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Mechanisms underlying T cell ageing

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Abstract

T cell ageing has a pivotal role in rendering older individuals vulnerable to infections and cancer and in impairing responses to vaccinations. Easy accessibility to peripheral human T cells as well as an expanding array of tools to examine T cell biology have provided opportunities to examine major ageing pathways and their consequences for T cell function. Here, we review emerging concepts of how the body attempts to maintain a functional T cell compartment with advancing age, focusing on three fundamental domains of the ageing process, namely self-renewal, control of cellular quiescence and cellular senescence. Understanding these critical elements in successful T cell ageing will allow the design of interventions to prevent or reverse ageing-related T cell failure.

Introduction

The adaptive immune system has been a prime area for researching the ageing process and its implications[1, 2]. Ageing-associated changes to the immune system are clinically important, leaving older individuals more vulnerable to new infections and to reactivation of latent viruses. Aggravating this problem is the fact that many of the current vaccine strategies only induce incomplete protection in older populations[3]. Improving vaccine responses is paramount for healthy ageing. This goal is achievable, as recently exemplified by the development of an adjuvanted varicella zoster virus (VZV) vaccine that is effective irrespective of age[4]. However, further progress will require approaches that are tailored to the ageing immune system and therefore a better knowledge of the specific immune defects. Strategies in young individuals cannot be simply translated to the older population, as shown by a recent meta-analysis of influenza virus vaccination studies[5]. In this analysis, biomarkers that were predictive of a superior vaccine response in the young were no longer informative in older individuals and an inflammatory signature had a positive effect in young individuals but was harmful in older adults[5].

In addition to the implications for immune system function, studies on T cell ageing provide a unique opportunity to explore the fundamental mechanisms that drive the ageing process in

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general[6]. The T cell system has unique mechanisms of replenishment, with the production of new T cells entirely dependent on thymic activity, which rapidly declines during adolescence and early adulthood[7]. In the absence of thymic output, naive T cells essentially function as their own stem cells. The T cell system is also an excellent model to study the influence of ageing on cell population dynamics[8]. Immune competence is determined by the frequencies of T cells that recognize one particular antigenic peptide. Therefore, the population has to establish a balance between maintaining a highly diverse set of T cell specificities in sufficient frequencies to be able to respond and increasing the clonal size of the T cell specificities that are needed to control acute, chronic and latent infections over the life time of the individual. Finally, T cells are a model system enabling studies of cellular states that are relevant for ageing, including cellular quiescence, senescence and exhaustion[9, 10, 11, 12].

Here, we review T cell ageing with respect to these mechanistic phases of the ageing process, focusing mainly on data available from human studies. By analogy to the stem cell theory, which postulates that the ageing process results from the inability of stem cells to replenish a tissue with functionally competent cells, we discuss whether and how the T cell population is maintained with age. Moreover, we discuss whether T cell ageing reflects cellular senescence or the failure to maintain quiescence and instead undergo differentiation. We highlight how the T cell ageing process is influenced by the accumulation of DNA damage and programmed pathways, in particular those that drive cell differentiation or senescence.

T cell replenishment in immune ageing

Naive T cell generation by peripheral T cell self-renewal.—One hallmark of ageing is the decline in homeostatic and regenerative capacity that is common to all tissues and organs and generally related to stem cell ageing[6, 13, 14, 15]. T cell replenishment in adult humans is special in that it is at least in part uncoupled from stem cells, relying less on thymic activity and more on homeostatic self-renewal of naive T cells. The generation of nascent T cells is entirely dependent on the thymus, where progenitor cells differentiate and are positively and negatively selected to generate the repertoire of self-restricted, self-tolerant and functional T cells. However, unlike any other organ, the thymus undergoes involution during childhood and adolescence, leading to reduced numbers of thymocytes and thymic epithelial cells and disruption of the tissue architecture[7, 16]. Thymic export rates decrease rapidly from the ages of one year to eight years and decrease at a more gradual rate in subsequent years[17].

The consequences of reduced thymic activity for maintaining a naive T cell compartment are remarkably species specific. In young mice, thymic output is at least 2 to 4 times higher than the daily production by peripheral T cell proliferation[18]. As a consequence, naive T cell numbers cannot be maintained with declining thymic activity. Moreover, molecular defects in aged haematopoietic stem cells (HSCs) in mice mirror those in aged peripheral T cells, and T cell function in mice can be improved by reversing HSC defects such as by inhibiting CDC42 activity in aged HSCs[19]. By contrast, even in young adult humans the majority of T cells derives from peripheral T cell proliferation and not from the thymus (Figure 1). The contribution of the thymus to T cell generation is estimated to decline from only ~16% to

<1% over the adult lifetime[20, 21], a decline that can be compensated by minimal adjustments in homeostatic proliferation. This is consistent with the observation that turnover of peripheral naive T cells does not noticeably increase with age, possibly with the exception of the very elderly[22, 23]. Moreover, in contrast to mice, so far there is no evidence that defects in HSCs translate to T cell defects in humans. For example, mutations of the epigenetic modifiers *TET2* and *DNMT3A* that have been associated with HSC clonality are found frequently in the elderly[24] and could be detrimental to T cell diversity, but they have not been reported in peripheral T cells, possibly because low thymic activity prevents the generation of such mutant T cells.

