Hematopoietic stem cell function in rheumatoid arthritis

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Abnormal innate and adaptive immune responses are critically involved in the pathogenesis of rheumatoid arthritis (RA). Anti-inflammatory and immunosuppressive therapies have been highly successful, but a cure for the disease has remained elusive, mostly due to lack of knowledge of disease instigators and the causative immune system abnormalities. RA is associated with premature aging of the immune system, which has been attributed to the chronic inflammatory milieu. However, recent data draw attention to processes of impaired immune regeneration as an underlying defect. Specifically, bone marrow hematopoietic stem cells (HSCs), permanently regenerating myeloid and lymphoid lineages, are functionally defective in RA, jeopardizing repopulation of the immune system. In RA, the pool of circulating HSCs is contracted and HSCs respond inadequately to hematopoietic growth factors. Most importantly, RA HSCs have age-inappropriate telomeric shortening, indicative of excessive proliferative stress. Defects in HSCs broaden the immunopathogenesis of RA to include early events in the shaping of the immune system. Restoring HSC function will be a necessary step in re-establishing immune health in RA patients.

Review
Future Rheumatology

All cells of the immune system ultimately derive from the bone marrow (BM), and the challenge of maintaining a functional immune system over a lifetime of many decades is critically determined by BM function. Blood cell production is a complex and tightly regulated process that depends upon a small number of hematopoietic stem cells (HSCs) that expand and differentiate in an ordered fashion; as they lose lineage potential, they give rise to the entire repertoire of mature hematopoietic cells. Mammals, including humans, possess 2×10^4 stem cells, and the number of HSC replications (self-renewal capacity) per animal lifetime is relatively conserved and constant across species [1]. Similar to other somatic cells, HSCs are not immortal, but undergo a finite number of replications (less than 100 times in humans [2]) before they enter cellular senescence, a status in which they are still alive but can no longer replicate [3,4]. Considering that only a small fraction of HSCs are cycling at any given time, and that approximately 4×10^{11} blood cells are produced each day (calculation based on the adult blood volume, the number of each of the blood cell types per µl of blood, and their circulatory half-life), a massive amplification process and a delicate balance between apoptosis, self-renewal and differentiation are needed to maintain homeostasis [5].

Whether the immune system is functional or dysfunctional, it depends upon constant input of precursor cells, which then enter a differentiation program. The size, diversity and turnover of the immune repertoire are ultimately regulated through the loss of differentiated cells and influx of novel cells that regenerate the pool. In humans, peripheral proliferation of mature lymphocytes contributes to immune homeostasis, even in the newborn [6]. However, loss of thymic T-cell production, likely a combination of declining stem-cell availability and deterioration of thymic epithelial function, profoundly affects the ability to avoid lymphopenia and sustain repertoire diversity [7].

Two major observations have given rise to the concept that early steps in immune homeostasis may be abnormal in rheumatoid arthritis (RA). First, hematologic manifestations, involving all three major blood cell lineages, are frequently encountered in RA patients [8]. In fact, anemia of chronic disease is the most common RA extra-articular manifestation, affecting up to 25% of patients during the first year of disease [9]. Leukopenia and thrombocytopenia in RA most frequently result from drug toxicity or the intercurrence of infectious processes. However, RA and systemic lupus erythematosus (SLE) are the systemic autoimmune diseases most often associated with secondary autoimmune neutropenias. Felty syndrome and large granular lymphocyte syndrome, disorders with a multifactorial pathogenesis involving humoral-mediated as well as cellular-mediated mechanisms, are specifically associated with RA [10]. Second, patients with RA have premature immunosenescence, essentially resulting in the accumulation of dysfunctional

Keywords
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T cells with a low threshold for activation, age-inappropriate telomeric shortening and restricted clonal expansion capacity. The accelerated aging of the RA immune system brings into question the intactness of immune regeneration [11–16]. This review will focus on whether HSCs in RA patients are competent, how functional competence of that cellular niche can be defined and how abnormalities in HSC function could relate to the pathogenesis of RA.

Defining 'competent' hematopoietic stem cells

In the mid-twentieth century, Metcalf and Moore, and Till and McCullough introduced the notion that multipotent cells present in BM are responsible for blood cell production [17–19]. Subsequently, the recognition that the cell surface transmembrane glycoprotein CD34 is expressed by immature hematopoietic progenitors, the validation that these cells are lineage-negative and the development of *in vivo* assays that allowed for their functional evaluation were key issues in the process of identifying human HSCs [20].

