Haematopoietic stem and progenitor cells in rheumatoid arthritis

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Abstract

RA is the prototypic chronic inflammatory disease, characterized by progressive articular cartilage and bone destruction. The systemic nature of RA is evidenced by the increased risk of atherosclerosis and lymphoproliferative disorders. Components of both the innate and adaptive immune system are implicated in the pathophysiology of the articular and extra-articular manifestations of the disease. A fundamental process in the onset of RA is the breakdown in self-tolerance. Accelerated ageing of immune cells (immunosenescence) appears to be a major mechanism favouring the disruption of tolerance. Telomere erosion, a hallmark of immunosenescence, is present in lymphoid (naïve and memory T cells) and myeloid (granulocytes) cells in RA. The premature ageing process also involves the haematopoietic stem and progenitor cells (CD34+ HSPC), thus extending the RA immunopathogenesis to include early events in the shaping of the immune system. This review summarizes current concepts of HSPC ageing and its impact on immune regeneration, highlighting the phenotypic and functional similarities between elderly and RA HSPC.

Key words: Rheumatoid arthritis, Haematopoietic progenitor cells, Telomeres, CD34+ cells, Ageing.

Introduction

Ageing is defined as a general loss of biological competence both at the individual cell and the organismal level [1]. The ageing process is driven by the accumulation of molecular damage in cells, resulting in the impairment of function and declining regenerative reserve of tissues and organs. The induction or acceleration of cellular ageing is a common feature in the pathogenesis of diverse chronic conditions, including chronic kidney disease, pulmonary fibrosis, emphysema, chronic hepatitis, diabetes, intervertebral disc degeneration, benign prostatic hyperplasia, chronic allograft rejection and cancer [2, 3].

Several lines of evidence support the association of RA with accelerated ageing. Epidemiological studies show that age is the strongest risk factor for the development of RA, and paralleling the global trends in population ageing, there is both an increase in the incidence and prevalence of RA [4]. It has been proposed that premature ageing (~2 extra years at the time of RA onset) and accelerated ageing (~1.4 years for each decade of actual time after diagnosis) may account for the increased mortality of RA, which is largely attributable to an increased risk of cardiovascular death [5, 6].

Pathogenic studies show that the immune system in RA patients recapitulates abnormalities observed in healthy older subjects. Changes in the frequency and phenotype of peripheral T-cell subpopulations (decrease of naïve T cells, increase in memory T cells, loss of CD28 costimulatory molecule, contraction of the TCR repertoire) [7, 8], the reduced output of TREC+ recent thymic emigrants and the erosion of telomeres (naïve and memory T cells and granulocytes) [9, 10] are hallmarks of the RA immunosenescence fingerprint [11]. These features, together with a variety of biochemical derangements of pathways integral to antigen responsiveness and immune regulation [12–14], are thought to (i) increase susceptibility to foreign pathogens (due to the restriction of the antigen repertoire to which T cells can respond); (ii) augment reactivity to self-tissue antigens, which are themselves modified post-translationally as a consequence of the ageing process; and (iii) generate a repertoire of lymphocytes that are defective in terms of tumour surveillance and more prone to cause tissue injury because of their high cytotoxic activity (perforin/granzyme) and excessive production of inflammatory cytokines (IFN-γ).
Recent evidence suggests that defects in telomere maintenance and faulty DNA repair are mechanisms that account for the premature T-cell ageing in RA, supporting the idea that complex genomic damage can drive autoimmunity [10, 15]. Of interest, age-inappropriate shortening of telomeres is not only limited to T cells, but also involves the myeloid lineage and the haematopoietic progenitor cell compartment of RA patients [9, 16]. The latter suggests a defect in the homeostasis of bone marrow (BM)-derived progenitor cells, which in turn questions the immune-regeneration capacity.