CD8$^+$ T cells: the Achilles' heel of T cell homeostasis.—Homeostatic proliferation in humans is sufficient to maintain a sizable naive CD4$^+$ T cell compartment (Figure 1). By contrast, loss of circulating naive CD8$^+$ T cells with age is more severe, in terms of both relative and absolute cell numbers[25, 26, 27]. In fact, reduced numbers of naive circulating CD8$^+$ T cells is the most consistent and prominent marker of immune ageing in healthy older adults, independent of comorbidities and chronic latent infections[28]. The diminution of naive CD8$^+$ T cells may even be underestimated, because CD8$^+$ central memory T cells lose CD45RO expression and regain CD45RA expression after resolved viral infections, thereby masquerading as naive T cells[29] (Figure 2). Consistent with this finding, a subset of naive CD8$^+$ T cells is able to produce effector cytokines upon stimulation, indicating that this subset is primed[30]. It is not clear why there is reduced resilience of the naive CD8$^+$ T cell compartment to age. It can be assumed that naive CD8$^+$ T cells receive more consistent survival signals than naive CD4$^+$ T cells, based on the facts that MHC class I molecules are ubiquitously expressed and CD8$^+$ T cells are responsive to IL-15 in addition to IL-7. It is possible that IL-2 supports better homeostatic survival of naive CD4$^+$ T cells, as CD4$^+$ T cells but not CD8$^+$ T cells gain expression of CD25 (the α-subunit of the high affinity IL-2 receptor) with age[31]. Because they are more easily activated, naive CD8$^+$ T cells may also be depleted through apoptosis or assume a memory phenotype. Such cytokine-activated CD8$^+$ T cells — known as virtual memory T cells — accumulate in mice with age and have been shown to develop features of cellular senescence[32, 33, 34]. Mouse naive CD8$^+$ T cells are more susceptible to this homeostatic proliferation-induced differentiation into virtual memory T cells than naive CD4$^+$ T cells. It is therefore possible that the generation of virtual memory T cells accounts for the preferential erosion of the naive CD8$^+$ T cell compartment. A cell population in humans that has similarities to virtual memory T cells in mice has been described[35], however, it is not known whether virtual memory T cells also accumulate in humans with age.

The tissue niche for homeostatic proliferation.—Peripheral homeostasis of T cells depends on their recruitment to secondary lymphoid organs where they encounter IL-7 produced by fibroblastic reticular cells (FRCs) and low-level T cell receptor (TCR) stimulation through antigen-presenting cells displaying self-antigens[36, 37]. Impaired access to or changes in the architecture of secondary lymphoid organs with age could therefore have a negative impact on the maintenance of the naive T cell compartment (Figure 2). This has indeed been found for aged mice, interestingly, independent of the production of IL-7 by FRCs[38, 39] The importance of FRC networks for T cell homeostasis has also been

demonstrated in humans, although not yet in the context of ageing. Initial studies showed that the inflammation associated with HIV infection leads to fibrosis of lymph nodes and contributes to peripheral T cell depletion[40]. In subsequent studies, this phenomenon was also seen in human populations that have a high incidence of chronic or recurrent infections other than HIV[41]. Lymph node fibrosis inversely correlated with peripheral naive T cell counts as well as with immune responses to yellow fever vaccination, indicating the functional relevance of this process.

In addition to failing FRC networks, diminished IL-7R expression on T cells contributes to a failure in T cell homeostasis with age. In genome-wide studies of ageing-associated epigenetic changes, reduced chromatin accessibility at the *IL7R* locus in $CD8^+$ T cells was identified as an ageing hallmark[42]. In addition, microRNA networks in $CD8^+$ T cells from older individuals are altered, reducing the activity of forkhead box protein O1 (FOXO1), the key transcription factor regulating *IL7R* gene expression[43]. Interestingly, compared with $CD8^+$ T cells, $CD4^+$ T cells show less epigenetic alteration with age, which may explain their higher resilience[42].

Maintaining T cell diversity: how much is enough?—T cell homeostasis is unique in that it needs to maintain not only population size but also high diversity of TCR specificities (Figure 1). The notion that T cell specificities are lost with age, resulting in a contraction of the TCR repertoire, has been an attractive explanation for defective immune responses. However, there is little evidence that ‘holes’ in the repertoire occur in humans without extreme reduction in population sizes. In computer simulations of human T cell homeostasis, only minimal contraction in diversity was observed over 50 years of homeostatic proliferation despite the absence of thymic production; a marked reduction in diversity was not even observed when the size of the naive T cell compartment shrunk by >50%, as is regularly the case for $CD8^+$ T cells[44]. Clonal extinction is more likely if the initial clonal size is very small, which does not appear to be the case in humans. Moreover, peripheral T cell selection implies that homeostatic proliferation occurs in TCR specificity-specific niches, where clones compete for the recognition of self-antigen in the context of self-MHC. Experimental evidence for the existence of such niches is lacking[21].

Given the enormous diversity of the human naive TCR repertoire, clonal size estimates are difficult. Theoretical estimates have been in the order of 10 cells per clone[45], which is likely at the lower end for humans, in particular for those clones that are generated early in life and have space to expand. Frequencies of naive T cells containing TCR excision circles in newborns suggest clonal sizes of 100, which would make complete extinction of a clone over a lifetime an unlikely event[46]. Several studies have used next-generation sequencing to estimate repertoire contraction with age. Due to undersampling and contamination with memory cells, estimates of naive T cell diversity are frequently misjudged[47, 48, 49, 50]. In part circumventing these limitations by analysing replicate samples of purified T cell subsets, we estimated there to be up to 10^8 unique TCR β-chain sequences in young adults[51]. TCR β-chain diversity declined with age, but only by a factor of 3 to 5 in healthy elderly, which still leaves a very diverse repertoire. Whether this decline is of functional significance is unclear.

Clonally expanded T cells with a fitness advantage challenge diversity.—*In silico* simulation of T cell homeostasis provided evidence that, under certain conditions, selection of peripheral T cell clones on the basis of "fitness" compromises repertoire diversity[44]. Rapid contraction in TCR diversity was observed if single clones changed their growth behaviour and gained the ability to rapidly expand (Figure 2). Acquired mutations conferring clonal growth advantage have been identified in HSCs with age, with *TET2* and *DNMT3A* being frequent drivers of clonal haematopoiesis[24, 52]. As discussed above, thymic T cell generation at older ages is very low, preventing the differentiation of such HSCs into T cells. However, similar to HSCs, aged T cells have an extensive replicative history due to homeostatic proliferation and antigen-induced clonal expansion. It has been recently estimated that there are three mutations for every cell division[53]. It is therefore likely that T cells, unrelated to HSCs, accumulate mutations that could lead to the generation of T cell specificities with a growth advantage and rapid contraction in repertoire diversity[44]. Indeed, somatic mutations were discovered in clonally expanded $CD8^+$ T cells in patients with rheumatoid arthritis[54].

