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Properties of end-stage human T cells defined by CD45RA re-expression

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Persistent viral infections, inflammatory syndromes and ageing all induce the accumulation of highly differentiated CD45RA re-expressing memory T cells. These cells increase during ageing, especially in individuals who are infected with cytomegalovirus (CMV). These cells have decreased proliferative capacity, increased activation of senescence signalling pathways and greater susceptibility to apoptosis *in vitro*. However these cells are capable of multiple effector functions and thus bear all the hallmarks of short-lived effector T cells. This indicates that senescence signalling may govern the unique characteristics of effector T cells. In this article, we address the functional and migratory properties of these T cells and mechanisms that are involved in their generation. Finally we assess the potential for manipulation of their activity and whether this may improve immune function during ageing.

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Introduction

CD45, the leukocyte common antigen is a glycoprotein that constitutes much of the cell surface of lymphocytes [1,2]. Multiple isoforms of this molecule have been identified and these are differentially expressed depending on cell type and activation status [2]. The large cytoplasmic domain of this molecule has protein tyrosine phosphatase activity [3]. The different CD45 isoforms are generated by alternate splicing [4] from exons 4, 5 and 6, generating CD45RA, RB and RC respectively [2]. It is now recognized that naive T lymphocytes express the high molecular weight isoform containing the A exon (CD45RA) and that is lost after activation and replaced by the low molecular weight isoform CD45RO [5]. Therefore CD45RA expressing T cells were initially considered to be naïve T cells that were the precursors of $CD45RO^+$ T cells that contained the primed/memory T cell pool [5]. However the division of T cells into $CD45RA^+$ naïve and $CD45RO^+$ memory T cells was found to be an oversimplification, as human T cells can revert from $CD45RO^+$ to $CD45RA^+$ *in vivo* [6–8], and *in vitro* [9,10].

Highly differentiated CD45RA re-expressing T cells within both the CD4 and CD8 compartments increase considerably during ageing and after CMV infection [11–14]. Numerous reports have suggested that these cells have characteristics of end stage differentiation [15–17]. However more recent studies have shown that they can be reactivated to proliferate when provided with appropriate co-stimulatory signals [18•,19•,20••]. In this article we question the relevance of highly differentiated CD45RA re-expressing T cells to immunity in general, the signalling processes that may govern their end-stage characteristics, and whether the functions of these cells are altered during ageing.

Primed CD45RA re-expressing T cells have end-stage characteristics

The process of repeated T cell stimulation that leads to differentiation may also result in a loss of replicative capacity as a consequence of telomere erosion and/or unrepaired DNA damage [21,22]. Both processes engage a complex set of proteins involved in DNA repair [23•]. If the perceived damaged DNA is not rectified, then growth arrest mediated by active cell signalling ensues [21,22]. There are a number of similarities between the senescence induced growth arrest in fibroblasts in and human T cells. For example signalling through the mitogen-activated protein kinase p38 MAP kinase and p53 occurs and the cyclin-dependent kinase inhibitors p16 and p21 [21] are involved in both cell types. However differences in senescence between these cells include the fact that senescent fibroblasts are viable but non-proliferative [24] whereas end stage T cells are highly susceptible to apoptosis [13] but may persist *in vivo* in the presence of appropriate survival signals [21]. Indeed, analysis of deuterated glucose uptake in proliferating T cells indicates that CD45RA re-expressing memory $CD8^+$ T cells in older individuals persist owing to a reduced rate of cell death and not accelerated proliferation *in vivo* [25]. Another difference is that fibroblasts do not upregulate the enzyme telomerase, which can add-back telomeres and compensate for telomere loss, whereas human T cells are able to do so [26,27]. It has been shown that CD45RA re-expressing T cells lose the ability to induce this

enzyme and this contributes to their senescence [20••,28,29].

T cell differentiation can be defined using the relative levels of expression of CD27, CD28 and CD45RA [17,21,30,31], with highly differentiated end-stage T cells being $CD45RA^{+}CD27^{-}CD28^{-}$. These end-stage T cells also express increased surface inhibitory receptors such as KLRG1 [19•] and CD57 [17,21,30,31]. $CD45RA^{+}CD27^{-}CD28^{-}$ T cells within both the CD4 and CD8 compartments also exhibit an increase in DNA damage visualized by phosphorylated H2AX (γH2AX), a member of the histone H2A family that is phosphorylated in response to double-strand breaks [23•]. In addition, highly differentiated human T cells within both CD4 and CD8 compartments have altered signalling pathways including defective Akt (PKB) ser473 phosphorylation [13,29] and increased p38 MAP kinase signalling [20••] and both these alterations may contribute to the defective telomerase activity in these cells [20••,29].