**Ageing impairs the functional capacity of haematopoietic stem and progenitor cells**

Haematopoietic stem cells (HSCs) are the best characterized type of adult stem cells and served as the paradigm for understanding stem cell biology. HSCs reside in BM and generate progenitors that become progressively restricted to several or single lineages. HSCs are currently conceptualized as groups of cells with varying developmental potentials based on intrinsic networks driven by transcription factors and inputs from the cellular niches in which they reside [17–19]. Like all other stem cells, HSCs have two defining properties: (i) they are capable of self-renewal by producing additional stem cells; and (ii) they can differentiate into mature blood cell lineages [20]. HSCs are defined functionally by their capacity to reconstitute the entire blood system of a recipient.

HSC and progenitor cells (HSPCs) in humans are enriched within the subset of CD34+ cells. CD34 is a type 1 transmembrane protein expressed on HSPCs with the capacity to repopulate BM for all lineages. Thus, in clinical practice, the cell subset characterized by CD34+ expression is considered to contain the pluripotent HSC population with the ability of self-renewal [18]. CD34+ HSPC can be isolated from cord blood, BM and peripheral blood (mean frequency in non-mobilized peripheral blood of healthy individuals: 2.16 cells/i.l). In the BM, HSPCs preferentially reside in two micro-environments: in association with osteoblasts near the trabecular bone (the osteoblast niche), and adjacent to blood vessels (the vascular niche) [20]. These niches are thought to represent a nurturing environment providing signals for HSPC survival, proliferation and self-renewal.

HSPCs exist in a relatively quiescent state in the BM micro-environment but can be activated to enter the cell cycle and thus drive haematopoiesis as physiological demands dictate [21]. Imbalances between quiescence and proliferation could lead to impaired haematopoiesis and BM failure in one extreme case or could lead to hyperproliferation with exhaustion of reserves and overproduction of various cell lineages on the other one.

In animals, HSPCs recirculate under physiological conditions. They migrate out of the BM to the blood, trafficking to non-lymphoid extramedullary tissues where they divide/differentiate and replenish tissue-resident leukocytes and finally enter the lymphatic system to return to the blood and eventually the BM [22]. Interestingly, it has been proposed that migration enables HSPC to participate in the sensing of pathogens at infection sites (through Toll-like receptors) and to rapidly produce innate immune-effector cells [23].

During ageing, HSPCs undergo quantitative, phenotypic and functional changes. The relative contributions of the BM micro-environment, cell-intrinsic processes and systemic factors to the ageing process of HSPCs are poorly defined. Animal studies support that each of these factors is involved in HSPC ageing. The existence of cell-intrinsic defects derives from transplantation assays comparing the behaviour of young and aged HSPCs in a single recipient. These studies show that the repopulation capacity of aged HSPCs on a per cell basis declines by a factor of 2–4 compared with young HSPCs and that these defects persist even if the cells are transplanted into young recipients [24–26]. *In vivo* parabiotic models support the contribution of the BM micro-environment and systemic factors to HSPC ageing. These studies suggest that ageing induces an alteration in the ability of niche cells to support HSPC function and that systemic factors from young animals can improve the engraftment and lineage differentiation potential of BM cells from aged mice [27].

In humans, both donor age and recipient age influence patients’ outcomes in response to HSC transplantation, with donor age being the only parameter significantly associated with survival of the transplant recipient [28–30]. The fact that donors’ age >45 years is a strong negative prognostic marker of survival is consistent with a cell-autonomous compromise in the function of HSPCs. These clinical data support the concept that both cell intrinsic and extrinsic mechanisms contribute to the functional decline of HSPCs during human ageing [31].

HSPC age-related changes described in humans include a decrease in BM haematopoietic tissue volume (adipose replacement [32]) with a decline in BM and circulating HSPC numbers [33, 34]. This explains the reduced number of CD34 harvested from peripheral blood in individuals >65 years and, together with an increase in the percentage of HSPC apoptosis, accounts for the increased risk of BM failure and anaemia in the elderly [34]. Aged HSPCs have reduced growth factor sensitivity, reduced ability to support erythropoiesis and T-cell generation capacity (irrespective of thymic involution), and are more prone to generate myeloid cells [31, 35, 36]. It has been proposed that the combination of these factors may increase the risk for inflammatory reactions in the elderly host. Studies on the mechanisms underlying HSPC ageing have been conducted in mice. It is critically relevant to evaluate whether similar factors contribute to human HSPC ageing and to establish the impact and reversibility of age-related defects.

