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Review

Significance of senescence for virus-specific memory T cell responses: rapid ageing during chronic stimulation of the immune system

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Abstract

There is a generalized age-related decline in immune responses which leads to increased susceptibility of elderly to infection and, possibly, to autoimmune disease and cancer. This is associated with phenotypic changes of CD8⁺ T lymphocytes that include the loss of costimulatory molecules CD28 and CD27, which are important for proliferation and cell survival of CD8⁺ T cells. Loss of these molecules is associated with less ability to respond to recurrent infection. Functional changes within T cells during ageing include a reduction in the number of naïve T cells and a progressively limited T cell repertoire. Furthermore, persistent life-long antigenic stress upon the memory pool leads to telomere erosion and concomitant loss of proliferative capacity, a phenomenon known as replicative senescence. In this review, we discuss that replicative senescence, or clonal exhaustion, may also occur in relatively young individuals, as evidenced from HIV-infected individuals and healthy Ethiopians. We discuss data suggesting that T cell defects may arise in individuals because of chronic antigen activation leading to rapid ageing of the memory CD8⁺ T cell pool.

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Keywords: CD8; Memory; Senescence

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1. Introduction

1.1. Immunological (T cell) memory

Upon recognition of viral antigens, CD8⁺ T cells are activated to expand and kill virus-infected cells [1–4], or inhibit viral replication by cytokine release [5,6]. After resolution of infection the majority of expanded T cells undergo apoptosis resulting in contraction, leaving a small pool of memory T cells [7,8]. Upon re-encounters with antigen, these memory CD8⁺ T cells will be more rapidly activated, the memory T cell pool will expand and is once again subjected to contraction once the secondary infection or reactivation is controlled [7].

While adequate numbers of anti-viral T cells are essential to maintain immunological memory, excessive numbers of cell divisions may lead to the generalized age-related decline in immune responses – particularly in the T cell compartment [9] – that has been described in elderly individuals [9,10]. This leads to increased susceptibility of old subjects to infection and, possibly, to autoimmune disease and cancer. In this review we will discuss functional and phenotypic changes that occur in the memory CD8⁺ T cell pool during ageing and provide indications that these changes may also occur in relatively young individuals, as evidenced from HIV-infected individuals and healthy Ethiopians, as a result of chronic antigen stimulation.

1.2. In vivo evidence for diminished memory T cell responses during ageing

In elderly individuals, an age-related decline in VZV cell-mediated immunity leads to an increase in the incidence of herpes zoster, which can be restored upon administration of live-attenuated varicella vaccine [11]. Since these individuals were previously immune to VZV, which they first encountered during childhood, suggests that immunological memory to this virus has been lost. Furthermore, diminished frequency and level of cellular immune reactivity to Tetanus Toxoid, another antigen to which elderly individuals were previously immune, further suggests a decline in memory T cell responses [12]. However, the most striking evidence for age-associated immune defects during virus-infections

comes from studies of elderly individuals with influenza infection. These individuals have a greater risk of morbidity and mortality from influenza [13] which may be due to diminished influenza virus-specific CTL [14–16] and lack of IL-2 [17]. The reduced CTL activity in elderly subjects is not due to a lower frequency of virus-specific T cells, but arises from impaired antigen-specific proliferation or lower lytic capacity of these specific CD8⁺ T cells [18]. In addition, older individuals have greater difficulty mounting a protective response to influenza vaccine [17,19]. Reduced activation of T cells ex vivo and decreased expansion of CTL cultured in vitro may explain these blunted responses to vaccines in the elderly [20]. The diminished memory CD8⁺ T cell responses in elderly may be explained by phenotypic and functional changes that occur during ageing.