The loss of telomeric repeats is another way of assessing T cell differentiation [26] and initial studies indicated that highly differentiated $CD8^{+}$ T cells that re-express CD45RA had the shortest telomeres [31,32]. However studies using larger numbers of donors showed that $CD45RA^{+}CD27^{-}$ T cells have relatively long telomeres compared to the $CD45RA^{-}CD27^{-}$ effector memory T cells but shorter telomeres than naïve and central memory populations in both $CD4^{+}$ and $CD8^{+}$ compartments [20••,28]. Therefore $CD45RA^{+}$ re-expressing T cells have multiple signatures of senescence and end-stage differentiation however these characteristics are not totally dependent on telomere erosion [21].

Primed CD45RA re-expressing T cells are a potent effector population

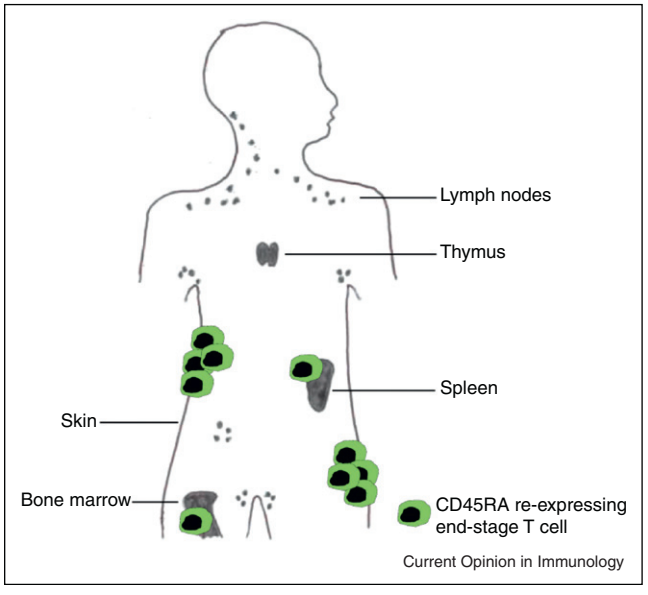
It has been shown that primed CD4 and CD8 T cells that re-express CD45RA can secrete multiple cytokines including IFN-γ and TNF-α and exhibit potent cytotoxic activity after activation [9,13,16]. However these cells have reduced capacity to secrete IL-2 [30,32]. This has been demonstrated for total T cells as well as populations that are specific for Epstein Barr virus [33] as well as cytomegalovirus [11,12,34]. Although these cells accumulate in older humans [11,13,14] it is not clear if these cells are fully functional since some reports show that CMV specific T cells have reduced capacity to synthesize IFN-γ [35]. The majority of studies of CD45RA re-expressing T cells have been performed with peripheral blood populations. However an important observation is that although these cells have poor proliferative activity they can be activated and proliferate if provided with appropriate co-stimulatory signals or accessory populations [9,29]. Once activated, these cells can mediate potent effector function but die rapidly [13]. This cell population may therefore have a role as a sentinel population of effector cells that patrol sites of frequent antigenic encounter. However the effector properties of these cells suggest that they may have a role in other tissues and it is therefore important to assess their migratory potential.

Migratory potential of CD45RA re-expressing T cells

The effectiveness of a T cell is closely related to its propensity to migrate to particular organs and tissues [36,37]. Naïve and central memory cells have a propensity to home to draining lymph nodes (LN) where dendritic cells can present cognate antigen from the site of infection [36–38] while effector memory cells require entry into peripheral tissues where they induce pathogen clearance through cytokine secretion and cytotoxic activity [36].

The chemokine receptors CXCR4, CCR7 and CD62L that are expressed by naïve and central memory T cells facilitate their migration into LNs [37,39]. CD45RA re-expressing memory T cells do not express these four homing receptors that strongly suggest an inability of the population to migrate into LN (Figure 1). Indeed, cytomegalovirus (CMV)-specific $CD8^{+}$ T cells that express CD45RA are absent from the LN of humans despite being present at high percentages in the peripheral blood [40••]. Instead, CD45RA re-expressing memory T cells constitutively express receptors that direct their migration to peripheral and/or inflamed tissue. These include high levels of CX3CR1, which binds fraktalkine

Figure 1



Migratory potential of CD45RA re-expressing T cells. The migratory marker expression of human CD45RA re-expressing T cells indicates their propensity for peripheral and inflamed tissues and not the lymph nodes. However human CD45RA re-expressing T cells are not strictly exiles from lymphoid organs as large amount of these cells are also found in the bone marrow and spleen.

that is expressed by stressed endothelium, thus allowing entry into inflamed vascular endothelium [41]; high levels of CD11a or CD18, the components of LFA-1 that facilitates recruitment to infection sites by binding ICAM-1; and CD49e that allows interaction with extracellular matrix [42•]. Upon stimulation with cognate antigen, CD45RA re-expressing memory T cells also upregulate other inflammation associated adhesion markers, such as CCR5 that binds RANTES or macrophage inflammatory proteins (MIP) that are both expressed by inflamed tissue [12]. CD45RA re-expressing memory T cells are also found in human spleen and bone marrow (BM) [13,43,44,45••].