**The aged HSPC phenotype**

Senescence seems to represent one of the several programmes that can be activated by cells when physiological stress is encountered, and serves as a tumour-suppressing mechanism. Senescent cells have reduced
proliferative responses and display a radically altered phenotype that is genetically, morphologically and behaviourally distinct from its growth-competent counterparts [37].Senescent cells are dysfunctional and are thought to contribute to disease development and progression in several ways: (i) by altering the behaviour of neighbouring cells; (ii) by stimulating chronic tissue remodelling and/or local inflammation through the secretion of inflammatory cytokines and tissue remodelling enzymes; and (iii) by reducing the pool of growth-competent mitotic cells, leading to a decreased regenerative reserve of tissues [38].

Haematopoiesis is a high-turnover process with an estimated output of >10¹¹ cells/day [39]. It is sustained by HSPCs that are subject to the effects of ageing [40, 41] opening the possibility that the pool of haematopoietic cells may dramatically change over a lifetime. HSPCs from old mice are less efficient at homing to and engrafting in the BM [42, 43]. In addition, their differentiation potential is biased towards myeloid vs lymphoid lineages [42] (Fig. 1A). Skewing towards myelopoiesis seems to be the result of the down-regulation of genes involved in lymphoid specification [26] and the selective expansion of clonal subtype myeloid-biased HSPCs [19]. The bias towards myeloid cells may favour the generation of a pro-inflammatory environment described as inflammaging [44]. However, the molecular mechanisms underlying such processes have not been defined.

HSPCs from old mice show a differential regulation of genes with increased expression of leukaemia-associated genes and decreased expression of genes contributing to DNA damage repair, genomic integrity and chromatin remodelling [25, 26, 45]. Similarly in humans, HSPC gene expression changes with ageing. In old human HSPCs, genes involved in DNA repair, regulation of transcription and chromatin remodelling are repressed, whereas genes involved in differentiation, plasma membrane and extracellular matrix are up-regulated [46]. The concept that the proliferative and regenerative capacity of human HSPCs diminishes with age is also consistent with the inverse association observed between advanced donor age and disease-free survival following BM transplant [30].

**Accumulation of DNA mutations and diminished DNA repair**

Accumulation of DNA damage (i.e. oxidation of nucleotides, single- and double-strand breaks, depurinations, depyrimidations, interstrand cross-links and telomere dysfunction) is believed to contribute to human ageing. A number of mechanisms, including the production of reactive oxygen species (ROS), as well as exposure to toxic metabolites, background irradiation, ultraviolet radiation and environmental toxins, have all been implicated in inducing DNA damage. Conversely, DNA repair pathways [i.e. O6-alkylguanine-DNA alkyltransferase (AGT) repair, nucleotide excision repair, base excision repair, mismatch repair, non-homologous DNA end-joining and homologous recombination] oppose the accrual of DNA damage and are critically involved in preserving genomic integrity [47]. Aberrant gene expression observed during ageing could possibly have connections to the loss of DNA repair capability and epigenetic deregulation.

Genomic stability of HSPCs is essential for self-renewal and differentiating capacity. The concept that DNA damage limits stress-induced haematopoiesis by diminishing the ability of HSPCs to proliferate and self-renew has been tested in DNA repair-deficient mice (Lig4/y/y, Csb/m/m, Xpa/m/m, Xpd/m/m, Xpa+/−) not exposed to exogenous genotoxic stress. In these models, there is an age-dependent accumulation of spontaneous or endogenous DNA damage lesions in quiescent cells suggesting that intracellular products such as ROS could be responsible for the damage in non-proliferating cells [48]. 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 (Kroc1, Rad52, Xab2)—has been implicated in age-dependent HSPC functional decline [25, 26, 45, 46] (Fig. 1B). Damage-induced phosphorylated histone H2Ax (γH2AX), a sensitive indicator for the existence of DNA double-strand breaks, accumulates in the genomes of HSPCs from old mice, which also show changes in chromatin structure and form senescence-associated heterochromatin foci (SAHF). Interestingly, depletion of one of the principal age-associated inflammatory cytokines,

