Senescence of T Lymphocytes: Implications for Enhancing Human Immunity

Arne N. Akbar[1], Sian M. Henson[2] and Alessio Lanna[1,3]

[1]Division of Infection and Immunity, University College London.

[2]William Harvey Research Institute, Barts & The London School of Medicine and Dentistry

Queen Mary, University of London, London.

[3]Nuffield Department of Medicine, University of Oxford, Oxford. United Kingdom.

Correspondence: Professor Arne N. Akbar, Division of Infection and Immunity, The Rayne Building, 5 University Street, London WC1E 6JF.

E-mail.akbar@ucl.ac.uk

Abstract

As humans live longer, a central concern is to find ways to maintain their health as they age. Immunity declines during ageing, as shown by the increased susceptibility to infection by both previously encountered and new pathogens and by the decreased efficacy of vaccination. It is therefore crucial to understand the mechanisms responsible for this decrease in immunity and to develop new strategies to enhance immune function in older humans. Here, we discuss how the induction of senescence alters leukocyte, and specifically T cell, function. An emerging concept is that senescence and nutrient sensing signalling pathways within T cells converge to regulate functional responses, and the manipulation of these pathways may offer new ways to enhance immunity during ageing.

What Is Senescence?

The word senescence is defined by the Oxford English Dictionary as “The process or condition of growing old”. Cellular senescence was first described in fibroblasts where the erosion of telomeres, repeating hexameric DNA sequences at the ends of chromosomes, occurs each time a cell divides that initiates a cascade of events culminating in growth arrest (Box 1), [1]. Cellular senescence can also be triggered independently of telomere attrition, when there is actual damage to DNA that can occur anywhere in the genome and this also activates a brake on cellular proliferation [2, 3]. The intracellular sensing of DNA damage leads to the initiation of DNA repair, which, if successful, enables the cell to continue its existence. If the DNA cannot be repaired, DNA damage repair foci persist within the nucleus, leading ultimately to growth arrest or senescence [4]. While the limit to the extent of proliferation of cells that have damaged DNA is considered to be a natural defense against malignancy, the accumulation of senescent cells is associated with the ageing of tissues [5, 6].

In this article, we will highlight recent information on the signalling processes that are involved in senescence-associated proliferative arrest in T cells, consider the potential impact of accumulation of senescent T cells on immunity during ageing and discuss ways by which proliferative activity may be reconstituted in these cells with the aim of enhancing immunity.

Senescence in T Lymphocytes

There is a central role for proliferation of antigen-specific T and B cells in the maintenance of immune memory [7, 8]. The restriction to the extent of proliferation of T cells, especially those that are specific for frequently encountered or persistent antigens may therefore compromise the maintenance of specific immunity in older humans as these specific cells may be progressively driven towards senescence [1, 9, 10] and this is highlighted further by the

increased susceptibility to infection induced by previously encountered pathogens [11-13]. Furthermore, decreased proliferative activity of T cells after vaccination will reduce vaccine efficacy, that is known to be suboptimal in older subjects [14]. However, there is confusion as to what senescence in the immune system actually refers to. In some studies the overall decline in immune function during ageing that involves both the innate and adaptive arms of the immune system, has been termed “immunosenescence” [15]. However, this term does not address specifically the characteristics of individual lymphocytes, and it is a misconception to assume that the function of all leukocytes is decreased during ageing. In this article and in other studies [16-18], T cell senescence refers to a low proliferative activity. This may result from excessive telomere erosion due to the induction of excessive proliferative activity, or to DNA damage due in part to mitochondrial dysfunction leading to increased production of reactive oxygen species (ROS) [18]. Senescent T cells can be found even in young individuals as a result of excessive proliferative activity [19], but they increase considerably during ageing, especially in individuals who are infected with persistent viruses such as cytomegalovirus (CMV) [9, 10, 20]. Recent evidence indicates that NK cells can recognize and eliminate senescent cells in vivo [21, 22]. Furthermore, the elimination of senescent (non-lymphoid) cells in many organs of a mouse model of premature ageing (progeria) can reduce age related pathology in many tissues [23]. The interplay between senescent leukocytes and other senescent cell types in tissues during ageing is an exciting area that remains to be explored.

