains largely unknown, but possibly involves epigenetic modifications [71].

Taken together, the available evidence supports a model wherein SIR starts at the level of aging of HSCs (Figure 1), and aging of HSCs contributes to multiple aspects of the clinical presentation of SIR.

Concluding Remarks

What can be done to overcome at least some of the problems of aging or disease-associated immune remodeling to improve therapeutic shortcomings such as vaccination failure or failure to clear systemic infections (see Outstanding Questions)? SIR correlates with multiple changes in the immune system, ranging from aging of stem cells to changes in the number and function of multiple types of effector and regulatory cells. So far, clear mechanistic and thus causal relationships in SIR are difficult to pinpoint. The identification of cellular and molecular mechanisms is a prerequisite for developing successful targeted therapies to attenuate SIR. Approaches that are proven to ameliorate SIR in the clinic therefore are currently primarily behavioral approaches such as exercise (Box 1 and [72]). Moderate exercise (5 days per week for 6 months) improved CD28 expression on T cells and improved the Th1/Th2 balance [73]. Regular exercise in older adults was also correlated with an improved Th1/Th2 cytokine balance, reduced levels of proinflammatory cytokines, changes in the naïve/memory cell ratio, and increased antibody titers upon influenza vaccination [74]. Interestingly, some nutritional interventions in the clinic have been reported to correlate with better overall immunity and improved Th1/Th2 balance in older adults, such as zinc, probiotics, and vitamin D (Table 2). However, how such interventions target the cellular or molecular mechanisms of SIR are unknown.

Cytokines crucial for thymic development {IL-7 [75], KGF (keratinocyte growth factor/fibroblast growth factor 7 FGF7) [76], IL-22 [77], and ghrelin [78]} have been already tested in mice for attenuating the loss of function of the thymus upon involution. Some of the factors (IL-7, KGF) demonstrated good success in attenuating at least in part the loss of thymic function with aging (summarized in [79]). Currently, however, no human trials are ongoing to test these factors for efficacy in preventing thymic loss of function with aging. This might at least in part also reflect the fact that it is still not fully clear whether thymic involution in humans makes a central contribution to SIR, as discussed earlier.

Box 1. Interventions Aimed at Attenuating Diseases Associated with SIR

Clear Evidence

Physical Activity/Exercise: can have strong positive effects on overall immunity. Activity was shown to reduce inflammation and prevent senescent cell accumulation in older adults [93].

Preliminary Evidence

Zinc: decreased inflammaging markers [94] and better T cell development, reducing the shift from Th2 to Th1 and improving vaccination response [95].

Vitamin D: associated with better overall immunity [96].

Probiotics: recent studies with particular strains of lactobacilli reveal less inflammation and more NK and immature T cells in older adults [97].

Future Therapies

Novel cellular and pharmaceutical interventions rejuvenating hematopoietic stem and progenitor cells via pharmacological compounds that target MTOR (mechanistic target of rapamycin) or CDC42 activity [54] or via a periodic diet that mimics fasting [98,99], which might be combined with IL-7 and IL-7-related therapies [75] to enhance thymic output.

Outstanding Questions

Is the SIR-associated reduced responsiveness to immune stimulation, infection, and vaccination a direct consequence of reduced numbers of naïve T and B cells? Or do higher levels of Tregs as well as changes in Th1/Th2 ratios also directly contribute to SIR? If these hallmarks of SIR are set to young levels in, for example, adoptive transfer experiments in aged mice, will they significantly improve SIR in these mice?

To what extent does thymic involution with aging counteract attempts to attenuate SIR? The role of thymic involution for SIR has been recently critically discussed. Does a rejuvenated immune system also function in an aged environment without thymic support? Can, for example, functional naïve T cells be generated from young T cell precursors when transplanted into aged (athymic) recipients?

To what extent will the hallmarks of SIR be reproduced in mice xenotransplanted with aged human HSCs? Do such mice also display reduced responsiveness to immune stimulation and vaccination, and will they thus serve as a valid model system to test additional approaches to attenuate SIR in humans?