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**Fig. 1** Aged HSPCs loose multi-lineage differentiation capacity (with myeloid skewing) and have reduced self-renewal capacity. The accrual of DNA damage and telomere shortening are mechanisms underlying the age-associated HSPC loss of function.
Telomere attrition

Telomeres are repetitive DNA sequences coated by capping proteins (shelterin complex) at the ends of linear chromosomes. Shelterin proteins serve as molecular signals to prevent the cellular DNA repair machinery from mistaking chromosome ends as DNA double-strand breaks [49], thus protecting chromosome ends from fusing to each other and ensuring the correct segregation of the genetic material into daughter cells during each division cycle. In addition to chromosomal protection, telomeres have also been involved in the regulation of gene expression (i.e. stress response genes such as IFN-stimulated gene 15 kDa) in human cells, a process known as telomere position effect [50].

During each cycle of cell division, telomeres shorten because DNA replication machineries incompletely copy 3’ DNA ends. Also, it has been suggested that the G-rich telomeric DNA is susceptible to oxidative damage and hence repair-mediated loss [51]. After several rounds of division, short telomeres activate cell-cycle checkpoints, leading to cellular dysfunction [52]; critically short telomeres signal cell-cycle arrest or apoptosis.

Telomere shortening occurs in human tissues during ageing. It constitutes an important barrier for the uncontrolled proliferation of tumour cells and it is accelerated in patients with chronic diseases. Several factors influence telomeric dynamics, including both genetic determinants (telomerase gene mutations) and acquired factors (i.e. chronic stress, increased cortisol levels, free radicals and oestrogen levels). Telomerase, a reverse transcriptase specialized in telomere replication, is the main mechanism responsible for telomere length maintenance. Telomerase activity is undetectable in somatic tissues, except in stem cell compartments, but the level of activity is not sufficient to prevent telomere shortening associated with cell division [49]. The generation of telomerase knockout mice has demonstrated in vivo that short telomeres are associated with decreased stem cell frequency and functionality as well as with haematopoietic failure [53]. In addition, the skewing of haematopoiesis (decreased lymphopoiesis and increased myelopoiesis) that occurs with age is accelerated and appears earlier in telomerase knockout mice [54]. In contrast, constitutive expression of telomerase reverse transcriptase (one of the components of telomerase) in mice engineered to be cancer resistant by preserving the proliferative potential of stem cells improves the fitness of tissues and extends the median life span [55, 56]. The concept that altered telomerase activity and reduced telomere length contribute to ageing by limiting tissue renewal is further supported by the fact that germline mutations in telomerase genes underlie human BM failure syndromes such as aplastic anaemia [57].

Specifically in HSPCs, telomerase appears to be the major salvage pathway. CD34+/CD38- HSPCs have higher telomerase activity and shorter telomeres than the CD34+/CD38+/low HSPCs that are more primitive non-proliferating cells with longer telomeres. However, elevated levels of telomerase activity alone are unable to prevent proliferation-associated telomere shortening and thus HSPCs have a finite replicative capacity. Telomere length is a surrogate of the proliferative history of HSPCs [58] and an indicator of their ageing [59].

How does telomere-shortening impact HSPC function?