Characterization of senescent T cells. Within primary human T cells, in both $CD4^+$ [24-26] and $CD8^+$ [18, 27] populations, there is a subset of poorly proliferative cells that exhibit characteristics that are very similar to senescent fibroblasts (Table. 1). These cells can be identified by surface marker expression ($CD27^-$, $CD28^-$, $CD45RA^+$, $KLRG1^+$, $CD57^+$) [24, 25, 27,

28]. Unlike senescent fibroblasts however, freshly isolated T cells from healthy individuals have characteristics of senescence are prone to cytokine deprivation induced apoptosis *in vitro* [16, 25] . However T cell clones and T cell lines generated from patients with rheumatoid arthritis are resistant to activation and cytokine deprivation induced apoptosis *in vitro* [29, 30]. Therefore there may be differences in the characteristics of senescent cells from healthy individuals in patients with different diseases. It is important to note that senescence and exhaustion are different processes that may be induced and regulated separately in T cells [11-13].

Senescent T cells increase during ageing. An important observation is that T cells within both $CD4^+$ and $CD8^+$ compartments that have senescent characteristics increase during ageing [18, 31-36] and are therefore likely to have an impact on altered immune function in older individuals. Furthermore, persistent infection with viruses including cytomegalovirus (CMV) and human immunodeficiency virus (HIV) can induce the accumulation of senescent T cells with all the characteristics described above, compared to age-matched seronegative donors [32, 37, 38]. Although this article focuses on the senescence of human T cells, this process may also govern the proliferation and function of other lymphocytes such as B cells and innate lymphoid cells during ageing, but this remains to be investigated.

The Secretory Nature of Senescent T Cells

Senescent T cells within both $CD4^+$ and $CD8^+$ subsets express high levels of intracellular granules containing the cytotoxic proteins perforin and granzyme B and these cells that are isolated immediately *ex vivo* can mediate antigen-specific cytotoxicity against infected target cells [39-41]. In comparison to less differentiated populations, senescent T cells also secrete high levels of inflammatory cytokines such IFNγ and TNFα after short term activation [24, 27, 42] Therefore, senescent T cells may have an adaptation of the senescence associated

secretory phenotype (SASP, Box 2) originally described in fibroblasts, as they are non-proliferative but have a high capacity for secreting immune active mediators. While the SASP in fibroblasts may be involved with tissue re-modelling, the secretory characteristics of senescent T cells are involved with immune effector functions (Table 1). Therefore, cell type-specific secretory characteristics of senescent cells may be specifically adapted to control different biological processes.

The Association between Cell Phenotype and T Cell Senescence.

For T cells in peripheral blood, there is a correlation between the sequential loss of co-stimulatory receptors on the cell surface and the progressive loss of telomeres. While undifferentiated T cells that have relatively long telomeres are $CD27^{+}CD28^{+}$, senescent T cells with the shortest telomeres are found within the $CD27^{-}CD28^{-}$ population [16, 24, 32, 43]. The highly differentiated $CD27^{-}CD28^{-}$ population of T cells can be further divided and the cells that re-express CD45RA within this subset have multiple characteristics of senescence including loss of telomerase activity, DNA damage, high levels of reactive oxygen species (ROS) and the lowest proliferative capacity [18, 27]. Intriguingly, the CD45RA re-expressing senescent T cells do not have critically short telomeres, suggesting that senescence in these cell may be induced by other mechanisms including DNA damage caused by increased ROS production [18, 27]. Senescent T cells with relatively short telomeres and the phenotypic characteristics described above are also found in young individuals, especially if they have genetic defects that lead to excessive proliferation after T cell activation [19]. Nevertheless, the numbers of senescent T cells in both $CD4^{+}$ and $CD8^{+}$ compartments increase significantly during ageing [20]. Ultimately, the characterization of T cells within tissues will require a full panel of markers for the simultaneous identification of leukocytes as well as the senescent population (Box 3).

T Cell Senescence Is Maintained by Active Signalling and Is Reversible

Involvement of p38MAPK signalling in T ell senescence. Until recently, it was not clear if senescent phenotypes (low proliferative potential and loss of ability to up-regulate telomerase activity) after activation were a passive response due to cellular dysfunction, or an actively maintained process involving the activation of inhibitory pathways. It was shown that senescent fibroblasts have constitutively activated p38 MAP kinase and the inhibition of this enzyme revives the proliferative activity of these cells [44, 45]. This was an early indication that senescence was an actively maintained and not passive process. However fibroblasts, unlike T cells, do not exhibit telomerase activity [46]. These observations led to the investigation of whether human senescent T cells, that also exhibit constitutive p38 MAPK activation, could be induced to proliferate upon p38 blockade. Using small molecule inhibitors [25, 27] or specific inhibitory shRNA [17] it was found that p38 inhibition reconstituted proliferation and also telomerase activity in T cells after activation. Therefore, the reduced proliferative activity in senescent T cells is an actively maintained process that is induced by the engagement of specific inhibitory signalling pathways. This raised the question of how p38 was activated in senescent T cells in the first place.