Cells with different repopulation and self-renewal potentials compose the HSC pool [21,22]. The capacity to durably regenerate hematopoiesis in a lethally irradiated animal remains the gold standard for the field. CD34 has emerged as an important marker in enriching HSC populations for clinical use. For hematopoietic stem cell transplantation (HSCT) in an autologous as well as allogeneic setting, and regardless of the source of the HSC (umbilical cord blood [UCB], BM or peripheral blood [PB]) [23], immunophenotypic isolation and characterization of HSCs is based on the expression of CD34 antigen. In fact, enumeration of cells that express CD34 on their surface by flow cytometry is the most frequent method to determine stem cell dose in apheresis and to evaluate PB stem cell engraftment. However, CD34 selection renders a heterogeneous population, and true HSCs most likely represent a subgroup of these cells [22]. Therefore, efforts to define better markers for HSCs (i.e., $CD133^+$ $CD34^+$ cells) are ongoing [24,25]. Moreover, $CD34^-$ cells were also recently shown to be competent for hematopoietic reconstitution with long-term engraftment potential in humans [26]. The precise analysis of the human $CD34^-$ population (which in mice includes the earliest stem cells) has been hindered by the lack of a positive marker and a simple and reliable assay system for these rare cells [27]. $CD34^-$ cells upregulate CD34 antigen expression as they proliferate into committed progenitors [28]. The gain and loss of CD34 raises the still unanswered question about the functional significance of the CD34 protein. There is evidence supporting the concept that CD34 negatively regulates cell proliferation [29]. Although CD34 silencing does not interfere with the proliferative capacity of HSCs, it enhances granulocyte and megakaryocyte differentiation at the expense of the erythroid lineage [29]. Also, CD34 has signal transducing capacity, and through the interaction with L-selectin can regulate the balance between adhesion/anti-adhesion [30].

Functionally, HSCs are characterized at the single cell level by their dual capacity of self-renewal and multilineage differentiation. Through self-renewal, HSCs can maintain their pool, ensuring the integrity of the compartment throughout life, while their multipotency property allows for the generation of mature differentiated blood cell lineages. Hematopoiesis is a hierarchically organized process in which multipotent HSCs give rise to progeny (oligo-potent and lineage-restricted progenitor cells) that progressively lose self-renewal potential while they become more restricted in their differentiation capacity [31,32]. Multiple players, amongst them cytokines, growth factors, transcription factors and cell-cycle regulators, are involved in the modulation and control of stem cell fate (self-renewal vs differentiation) [33].

'Incompetent' hematopoietic stem cells in RA

Diminished HSC reserve in RA

Guided by the clinical observation that hematopoiesis may be suppressed in RA patients, Papadaki and colleagues have tested whether the BM stem cell compartment and/or the microenvironment are affected by the RA inflammatory process [34]. The authors evaluated patients with severe, active RA in whom they found low HSC frequency, accelerated Fas-mediated apoptosis of $CD34^+$ cells, defective clonogenic potential of BM stem cells and impaired hematopoiesis-supporting capacity of the BM stroma. They implicated TNF-α production by inflammatory cells in the BM microenvironment in mediating the apoptotic depletion of patient stem cells since *in vitro* anti-TNF treatment restored stromal cell function. Furthermore, they showed a 'pseudo-normalization' of the RA BM findings after initiation of anti-TNF therapy. In fact, anti-TNF restored the number of $CD34^+$ cells, the percentage of Fas^+ cells within the CD34 cell compartment and the hematopoiesis-supporting capacity of patient stroma [35].

We have recently reported that the circulating HSC frequencies in RA patients are significantly reduced compared with demographically matched controls [36]. Whereas the size of the circulating CD34 pool in healthy controls is strictly age-dependent, frequencies of $CD34^+$ cells in RA patients are depressed even in those who are young. The HSC frequencies of 30- versus 70-year-old RA patients are similar and comparable with those of 70-year-old controls (Figure 1). Previously, another group showed that the CD34 yields in PB stem cell harvests from RA patients with severe disease were lower than those in controls [37]. We explored the possibility that loss of circulating HSC is a direct consequence of chronic inflammation and reasoned that increasing disease duration or disease activity should predict lower levels of $CD34^+$ cells. However, that is not the case; CD34 frequencies are similar in patients with early and late RA and are equally depressed in those with active and inactive disease. The fact that even after correcting for age, disease duration is not a predictor of low HSC counts in RA raises the still unanswered question of whether the accelerated loss of HSCs starts during the preclinical phase of the disease [38], or even precedes it.