The consequence of telomere shortening/dysfunction is to elicit a DNA damage response similar to that induced by DNA double-strand breaks [60]. Telomere attrition can signal cell-cycle arrest, cellular senescence and apoptosis, and thus lead to impairment of HSPC self-renewal and proliferative capacity [61]. Studies in mice with telomerase gene mutations have shown that the activation of intrinsic cell-cycle checkpoints in response to telomere dysfunction limits the repopulating capacity of HSPCs [54]. In these mice, HSPCs also have defects in their differentiation potential (impairment in B lymphopoiesis and acceleration of myelopoiesis). In addition, telomere dysfunction impairs the maintenance of the BM stroma by reducing the proliferative reserve and the number of early mesenchymal progenitor cells [54]. Importantly, the expression of hTERT eliminates the DNA damage signalling and stops the replicative senescence induced by short telomeres [50]. Telomere dysfunctional and DNA repair-deficient mice show a reduction in haematopoietic progenitor cells, which is more pronounced than the reduction of stem cell number, suggesting either that quiescent cells are more protected from DNA damage or that DNA damage responses are reduced in stem cells compared with progenitor cells. The accumulation of damage in stem cells has been postulated as a mechanism through which stem cell ageing can lead to cancer [62].

The purpose of the activation of stem cell intrinsic checkpoints is to impose a developmental barrier limiting the clonal expansion of these cells and thereby acting as a tumour-suppressor mechanism [63]. However, in cells that must sustain proliferation over a lifetime, such as HSPCs, the telomeric shortening and DNA damage can result in a diminished capacity to maintain homeostasis and can be horizontally propagated to other HSPCs (through self-renewal) and vertically conveyed to downstream progenitors [43, 64]. Therefore, telomere length in mature haematopoietic cells reflects the telomere length of the HSPC from which the cells are derived, the number of cell divisions occurring during haematopoietic maturation and the regulation of telomere shortening during cell division [65–67].

In humans, the connection between HSPC-replicative capacity and telomere dynamics has been recently
established by showing that cord blood HSPCs with longer telomeres have a replicative advantage in comparison with peripheral blood HSPCs during allogeneic stem cell transplantation [45]. Transplant studies have also shown that the telomere length of marrow cells from allogeneic recipients is shorter than the telomere length of the donor cells, likely reflecting increased replicative demand after transplant [66]. Moreover, accelerated telomeric shortening seems to precede the development of therapy-related myelodysplasia or acute myelogenous leukaemia after HSC transplant, supporting an important pathogenic role for telomere dysfunction in the development of these malignancies [66]. Another model that highlights the telomere attrition–DNA damage–stem cell deficiency link is Down’s syndrome. Children with this syndrome have a stem cell ageing signature characterized by accelerated telomere shortening, decreased ability to repair DNA damage, enhanced apoptosis and a reduction in CD34+ cells [68].

**Elevation of intracellular ROS levels**

Intracellular ROS levels modulate functional properties of HSPCs. ROS overproduction results in oxidative stress and compromises HSPC function. The DNA-damage response protein ataxia telangiectasia mutated (ATM) and the FOXO transcription factors have been implicated in the regulation of ROS levels [69]. HSPCs from ATM-deficient mice, similar to those from mice lacking FOXO proteins, have elevated intracellular levels of ROS with decreased size and functional capacity of the stem cell compartment. Activation of p38 mitogen-activated protein (MAP) kinase and induction of p16 seem to mediate these defects, which can be restored upon treatment with the antioxidant N-acetyl-L-cysteine [69–72]. Two subtypes of HSPCs have been defined in mice based on the intracellular ROS levels: the ROS-low population, which has better self-renewal ability, and the ROS-high HSPCs, which have a skewed differentiation potential towards myeloid lineage [73].

**HSPCs in RA**

**Diminished HSPC reserve**

Postulating that haematopoiesis suppression could account for some of the haematological manifestations of RA patients, Papadaki et al. [74] evaluated the RA stem cell compartment and BM micro-environment. Low HSPC frequency, accelerated Fas-mediated apoptosis of CD34+ cells, defective clonogenic potential of BM stem cells and impaired haematopoiesis-supporting capacity of the BM stroma characterize the BM of RA patients. TNF-α production by inflammatory cells of the BM micro-environment was implicated in mediating the apoptotic depletion of patient stem cells. Initiation of anti-TNF therapy resulted in improved CD34+ cell frequency, percentage of CD34+ Fas+ cells and haematopoiesis supporting capacity of the stroma [74].