Oligoclonality is most prevalent in the subset of the T effector memory CD45RA cells (T_{EMRA} cells), which are populations of T cells associated with memory inflation following certain viral infections[55]. Clonally expanded T_{EMRA} cells are enriched in the bone marrow[56] and therefore do not seem to compete with naive T cells for lymph node niches. In studies comparing memory T cells from the blood, spleen, bone marrow, lymph nodes and lungs, those in the lymph nodes were found to be less differentiated than those in other compartments[57]. It is therefore unlikely that T_{EMRA} cells outcompete normal T cell self-renewal in the lymph nodes.

Memory T cell homeostasis: a surprisingly dynamic process to maintain long-term memory.—Regulation of memory T cell populations is highly complex and more information on the dynamics of the memory T cell compartment is required, particularly in humans, to fully understand how it is affected by age. In the most simplified model, memory T cells that are maintained in the absence of antigen have to be distinguished from those that re-encounter antigen. Antigen re-encounter may be periodic, for example in the setting of reinfection or reactivation of latent viruses, or chronic, for example by antigens expressed during viral latency or chronic active viral infection.

Studies in settings in which antigen re-exposure was excluded concluded that immune memory lasts for 40–60 years in the absence of antigen and therefore may be lifelong[58]. Frequencies of antigen-specific memory T cells after small pox vaccination decreased by 50% every 8–15 years[59]. However, the lifetime of individual memory T cells is much shorter; in humans, it is generally ~6 months as determined by deuterium labelling studies[60]. Thus, maintenance of immunological memory is a dynamic process that is not only determined by the survival of individual memory T cells, but also by homeostatic proliferation of these cells. Recent studies of antigen-specific $CD8^+$ T cell kinetics after yellow fever vaccination have shown that by 30 days the cells have entered a quiescent state, which is characterized by a long intermitotic phase and division rate of once every 485 days[29]. The division rate of this antigen-specific $CD8^+$ T cell population was lower than previous estimates for unfractionated $CD4^+$ or $CD8^+$ memory T cells (both ~180 days);

however, it should be noted that the yellow fever-specific $CD8^+$ T cells no longer express the classical memory cell markers, but are contained within the compartment of cells expressing naive T cell phenotypic markers and therefore resemble the subset of cells that have recently been coined stem cell-like memory T cells[61, 62, 63]. Recent studies of total stem cell-like memory $CD4^+$ and $CD8^+$ T cell populations yielded self-renewal estimates of ~430 days[64], which is similar to estimates for yellow-fever-specific $CD8^+$ T cell self-renewal[64].

In summary, T cell memory appears to be maintained less by longevity of individual T cells than by homeostatic proliferation, which occurs at a slow rate, but is still faster than self-renewal of naive T cells. Based on data from mice, this proliferation is cytokine driven and has less requirement for tonic TCR signalling[65, 66, 67]. Limited kinetic data from in vivo deuterium labelling of healthy young and old individuals suggest similar turnover rates of central memory and effector memory populations, suggesting that this process is intact in older individuals, again with the caveat that only healthy individuals were studied[23, 68]. Moreover, TCR repertoire studies of memory cells only showed a small contraction in diversity[51].

However, analysis of turnover of total T cell populations does not take into account possible heterogeneity in population dynamics. Indeed, analytical approaches to deuterium labelling data performed better using multi-exponential rather than single-exponential models indicating kinetic heterogeneity that is not reflected in conventional phenotypic markers[69]. Such heterogeneity is typical for memory T cells but not for naive T cells. One possible and intuitively appealing explanation is temporal kinetic heterogeneity — that is, a temporally limited increase in proliferative rate of a subpopulation due to antigenic re-exposure in the absence of clinical symptoms. Such proliferative burst could lead to the extinction of unrelated memory T cells and therefore the loss of T cell memory with age. Surprisingly, the computational analysis of available data is more consistent with the alternative model of kinetic heterogeneity — that is, the existence of memory cell subpopulations with stable distinct homeostatic proliferation kinetics[60]. Such population heterogeneity may in part challenge the concept that immune memory is maintained by the division of short-lived memory cells rather than the persistence of long-lived cells. Kinetic heterogeneity has also been described among stem cell-like memory T cells. Although most stem cell-like memory T cells are frequently dividing, a small subpopulation of these cells showed self-renewal dynamics that are consistent with the decline in memory[64].

Recent studies of mice that were kept in a clean environment and were not infected indicated an even higher complexity of the kinetic structure of the $CD4^+$ memory T cell compartment[70]. These studies revealed two central memory and two effector memory $CD4^+$ T cell subpopulations of equal sizes, but with vastly different kinetics; one dividing and dying every three days, the other dividing every 170 days (of note, the average turnover rates of memory T cells in mice are tenfold higher than in humans). These studies also documented a constant influx of naive T cells into the memory cell compartment, with a surprisingly high replacement of up to 10% of cells per week in young mice in the absence of any obvious antigenic challenges. The replacement rate declined with age, which may reflect declining rates of memory T cell differentiation due to reduced numbers of naive T cells or higher resistance to replacement by memory T cells. Computational modelling could

not clearly distinguish between these two models, but favoured the resistance model. These high replacement rates, as well as the existence of a highly proliferative memory T cell subset, represent major challenges to preserving memory.

It will be important to determine whether humans also have a pool of memory T cells that is rapidly and constantly replaced, whether and how this pool contributes to immune memory and, perhaps most importantly for immune health in older individuals, the nature of those memory T cells that appear to resist replacement. Longevity of T cell memory can differ depending on viral infection or modes of vaccination, which is currently not well understood. The most marked example in the context of ageing is the difference in immune memory to latent herpes viruses. Frequencies of VZV-specific T cells decline with age, resulting in frequent VZV reactivation presenting as shingles[71, 72], whereas the T cell memory to cytomegalovirus (CMV), and in part to Epstein–Barr virus, tends to inflate, contributing to the age-associated increase of effector memory T cells and T_{EMRA} cells[26, 73]. Further insights into the dynamics of the memory T cell population are needed to better understand what determines the longevity of memory.