Another consideration is the potential 'deleterious' effect of DMARDs, in particular methotrexate, on HSC function. In two different reports [34,36], HSCs from RA patients studied prior to exposure to cytotoxic agents (patients naïve to DMARDs) had the same defects as treated patients. This finding can be related to studies showing that the mechanism by which folate analogs exert their hematological toxicity is through the depletion of relatively mature, nonclonogenic precursor cells, and not by killing stem cells [39,40]. Taken together, current evidence suggests that drug-induced damage is not the major factor affecting hematopoiesis in patients with RA.

Depletion of circulating HSCs in RA patients could result from multiple factors, including a reduced BM reserve [34,41], increased HSC attrition [35,41] and a 'pseudo-reduction' as a result of relocation of HSCs to peripheral tissues [42]. Regardless of the cause, considering that disease duration, activity and severity are not predictors of low HSC numbers in RA patients [36], the loss of circulating HSCs seems to result from an intrinsic defect in this cell population.

In order to self-renew or differentiate, HSCs need to replicate; therefore, assessing the ability of $CD34^+$ cells to proliferate should provide critical information about their functional integrity. In RA, not only is the proliferative capacity of HSCs impaired (reflected in the decreased number of cell cycles that RA HSCs achieve after hematopoietin expansion) (Figure 1), but also a significant percentage of HSCs (10–15%) could not be driven into proliferation, and a delay in the lineage-committed cell differentiation following hematopoietin stimulation is observed [36]. This is in agreement with previous studies showing that the proliferative activity of BM myeloid progenitors is decreased in RA patients [41]. Together, all of these findings support the concept that a critical HSC function, the ability to replicate, is no longer intact in RA.

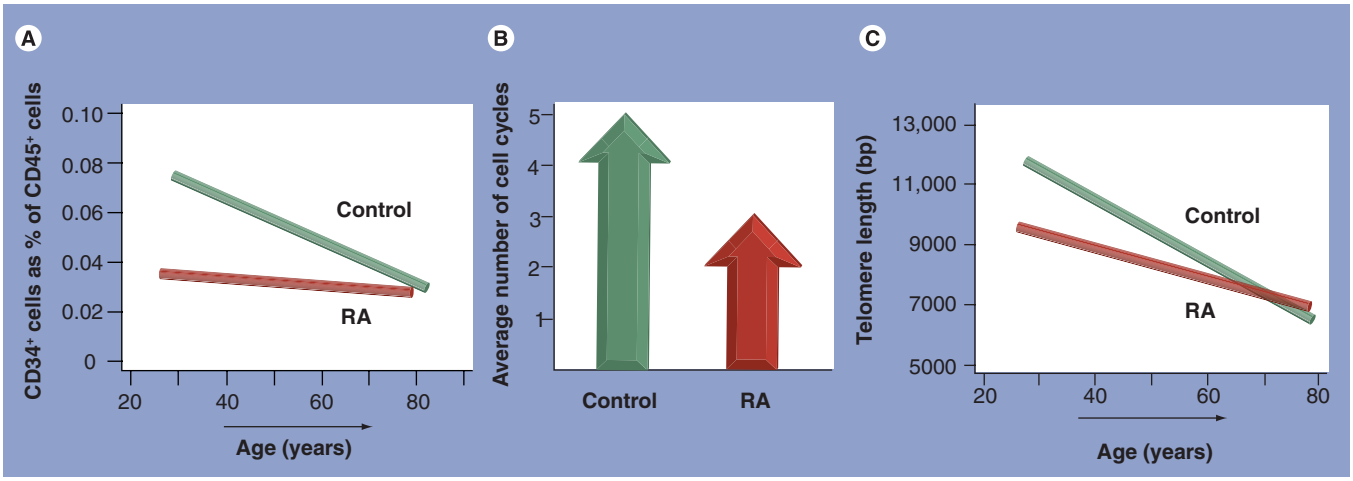

Figure 1. Defects in HSCs in rheumatoid arthritis. (A) Reduction in pool size, **(B)** Impaired proliferative capacity, **(C)** Premature telomere attrition. In RA patients, the frequencies of HSCs are age-inappropriately reduced compared with demographically matched controls. HSC proliferation when driven with hematopoietins is impaired in RA. The lengths of telomeres, a surrogate of the proliferative history of a cell, show premature shortening in RA $CD34^+$ cells.
HSC: Hematopoietic stem cell; RA: Rheumatoid arthritis.