Peripheral blood HSPC frequencies are also reduced in RA patients by almost 50% compared to age-matched controls [16] (Fig. 2). Furthermore, while the size of the circulating CD34+ pool in healthy controls is strictly age dependent, the number of CD34+ cells—even in young RA patients—is depressed. Counts of circulating CD34+ cells of 30- vs 70-year-old RA patients are similar, and both are comparable with those of 70-year-old healthy controls. This is consistent with the finding that CD34 yields in peripheral blood stem cell harvests from RA patients with severe disease are lower than those in controls [75]. CD34+ cell numbers do not differ 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 HSPC counts in RA raises the unanswered question of whether the accelerated loss of HSPCs starts during the pre-clinical phase of the disease or even precedes it.

Another consideration is the potential deleterious effect of disease-modifying anti-rheumatic drugs (DMARDs), in particular MTX, on HSPC function. Two different reports show that HSPCs from RA patients naïve to DMARDs had the same defects as treated patients [16, 74], suggesting...
that drug-induced damage is not the major factor affecting haematopoesis in RA.

Depletion of circulating HSPCs in RA patients could result from multiple factors, including reduced BM production, increased HSPC attrition and a pseudo-reduction secondary to relocation of HSPCs to peripheral tissues [75–77]. Since disease duration, activity and severity are not predictors of low HSPC numbers in RA patients, an intrinsic defect of this population should be considered [16].

In order to self-renew or differentiate, HSPCs 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 HSPCs impaired (reflected by the decreased number of cell cycles that RA HSPCs achieve after haematopoietin expansion), but also a significant percentage of HSPCs (10–15%) cannot be driven into proliferation, and a delay in the lineage-committed cell differentiation following haematopoietin stimulation is observed [16]. This is consistent with previous studies showing that the proliferative activity of BM myeloid progenitors is decreased in RA patients [76]. Taken together, these findings support the concept that a critical HSPC function—the ability to replicate—is not as efficient in RA patients as in healthy individuals. The slower rate of proliferation can also be seen as a compensatory mechanism to preserve stem cell quiescence and maintain stem cell pools [55].

Accelerated HSPC telomeric loss

Recent studies have confirmed that the premature telomeric loss in RA not only affects committed immune cells, but also involves HSPCs. RA CD34+ cell telomeres are 1600-bp shorter than those of age-matched controls, and this feature is independent of disease activity [16]. It remains unclear 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. Of interest, healthy HLA-DRB1*04-positive individuals have attrition of HSPC telomeric sequences, suggesting that the rate of telomeric loss and HSPC ageing are under the control of genes within the disease-associated major histocompatibility complex (MHC) region [7, 9]. Aged HSPCs could originate limited frequencies of pre-aged precursors that insufficiently seed the thymus. This, together with other factors, such as reduced thymic activity, may contribute to the accumulation of prematurely aged T cells that are phenotypically and functionally altered and prone to auto-reactivity (Fig. 3).

Potential implications of HSPC ageing to RA immune dysfunction

The decline in regenerative capacity and organ homeostasis is a major feature of human ageing and is associated with impaired stem cell function. Altered differentiation and function of aged HSPCs contribute to the complex process of immunosenescence [24]. The age-associated change in lineage commitment with the expansion of myeloid-biased HSPC clones (in mice) is believed to favour the decline of the adaptive immune system with age [19]. This change in differentiation potential can also account for the increased risk of myelogenous diseases observed in the elderly [43]. The question of whether rejuvenation of the stem cell niche could ultimately improve immunocompetence needs to be experimentally addressed.

**Fig. 3** The shortening of telomeres is a hallmark of HSPC ageing. In RA, this process is accelerated and affects committed immune cells (lymphoid and myeloid lineage) as well as HSPCs. Telomere attrition has been associated with decreased haematopoietic tissue and homeostatic imbalance of HSPCs.
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Acknowledgements

Rheumatology key messages

- Accelerated ageing of immune cells favours the disruption of tolerance.
- Age is the greatest risk factor for the development of RA.
- The premature ageing process involves haematopoietic progenitor cells in RA.

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