Do available pharmacological approaches that rejuvenate hematopoietic stem cells (HSCs), such as treatment with CASIN or rapamycin, also result in functionally 'young' immune systems driven by these HSCs with respect to immune stimulation and vaccination? If yes, will such a younger system also successfully respond to infections and suppress autoimmunity in experimental model systems?

Because SIR is already initiated by aging of HSCs, rejuvenating HSCs to ultimately increase the number of functionally young and thus relevant adaptive effector cells from the B and, probably more importantly, T cell lineages might be a novel approach to attenuate SIR. Whether intervention to increase the production of younger lymphocyte progenitors, and later of T cells, would result in sufficient naïve cells that are functional for stimulating T cell-dependent responses to antigens in the absence of a fully functional thymus in older adults will need to be evaluated in detail. To date, pharmacologic interventions that target intrinsic mechanisms of HSC aging are limited to the use of rapamycin [80] and the CDC42 activity inhibitor CASIN [54] while, more recently, temporary fasting in mice was shown to attenuate aging of HSCs *in vivo* [81]. For CASIN, selective CDC42 inhibition restored not only HSC polarity but also their lymphopoietic potential, suggesting that this could also be a promising approach to attenuate SIR. CASIN also reverted levels and patterns of histone H4 acetylation from old HSCs to patterns that resembled young HSCs [54], which implies epigenetics as a novel driving force in stem cell aging, and suggests also that specific histone methylation or acetylation patterns might someday represent therapeutic targets in SIR. Because SIR is an important factor in the elevated morbidity and mortality of older adults, as well as frailty in the clinic, there is continued need to better understand the molecular mechanisms of aging of the immune system. Novel animal model systems, such as mice humanized with respect to hematopoiesis and the immune system, might further support the translation of novel findings into the clinic to promote healthy aging.