Canonical and alternative pathways for p38 activation in T cells. Two separate pathways for p38 MAPK activation in T cells had been previously described [17, 47]. The canonical MAPK cascade relies on a cascade of upstream kinases, activated by cytokines or co-stimulatory receptor engagement, that phosphorylate and activate downstream kinases culminating ultimately in p38 activation. In this context, inflammatory cytokines such as IFN-α can activate p38 in non-senescent T cells and this can inhibit proliferation and telomerase activity [26, 32, 48]. Thus in addition to intrinsic signals resulting from telomere erosion or DNA damage due to

ROS, extrinsic signals such as cytokines can also trigger senescent pathways within a cell. This raises the question of whether p38 is activated in senescent T cells by inflammation *in vivo*? However, it is important to note that senescent T cells lose expression of co-stimulatory receptors such as CD27 and CD28 or upstream kinases that activate the canonical pathway, suggesting that p38 may be activated an alternative mechanism in these cells [17, 47].

Novel mechanism for p38 activation in senescent T cells. An alternative p38 activating pathway that requires TCR ligation that induces p38 auto-phosphorylation independently of upstream canonical MAPK activity, has been described [60]. However, it was found that senescent T cells do not express several kinases and scaffold molecules that are involved in this alternative p38 activation pathway and are thus unlikely to engage this second pathway for p38 activation [17, 47]. This suggested the existence of a third unique mechanism for the activation of p38 in senescent T cells. This third p38 activation pathway in T cells was identified recently and involved the activation of a molecular complex that contains AMP-responsive protein kinase (AMPK), a low nutrient and energy sensor, a scaffold molecule TAB 1 and p38 itself [17] (Fig. 1). This complex induces p38 activation through auto-phosphorylation. The critical point is that AMPK is not only activated by sensing low intracellular glucose but also by endogenous DNA damage (senescence) signalling in T cells [17]. This suggests that there is a convergence of senescence and nutrient sensing pathways in T cells to activate p38 via AMPK/TAB1. Correspondingly, the inhibition of either AMPK, TAB1 or p38 itself reconstitutes proliferation as well as telomerase activity in the senescent T cell population [17]. This finding provides points for intervention, in addition to targeting p38 itself, to enhance the function of senescent T cells. Therefore, while senescence may be induced by intrinsic events such as DNA damage or telomere erosion, extrinsic signals such as cytokines and nutrient availability can also activate

senescence related pathways. This applies to T cells as well as other cell types such as fibroblasts. Undoubtedly other pathways that regulate the senescence characteristics of T cells will be identified in the future.

Metabolism of Senescent Human T cells

Dysfunctional mitochondria and increased reactive oxygen species production in senescent T cells.

Senescent T cells utilize glycolysis to generate energy. The regulation of T cell function by senescence and nutrient sensing raises the question of how senescent T cells obtain their energy for survival and secretory functions. It was shown that human senescent $CD8^+$ T cells preferentially utilise glycolysis to generate ATP, as opposed to the effector memory subset which are much more metabolically flexible and can either glycolysis or oxidative phosphorylation (OXPHOS) for functional activities (Fig. 2). Senescent $CD8^+$ T cells exhibit mitochondrial dysfunction, increased production of reactive oxygen species (ROS) and impaired mitochondrial biogenesis, which may explain their dependence on glycolysis for energy [18]. The lack of mitochondrial biogenesis may well be a consequence of the failure of senescent T cells to activate mTOR (mammalian target of rapamycin), a central integrator of immune signalling and cell metabolism [49], as demonstrated by their inability to phosphorylate the mTOCR1 [18] or the mTORC2 complex [50]. mTOR regulates multiple processes, including autophagy, a degradation pathway that removes damaged or unwanted organelles as well as provides metabolites during periods of starvation [51]. In non-lymphoid cells, the decline in autophagy during ageing is due to increased mTORC1 activity. However senescent $CD8^+$ T cells displayed low autophagic activity [18, 52] but also lack mTORC1 activity [18].. Regardless

of the mechanism, the defective autophagy results in a failure to clear the large giant dysfunctional mitochondria that are linked to increased ROS production by senescent $CD8^+$ T cells [18].