2. Phenotypic changes in CD8⁺ T cells during ageing

Naïve CD8⁺ T cells express CD45RA, a lymphocyte cell surface glycoprotein which plays a role in signal transduction [21], and high levels of co-stimulatory receptors like CD27, a member of the tumor necrosis factor (TNF) receptor family and CD28, which is a Ig superfamily member family [22–24]. Initial activation of unprimed CD8⁺ T cells results in the loss of CD45RA expression and gain of CD45RO expression [25]. Thus, recently primed virus-specific memory T cells are mostly of the CD45RO⁺CD28⁺CD27⁺ phenotype [26]. Repeated stimulation of CD8⁺ T cells by antigen induces downregulation of CD28 [27–30] and subsequently CD27 [31]. Using these markers, CD8⁺ T cells at different stages of differentiation can be identified (Fig. 1). Finally, CD8⁺ T cells may re-express CD45RA and acquire the CD45RA⁺RO[−]CD27[−]CD28[−] effector phenotype [32–36]. Although armed with high cytotoxic activity due to the presence of perforin, granzyme B and CD95 ligand, these highly differentiated cells were considered to be non-proliferating end-stage effector cells [26,35,37]. However, more recent studies suggest these cells are quiescent, relatively resistant to apoptosis and can reinduce CD45RO-expression. In addition, they were shown to have considerable replicative capacity. This all indicates that they may represent a stable memory CD8⁺ T cell pool [38–40].

Antigen-specific T cell differentiation

Fig. 1. Differentiation scheme of CD8⁺ T cells. Based on CD45RO, CD27 and CD28 expression the maturation pathway in CD8⁺ T-cell differentiation has been proposed to go from naïve CD45RA⁺CD28⁺CD27⁺ to memory CD45RO⁺CD28⁺CD27⁺ via downregulation of CD28 and CD27 to effector CD45RA⁺CD28⁻CD27⁻ T cells [34]. The CD45RO⁺CD27⁺ memory T-cells have high proliferative capacity, but cytolytic activity only after restimulation *in vitro* [26]. The highly differentiated T-cells have low proliferative capacity with strong effector functions like direct cytolytic capacity due to high expression of effector molecules like perforin and Granzymes [26]. With differentiation from memory to effector, the T cell population shows increased T cell clonality and decreasing telomere length.

With increasing age there is a decrease in numbers of CD45RA⁺ naïve CD8⁺ T cells and a concomitant increase in CD45RO⁺ memory CD8⁺ T cells [41]. In addition, CD8⁺ T cells in elderly individuals show reduced expression of both CD28 and CD27 molecules indicating that they are highly differentiated [42–44].

3. Functional changes in CD8⁺ T cells during ageing

The loss of CD28 on CD8⁺ T cells in elderly individuals may have considerable impact on the functional activity of these cells, since this molecule is essential for delivery of the second activation signal to T cells that leads to IL-2 gene transcription [24], adhesion, and enhanced telomerase activity [23,27,45]. The main feature of senescent cells is impaired IL-2 production [46] leading to impaired proliferative capacity and decreased responsiveness to TCR stimulation. Thus, activation of highly differentiated CD28⁻ T cells in elderly subjects will be suboptimal. Interestingly, CD28⁻ cells

are more apoptosis resistant than their CD28⁺ counterparts, explaining their relative accumulation *in vivo* [47]. Furthermore, the loss of CD27, which is important as a costimulatory signal for T cell proliferation and survival [48,49] will further compromise the reactivation of CD8⁺ T cells in elderly individuals. Although CD8⁺ T cells that have downregulated the CD28 and CD27 molecules accumulate with age, they are still able to proliferate and are not necessarily senescent [47].

3.1. Clonal expansions

Oligoclonal expansions of CD8⁺ T cells, which are uncommon in children, increase after childhood after encounter with viruses that establish chronic infections (EBV, CMV and HIV) [75] or repeatedly infect their host (influenza virus). These T cell clones persist at high frequencies in both mice and men [50–53] and are significantly increased in older persons (Fig. 2). These expansions predominate in the CD8⁺ T cell pool, probably due to the fact that more moderate expansions occur in CD4⁺ T cells [54]. It has been suggested that the accumulation of highly focussed T cells consisting of a few expanded clones directed to a restricted number of epitopes leads to filling up of the immunological space resulting in a shrunken repertoire [55] (Fig. 2B). This results in the inability of CD8⁺ T cells from elderly individuals to respond to new antigens and they may also lose the ability to respond to secondary antigens because these cells have been lost through competition with the expanded clonal populations for survival signals [55]. The expanded clones that have been identified in elderly individuals have lost both CD28 and CD27 expression, however, there is no evidence whether these expanded clones of cells are senescent or retain the capacity to proliferate.