Accelerated telomere loss in rheumatoid HSC

Telomeric shortening is one of the best-known cell-intrinsic events associated with aging. Telomeres are guanine-rich tandem DNA repeats located at the ends of eukaryotic chromosomes that prevent the chromosome ends from being recognized as a DNA break. In humans, the telomere length is in the range of 2–15 kb. Since conventional DNA polymerases are not able to completely replicate the 3′ end of linear duplex DNA (end-replication problem) [4], 30–100 base pairs of telomere repeats are lost with every cycle of cell division [43].

HSCs have a finite replicative capacity, and telomeres shorten during HSC replicative aging; therefore, telomere length is a useful surrogate of the proliferative history of these cells [44–46]. Recently, it has been shown that the expression levels of marker proteins secreted from telomere-dysfunctional BM cells of late-generation telomerase knockout mice increase with age in contrast to aged mice with long telomere reserves [47]. Similar biomarkers were found to be elevated in the blood of patients with myelodysplastic syndrome and cirrhosis, chronic diseases associated with increased rate of cell turnover and telomere attrition.

The immune system strongly depends on clonal expansion and cell division, and to homeostatically accomplish these purposes has evolved mechanisms of telomere maintenance. Among these, specifically in HSCs, telomerase appears to be the major salvage pathway. In fact, higher telomerase activity and shorter telomeres can be detected in the $CD34^+CD38^+$ cell fraction when compared with the $CD34^+CD38^{-/low}$ fraction, which contains more primitive nonproliferating cells with longer telomeres. However, elevated levels of telomerase activity alone are unable to prevent proliferation-associated telomere shortening in HSCs [48,49]. This is illustrated *in vitro* by human telomerase reverse transcriptase (hTERT) overexpression studies in HSCs, which result in a significant upregulation of telomerase activity that nevertheless cannot prevent overall telomere shortening, nor increase the replicative capacity of these cells [49,50].

Telomeres with critically short length behave as double-stranded DNA breaks, therefore activating DNA damage responses [43,51]. Accrual of DNA damage, together with age-related changes in epigenetic regulation, particularly with a decline in the expression of genes involved in chromatin regulation and DNA repair, are suspected to underlie the age-dependent HSC functional decline [46,52–54]. In fact, telomere attrition through DNA damage can signal cell-cycle arrest, cellular senescence, apoptosis or genome instability, leading to impairment of HSC self renewal and proliferative capacity, and, ultimately, to tissue failure [2]. In mice, telomere shortening contributes to the replicative exhaustion of HSCs evident upon serial BM transplantation [55]. In humans, the connection between replicative capacity and telomere dynamics has been recently studied by Pipes and colleagues [56], who showed that cord blood HSCs with longer telomeres have a replicative advantage in comparison with PB HSCs during allogeneic stem cell transplantation.

The purpose of telomere-dependent cell growth arrest is to act as a developmental barrier, therefore suppressing cancer *in vivo* [51]. However, in cells that must sustain proliferation over a lifetime, such as HSCs, the consequences of telomeric shortening and DNA damage can result in a diminished capacity to maintain homeostasis, and can be horizontally propagated to other HSCs (through self-renewal) and vertically conveyed to downstream progenitors [57]. Therefore, short telomeres of T and B cells could reflect the progressive telomere shortening in HSCs and/or be acquired during the process of differentiation or activation of mature lymphocyte populations [58].

In RA patients, premature telomeric shortening affects $CD4^+$ and $CD8^+$ T cells. Notably, age-inappropriate loss of telomeres is most pronounced in the naïve compartment, thus affecting T cells that have not yet been involved in the chronic inflammatory process [59]. Further evidence for this abnormality to precede instead of follow RA comes from data demonstrating that normal, healthy individuals who share the HLA-DR4 haplotype, the major genetic risk factor for RA, are similarly affected by telomere attrition [13]. Mechanistic insights as to the stage of hematopoietic development at which telomeres are prematurely lost in RA patients have derived from experiments examining the myeloid lineage. In RA, not only T cells have shortened telomeres; telomeric sequences are similarly shortened in granulocytes [12,13]. Granulocytes are a lineage that undergoes few constant divisions during differentiation from HSCs, and they do not divide as mature cells [12,13]. In contrast, telomeric lengths of sperm cells from $HLA\text{-}DR4^+$ individuals do not differ from those of DR4-negative ones [13]. Taken together, inappropriate telomeric erosion in RA patients and in healthy $DR4^+$ donors involves multiple blood lineages, but not gametes.