After P38 blockade senescent T cells still use glycolysis, despite enhanced autophagy and clearance of defective mitochondria. Since the blockade of p38 increases the proliferation of senescent T cells, it raises the question of how the energy for this enhanced activity is obtained. It was found that the p38 signalling inhibited autophagy in senescent T cells via an mTORC1-independent mechanism that involved increased trafficking of the autophagy regulating protein Atg9 from the endosomes to the lysosomes [18]. Blocking p38 enhanced autophagy, increased mitochondrial biogenesis, cleared dysfunctional mitochondria and reduced ROS production in senescent $CD8^+$ T cells [18], indicating an overall improvement of mitochondrial fitness. However, despite the increased mitochondrial function, senescent T cells still preferentially utilize glycolysis and not OXPHOS for increased proliferation after p38 blockade. Furthermore, the recycling of metabolic precursors resulting from increased autophagy and not greater glucose uptake fuels the enhanced proliferative activity of senescent T cells after p38 inhibition. These results reinforce the observations that senescence and nutrient sensing pathways are inextricably linked and control the function of T cells.

Inflammation and Senescence

Cellular senescence in different cell types can cause chronic inflammation through the SASP and this chronic non-microbial (sterile) inflammation is thought to be the prime cause of many age-related disorders such as atherosclerosis, autoimmunity, cancers and dementias [53].

Recent investigations have been focused on defining the relationship between inflammageing (association between ageing and increased inflammation) and inflammatory diseases [54]. Several studies have reported a link between senescence-associated inflammation and a 2-4 fold rise in the levels of acute-phase markers, such as C-reactive protein, VCAM-1 and IL-6 [55]. Many inflammatory disease states exhibit a shortening of telomeres in peripheral blood leukocytes. A meta-analysis of 27 studies on 13 different cancers found a significant inverse correlation between immune cell telomere length and cancer incidence, itself associated with inflammation [56]. In addition, epidemiological studies showed an increased telomere shortening among patients with cardiovascular disease, atherosclerosis, and myocardial infarction [57]. These studies are correlative with no information as to whether telomere shortening increases the disease risk or whether short telomeres are a secondary consequence of chronic systemic inflammation associated with all these diseases [57]. The fact that cytokines such as IFNα can inhibit telomerase in T cells and induce telomere erosion in these cells [26, 32, 48] lends support to the latter possibility. Molecular studies in rheumatoid arthritis have identified alternative defects affecting telomeric stability. It was found that T cells from rheumatoid patients accumulate DNA double strand breaks in non-telomeric DNA, indicating more generalised abnormalities in sensing, repairing and tolerating DNA damage [55]. Furthermore healthy individuals carrying the HLA-DR4 haplotype share with rheumatoid patients the age-inappropriate telomere loss, implying that there is a genetic mechanisms in the premature deterioration of chromosomal ends thus identifying a possible link between cellular senescence and autoimmunity [58]. Collectively, these data suggest that in addition to a link between nutrition and senescence signalling pathways (Figs 1, 2), inflammation related pathways may be a third interacting axis that is involved in controlling of the function of senescent T cells.

Implications of Blocking Senescence by Targeting p38 to Enhance Immunity

The emerging picture is that senescence in T cells is actively regulated and that by blocking cell signalling pathways, the inhibition of proliferation and telomerase activity in these cells can be reversed. The question that arises is whether p38 blockade to alter T cell function would be beneficial or dangerous *in vivo*. Based on data that has been obtained *in vitro*, it seems that blocking agents that directly target p38 could be used *in vivo* to enhance certain T cell functions [17, 18, 25, 27]. However since the process of cellular senescence is considered to be a mechanism to safeguard against malignancy by preventing the excessive proliferation of cells with either short telomeres or damaged DNA [59], the possible danger is that the inhibition of senescence may promote cancer. Serendipitously, many pharmaceutical companies have already tested small molecule p38 inhibitors in humans *in vivo* in phase I,II and II trials to block inflammatory cytokine production in diseases that include rheumatoid arthritis, chronic obstructive pulmonary disease and diabetes [60]. Clinical trials with p38 inhibitors in patients indicate that it is safe in the short term (weeks) to treat humans *in vivo* with p38 inhibitors as there have been no reports of increased risk of malignancy in individuals treated in this for period [60]. However senescent T cell function was not tested before or after treatment with these drugs. These trials have were discontinued because of hepatotoxicity after long term treatment (>3 months) and also adaptation of cell signalling pathways leading to reduced drug efficacy [61]. An example of a drug that is already in use to target T cell senescence is anti-lymphocyte globulins that have been shown to be effective in depleting senescent like peripheral blood CD3(+)CD4(+)CD28(-) T-cells in vivo [62], however the mechanism involved were not clarified nor were the long term effects of depleting these cells. Therefore, certain drugs that may target T cell senescence may have already been developed for clinical use and it may be feasible to treat older humans safely, at least in the short term, to boost immunity.