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References

1. WHO (2015) *World Report on Ageing and Health,* World Health Organization
2. West, L.A. *et al.* (2014) *65+ in the United States: 2010,* United States Census Bureau
3. Lucado, J. *et al.* (2012) *Clostridium difficile Infections (CDI) in Hospital Stays, 2009 Statistical Brief 124,* Agency for Healthcare Research and Quality
4. Loo, V.G. *et al.* (2005) A predominantly clonal multi-institutional outbreak of *Clostridium difficile*-associated diarrhea with high morbidity and mortality. *N. Engl. J. Med.* 353, 2442–2449
5. Jain, S. *et al.* (2015) Community-acquired pneumonia requiring hospitalization among u.s. adults. *N. Engl. J. Med.* 373, 415–427
6. Vergis, E.N. *et al.* (2001) Pneumonia in long-term care: a prospective case-control study of risk factors and impact on survival. *Arch. Intern. Med.* 161, 2378–2381
7. Simonsen, L. *et al.* (2005) Impact of influenza vaccination on seasonal mortality in the US elderly population. *Arch. Intern. Med.* 165, 265–272
8. Nguyen, A.M. and Noymer, A. (2013) Influenza mortality in the United States, 2009 pandemic: burden, timing and age distribution. *PLoS ONE* 8, e64198
9. Skowronski, D.M. *et al.* (2011) Immuno-epidemiologic correlates of pandemic H1N1 surveillance observations: higher antibody and lower cell-mediated immune responses with advanced age. *J. Infect. Dis.* 203, 158–167
10. Hottes, T.S. *et al.* (2011) Influenza vaccine effectiveness in the elderly based on administrative databases: change in immunization habit as a marker for bias. *PLoS ONE* 6, e22618
11. Darvishian, M. *et al.* (2014) After adjusting for bias in meta-analysis seasonal influenza vaccine remains effective in community-dwelling elderly. *J. Clin. Epidemiol.* 67, 734–744
12. Makinodan, T. (1998) Studies on the influence of age on immune response to understand the biology of immunosenescence. *Exp. Gerontol.* 33, 27–38
13. Vallejo, A.N. (2007) Immune remodeling: lessons from repertoire alterations during chronological aging and in immune-mediated disease. *Trends Mol. Med.* 13, 94–102
14. Lang, P.O. *et al.* (2013) Reversing T cell immunosenescence: why, who, and how. *Age* 35, 609–620
15. Ferguson, F.G. *et al.* (1995) Immune parameters in a longitudinal study of a very old population of Swedish people: a comparison between survivors and nonsurvivors. *J. Gerontol. A Biol. Sci. Med. Sci.* 50, B378–B382
16. Strindhall, J. *et al.* (2007) No Immune Risk Profile among individuals who reach 100 years of age: findings from the Swedish NONA immune longitudinal study. *Exp. Gerontol.* 42, 753–761
17. Franceschi, C. *et al.* (2000) Inflamm-aging. An evolutionary perspective on immunosenescence. *Ann. N. Y. Acad. Sci.* 908, 244–254
18. Hayashi, M. *et al.* (2015) Whole-body in vivo monitoring of inflammatory diseases exploiting human interleukin 6-luciferase transgenic mice. *Mol. Cell Biol.* 35, 3590–3601
19. Sansoni, P. *et al.* (1993) Lymphocyte subsets and natural killer cell activity in healthy old people and centenarians. *Blood* 82, 2767–2773
20. Ginaldi, L. *et al.* (2000) Immunophenotypical changes of T lymphocytes in the elderly. *Gerontology* 46, 242–248
21. Ventevogel, M.S. and Sempowski, G.D. (2013) Thymic rejuvenation and aging. *Curr. Opin. Immunol.* 25, 516–522
22. Douek, D.C. *et al.* (1998) Changes in thymic function with age and during the treatment of HIV infection. *Nature* 396, 690–695
23. Dutilh, B.E. and de Boer, R.J. (2003) Decline in excision circles requires homeostatic renewal or homeostatic death of naive T cells. *J. Theor. Biol.* 224, 351–358
24. den Braber, I. *et al.* (2012) Maintenance of peripheral naive T cells is sustained by thymus output in mice but not humans. *Immunity* 36, 288–297
25. Westera, L. *et al.* (2015) Lymphocyte maintenance during healthy aging requires no substantial alterations in cellular turnover. *Aging Cell* 14, 219–227