T cell homeostasis: vulnerable to failure but generally robust.—In summary, $CD4^+$ T cell homeostatic mechanisms can be surprisingly resilient to withstand the challenges of ageing in healthy individuals, such as thymic involution, peripheral selection, changing environments and recurring antigenic stimulation. The notion that T cell memory is maintained by unbiased homeostatic proliferation of memory T cells appears to be an oversimplification as the dynamics in the memory compartment are more complex than previously thought. The influence of age on memory cell dynamics and the implications for interventions to prolong immune memory have not been determined.

Most of the more mechanistic studies done so far have focused on healthy individuals, concluding that the ageing process does not necessarily induce major distortions. How far these findings can be generalized to less healthy individuals is currently unclear. While there is an association between frailty and a state of chronic activation of the innate immune system known as inflamm-ageing, it is unclear whether a similar relationship exists for adaptive immune defects. Poorer vaccine responses and higher risk of latent virus reactivation in frail individuals have been reported by some but not all studies[74, 75, 76, 77]. Mechanistic studies of T cell homeostasis in populations including frail elderly are needed. Larger population studies may also capture more infrequent and stochastic events, such as mutations that drive clonal expansion or cause massive contraction in diversity. Moreover, disease states are likely to have a major influence over T cell homeostasis, as shown by the effects of chronic or recurring inflammation on FRC niches.

Failed quiescence in ageing

T cells are quiescent but poised.—Naive and memory T cells exist in a quiescent state, poised to proliferate and differentiate upon antigen stimulation. Maintaining quiescence is vital to retain self-renewal potential and differentiation plasticity throughout life (Figure 1). In quiescence, cell division and growth are coordinately downregulated, cells are arrested in their cell cycle, and they have low metabolic and mammalian target of

rapamycin complex (mTORC) activity resulting in reduced ribosome biogenesis and protein synthesis[78]. Disruption of quiescence has been implicated in stem cell ageing[15]. For example, activation of transcription factor networks including basic leucine zipper transcriptional factor ATF-like (BATF) induce clonality in lymphoid precursors and a preference for myeloid lineage differentiation with age[79]. As observed for FRC networks in the aged lymph node, stem cell niches in young mice generally promote stem cell proliferation more efficiently than those from old mice. However, the aged niche can also become more stimulatory, driving cells out of quiescence. For example, elevated fibroblast growth factor 2 (FGF2) signalling from the aged niche has been associated with depletion of the resident stem cell population in the muscle and diminished muscle regenerative capacity[80]. This model may also apply to HSCs that have been found to be more activated in the aged niche[81, 82]. Similarly, the favourable quiescent state of T cells is continuously challenged by environmental stimuli, including those that drive homeostatic proliferation, in particular under conditions of lymphopenia.

Differentiation of aged T cells due to failed quiescence.—Human T cell ageing shares many features with T cell differentiation, presumably due to a loss of quiescence (Figure 3). The clearest evidence for this is the accumulation of virtual memory $CD8^+$ T cells in aged mice (Figure 2). These cells develop in response to cytokine stimulation in the absence of cognate antigen encounter and they express the same marker profile as cells undergoing lymphopenia-induced homeostatic proliferation[83]. The generation of virtual memory T cells has been considered to be beneficial, because these cells show innate properties and provide bystander protective immunity in response to secreted cytokines[84, 85]. However, being more-differentiated memory T cells, they have lost proliferative potential and therefore may account for the decline in primary $CD8^+$ T cell responses with age[34]. How far human virtual memory T cells accumulate with age is not firmly established. If virtual memory T cells constitute a major proportion of the memory T cell compartment, TCR sequence diversity in the memory T cell population should increase with age, which is not the case. In fact, although virtual memory T cells are mostly $CD8^+$ T cells, TCR diversity is 5–10 fold less for $CD8^+$ memory T cells than for $CD4^+$ memory T cells[51].

Epigenetic evidence for loss of stemness in aged T cells.—Epigenetic studies have provided evidence that the quiescent state is not fully maintained in human T cells with ageing[86, 87]. Age-related changes in T cell chromatin accessibility are very similar to those that are seen with T cell activation and differentiation (Figure 3). These changes are much more prominent in $CD8^+$ T cells than in $CD4^+$ T cells. Sites of increased accessibility in naïve $CD8^+$ T cells include motifs for basic leucine zipper domain-containing transcription factors, such as BATF and AP-1, but not motifs of the T-box or RUNX transcription factors, which is consistent with incomplete differentiation. Progression towards a more differentiated state with age is also observed for $CD8^+$ memory T cells and, to some extent, for $CD4^+$ naive T cells. At the single cell level, older T cells have increased population heterogeneity in histone acetylation, indicating diversity in activation and differentiation states[88]. Differentiation of naive T cells and/or phenotypic conversion of memory T cells could explain this finding.

Functional evidence for differentiation of aged naive T cells.—The naive T cell compartment is also increasingly recognized as being more heterogeneous than previously thought[89]. Gene expression studies of naive T cells suggest that T cell ageing, at least in part, involves the same pathways that are seen in T cell activation and differentiation[31, 90]. Single cell RNA-sequencing studies have shown that ageing increases the cell-cell variability in gene expression during early activation of mouse $CD4^+$ T cells, possibly due to increased heterogeneity in differentiation states[91]. Age-associated changes in microRNA networks in part resemble those of differentiation[43, 92, 93]. For some key microRNAs, such as miR-146a, which increases with ageing and differentiation, and miRNA-181a, which decreases with ageing and differentiation, flow cytometry studies have shown that the microRNA expression levels follow a Gaussian distribution, supporting the idea that the entire population is affected and the observed changes are not due to subset contamination[43]. Transition to a more differentiated state is sufficient to change cell behaviour, without causing typical senescence (Figure 3). Most interesting is a bias in the differentiation potential of older T cells[94]. For example, increased expression of miR-21 in naive $CD4^+$ T cells from older individuals targets for degradation negative regulators of several signalling pathways including PTEN, SPRY1 and PDCD4. The ensuing sustained activation of these signalling pathways favours the generation of inflammatory effector T cells over that of T follicular helper cells and memory precursor cells[94]. Also, transcription factor networks in aged memory $CD4^+$ T cells are poised to favour effector states and expression of the ectoATPase CD39[95], which has been associated with defective vaccine responses and effector states including T cell exhaustion[95, 96].