The existence of at least three different p38 activation pathways in T cells ('Canonical', 'Alternative' and the recently identified AMPK/TAB1 mode) suggests several potential interventions to block p38 and thus boost immunity. Since p38 is activated through an AMPK/TAB1 dependent pathway in senescent T cells [17], it may be feasible to inhibit one of these molecules to block p38 signalling. However AMPK is also a central regulator of cell function and many different cell types including T cells may be affected [63]. Therefore, AMPK inhibition may have too broad an effect to be therapeutically viable for enhancing immunity. Regarding T cell senescence, a more selective way to inhibit p38 would be to block the scaffold molecule TAB1 required for p38 activation in senescent cells, however, further studies are required to indicate the feasibility of this. Moreover, blocking the p38 pathway can only rescue certain features of T cell senescence. A global approach for boosting immunity by targeting multiple MAP kinases during ageing may thus provide better results than the targeting of p38 alone.

Inhibiting Senescence to Enhance Immunity

One situation where blocking the p38 pathway may be advantageous is during vaccination. The efficacy of many vaccines is reduced in older subjects [14] and the short-term blockade of p38 may open a therapeutic window for enhancement of antigen-specific T cell proliferation, that would increase the number of antigen specific cells. This increase is considered to be one of the end-points of successful vaccination [64]. Another situation for immune enhancement induced by p38 blockade may be in tumour immunotherapy. T cell proliferation and function can also be enhanced by blocking signalling through the inhibitory receptors such as PD1 [65]. This has

been exploited in the therapy of tumours, especially melanoma, where PD1 blockade in patients enhances tumour eradication by T cells and improves the survival of these patients [66]. The pathway involved in PD1 engagement in senescent human T cells is different for that induced by p38 MAP signalling as there is an additive enhancement of proliferation when both PD1 and p38 pathways are blocked simultaneously [27]. Therefore, it may be possible to manipulate senescence signalling pathways in conjunction with other signalling pathways to enhance T cell function. While this may be beneficial for older humans, who have decreased overall immunity, it may also be useful for the treatment of younger patients with malignancies and/or other immune-mediated diseases.

The targeting of AMPK may also be useful in the setting of anti-tumoral responses. It is well recognized that tumours grow even when there are large numbers of T cells surrounding or infiltrating them, indicating impaired T-cell killing activity *in situ*. Recent studies showed indeed that tumour infiltrating lymphocytes (TILs) possess attributes of glucose-deprived cells, resulting in T cell dysfunction [67]. Strikingly, administration of blocking antibodies to CTLA4/PD1 can restore glucose metabolism in mouse TILs contributing to tumour eradication [68]. This indicates that T cell activity is actively inhibited within the tumour microenvironment, in part through the engagement of inhibitory receptor signalling that leads to metabolic remodelling. Another possible mechanism of cancer-mediated immunosuppression is the induction of T regulatory cells (Tregs), an inhibitory T cell population, highly represented at the tumour site and characterized by AMPK-dependent metabolic requirements [68]. An exciting possibility for tumour persistence despite the presence of T cells is that the tumour-driven deprivation of glucose would trigger the AMPK/TAB1 pathway for p38 activation even in non-senescent T cells. The ‘Intra-Sensory’ activated p38 would in turn restrict T cell responses. Since senescence and nutrient sensing signals converge to activate AMPK [17], it may be possible to perform nutrient manipulation as well as anti-senescence therapy by targeting other molecules

in the AMPK activation pathway to promote anti-tumour activity by T cells. Future work is needed to investigate the role of T cell senescence in cancer, and to characterize the metabolic landscape of TILs especially in humans.

Nutrient deprivation pathways may also be targetable for boosting responsiveness to vaccination in malnourished individuals, for instance in developing countries. Immune-activation is an energy-demanding process, and it is thus possible that diminished immune function in such individuals may be direct consequence of activation of AMPK-dependent mechanisms. Correspondingly, increased risks for opportunistic infections affect third world populations. This also opens for possible drug-free interventions to boost immune-responsiveness and implement cost-effective, pan-immunization profiles in these people. Simple and immediate interventions, i.e. addition of glucose to vaccine formulations, may unveil potent yet underestimated immune-stimulatory effects disrupting AMPK activation and allowing vaccination of malnourished subjects. In the future, exploring nutrient-based strategies and other food-related changes in dietary intake either alone or in combination with drug-based interventions may be a cost effective way to cope with large-scale immunizations.