26. Sauce, D. *et al.* (2012) Lymphopenia-driven homeostatic regulation of naive T cells in elderly and thymectomized young adults. *J. Immunol.* 189, 5541–5548
27. Britanova, O.V. *et al.* (2014) Age-related decrease in TCR repertoire diversity measured with deep and normalized sequence profiling. *J. Immunol.* 192, 2689–2698
28. Johnson, P.L. *et al.* (2014) A population biological approach to understanding the maintenance and loss of the T-cell repertoire during aging. *Immunology* 142, 167–175
29. Sikora, E. (2015) Activation-induced and damage-induced cell death in aging human T cells. *Mech. Ageing Dev.* 2,Published online April 2015. http://dx.doi.org/10.1016/j.mad.2015.03.011
30. Merino, J. *et al.* (1998) Progressive decrease of CD8high$^+$ CD28$^+$ CD57$^-$ cells with ageing. *Clin. Exp. Immunol.* 112, 48–51
31. Brzezinska, A. *et al.* (2004) Proliferation and apoptosis of human CD8$^+$CD28$^+$ and CD8$^+$CD28$^-$ lymphocytes during aging. *Exp. Gerontol.* 39, 539–544
32. Wikby, A. *et al.* (2002) Expansions of peripheral blood CD8 T-lymphocyte subpopulations and an association with cytomegalovirus seropositivity in the elderly: the Swedish NONA immune study. *Exp. Gerontol.* 37, 445–453
33. Vescovini, R. *et al.* (2010) Intense antiextracellular adaptive immune response to human cytomegalovirus in very old subjects with impaired health and cognitive and functional status. *J. Immunol.* 184, 3242–3249
34. Pawelec, G. *et al.* (2005) Human immunosenescence: is it infectious? *Immunol. Rev.* 205, 257–268
35. Cicin-Sain, L. *et al.* (2012) Cytomegalovirus infection impairs immune responses and accentuates T-cell pool changes observed in mice with aging. *PLoS Pathog* 8, e1002849
36. Sansoni, P. *et al.* (2014) New advances in CMV and immunosenescence. *Exp. Gerontol.* 55, 54–62
37. Bartlett, D.B. *et al.* (2012) The age-related increase in low-grade systemic inflammation (inflammaging) is not driven by cytomegalovirus infection. *Aging Cell* 11, 912–915
38. Derhovanessian, E. *et al.* (2015) CCR4$^+$ regulatory T cells accumulate in the very elderly and correlate with superior 8-year survival. *J. Gerontol. A Biol. Sci. Med. Sci.* 70, 917–923
39. Valmori, D. *et al.* (2005) A peripheral circulating compartment of natural naive CD4 Tregs. *J. Clin. Invest.* 115, 1953–1962
40. Sakata-Kaneko, S. *et al.* (2000) Altered Th1/Th2 commitment in human CD4$^+$ T cells with ageing. *Clin. Exp. Immunol.* 120, 267–273
41. Sandmand, M. *et al.* (2002) Is ageing associated with a shift in the balance between type 1 and type 2 cytokines in humans? *Clin. Exp. Immunol.* 127, 107–114
42. Uciechowski, P. *et al.* (2008) TH1 and TH2 cell polarization increases with aging and is modulated by zinc supplementation. *Exp. Gerontol.* 43, 493–498
43. Goronzy, J.J. and Weyand, C.M. (2013) Understanding immunosenescence to improve responses to vaccines. *Nat. Immunol.* 14, 428–436
44. Gibson, K.L. *et al.* (2009) B-cell diversity decreases in old age and is correlated with poor health status. *Aging Cell* 8, 18–25
45. Wang, C. *et al.* (2014) Effects of aging, cytomegalovirus infection, and EBV infection on human B cell repertoires. *J. Immunol.* 192, 603–611
46. Franceschi, C. and Campisi, J. (2014) Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. *J. Gerontol. A Biol. Sci. Med. Sci.* 69 (Suppl. 1), S4–S9
47. Michaud, M. *et al.* (2013) Proinflammatory cytokines, aging, and age-related diseases. *J. Am. Med. Dir. Assoc.* 14, 877–882
48. Collerton, J. *et al.* (2012) Frailty and the role of inflammation, immunosenescence and cellular ageing in the very old: cross-sectional findings from the Newcastle 85$^+$ Study. *Mech. Ageing Dev.* 133, 456–466
49. Wikby, A. *et al.* (2006) The immune risk phenotype is associated with IL-6 in the terminal decline stage: findings from the Swedish NONA immune longitudinal study of very late life functioning. *Mech. Ageing Dev.* 127, 695–704