4. Telomere erosion in CD8⁺ T cells during ageing

It has been hypothesized that memory CD8⁺ T cells may lose their replicative capacity as a result of telomere loss and that this may restrict their function or persistence during ageing [56]. Telomeres are repeating hexameric units of repeating nucleotide sequences that are found at the ends of chromosomes that act to cap and stabilize these structures. Loss of 50–100 base pairs of telomeric DNA occurs with each cell division. Critically short telomeres trigger cell cycle arrest and replicative senescence after approximately 50 population doublings (as reviewed in [57]). Indeed, both naïve and memory CD8⁺ T cells from elderly individuals have significantly shorter telomeres than the corresponding subsets from young individuals [58,59].

Telomeres are not automatically lost when T cells are induced to proliferate. Despite excessive proliferation during acute infections, telomeres do not shorten because of upregulation of the enzyme telomerase which adds telomeric end repeats [50–61]. Lack of this enzyme in telomerase deficient

Fig. 2. Model of senescence induction. (A) During first encounter with a virus (pathogen A), virus-specific T cells from the naïve T cell pool, a polyclonal T cell population with expression of the molecules CD27 and CD28 (+) (left panel), will be induced to proliferate, leading to an expansion of virus-specific T cells (and the total T cell pool, middle panel). This leads to an increase in T cell clonality, because cells with a certain T cell receptor will be induced to proliferate. A certain amount of these expanded T cells will remain CD28/27⁺, a certain proportion of these cells could already loose expression of these markers (as indicated by the + and -). After the expansion phase, the T cell pool will contract again. By AICD most of the activated T cells will die, leaving a small pool of memory T cells (which is larger than the initial frequency of virus-specific T cells in the naïve population), consisting of an oligoclonal population of T cells with either expression of CD28/CD27 (+) or not (-). (B) Infection with a different virus (B), subsequently leads to expansion of virus-B specific T cells, of which most cells die by AICD, leaving a small pool of virus-B specific memory T cells. The clonality of the total T cell pool has narrowed concomitantly and a higher proportion of T cells are of the CD28/CD27⁻ phenotype. (C) Chronic antigen stimulation leads to repeated expansion of T cells, which will be derived from the naïve pool and the memory pool. The largest expansion will be due to CD28/CD27⁺ T cells, which have the highest proliferative capacity, leading to a certain proportion of CD27/28⁺ and CD27/28⁻ T cells. The net result will be a more focused T cell pool (more oligoclonal) with less expression of the CD28 and CD27 molecules (C right panel), which will eventually lead to clonal exhaustion and less ability to respond to reinfection/reactivation.

mice [62] and reduced concentrations of the enzyme in humans suffering from Dyskeratosis Congenita, an inherited disorder associated with premature ageing, leads to accelerated telomere shortening resulting in various age-related diseases affecting highly proliferative tissues like T lymphocytes and eventually early death [63]. However, the ability of T cells to upregulate telomerase activity progressively decreases upon repeated stimulation [64–66] and this results in telomere shortening in virus-specific CD8⁺ T cells in the same individuals studied up to 14 years after acute infection [60]. Interestingly, signaling through CD28 has been implicated in telomerase activation especially since this molecule is lost after repeated stimulation of CD8⁺ T cells and is down-regulated on memory CD8⁺ T cells from elderly individuals [64].

Thus, telomere erosion on memory CD8⁺ T cells, especially those specific for persistent antigens, may eventually restrict the capacity of these cells to expand and this may become a problem for elderly individuals who have experienced a lifetime of antigen re-encounter. Therefore, chronic virus infections may play a major role in establishment of replicative senescence.