Concluding Remarks and Future Perspectives

There is an increasing need to understand how cellular processes, that are usually investigated in isolation from each other, are linked. A case in point is the new awareness of the interrelationship between senescence, nutrition and inflammation in regulating T cell function [18, 26, 48]. Although a supramolecular complex containing AMPK, TAB1 and p38 is involved in regulating the function of senescent T cells, it is possible that this complex also contains other

molecules that may be targeted to alter T cell function. This raises the question of whether other cellular processes also feed into this interactive network. A key unanswered question is whether the same pathways are linked and control the function of other leukocytes and also cells outside the immune system in the same way (see Outstanding Questions). The fact that telomere erosion in leukocytes correlates with the severity of a vast array of disease states that are associated with many different cell types/organs suggests that this may be the case. A crucial requirement would be the identification of an appropriate animal model for testing interventions and improve the understanding of the cellular processes involved (see Outstanding questions), using clinically tested drugs that are already available to target senescent T cells boost immunity, as ethical constraints preclude most of such studies in humans. Nevertheless, the key message at present is that the reduced proliferative function of senescent primary human T cells that accumulate during ageing is globally controlled by divergent interacting pathways, and that it may be reversible.

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Figure 1. AMPK activates p38 MAPK in senescent T cells.

The canonical MAPK cascade is the main mechanism for activation of p38 in mammalian cells, including T cells. However senescent T cells have an engage a distinct mechanism, whereby under low nutrient conditions p38 is phosphorylated via AMPK and the scaffold protein TAB1β, an isoform only expressed in senescent T cells.

Figure 2. Senescent T cells lose their metabolic flexibility

T cells can utilise either oxidative phosphorylation (OXPHOS) or glycolysis to meet their energy requirements. However senescent $CD8^+$ T cells display a profound mitochondrial dysfunction causing the cells to switch to glycolysis in order to fuel their effector functions. This switch renders the senescent $CD8^+$ T cells metabolically unstable.

Outstanding Questions Box

- Does senescence occur at the same rate in cells of different organs?
- Will removal of senescent T cells enhance or reduce immunity *in vivo*?
- Can the inhibition of systemic inflammation boost T cell immunity, vaccination efficacy and against tumours during ageing ?
- Can dietary intervention during ageing alter nutrient signalling pathways to increase T cell function in older individuals?

Trends Box

- Senescent T cells increase during ageing
- These cells have poor proliferative activity but are cytotoxic and secrete cytokines
- Senescence and nutrient signalling pathways converge to activate AMPK and p38 MAP kinase in these cells
- Blocking either of these molecules enhances T cells proliferation indicating that aspects of senescence are reversible

Figure 1.

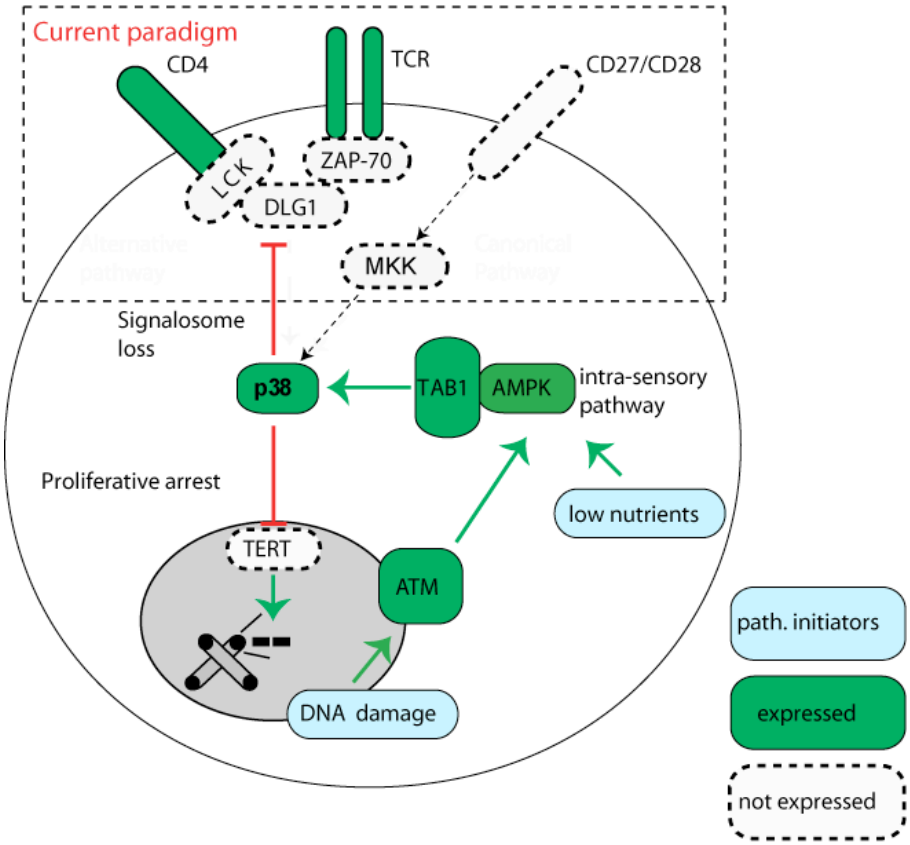
Current paradigm
CD4
TCR
CD27/CD28
LCK
ZAP-70
DLG1
MKK
Signalosome
loss
p38
TAB1
AMPK
intra-sensory
pathway
Proliferative arrest
low nutrients
TERT
ATM
DNA damage
path. initiators
expressed
not expressed