50. Giunta, B. *et al.* (2008) Inflammaging as a prodrome to Alzheimer's disease. *J. Neuroinflammation* 5, 51
51. Youm, Y.H. *et al.* (2013) Canonical Nlrp3 inflammasome links systemic low-grade inflammation to functional decline in aging. *Cell Metab.* 18, 519–532
52. Baker, D.J. *et al.* (2011) Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders. *Nature* 479, 232–236
53. Dykstra, B. *et al.* (2011) Clonal analysis reveals multiple functional defects of aged murine hematopoietic stem cells. *J. Exp. Med.* 208, 2691–2703
54. Florian, M.C. *et al.* (2012) Cdc42 activity regulates hematopoietic stem cell aging and rejuvenation. *Cell Stem Cell* 10, 520–530
55. McKerrell, T. *et al.* (2015) Leukemia-associated somatic mutations drive distinct patterns of age-related clonal hemopoiesis. *Cell Reports* 10, 1239–1245
56. Kim, H.R. *et al.* (2006) Altered IL-7Ralpha expression with aging and the potential implications of IL-7 therapy on CD8$^+$ T-cell immune responses. *Blood* 107, 2855–2862
57. Gruver, A.L. *et al.* (2007) Immunosenescence of ageing. *J. Pathol.* 211, 144–156
58. Lescale, C. *et al.* (2010) Reduced EBF expression underlies loss of B-cell potential of hematopoietic progenitors with age. *Aging Cell* 9, 410–419
59. Li, G. *et al.* (2012) Decline in miR-181a expression with age impairs T cell receptor sensitivity by increasing DUSP6 activity. *Nat. Med.* 18, 1518–1524
60. Bissels, U. *et al.* (2012) MicroRNAs are shaping the hematopoietic landscape. *Haematologica* 97, 160–167
61. Rossi, L. *et al.* (2012) The sixth sense: hematopoietic stem cells detect danger through purinergic signaling. *Blood* 120, 2365–2375
62. Esplin, B.L. *et al.* (2011) Chronic exposure to a TLR ligand injures hematopoietic stem cells. *J. Immunol.* 186, 5367–5375
63. Baldridge, M.T. *et al.* (2011) Inflammatory signals regulate hematopoietic stem cells. *Trends Immunol.* 32, 57–65
64. Pronk, C.J. *et al.* (2011) Tumor necrosis factor restricts hematopoietic stem cell activity in mice: involvement of two distinct receptors. *J. Exp. Med.* 208, 1563–1570
65. Walter, D. *et al.* (2015) Exit from dormancy provokes DNA-damage-induced attrition in haematopoietic stem cells. *Nature* 520, 549–552
66. Shao, L. *et al.* (2014) Total body irradiation causes long-term mouse BM injury via induction of HSC premature senescence in an Ink4a- and Arf-independent manner. *Blood* 123, 3105–3115
67. Khoo, M.L. *et al.* (2014) Gene profiling reveals association between altered Wnt signaling and loss of T-cell potential with age in human hematopoietic stem cells. *Aging Cell* 13, 744–754
68. Cairney, C.J. *et al.* (2009) A systems biology approach to Down syndrome: identification of Notch/Wnt dysregulation in a model of stem cells aging. *Biochim. Biophys. Acta* 1792, 353–363
69. Florian, M.C. *et al.* (2013) A canonical to non-canonical Wnt signalling switch in haematopoietic stem-cell ageing. *Nature* 503, 392–396
70. Aoyama, K. *et al.* (2007) The interaction of the Wnt and Notch pathways modulates natural killer versus T cell differentiation. *Stem Cells* 25, 2488–2497
71. Li, J. *et al.* (2010) WNT5A antagonizes WNT/beta-catenin signaling and is frequently silenced by promoter CpG methylation in esophageal squamous cell carcinoma. *Cancer Biol. Ther.* 10, 617–624
72. Muller, L. and Pawelec, G. (2014) Aging and immunity – impact of behavioral intervention. *Brain Behav. Immun.* 39, 8–22
73. Shimizu, K. *et al.* (2008) Effect of moderate exercise training on T-helper cell subpopulations in elderly people. *Exerc. Immunol. Rev.* 14, 24–37
74. Kohut, M.L. and Senchina, D.S. (2004) Reversing age-associated immunosenescence via exercise. *Exerc. Immunol. Rev.* 10, 6–41
75. Phillips, J.A. *et al.* (2004) IL-7 gene therapy in aging restores early thymopoiesis without reversing involution. *J. Immunol.* 173, 4867–4874
76. Min, D. *et al.* (2007) Sustained thymopoiesis and improvement in functional immunity induced by exogenous KGF administration in murine models of aging. *Blood* 109, 2529–2537

