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HSC aging and senescent immune remodeling

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Abstract

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 that 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 of age) [7, 8]. Contributing to higher mortality rates in

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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 to approximately 50% of the elderly population (age 65+), compared to 70% in adults less than 65 years of age [[10, 11].

These aging-associated changes in the immune system have been referred to as immunosenescence [12], or perhaps more accurately as immune function is not only impaired by changed, as senescent immune remodeling (SIR) ([13, 14]). SIR impacts 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].

Here we review the current understanding of the cellular and molecular mechanisms that underlie SIR. In particular we examine the contribution of aging of hematopoietic stem cells (HSCs) to SIR, and discuss rejuvenation of aged HSCs as a potential approach towards the amelioration of SIR.

Senescent immune remodeling: cellular mechanisms

A hallmark of aging-associated SIR is a reduction in the number of naïve T-cells [19] and an 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-DR+) and T lymphocytes expressing 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]. A 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 critical, but does only start to contribute to this peripheral pool in older adults [26]. This proliferation in the periphery is probably driven by the age-constant levels of IL-7, tonic 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 T-cell receptor repertoire upon aging might have been overestimated [27]. Finally, the peripheral repertoire in the elderly is also impacted 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 the 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 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 for 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 infection is considered an environmental contribution to SIR, as latent CMV infection in the very elderly (86–94 years old) 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 the memory compartment, and that these effects may be relevant to SIR [34]. In support of this notion, latent CMV infection in mice resulted indeed 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 to SIR remains to be further defined.

With regards to the regulatory T cell (Treg) compartment, multiple studies provide evidence for the accumulation of FoxP3⁺ 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 with the general accumulation of Treg cells with aging

[39]. Whether this population of Treg cells exerts 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 Th1 and Th2 immune responses, as determined by measurement of cytokine profiles of peripheral blood mononuclear cells (PBMCs), with the very elderly presenting a skewing towards a Th2 profile, although both IFN- γ and IL-4 was 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 alteration 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 pro-inflammatory cytokines (IL-1, IL-6, IL-8, TNF-alpha 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 85+ adults; interestingly this study revealed no correlation between frailty and CMV serum-positivity or IRP [47, 48] [49]. Pro-inflammatory 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 inflammasome-dependent proinflammatory cascade [51] and inducible deletion of p16⁺ senescent cells [52] impact systemic inflammation associated with aging in mice. How 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) 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 to the clonality seen in the HSCs 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 the differentiation potential HSCs themselves [53]. Further downstream, impaired differentiation in the lymphoid lineage upon aging is linked to impaired IL-7-stimulation of CLPs due to lower levels of both IL7, reduced expression on IL7-R on T-cell progenitors [56] and reduced expression of differentiation regulators such as Notch-1 and GATA-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 Pax5); transduction of CLP from older mice with a constitutively active form of STAT5 restored both EBF and Pax5 expression and increased B-cell potential [58].

Aging has also been shown to impact 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, a cytoplasmic phosphatase that targets phosphor-ERK 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 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 TNF α , 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 polyIC, 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) in the aging of HSCs have been reported [66]. Thus, SIR may result from a cycle that involves inflammation and HSC aging, but the relative contribution 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 signaling appear directly related to T-cell lineage differentiation. Human HSCs exhibit reduced levels of beta-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, 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 has been identified as a critical 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, 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 ageing or disease-associated immune remodeling in order to improve therapeutic shortcomings such as vaccination failure or failure to clear systemic infections? SIR correlates with multiple changes in the immune system, ranging from aging of stem cells to changes in 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 though 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 a 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 level of pro-inflammatory cytokines, and a changes in naïve/memory cell ratio and increased antibody titers upon influenza vaccination [74]. Interestingly, there are also nutritional interventions reported from the clinic that have been shown to correlate with better overall immunity and improved Th1/Th2 balance in older adults such zinc, probiotics or vitamin D (see infobox 2). How such interventions target cellular or molecular mechanisms of SIR are though unknown.

Cytokines critical for thymic development (IL-7[75], KGF[76], IL-22 [77], 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 attenuation at least in part loss of thymic function upon aging (summarized in [79]). Currently though,

there are no human trials running to test them for loss of function of the thymus upon aging. This might at least in part also owe to the fact that it is still not fully clear whether in humans thymic involution contributes to a critical extent to SIR, as discussed before.