Entry into a more differentiated state may also imply a bias in lineage commitment and therefore a loss in plasticity to respond. For example, a decrease in variation of CD38 expression with age among naïve $CD4^+$ T cells was observed, and it was proposed that the increased heterogeneity in young T cells endows more plasticity in response patterns[97]. Similarly, we observed increased responsiveness to transforming growth factor-β in the presence of transcription factor such as BATF and IRF4 that favoured the generation of T helper 9 cells in older individuals[98].

Cellular senescence in T cell ageing

Mechanism of cellular senescence.—Cellular senescence is generally implicated as a major mechanism of ageing-associated dysfunction[6, 99]. It is a form of irreversible growth arrest induced in response to telomere shortening or a variety of stress responses, mostly involving DNA damage[100, 101]. To avoid chromosomal instability and to safeguard genomic stability, DNA damage responses are triggered leading to permanent cell cycle arrest[102]. T cells are exposed to short-term and long-term stressors that could induce senescence[103]. Telomere shortening in naive T cells is generally thought to be a consequence of homeostatic proliferation[104]. Consistent with their increased replicative history, memory T cells have shorter telomeres than naive T cells[105]. Moreover, the DNA damage response is activated in T cells proliferating in response to antigen recognition[106, 107] and is predictive for less successful vaccine responses in older individuals[108].

Lack of typical cellular senescence features in aged naive and memory T cells.—Is cellular senescence functionally relevant for immune ageing in humans? Expression of the DNA damage response component p16INK4A (also known as CDKN2A) in human peripheral T cells has been described as a biomarker of ageing[109]. However, p16INK4A is also expressed during normal differentiation into effector T cells and is therefore not in itself sufficient to indicate senescence. Chromatin accessibility mapping has shown increased accessibility at the *INK4A-ARF* locus in memory and effector T cells compared with $CD8^+$ naive T cells, but this is only slightly more so in older individuals[90]. Also, ageing-associated changes in heterochromatin accessibility (similar to changes occurring in senescent cells[110, 111, 112]) are subtle and occurred in $CD8^+$ T cells but not in $CD4^+$ T cells[42]. Moreover, most aged naive and memory T cells do not display the phenotypic hallmarks of senescent cells[78], such as a secretory phenotype, increased size, increased lysosomal content and function, and vacuolated and granular morphology. Most importantly, the vast majority of aged naive and central memory T cells are able to proliferate, when appropriately activated. In fact, aged naive $CD4^+$ T cells from patients with rheumatoid arthritis divide even faster although they show accelerated ageing (based on increased telomere attrition and DNA damage levels) compared with T cells from age-matched healthy controls[107, 113, 114, 115].

T_{EMRA} cells: effector T cells or senescent T cells?—Although most aged T cells do not exhibit functional defects due to cellular senescence, rare senescent T cells may still contribute to immune defects. In non-immune aged tissues, senescent cells only represent a small fraction of cells. Whether and how such infrequent cells have an impact on tissue function has been the basis for debate. It is now generally accepted that senescent cells, triggered by DNA damage, secrete a range of mediators, such as pro-inflammatory cytokines, that have potent paracrine and endocrine effects, a property known as senescence-associated secretory phenotype (SASP)[116, 117, 118, 119, 120]. This function of senescent cells can be beneficial, as long as it is of limited duration and senescent cells are rapidly cleared by the immune system. Long-term persistence of SASP can result in chronic inflammation and disease.

T_{EMRA} cells meet several of the criteria for cellular senescence[121] (Figure 4). These cells have short telomeres, exhibit cell cycle arrest, express DNA damage foci and have a secretome reminiscent of SASP. In common with exhausted T cells, T_{EMRA} cells develop in the setting of chronic viral infection and exhibit cell cycle arrest, however unlike exhausted T cells, they maintain high effector functionality and actually increase their production of inflammatory mediators[12]. How these T cells accumulate in chronic viral infection is not entirely clear, but it may be due to failure in the attrition of effector T cells owing to increased expression of survival factors or reduced clearance by innate immune cells. Consistent with the concept that senescent cells exert systemic detrimental effects, T_{EMRA} cells have been implicated in several chronic disease states as well as poor vaccine responses[122, 123, 124]. However, expanded T_{EMRA} cells have been shown to have both positive and negative effects on life expectancy[125].

Nevertheless, it should be noted that T_{EMRA} cells exhibit clear differences to true senescent cells. Most importantly, their cell cycle arrest is reversible[126]. In support of the notion that

they are a form of terminal differentiation, they gain not only secretory features, but also the expression of regulatory cell surface receptors such as leukocyte immunoglobulin-like receptors and killer immunoglobulin-like receptors[127, 128, 129]. Their function depends on several biological pathways, including a reduced expression of NAD-dependent protein deacetylase sirtuin 1 that leads to increased lysosomal degradation of FOXO1 and metabolic reprograming that favours glycolytic activity and granzyme B production[130]. A characteristic hallmark of these cells is the activation of p38 mitogen-activated protein kinase (MAPK) downstream of AMPK and TAB1 and not downstream of TCR signalling[131, 132], uncoupling T cell activation from antigen recognition (Figure 5). Inhibition of p38 MAPK restores telomerase activity and proliferative potential of T_{EMRA} cells. More recent studies have shown that in old T_{EMRA} JNK and ERK are activated downstream of sestrins and this activation was independent of regulators of the upstream MAPK cascade[131, 133].

Targeting senescent T cells.—The idea that even infrequent senescent immune cells could have harmful system-wide effects and that clearance of senescent cells may be ineffective in ageing has led to the development of therapeutic approaches to eliminate senescent cells[134]. Several compounds — known as senolytics — have been developed that exhibit various degrees of selectivity in inducing death of senescent cells, by targeting apoptosis, chaperones and histone modifications[99, 134]. The best-examined senolytics are a combination of dasatinib and quercetin, which has shown benefits in model systems of cardiovascular disease and osteoarthritis and increases in lifespan[135, 136, 137]. Senolytics have not been explored to improve immune function; and it is unclear whether they would deplete T_{EMRA} cells and, if they do so, whether they would be harmful or beneficial. As T_{EMRA} cells maintain effector functionality and are specific for latent viruses, their depletion may enable viral reactivation. In fact, the memory inflation by T_{EMRA} cells may account for the successful control of latent CMV infection in the elderly. In line with this, infection with VZV, which is also a herpes virus, does not induce memory inflation of T_{EMRA} cells and shows frequent reactivation, presenting as shingles[138].