77. Dudakov, J.A. *et al.* (2012) Interleukin-22 drives endogenous thymic regeneration in mice. *Science* 336, 91–95
78. Dixit, V.D. *et al.* (2007) Ghrelin promotes thymopoiesis during aging. *J. Clin. Invest.* 117, 2778–2790
79. Lynch, H.E. *et al.* (2009) Thymic involution and immune reconstitution. *Trends Immunol.* 30, 366–373
80. Chen, C. *et al.* (2009) mTOR regulation and therapeutic rejuvenation of aging hematopoietic stem cells. *Sci. Signal.* 2, ra75
81. Villeda, S.A. *et al.* (2014) Young blood reverses age-related impairments in cognitive function and synaptic plasticity in mice. *Nat. Med.* 20, 659–663
82. Gupta, S. and Gollapudi, S. (2008) CD95-mediated apoptosis in naive, central and effector memory subsets of $CD4^+$ and $CD8^+$ T cells in aged humans. *Exp. Gerontol.* 43, 266–274
83. Desai, A. *et al.* (2010) Leukocyte function in the aging immune system. *J. Leukoc. Biol.* 87, 1001–1009
84. Lages, C.S. *et al.* (2008) Functional regulatory T cells accumulate in aged hosts and promote chronic infectious disease reactivation. *J. Immunol.* 181, 1835–1848
85. Rossi, D.J. *et al.* (2005) Cell intrinsic alterations underlie hematopoietic stem cell aging. *Proc. Natl. Acad. Sci. U.S.A.* 102, 9194–9199
86. Kamminga, L.M. *et al.* (2005) Impaired hematopoietic stem cell functioning after serial transplantation and during normal aging. *Stem Cells* 23, 82–92
87. Xing, Z. *et al.* (2006) Increased hematopoietic stem cell mobilization in aged mice. *Blood* 108, 2190–2197
88. Ciocca, M.L. *et al.* (2012) Cutting edge: asymmetric memory T cell division in response to rechallenge. *J. Immunol.* 188, 4145–4148
89. Satoh, Y. *et al.* (2013) The Satb1 protein directs hematopoietic stem cell differentiation toward lymphoid lineages. *Immunity* 38, 1105–1115
90. Tzeng, Y.S. *et al.* (2011) Loss of Cxcl12/Sdf-1 in adult mice decreases the quiescent state of hematopoietic stem/progenitor cells and alters the pattern of hematopoietic regeneration after myelosuppression. *Blood* 117, 429–439
91. Bayod, S. *et al.* (2014) Wnt pathway regulation by long-term moderate exercise in rat hippocampus. *Brain Res.* 1543, 38–48
92. Notani, D. *et al.* (2010) Global regulator SATB1 recruits beta-catenin and regulates T_H2 differentiation in Wnt-dependent manner. *PLoS Biol.* 8, e1000296
93. Senchina, D.S. and Kohut, M.L. (2007) Immunological outcomes of exercise in older adults. *Clin. Interv. aging* 2, 3–16
94. Costarelli, L. *et al.* (2010) Distinctive modulation of inflammatory and metabolic parameters in relation to zinc nutritional status in adult overweight/obese subjects. *J. Nutr. Biochem.* 21, 432–437
95. Putics, A. *et al.* (2008) Zinc supplementation boosts the stress response in the elderly: Hsp70 status is linked to zinc availability in peripheral lymphocytes. *Exp. Gerontol.* 43, 452–461
96. Leow, L. *et al.* (2011) Vitamin D, innate immunity and outcomes in community acquired pneumonia. *Respirology* 16, 611–616
97. Mane, J. *et al.* (2011) A mixture of *Lactobacillus plantarum* CECT 7315 and CECT 7316 enhances systemic immunity in elderly subjects. A dose-response, double-blind, placebo-controlled, randomized pilot trial. *Nutr. Hosp.* 26, 228–235
98. Cheng, C.W. *et al.* (2014) Prolonged fasting reduces IGF-1/PKA to promote hematopoietic-stem-cell-based regeneration and reverse immunosuppression. *Cell Stem cell* 14, 810–823
99. Brandhorst, S. *et al.* (2015) A periodic diet that mimics fasting promotes multi-system regeneration, enhanced cognitive performance, and healthspan. *Cell Metab.* 22, 86–99