Recent studies have confirmed that the 'premature' telomeric loss can be traced back to HSCs, thus affecting the hematopoietic system at a very early stage. RA CD34$^+$ cell telomeres are 1600 bp shorter than those of age-matched controls (Figure 1), and this feature is independent of disease activity [36]. Determining whether telomere attrition in RA is a primary event or alternatively results from a compensatory cell turnover in response to an increased demand, similar to the accelerated telomere shortening that occurs in recipients of HSC transplants, is elusive. In any case, aged HSCs could ultimately lead to the accumulation of prematurely aged T cells that are phenotypically and functionally altered and prone to autoreactivity (Figure 2) [11,60]. Interestingly, a recent study in patients with juvenile idiopathic arthritis (JIA) found that abnormalities in T-cell homeostasis were evident very early in the disease process, and did not progress over the course of disease, suggesting a primary defect in T-cell generation and/or maturation in the pathogenesis of JIA [61].

Potential mechanisms of accelerated HSC aging in RA

Hematopoietic stress can accelerate the aging of the hematopoietic system, and therefore impair its ability to maintain homeostasis [62]. In the case of RA, the inflammatory milieu would be a prime candidate. However, it is incompletely understood through which molecular pathways inflammatory cytokines can regulate cellular turnover. Pro-inflammatory cytokines could enhance proliferation of mature lymphocytes, thus prematurely exhausting their proliferative reserve. Alternatively, cytokines could directly influence the threshold setting of apoptosis, leading to enhanced cell loss and the need for compensatory proliferation.

The BM stroma consists of a heterogeneous population of cells that provide the structural and physiological support for hematopoietic cells; therefore, a dysfunctional stroma could result in HSC defects. The BM stromal cell hematopoiesis-supporting capacity in patients with severe, active RA was assessed by Papadaki and colleagues. In this study, the authors show that RA stromal cells produce abnormally high amounts of TNF-α, which reduces their capacity to support the growth of autologous or allogeneic normal HSC. These defects can be reversed by anti-TNF therapy [34]. It is possible that early senescence can affect stromal cells, altering their pattern of gene expression, upregulating inflammatory cytokines and affecting the behavior of