5. Role of chronic virus infections in induction of replicative senescence

High proportions of CD28[−] T cells [73] and CD45RA⁺CD27[−] T cells were shown to correlate with CMV-seropositivity.[74] Furthermore, CMV-seropositivity has been suggested to drive the CD8⁺ T cell repertoire towards greater clonality in healthy elderly [75] and large clonal expansions of EBV-specific T cells have been observed during acute EBV infection, which persist over time [76]. Moreover, in other chronic underlying diseases, like B-CLL, also large expansions of CMV-specific CD45RA⁺CD27[−] T cells are induced [77]. Recently, these clonal expansions have been shown to be poorly functional CMV- and EBV-specific T cells expressing little CD28 [78]. Furthermore, the frequency of EBV-specific T cells is higher in very old persons (>87) compared to young (20–40 years) EBV carriers, suggestive of continuous stimulation. Interestingly, in old persons the frequency of IFN- γ producing EBV-specific T cells is lower [78]. These data suggest that clonal CD8⁺ T cell expansions occur in the elderly, resulting in accumulation of dysfunctional virus-specific T cells which may lead to reduction of the repertoire of functional cells available for responses to novel antigens [79]. Interestingly, the clonally expanded CMV-specific CD8⁺ T cells from young individuals also express a CD28[−]CD27[−] phenotype [80,81] suggesting that highly differentiated CD8⁺ T cells are not only found in old subjects, but can also be induced by chronic infection in young individuals.

Recently, CD57 has been suggested as a marker for replicative senescence [67]. Although CD57 is often upregulated when CD27 is downregulated [68,69] (+own data),

these cells were shown to have short telomeres and not to proliferate in vitro even in the presence of IL-2. In addition, an inhibitory killer cell lectin-like receptor has been described (KLRG1) with similar features as CD57. Both molecules are upregulated with age [70] and are associated with lack of proliferative capacity [71]. This suggests the possibility that CD8⁺ T cells that have reached replicative senescence may indeed accumulate during ageing [71,72]. As the expression of KLRG1 also increases after viral infection [70] and an age-associated increase in CMV-specific CD8⁺ T cells expressing KLRG1 was shown [72], chronic viral infections may play a role in the induction of senescence.

5.1. Phenotype and function of virus-specific T cells in HIV-infection and ageing

In parallel to what can be seen in elderly individuals over 80 years, in HIV-infected individuals (between 30 and 40 years of age) the total CD8⁺ T cell pool also lacks CD28 and CD27 [29,82,83] and increased numbers of CD28[−] T cells correlate with HIV-disease progression [84,85]. Analysis of telomere length in HIV-infected individuals showed that CD28[−] T cells from 40-year-old HIV⁺ persons have identical telomere length to that of centenarians [86], suggesting that CD8⁺ T cells that have undergone considerable proliferation [87] resemble those that are found in elderly persons. Moreover, a longitudinal study in young HIV-infected individuals showed a decrease in telomere length in CD8⁺ T cells over time [88]. In addition, T cells that are found in HIV-infection has been shown to be more oligoclonal than in control subjects suggesting that the T cell repertoire of these individuals begins to resemble that of elderly subjects [89]. Interestingly, some of the TCR repertoire perturbations were shown to be caused by clonal expansion of HIV-1 RT-specific CTL [90] and anti-retroviral therapy is able to restore part of the perturbations [91,92].

Functional defects that have been described in HIV⁺ individuals with respect to HIV-specific T cells include reduced numbers of IFN- γ producing T cells upon antigen-stimulation late in infection compared to HIV-specific responses early in HIV-1 infection (HIV) [93]. Furthermore, despite the presence of high numbers of HIV-specific CTL in both progressors and nonprogressors to AIDS, only T cells from nonprogressors maintained high proliferative capacity. Proliferation is coupled to increases in perforin expression suggesting that proliferation is required for obtaining efficient effector T cells. Thus, diminished proliferative capacity, which is a feature of immunosenescence, leads to a memory T cell population which is less able to control infections because of lower effector functions and thus may be called exhausted [94]. In HIV-infected individuals, T cell functional defects have been described with respect to other T cells specific for other viruses. Specifically, less IFN- γ producing EBV-specific T cells were observed upon antigen-stimulation compared to healthy controls [95]. In parallel with the observation that elderly people also have lower

EBV-specific responses (IFN- γ), this suggests that with respect to other virus-specific T cell responses, HIV⁺ individuals seem to display a more ‘senescent’ T cell phenotype.