Collectively the available data suggest that although CD45A re-expressing T cells are highly differentiated, close to senescence and have low proliferative capacity, they migrate preferentially to sites of inflammation where they may exert their potent effector activity [42•]. However it is also possible that the spleen and bone marrow may be sites where these cells are generated (Figure 1) [13].

CD45RA re-expressing T cells may accumulate via antigen-independent cytokine driven homeostasis

Memory T cells are maintained by and can differentiate in response to both antigen activation and homeostatic cytokine stimulation [36]. It is unlikely that CD45RA re-expressing memory cells have experienced a recent encounter with antigen since TCR ligation induces CD45RO expression both *in vitro* and during primary responses *in vivo* [12,15,46,47••]. However after resolution of a primary infection with Epstein-Barr virus (EBV) some antigen specific T cells express CD45RA [8]. In addition, $CD45RA^+$ re-expressing $CD8^+$ T cells were found to be the dominant (quiescent) memory population that was generated following vaccination against yellow fever despite the fact that these cells were $CD45RO^+$ initially [47••]. This highlights that CD45RA re-expressing memory cells may arise without the requirement of repeated antigen exposure, since these individuals were only vaccinated once, and can survive *in vivo* in the absence of antigen [47••].

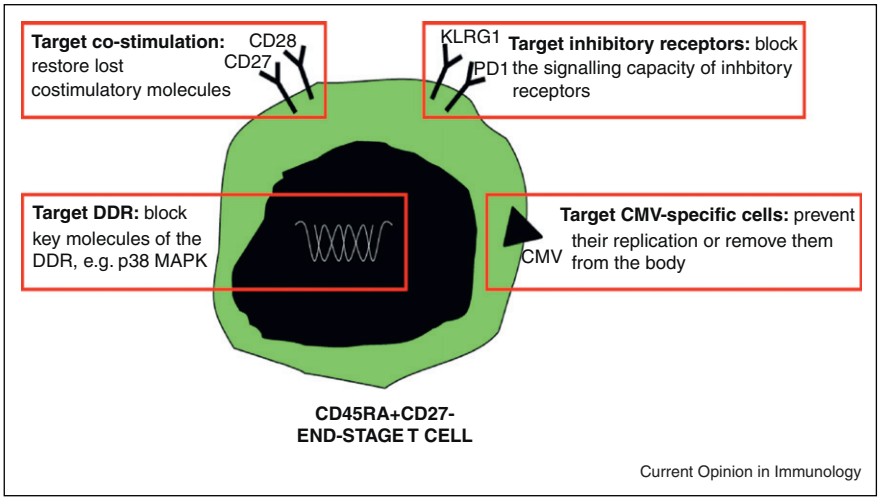
It has been shown that CD45RA re-expressing memory cells can be generated from the CD45RO population of T cells by the addition of the homeostatic cytokines IL-7 and IL-15, in the absence of antigen *in vitro* [9,10,13,15]. Isolated EBV-specific $CD8^+$ T cells that were $CD45RO^+$ could be induced to re-express CD45RA by incubation with IL-15, and these cells exhibited potent cytotoxic activity against EBV infected target cells [9]. Furthermore, IL-15 injections can induce massive expansions of $CCR7^-CD28^-CD8^+$ memory T cells in rhesus macaques [48] however it is not clear if these cells are equivalent to the CD45RA re-expressing T cells in humans as reagents to this molecule is not available in these primates. It would therefore be predicted that these cells may be generated in tissues that contain large amounts of homeostatic cytokines or cytokine producing cells [49,50]. This is supported by the observation that $CD4^+$ $CD45RA^+CD27^-$ T cells of different antigen specificities were found at significantly higher proportions in the bone marrow, a recognized site of IL-7 production [51], compared to the blood of the same individuals [13]. Finally, the fact that IL-15 stimulation can maintain human memory cells without telomere erosion *in vitro* may explain how $CD8^+CD45RA$ re-expressing memory cells become highly differentiated without chronic reduction in telomere length *in vivo* [52], since these cytokines can induce telomerase activity [52,53]. Important but unanswered questions are whether these cytokines engage senescence signalling pathways in the cells that re-express CD45RA and whether the CD45RA molecule itself is essential for the function of these cells. Furthermore since large populations of CD45RA re-expressing T cells dominate the T cell compartment of older humans, the role of homeostatic proliferation in shaping the T cell repertoire during ageing has to be addressed.