As SIR is already initiated by aging of HSCs, rejuvenating HSCs to ultimately increase the number of functionally young and thus relevant adaptive effectors cells from the B- and, probably more important – T-cell lineage, might be a novel approach to attenuate SIR. Whether though an intervention to increase the production of younger lymphocyte progenitors and later of T-cells would result in enough naïve cells that are functional for stimulating T-cell dependent responses to antigens without a fully functional thymus in the older adults would need to be determined 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 has restored not only HSC polarity but also their lymphopoietic potential, thus rendering that a likely approach that might also 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 that consequently also specific histone methylation or acetylation patterns might someday represent therapeutic targets in SIR. As 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 molecular mechanisms of aging of the immune system. Novel animal model systems like mice humanized with respect to hematopoiesis and the immune system might further support the translation of novel findings into the clinic to allow for healthy aging.

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Box 1**Interventions aimed attenuating diseases associated with SIR****Clear evidence**

- *Physical activity/exercise* can have strong positive effects on overall immunity. It was shown to reduce inflammation and prevent senescent cell accumulation in older adults [89]

Preliminary evidence

- *Zinc*: less inflammaging markers [90] and better T-cell development reducing the shift from Th2 to Th1 and improving vaccination response [91]
- *Vitamin D*: associated with better overall immunity [92]
- *Probiotics*: recent studies with certain strains of lactobacilli reveal less inflammation and more NK and immature T-cells in older adults [93]

Future therapies

- Novel cellular and pharmaceutical interventions rejuvenating hematopoietic stem and progenitor cells via pharmacological compounds that target mTOR or Cdc42 activity [54] or via a periodic diet that mimics fasting [94, 95], which might be combined with IL7 and IL7-related therapies [75] to enhance thymic output.

Outstanding Questions Box

1. 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?
2. To what extent does thymic involution upon aging counteracts 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 out of young T-cell precursors when transplanted into aged (athymic) recipients?
3. To which extent will hallmarks of SIR also present in mice xenotransplanted with aged human HSCs? Do such mice also present with reduced responsiveness to immune stimulation and vaccination, and will they thus serve as valid model system to test for additional approaches to attenuate SIR in humans?
4. Do available pharmacological approaches that rejuvenate hematopoietic stem cells (HSCs), such as treatment with CASIN or rapamycin, also result in a 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?

Trends Box

- Thymic involution in senescent immune remodeling (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 does not require attenuating of aging of the thymus.
- 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 very critical role of inflammaging for the clinical manifestations of SIR.
- SIR is already initiated by aging of the hematopoietic stem cell (HSC), the stem cell that forms most immune cells. Aged HSCs present, among others, with enhanced differentiation into myeloid 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.

Figure 1. Senescent immune remodeling starts with aging of HSCs

Wnt signaling in old HSCs switches from a canonical β -catenin-dependent pathway to a non-canonical β -catenin-independent pathway associated mainly to Wnt5a [69]. This again increases the activity of the small RhoGTPase CDC42 and results in an increase of Ca^{2+} (or induction of JNK pathways) to regulate transcription factors such as NFAT. There seems to be a 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 the down-regulation of the global

chromatin regulator *Satb1* upon aging [96] and changes of cytokines in the niche (i.e. CXCL12) [97]. Potential modification targets that have been identified so far are CDC42 inhibitor (CASIN)[54], *Sat1b* [96], Wnt pathways [67, 69, 98] or Calcium signaling (i.e. via for example physical activity [98]). With respect to later stages in T-cell development, certain cytokines such as IL-7, IL-12, IL-15 or IL-22 and growth factors such as KGF (important for the stability of the thymic microenvironment) have been introduced 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 [99]. Potential novel and currently used targets for attenuation of SIR are marked in green.

Table 1

Age-related changes in the human adaptive immune system on a cell-level

Compartment	Age-associated changes	Clinical implications (selected)	Ref.
T-cells	- decrease in naive T cells narrowing the T cell receptor repertoire	- less responsive to immune stimulation / infection and vaccination	[22, 31, 38, 41, 82–84]
	- TRECs in peripheral blood decline throughout adulthood	- predisposition to cancer	
	- imbalance between Th1 and Th2 response	- reactivation of chronic infections	
	- increased sensitivity to CD95-mediated apoptosis		
	- decreased ratio of CD8 ⁺ CD28 ⁺ /CD28 ⁻ T cells, T cell replicative senescence?		
	- increase in Tregs with age		
B-cells	- no change in the total number of B cells	- autoimmunity	[43–45, 83]
	- changes in B cell generation and repertoire	- impaired responses to infection, cancer cells and vaccination	
	- decline in antibody diversity	- potential for late-life b-cell lymphomas	
	- reduced number of specific antibodies, increased number of non-specific antibodies		
	- B cell clonal expansions, appearance in monoclonal antibodies		

Table 2

Aging related changes of hematopoietic stem cells 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 in 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 [54] [53, 85]
Stem cell self-renewal potential	Both aged murine and human HSCs possess reduced serial transplantation abilities 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 for functional memory T-cells [88]