5.2. Role of chronic immune activation in replicative senescence

Functional defects may be caused by chronic exposure to antigens. Immunosenescence can be envisaged as the result of continuous challenge represented by exposure to a variety of potential antigens (viruses, bacteria, but also food and self-molecules). The induction of memory responses indicate successful reaction of the immune system, but will also lead to progressive accumulation of clones of memory cells. Indeed, HIV-infection has been suggested to lead to chronic activation of the immune system as evidenced by high levels of activation markers and high expression of markers for cell division like Ki67 [87]. This leads to enhanced differentiation of T cells, resulting in diminished naïve T cell numbers and eventually loss of CD4⁺ T cell numbers, the hallmark of HIV-infection. Also EBV-DNA levels, which are below the detection level in healthy EBV-carriers, has been shown to be persistently high in HIV⁺ individuals [96]. Recent insights show that the increase in measurable EBV particles in PBMC is associated with HIV-seroconversion [97].

5.3. No evidence for enhanced ageing of HIV-specific CD8⁺ T cells?

In contrast to the phenotypic characteristics of the total CD8⁺ T cell pool in HIV-infected subjects, showing evidence of decreased CD27 and increased CD57 expression, surprisingly, HIV-specific T cells do not accumulate in the CD27[−] pool (although CD28 is downregulated) [98,99]. However, their function (IFN- γ production) [93,100], cytotoxic capacity [99] and proliferative capacity [94] is reduced, suggesting that cells may become functionally senescent without phenotypic changes. Alternatively, other factors may play a role in phenotypic senescence. Not only antigen, but also provision of help (CD4⁺ T cells producing IL-2) may play a role [101]. Also, CD28[−]CD27[−] T cells (HIV-specific) may not be maintained in HIV-infection leading to rapid death of these cells resulting in the preferential maintenance of CD28[−]CD27⁺ T cells. In order to obtain more insight into the ‘block’ in differentiation of HIV-specific T cells, other markers for replicative senescence have to be investigated in the near future.

6. Increased (life-long) exposure to antigens leads to increased immune activation and more rapid immune ageing?

As a model for immunosenescence due to chronic antigen-stimulation, healthy Ethiopians, known to have low average life-expectancy of approximately 41 years [102] and to live in an environment in which the immune system is continuously

triggered, were studied. Among these individuals infectious diseases are most dominant in the medical scene, in particular TB infection. In addition, many individuals suffer from intestinal parasites.

6.1. Phenotypic differences between healthy Ethiopians and Caucasians

An interesting finding is the severely decreased CD4⁺ T cell number (median at 749 μl^{-1}) in healthy Ethiopians compared to Dutch age-matched controls [103] and a higher CD8⁺ T cell number in adult HIV-negative Ethiopians compared to Israelis [104], Swedish [105] and Dutch [103,106]. Interestingly, HIV-negative Ethiopians also have a lower proportion of naïve and increased percentage of memory/effector (RO⁺CD28[−]27^{+/−}) CD4⁺ and CD8⁺ T-cells compared to HIV-negative Dutch [103,107,108]. Additional analysis recently performed in neonates and children showed that the fraction of naïve T cells drops dramatically during the first 20 years of life [109]. Since naïve cells are responsible for the initiation of immune responses against newly encountered antigens, their lower proportion in Ethiopians suggest that this could result in a diminished capacity to react against new infections with microbial pathogens in the long run.

6.2. Evidence for increased immune activation in healthy Ethiopians

T cells were further characterized by studying expression of molecules suggesting recent activation. Indeed, activation of both CD4⁺ and CD8⁺ T cells, as determined by the presence of HLA[−]DR and CD38, was already observed in children under the age of 16 and remained increased in adults, indicating increased immune activation in Ethiopians from birth onwards [109]. Other signs of increased chronic immune activation like increased numbers of dividing (Ki67⁺) naïve T cells were also demonstrated in HIV-negative Ethiopians compared to Dutch subjects [110]. In addition, T cell receptor excision circles (TRECs) were measured, which are formed during T cell receptor rearrangement in the thymus and are at the highest level when naïve T cells leave the thymus. Dilution of TRECs occurs during ageing through cell division and differentiation to memory T cells. The average content of TRECs within the naïve cells was also observed to be decreased in healthy Ethiopians compared to Dutch age-matched controls. Because enhanced activation such as in HIV infection, has been shown to be responsible for dilution of TRECs [110], this decreased TREC content should also be taken as a sign of continuous immune activation in Ethiopians.