Figure 2.

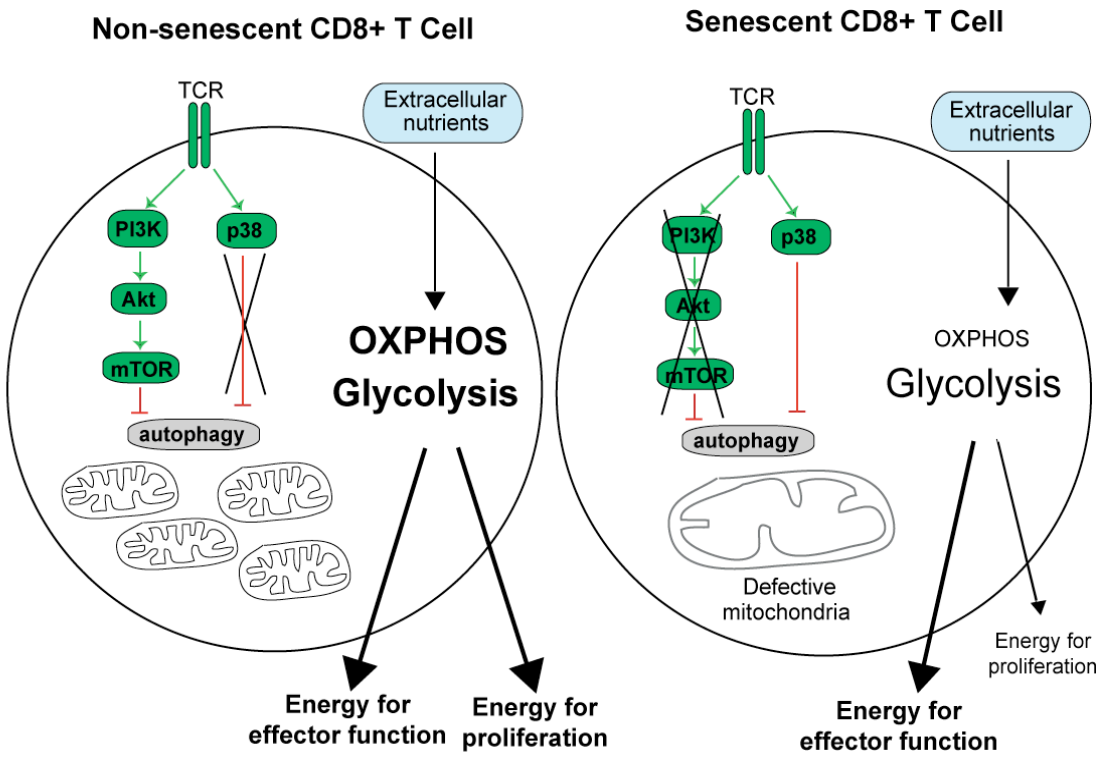
Non-senescent CD8+ T Cell
TCR
Extracellular nutrients
PI3K
p38
Akt
mTOR
autophagy
OXPHOS
Glycolysis
Energy for effector function
Energy for proliferation
Senescent CD8+ T Cell
TCR
Extracellular nutrients
PI3K
p38
Akt
mTOR
autophagy
OXPHOS
Glycolysis
Defective mitochondria
Energy for proliferation
Energy for effector function

Table 1. Characteristics of Fibroblast vs T Cell Senescence.