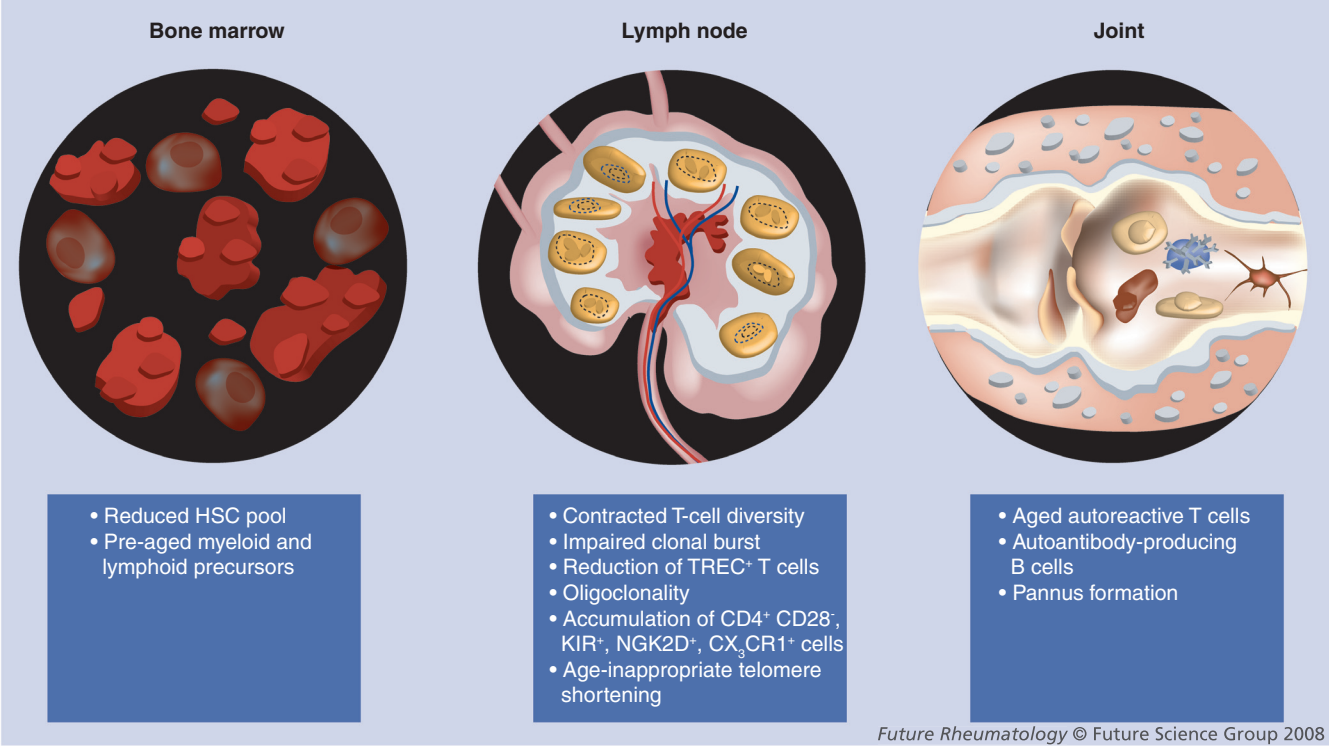

Figure 2. Abnormal T-cell homeostasis in rheumatoid arthritis. In RA, multiple stages of T-cell generation and regeneration are defective. Bone marrow stem cells are prematurely aged. The peripheral T-cell pool is remodeled, even for unprimed T cells, favoring autoreactivity. Senescent T cells accumulate in the joint, where they maintain chronic inflammation.
RA: Rheumatoid arthritis.

neighboring HSCs. This idea has found interest in the cancer field [63], but still needs to be tested in the context of RA.

Another mechanism through which the microenvironment regulates cell survival and longevity is the integrity of the DNA. Accrual of genomic damage is a proposed mechanism that mediates age-related HSC impairment. Cells under proliferative stress, for example HSCs, are equipped with powerful DNA damage recognition and repair machineries. The concept that DNA damage limits stress-induced hematopoiesis by diminishing the ability of HSCs to proliferate and self-renew has been tested in DNA repair-deficient mice ($Lig4Y^{288C}$, $Csb^{m/m}$, Xpd^{TTD} and $Xpa^{-/-}$) not exposed to exogenous genotoxic stress. In these models, there is an age-dependent accumulation of spontaneous or endogenous DNA damage in quiescent cells, suggesting that intracellular products (such as reactive oxygen species) that are able to induce DNA damage would be responsible for the damage in nonproliferating cells [57,62].

Recently, we have addressed the question of whether HSCs from RA patients show signs of advanced aging by accumulating damaged DNA. Freshly isolated HSCs from RA patients show a significantly higher degree of DNA damage than matched controls [64]. Again, the question arises as to whether this is a consequence of amplified hematopoietic stress or a primary HSC defect.

Similarities between RA & bone marrow failure syndromes

The concept that HSCs, like other somatic cells, are ultimately not immortal but are subject to telomeric loss and subsequent failure of their proliferative capacity, is not unique to RA [65,66]. Dyskeratosis congenita (DC) is a genetically heterogeneous multisystemic disorder with features of premature aging. Similar to patients with Fanconi anemia, aplastic anemia and paroxysmal nocturnal hemoglobinuria, DC patients have reduced BM hematopoiesis, contracted CD34 frequencies, shortened CD34 telomeric lengths and limited proliferative capacity of the hematopoietic stem cell compartment [65–68].

Telomeric erosion has also been linked to an increased risk for tumorigenesis, especially lymphomagenesis. The proposed mechanism is that damaged or short telomeres can be recognized as DNA double-stranded breaks, activate the DNA-repair machinery and become the targets of nonhomologous end-joining or homology-directed repair, potentially leading to inversions, translocations and terminal deletions [66]. Of note, following autologous HSCT, telomeric length is especially short in those patients who eventually developed post-transplantation myelodysplasia [66]. Genomic instability resulting from dysfunctional telomeres might be a hypothesis to test as a plausible explanation for the higher rate of lymphoproliferative diseases in RA patients [69].