An alternative approach to interfere with the harmful functions of senescent cells is to inhibit the production or activity of pro-inflammatory mediators (Figure 5)[139]. Treatment of senescent preadipocytes with a pan-JAK inhibitor reduced the production of inflammatory mediators in vivo, possibly by interfering with a positive feedback loop from secreted cytokines. Moreover, treatment of aged mice with a JAK1/JAK2 inhibitor alleviated SASP and frailty. However, T cell homeostasis depends on JAK-STAT5 signalling, and other JAK-STAT pathways are involved in T cell differentiation and function, indicating the limitation of this intervention. Indeed, the risk of developing shingles due to VZV reactivation was increased in patients with rheumatoid arthritis who were treated with the JAK1/JAK3 inhibitor tofacitinib[140, 141]. A similar increase in incidence of shingles was seen in patients treated with the JAK1/JAK2 inhibitor baricitinib[142].

SASP inhibitors mostly target the nuclear factor-κB (NF-κB), mTORC and p38 MAPK pathways that are active in senescent cells. In *in vitro* studies, low dose inhibition of both mTORC1 and mTORC2 using the mTOR-specific ATP mimetic AZD8055 reversed major phenotypes of senescence in near-senescent fibroblasts[143]. In a recent Phase 2a study, a

cohort of old individuals was treated for 6 weeks with a combination of a catalytic and an allosteric mTOR inhibitor in low doses to selectively inhibit mTORC1[144]. Treated individuals had an improved response to influenza virus vaccination, without any major side effects including no reactivation of latent viruses. It is possible that mTORC1 inhibition only modified the vaccine response without targeting senescent cells. In animal experiments, inhibition of the mTORC pathway has been shown to favour $CD8^+$ T cell memory over effector T cell generation[145]. Moreover, mTORC inhibitors could counteract the more sustained signalling in $CD4^+$ T cell responses from older individuals that is due to repression of the negative regulators PTEN and SPRY1, thereby favouring transcription factor networks involved in T follicular helper cell and memory T cell differentiation[94]. However, treated individuals also had significantly fewer infections over the subsequent year; this extended benefit following a short treatment period is surprising and indicated a sustained effect, possibly by eliminating senescent cells.

Other recent approaches are based on AMPK-dependent MAPK activation being central for senescence in T_{EMRA} cells[131]. This implies that inhibition of mTORC1, which is in many aspects antagonistic to AMPK, could be harmful; the more appropriate treatment approaches would be the transcriptional repression of sestrins or inhibition of downstream p38 activation. Inhibition of p38 MAPK has been shown to partially restore proliferative capacity in T_{EMRA} cells by inducing telomerase activity[146].

Conclusions and future perspectives

Pathways implicated in the ageing process, and in particular in stem cell ageing, are highly relevant for the T cell system. T cells depend on well regulated replenishment and homeostasis; they need to maintain quiescence while being poised to respond; and, as highly proliferative cells, they are at risk of developing cellular senescence. However, studies in healthy elderly have shown that ageing-associated differences are not extreme and are frequently within the range of general population variation; accordingly, many healthy older individuals are immunocompetent. A recent extensive immune profiling study in a population selected for absence of major comorbidities and low frailty indices identified only a few immune markers that correlated with age[28]. Major immune defects therefore do not appear to be an inevitable consequence of ageing.

Studies into T cell ageing therefore provide an opportunity to understand how challenges can be overcome and healthy T cell ageing can be supported. The central theme that emerges is to promote cellular quiescence — for example, by reducing infectious burden or by lessening endogenous and exogenous inflammatory stimuli. Many of the T cell-intrinsic defects compromising T cell activation and differentiation in ageing arise from the activation of normal differentiation pathways rather than from the induction of irreversible cellular senescence. If the overall T cell compartment structure is largely intact, as appears to be the case in many healthy older individuals who have reduced but sufficient T cell numbers and diversity, these molecular defects represent druggable targets to improve the efficacy of vaccinations. However, we have a dearth of data on whether this also applies to T cells in less healthy old individuals. It is possible that frail individuals have major immune defects, such as a major diminution of cell numbers or contraction of diversity, or have many small

changes that might act together to cause a clinical immune defect. Studies into systems immunology aimed at understanding the synergistic impact of small effects such as naturally occurring variation in immune parameters on immune responses, are still in their infancy, but eventually will provide insight into how to interpret and target ageing-associated changes.

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GLOSSARY

Homeostatic proliferation
A process of activation and proliferation of lymphocytes in the lymphopenic environment. T cell homeostatic proliferation is driven by T cell receptor interactions with self-peptide–MHC complexes and T cell responses to cytokines such as interleukin-7 (IL-7), IL-15 and possibly IL-21.

Quiescence
A phase in the cell cycle in which the cell is not dividing or preparing to divide but still has the ability to do so in the presence of an appropriate signal. Quiescent cells have low metabolic activity and reduced protein synthesis.

Senescence
A cellular state in which an irreversible growth arrest programme has been initiated that limits the lifespan of the cell and prevents unlimited cell proliferation. It can be caused by replication-induced telomere shortening or DNA damage. In contrast to quiescent cells, senescent cells have a secretory phenotype and upregulated protein synthesis.

Exhaustion
Refers to an impaired ability of effector T cells to carry out their functions such as cytotoxicity and cytokine secretion owing to chronic stimulation by antigen.

T effector memory CD45RA cells
(T_{EMRA} cells). Terminally differentiated antigen-specific memory T cells that re-express CD45RA. These cells have been identified in both $CD4^+$ and $CD8^+$ T cell compartments, have short telomeres, exhibit cell cycle arrest, express DNA damage foci and have a secretome reminiscent of senescent cells.