The majority of data on cellular senescence is derived from experiments on fibroblasts. While T cells also undergo senescence, some molecular features are distinct. The key features of the senescence phenotype are common to fibroblast and T cells, such as the lack of proliferation, short telomeres and the inability to upregulate telomerase. However, a major difference is the susceptibility to apoptosis with fibroblasts being long lived and T cells succumbing to death by apoptosis. Furthermore, it is not known whether senescent T cells express high levels of β-galactosidase or γH2AX. For reviews see [4, 9]

Molecular feature[a]	Fibroblast	T cells
Proliferation	↓	↓
Telomere length	↓	↓
Telomerase activity	↓	↓
Apoptosis	↓	↑
B-galactosidase	↑	?
γH2AX	↑	↑
p16	↑	?
p53	↑	↑
p38	↑	↑
Cytokine production	↑	↑

[a] For reviews, see [4, 10]

Table 1.

Text Box 1

The Discovery of Cellular Senescence. Leonard Hayflick and co-workers showed that fibroblasts have a finite capacity to replicate when cultured *in vitro*, after which they stop dividing but remain metabolically active for extended periods [69]. This constraint on the excessive proliferative activity, i.e. "growing old" of fibroblasts in tissue culture, is known as the Hayflick Limit, that is widely considered to be an anti-cancer mechanism [59]. It was subsequently shown that the molecular basis for the Hayflick Limit is the erosion of telomeres, repeating hexameric DNA sequences at the ends of chromosomes, that occurs each time a cell divides [70-72]. Senescent fibroblasts that are generated by continuous culture until they reach the Hayflick limit stop proliferating but are still metabolically active and perisist as long as they are supplied with nutrients *in vitro* [69, 73]. These cells have short telomeres, high activity of the enzyme β–galactosidase, show high expression of the DNA repair complex associated protein γH2Ax and constitutive activation of p53 and p38 map kinase (p38 MAPK), as well the cyclin inhibitor p16 [70, 74] (Table 1).

Text Box 2. ***The Senescence Associated Secretory Pphenotype.*** An unexpected property of senescent fibroblasts was their ability to secrete a wide array of pro-inflammatory cytokines including IL-6 and IL-1 and also other biological mediators such as matrix metalloproteinases [75]. This is known as the senescence associated secretory phenotype or SASP and is considered to be important for remodelling of ageing tissues [53, 75]. In addition, the secretion of chemoattractants including IL-8 and MIP-1α recruit immune cells to ageing tissue [6, 21, 22]. Furthermore, the elimination of senescent cells in a progeroid mouse model can reduce age related pathology in many tissues [23]. The SASP may therefore also direct immune cells towards senescent cells in tissues to facilitate their removal. Although the growth promoting,

tissue remodelling and senescent cell surveillance inducing activity of the SASP may be beneficial to maintain the integrity of ageing tissue, it is also associated with the development of malignancy [75].

Text Box 3. ***How Can We Detect Senescent T Cells?*** The emerging importance of T cell senescence in the maintenance of immunity during ageing and also the role of the immune system in the elimination of senescent cells in tissues has necessitated the development of new methods to identify senescent cells both within and outside the immune system. In T cells, telomere length can be assessed by measuring telomere restriction fragments (TRF) after restriction enzyme digestion of DNA and by qPCR (Q-FISH) [76]. However, these techniques are labour intensive, display variation between batches and require large amounts of DNA and prior subset isolation [76]. Combining flow cytometry with fluorescent *in-situ* hybridisation (flow-FISH) provides a quick and reproducible technique for telomere length analysis using a flurochrome-labelled telomere probe together with surface and intracellular parameter staining of different leukocyte populations [32, 77, 78]. The flow-FISH technique has been refined to enable the investigation of telomere length, surface phenotype and cytokine production in individual T cells [43] within a mixed cell population. This obviates the need for the isolation of specific subsets of cells and increases the extent of information that can be obtained from small samples of blood [43] or other biological samples. To identify senescent cells in histological section by immunocytochemistry or immunofluorescence, the most common markers used are senescence associated β−galactosidase (SAβ−gal) expression, the cyclin inhibitor p16/INK or the DNA damage response protein γH2AX however these techniques are rarely combined [79]. A new technique for the robust detection of senescent cells combines the staining for DNA damage foci within telomere may also prove useful [80].