Are HSC defects specific to RA?

Given the central role of hematopoietic processes in shaping the immune system, HSC biology may have an impact in autoimmune syndromes other than RA. The concept that murine SLE could be related to HSC defects was elegantly tested by Ikehara and colleagues in the early 1990s. This group was able to induce lupus nephritis and idiopathic thrombocytopenic purpura by transplanting T-cell-depleted BM cells from lupus-prone mice (NZW x BXSB F1) to normal mice [70]. The authors suggested that the adoptive transfer of the autoimmune disorder may result from the interaction between the genetic makeup of the recipient and the donors' lymphohematopoietic system (reviewed in [71]).

Similarly to RA, patients with SLE, even when the disease is in clinical remission, have decreased levels of circulating $CD34^+$ HSCs and impaired BM reserve compared with healthy controls [72]. The proposed underlying mechanism is an increased Fas-mediated apoptotic propensity of HSC, coupled with a limited capacity for hematopoietic renewal [72,73]. At least in part, the upregulation of Fas on HSCs and their subsequent apoptotic death is induced by interferon-γ and Fas ligand-producing T cells in the BM microenvironment [74]; a mechanism also relevant for patients with aplastic anemia [75]. In fact, in transfer experiments where HSCs from healthy donors were cultured in serum from SLE patients with leucopenia, or with T cells from SLE patients, HSC apoptosis occurred and the HSC colony-forming capacity was limited [76,77].

Finally, monocytes and lymphocytes from SLE patients have significantly shorter telomeres than controls [78], but the telomeric sequences in SLE HSCs have not yet been evaluated.

'Incompetent' HSCs in RA: can we replace them?

If the abnormalities in the immune system of RA patients can be traced back to the very early steps of hematopoiesis, then all of our efforts to reset the dysfunctional immune system in RA

will be superficial and transient, unless we can repair the original defect. The obvious question is whether HSC reconstitution could 'heal' the BM and restore all dependent functions. The success of BM transplantation in cancer patients with concurrent autoimmune disease in improving both conditions has pointed to this direction. Similarly, HSCT has been successfully applied in animal models to treat and/or avoid autoimmunity [23]. However, infections and secondary malignancies, and in particular autoimmunity, following HSCT, are serious side effects to be considered. Autoimmunity occurs during the vigorous phase of homeostatic expansion after conditioning-induced lymphopenia, and is usually organ-specific and not multisystemic. The mechanisms that can cause or mimic post-transplant autoimmunity (homeostatic proliferation, transfer of autoimmunity, autoimmunity associated with conditioning regimes and infections) have been recently reviewed [79,80].

As a general rule, HSC transplantion can only work if the cellular substrate that is transplanted is not itself faulty. The rationale for autologous HSCT is based on the concept that the existing autoimmune response is totally or partially ablated by the preconditioning regime, and that the transfer of HSCs will engraft and regenerate a self-tolerant immune system [81]. Autologous transplant is currently preferred because of its ability to reconstitute the immune system with low toxicity relative to allogeneic HSCT. The experience with autologous HSCT in RA shows that only 4% of patients achieved remission and only 12% of the transplanted patients had sustained response. Up to 92% of the patients required re-institution of DMARDs because of disease relapse [23,82,83]. This clearly indicates that autologous HSCT cannot reintroduce self-tolerance in patients suffering from RA. Rather, it appears that dose-intensive immunosuppression may transiently reset the immune system in a few patients and, in some cases, improve sensitivity to other drugs [84,85]. Since the persistence of autoreactive cells in spite of the conditioning regime could be responsible for the relapses, graft manipulation with further $CD34^+$ cell purification is being explored as an alternative [81]. In a pilot study, T-cell depletion by CD34 selection of the stem cell graft intended to reduce the re-infusion of autoreactive T cells did not provide a more durable or significant response [86]. With the ideal of 'cure the disease', other options, including HSC-based gene therapy to promote antigen-specific tolerance and intensified conditioning or post-transplant immunosuppressive regimes, are currently under investigation [87]. However, the recent recognition of defective HSCs in RA predicts that transplantation of such cells may sustain the problem.