6.3. Role of parasites in enhanced immune activation

A possible explanation for the old immune system in Ethiopians may be the increased number of pathogens they are exposed to as mainly parasitic infections are very

common. The effect of parasitic infection and treatment on T cell differentiation and activation markers expressed on CD4⁺ and CD8⁺ T cells in HIV-1 infected and uninfected adult Ethiopians was recently investigated [111]. Intestinal parasitic infections resulted in a significant increase of memory CD4⁺ T cells both in HIV-negative and HIV-positive subjects and a significant increase in percentage of CD8⁺HLA⁺DR⁺ T cells in HIV-positive subjects. In HIV-negative subjects, a significant decline in activated cells and a significant increase in resting CD8⁺ T cells was observed after treatment. In concordance, Ethiopian Jews who emigrated to Israel and underwent treatment for intestinal parasites, also showed decreased levels of activated T cells [12]. Moreover, the number of helminthes, known to cause chronic immune activation, present in HIV-infected Ethiopians correlated with HIV viral load. Treatment of intestinal worms (helminthes) led to a decrease in HIV plasma viral load [112]. Interestingly, a mathematical model of immunosenescence in Caucasians showed that decreasing the antigenic load by 50% led to a considerable increase in resistance to immunosenescence in middle ages, as reflected in immunosenescence parameters [113].

Collectively these results indicate that changes that are found in CD8⁺ T cells from old individuals, such as loss of co-stimulatory receptors and accumulation of CD8⁺ clones are likely to arise from chronic stimulation by persistent antigen (as summarized in Fig. 2) and not ageing in these cells per se. Furthermore, although CD8⁺ memory T cells from young individuals resemble CD8⁺ T cells that are found in old individuals in that they have expanded considerably and are highly differentiated, there is no data available at present to suggest that either population has reached replicative senescence. In this light recent data from our lab (Tsegaye et al. submitted for publication) do not show a difference in the telomere lengths of T cells from healthy Ethiopians compared to T cells from Dutch individuals, although 40% of the subjects showed clearly shortened CD4⁺ T cell telomeres.

7. Conclusion

7.1. Healthy ageing and possibilities for therapeutic intervention

Increasing evidence indicates that persistent exposure to infectious agents leads to more rapid senescence of our immune system. This leads to downregulation of molecules important for activation of T cells leading to lower functional capability, the occurrence of clonal expansion filling immunological space and shortening of telomeres which subsequently leads to less proliferative capacity. The fact that humans reach older age, most likely leads to problems with dealing with infectious agent later in life due to immunosenescence. Interestingly, healthy centenarians seem to have better preserved immune functions, more similar to young individuals [114], suggesting that individuals that become very old, may show less immunosenescence. The cause for this still

needs to be revealed, but it may be due to less immune activation, as has been shown to lead to slower progression to AIDS [87].

As loss of telomere length is the most important determinant of replicative senescence, telomerase induction may be a helpful tool to maintain telomere lengths and thereby preserve replicative capacity and maintain efficient immune responses [115]. In HIV-specific T cells, telomerase induction leads to enhanced proliferative capacity with subsequent enhanced cytolytic and anti-viral capacity [116]. Furthermore, impaired immune responses and increased susceptibility to infection may be overcome by vaccination strategies [117], Vitamin E supplements [118] and immunomodulators that induce certain cytokines or costimulatory molecules to be expressed at higher levels.

As chronic immune activation seems to play an important role in early senescence of the immune system, it seems worthwhile to reduce chronic stimulation of the immune system by treating infections causing chronic antigen-stimulation. This includes treatment of intestinal parasites and vaccination against the most common human persistent virus-infections, such as EBV- and CMV-infection.

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