Memory inflation
The gradual accumulation of peptide-specific $CD8^+$ T cells with an effector memory phenotype that occurs after the resolution of certain acute viral infections during viral latency (for example, cytomegalovirus infection). Induction in the setting of chronic antigen persistence suggests the clonal expansion is antigen-driven and not mutation-driven.

Fibroblastic reticular cells

(FRCs). Specialized reticular fibroblasts located in the T cell areas of lymph nodes and other secondary lymphoid organs. They provide IL7 for T cell survival and produce collagen-rich reticular fibres and form stromal networks and conduits that are important for the trafficking of immune cells.

Virtual memory T cells
Antigen-inexperienced memory-phenotype T cells, which may be induced by T cell receptor cross reactivity, low-affinity peptide and/or MHC ligands and certain cytokines.

TCR excision circles
(TRECs). Small, stable circles of DNA excised during T cell receptor (TCR) gene rearrangement in the thymus.

Stem cell-like memory T cells
Subset of memory T cells that has naïve-like features and phenotypes including enhanced self-renewal and multifunctional capacity.

DNA damage responses
A cell response triggered by DNA damage, such as single or double strand breaks. The DNA damage response stops cell cycle progression to enable repair before the damage is transmitted to progeny cells. Checkpoints in the mammalian DNA damage response are controlled by the PI3K-related kinases ATM and ATR.

Senolytics
Pharmacological compounds that preferentially deplete senescent cells.

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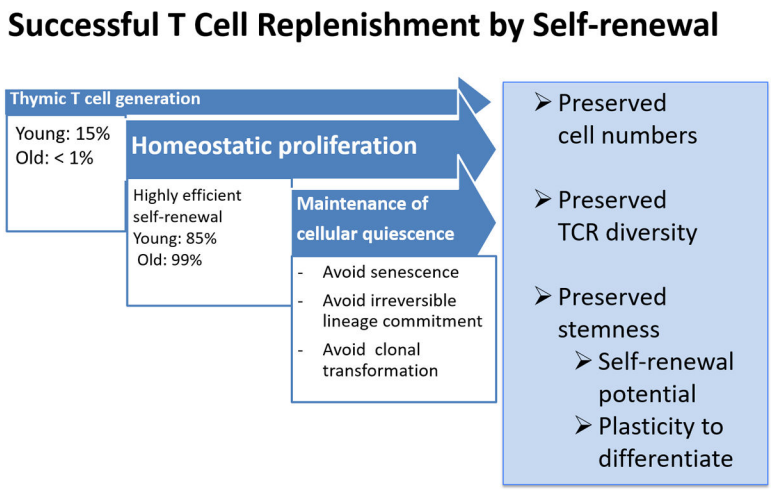

Figure 1 |. Successful T cell replenishment by self-renewal.

Throughout adult life, most T cell replenishment occurs by self-renewal of peripheral T cells rather than de novo generation by thymic activity. In healthy ageing, homeostatic proliferation is efficient to maintain a large compartment of naive T cells and is sufficiently stochastic to preserve a highly diverse T cell receptor (TCR) repertoire. Signals driving homeostatic proliferation should be below the threshold that interferes with cellular quiescence and initiates differentiation.

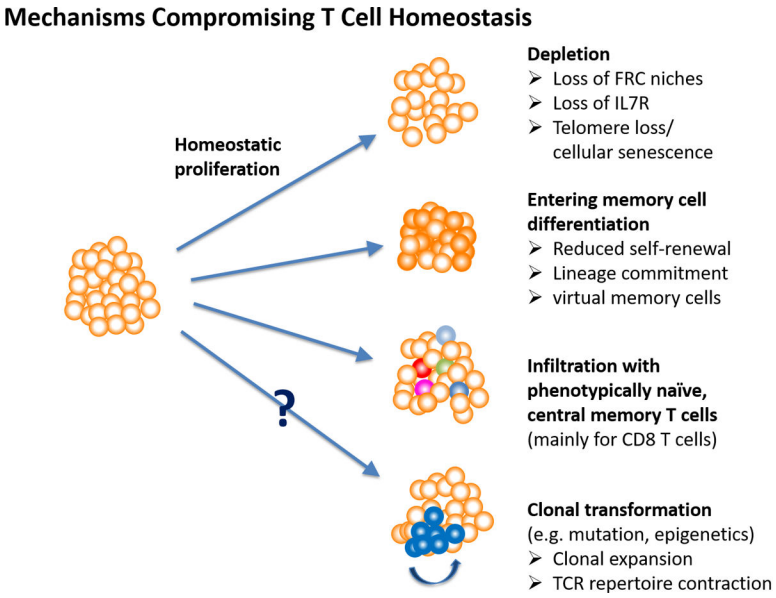

Figure 2 |. Mechanisms compromising T cell homeostasis.

Maintenance of a large, diverse and functional naive T cell compartment can be compromised by several mechanisms. Homeostatic proliferation can be impaired through T cell-intrinsic defects or failing niches formed by fibroblast reticular cell (FRC) networks in the T cell zones of lymph nodes, leading to a reduction in compartment sizes. For unknown reasons, $CD8^+$ naive T cells are more prone for homeostasis failure. If quiescence is not maintained, naive T cells undergo differentiation towards memory T cells. Whether differentiation proceeds as far in humans as seen with virtual memory cells in mice is unclear, but markers of early differentiation states are frequently observed, even in healthy old adults. Again, aged $CD8^+$ T cells appear more susceptible to differentiation. Moreover, the naive T cell compartment can be infiltrated by memory T cells that masquerade as naive T cells and compete for niches. Phenotypic switches regularly occur in $CD8^+$ central memory T cells, but are uncommon for $CD4^+$ T cells. Finally, clonal expansion of selected T cells may occur because of changes in growth behaviour. Such transformations are common for haematopoietic stem cells, but have not been widely studied for T cells. Computer modelling predicts that they could result in rapid contraction of repertoire diversity. TCR, T cell receptor.