Can T cell senescence be reversed?

The data that have been discussed above indicates that CD45RA re-expressing T cells exhibit characteristics of senescence. However, these characteristics of senescence are maintained by multiple distinct signalling processes and some of these changes are reversible. For example, as T cells differentiate they lose the expression of the surface co-stimulatory molecule CD28, which may result in some of the functional changes in highly differentiated populations [27,54]. However, the induction of CD28 re-expression or the ligation of alternative co-stimulatory molecules such as ICOS, CD137 and CD134, enhance the proliferation [18•,29] in certain subsets of $CD8^+CD28^-$ T cells [29]. Highly differentiated T cells also express multiple inhibitory receptors [55]. The defective proliferation of these cells can be reversed by blockade of KLRG1 and PD-1 signalling, which induces AKT Ser473 phosphorylation that is defective in these cells [19•,56]. However, neither the blockade of KLRG1 or PD-1 signalling reverses the telomerase defect in these cells [19•,56].

By contrast, it has been shown that CD45RA re-expressing $CD4^+$ T cells have increased expression of both total and phosphorylated forms of p38 MAP kinase, that is involved in the senescence process in fibroblasts [57,58••]. The inhibition of p38 signalling using a specific inhibitor in $CD45RA^+CD27^-CD4^+$ T cells significantly enhanced the proliferation, telomerase activity and survival of these cells after TCR activation [20••]. Similar observations have also been made in $CD8^+$ $CD45RA^+CD27^-$ T cells (Henson and Akbar, unpublished observations). An additional way to reverse senescence in human T cells is by transducing these cells with

Figure 2



Potential routes for functional manipulation of CD45RA re-expressing T cells. CD45RA re-expressing T cells display both proliferative and signalling defects but remain highly polyfunctional, is it therefore possible to enhance immunity by boosting the function of CD45RA re-expressing T cells. Potential avenues for manipulating function include replacing lost co-stimulatory molecules, such as CD28 or TNF family members or blocking inhibitory receptor signalling. Intervention may be targeted at either the prevention of CMV replication by vaccination or anti-viral therapy or the removal of CMV-specific cells. Finally DNA damage response could be manipulated, such as blockade of p53 or p38 MAPK signalling.

hTERT, the catalytic component of telomerase and many laboratories have achieved this goal [59]. Alternatively, the activity of this enzyme can be enhanced using ERK kinase activators [60].

Although there are ways available to reverse T cell senescence the key questions that arise are whether senescence restricts immune function in older humans in the first place and second whether it is safe to do so. Highly differentiated CD45RA re-expressing T cells have been shown to accumulate in older adults [27,28] patients with persistent viral infections [11,16,61] and those with inflammatory syndromes such as rheumatoid arthritis [42,62]. As T cell function is reduced in patients with chronic viral infections and in older adults [35], it raises the possibility that enhancement of function of the CD45RA re-expressing population may boost immunity (Figure 2). However the reversal of senescence by enhancing telomerase by a variety of ways has risks, since senescence-induced proliferative arrest is considered to be a first line of defence against cancer [22] and blocking this proliferative checkpoint may pose the risk of propagating T cells with DNA damage and thus malignancy. In addition reversal of the proliferative defect in T cells by blocking p38 may lead to decreased rather than increased effector functions as this molecule is important for pro-inflammatory cytokine secretion [20••,63]. Ideally, the enhancement of immunity during ageing would require a strategy that enhances the function of T cells with minimal risk of malignancy or inflammation. Although progress has been made about how T cell function is constrained during differentiation and ageing, the current state of expertise is somewhat short of this goal. Nevertheless since the proliferation of T cells can be enhanced temporarily by p38 inhibition or by blocking inhibitory receptors, this may be a way to boost specific T cell populations intermittently, for example during vaccination.

Conclusions

CD45RA re-expressing memory T cells display senescence-related proliferative defects that are reversible. In young individuals these cells are capable of efficient cytokine secretion and cytotoxicity however it is not completely clear if these cells lose effector function during ageing. The CD45RA re-expressing T cells have relatively long telomeres, suggesting that these cells are governed by telomere-independent senescence. The temporary reversal of proliferative defects in highly differentiated T cells may be a way to increase specific T cell numbers during vaccination, but long term reversal of senescence carries the risk of malignancy.

Acknowledgements

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- •• of outstanding interest

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