Allogeneic transplantation eradicates the host lympho-hematopoietic system and provides a new donor-derived immune system able to exert a graft-versus-host effect (graft versus autoimmunity [88,89]). Considerations specific for this modality are the high treatment-related mortality and the limited experience in patients with severe autoimmune diseases (the first report of an allogeneic-HSCT performed in an RA patient dates from 2004) [90]. Moreover, RA relapses, occurring even after HLA-identical allogeneic BM transplant with complete chimerism (full donor engraftment), have been reported [91,92]. RA patients with severe therapy-resistant disease, including patients that relapsed after autologous HSCT, might be considered candidates for allogeneic HSCT. However, the current availability of several effective alternative therapies seldom makes it necessary to expose RA patients to the risks of BM transplant [84].

Experience from transplanted RA patients showed that T-cell reconstitution in these patients, particularly the $CD4^+$ subset, is severely impaired, confirming that insufficient repopulation of lymphocytes may be a basic defect in this disease [93]. These limitations can ultimately only be overcome if functionality of HSCs is restored.

Conclusion

The hematopoietic system is under enormous proliferative demand, as millions of cells need to be replaced daily. Normal aging impairs hematopoietic homeostasis with one of the major consequences being the loss of immune competence. RA patients share features of immunosenescence with the normal elderly, including T-cell diversity contraction, oligoclonal proliferation with loss of the CD28 molecule and T cell and granulocyte telomere attrition [7,11,12,16,59,94]. Recent studies in RA HSCs suggest that these BM cells might also be involved in the accelerated aging process. As a result, RA HSCs have shortened telomeres and, more importantly, their proliferative capacity is impaired. This can ultimately lead to their inability to efficiently sustain hematopoiesis and/or regenerate immune cells [36]. Moreover, dysfunctional HSCs through the accumulation of DNA damage can underlie the increased lymphoma risk of RA patients. Ongoing studies are looking at the mechanisms

that lead to HSC functional exhaustion in RA and at interventions that ideally could restore 'HSC youth'.

Future perspective

Current knowledge in the field of HSC aging comes from murine models, which are invaluable in generating hypotheses and for testing concepts but, unfortunately, poorly resemble certain aspects of HSC and telomere biology in humans. Also, the human lifespan extends over almost 10 decades, whereas mice live not much longer than 2 years. Interspecies translational research (mouse to human) and the evaluation of human HSCs in health and disease is the big challenge. Dedicated studies need to provide the tools to overcome technical limitations associated with studying the small populations of BM stem cells. The number of $CD34^+$ cells that can be obtained from humans is limited. As proliferation and differentiation are intimately coupled, HSCs cannot be expanded without losing their intrinsic properties. Finally, the heterogeneity of cell subsets even after selecting for a CD34 surface marker poses a challenge. However, the potential to unravel the biology of HSCs greatly impacts a number of fields in medicine. Regenerative medicine builds upon the understanding of how pluripotent cells develop into sophisticated organ structures. Overcoming the limitations introduced by the aging process needs to include the ability to rebuild the hematopoietic and immune system. The promise of understanding fundamental abnormalities leading to autoimmune disease, such as RA, will drive the field forward. Here, deciphering pathogenic events in an inflammatory joint disease could enormously cross-fertilize the knowledge base in medicine as a whole.

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Executive summary

Hematopoietic stem cell defects in rheumatoid arthritis

- Age-inappropriate contraction of the hematopoietic stem cell (HSC) pool.
- Lack of correlation between clinical parameters of disease activity, severity or DMARD use, and the loss of HSCs.
- Impaired proliferative burst following stimulation with early hematopoietins (reduced number of cell cycles performed after 4 days of expansion, and 10–15% of HSCs are growth factor nonresponsive).
- Delayed lineage-committed cell differentiation.
- Premature telomeric erosion indicative of excessive proliferative stress in the bone marrow HSC pool.

Implications of HSC defects in rheumatoid arthritis pathogenesis

- Restricted generation of mature progeny.
- Insufficient regeneration of lymphocytes, necessitating homeostatic proliferation of peripheral T cells.
- Premature immune senescence with increased frequency of autoreactive immune cells.
- Accumulation of end-differentiated T cells with proinflammatory effector functions in the synovial lesions, as well as the atherosclerotic plaque.

Questions that still need to be answered

- Why do rheumatoid arthritis (RA) patients prematurely lose HSCs?
- What are the mechanisms responsible for the impaired proliferative function of RA HSCs?
- Is the premature aging of RA HSCs associated with lineage differentiation skewing?
- Does the modulation/attenuation of HSC defects impact RA chronicity?

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