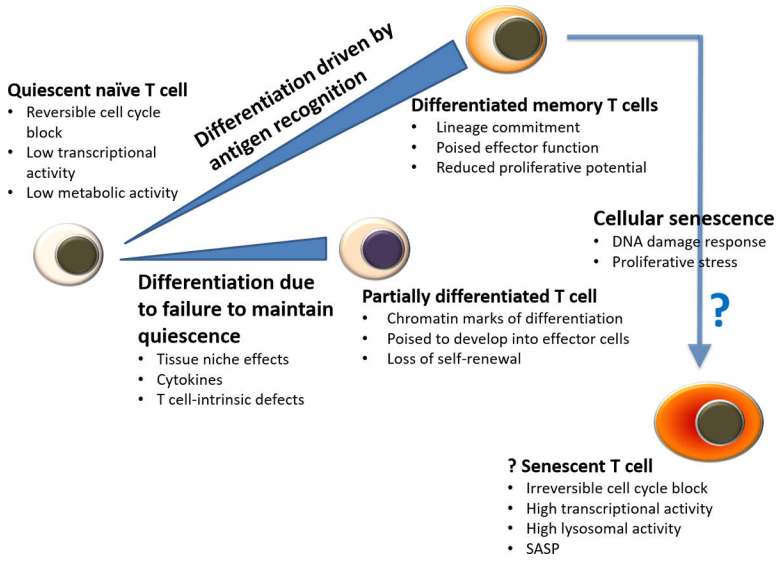

Figure 3 |. Activation of differentiation pathways in T cell ageing.

Many findings in T cell ageing are related to differentiation. Cumulative antigenic experience leads to the accumulation of differentiated cells. Even in the absence of antigen stimulation, T cells can leave their usual quiescent state and accumulate as partially differentiated cells. These cells, as well as functionally differentiated memory T cells, regain quiescence. Cellular senescence is fundamentally different from quiescence. SASP, senescence associated secretory phenotype.

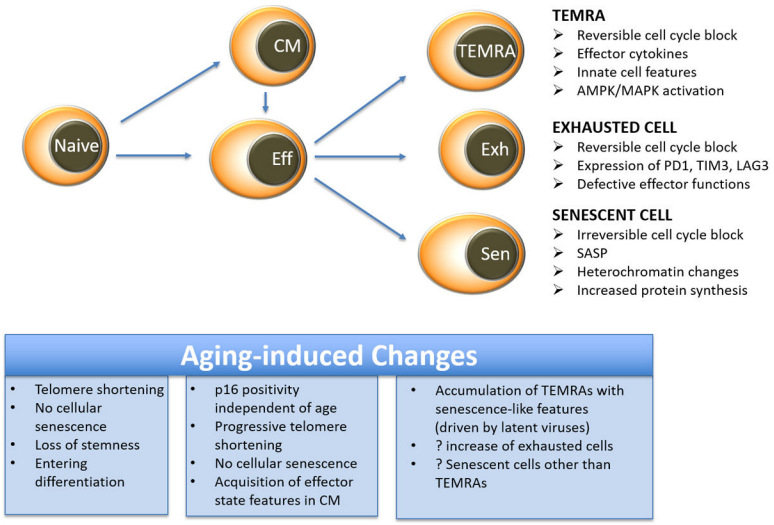

Figure 4 |. Relationship between T cell differentiation and cellular senescence in T cell ageing. Following activation and differentiation, naive and memory T cells activate pathways that are also involved in developing cellular senescence, such as telomeric erosion, activation of DNA damage responses and expression of cell cycle inhibitors. However, true cellular senescence is not observed in most naive and memory T cells. T effector memory CD45RA cells (T_{EMRA} cells) and exhausted T cells exhibit cell cycle blocks that are still, at least in part, reversible, distinguishing them from truly senescent cells. The excessive cytokine production by T_{EMRA} cells is reminiscent of the senescence associated secretory phenotype (SASP), whereas exhausted T cells lack effector functions, setting them apart from T_{EMRA} cells and senescent cells.

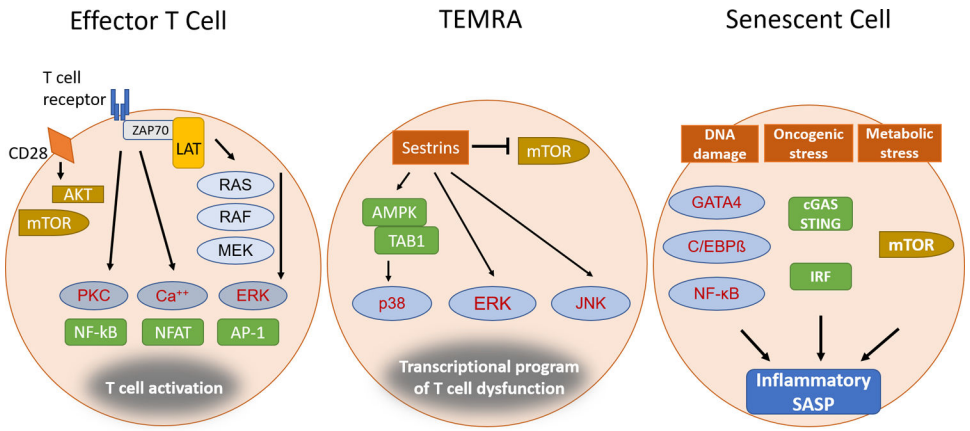

Figure 5 |. Regulation of cytokine transcription in effector T cells, T_{EMRA} cells and senescent cells.

Effector T cells generated during normal immune responses, terminally differentiated T effector memory CD45RA cells (T_{EMRA} cells), which are mostly responsive to latent viruses, and senescent T cells are all characterized by their excessive production of pro-inflammatory mediators. However, the signalling and transcriptional control mechanisms in the different cell states differ. For effector T cells, ligation of T cell receptor (TCR) and CD28 induces a signalling cascade that activates the mammalian target of rapamycin complex 1 (mTORC1) pathway as well as several transcriptional activators. For T_{EMRA} cells, sestrins provide a scaffold for broad mitogen-activated protein kinase (MAPK) activation, in particular activation of p38 MAPK by the AMPK–TAB1 complex, whilemTORC1 is inhibited. For senescent T cells, DNA damage and cellular stress activate signalling pathways, with nuclear factor-κB (NF-κB), p38, C/EBPβ and mTORC1 playing critical roles in regulating the senescence associated secretory phenotype (SASP).