Age-associated T-cell Clonal Expansions (TCE) in vivo—Implications for Pathogen Resistance

Cellular Immunosenescence – T cells

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Contents

Abbreviations

pMHC	peptide MHC complex
RTE	recent thymic emigrants
SPF	specific pathogen-free
TCE	T-cell clonal expansions

Abstract: Age-related T-cell clonal expansions (TCE) are an incompletely understood disturbance in T-cell homeostasis found frequently in old humans and experimental animals. These accumulations of CD8 T-cells have the potential to distort T-cell population balance and reduce T-cell repertoire diversity above and beyond the changes seen in the aging of T-cell pool in the absence of TCE. This chapter discusses our current knowledge of the role of these expansions in health and disease, with a special focus on their influence upon immune defense against infectious diseases.

Keywords: Ageing • Clonal expansions • Homeostasis • Infectious diseases • T-cells

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T. Fulop et al. (eds.), *Handbook on Immunosenescence*,
DOI 10.1007/ 978-1-4020-9062-2_12,

1 Introduction

As was extensively discussed in other chapters of this handbook, immunosenescence encompasses a number of diverse age-related cellular and extracellular milieu changes that affect cells and molecules of the immune and inflammatory system. The very definition of immunosenescence, however, operationally includes not only the decline of immunity with age by itself, but also its most important clinical manifestation, the increased susceptibility to infection and decreased immunosurveillance of cancer. Other factors can contribute to the increased exposure to infectious diseases and increased colonization with infectious pathogens (e.g., reduced barrier function of skin and mucosal membranes) with age, and multiple factors certainly strongly contribute to the age-related increase in incidence of cancer. However, it is clear that the inability to mount rapid and vigorous immune defense once an infectious invasion (and, likely, detectable malignant transformation) had occurred lies at the heart of many of the clinical manifestations of immunosenescence. Due to the involvement of numerous other nonimmunological factors in the age-related increase of cancer-related morbidity and mortality, this review will solely deal with infectious diseases.

It has long been known that aging is accompanied by an increase in mortality and morbidity from a number of common respiratory infections such as influenza (20,000–40,000 annual deaths in the USA alone) (Bender 2003; Betts and Treanor 2000; Couch et al. 1986; Glezen and Couch 2003; High 2004; Yoshikawa 2000), pneumococcal pneumonia (Bender 2003; High et al. 2005; Yoshikawa 2000) and RSV (Glezen and Couch 2003; High et al. 2005; Yoshikawa 2000), and urinary infections (Bender 2003; Hazelett et al. 2006). Moreover, this vulnerability extends to dangerous established pathogens such as variola (Hanna 1913) as well as the newly emerging pathogens that disproportionally affect the elderly such as the West Nile virus (Murray et al. 2006) the Severe Acute Respiratory Syndrome-causing Coronavirus (SARS-CoV) (Chan et al. 2007; Leung et al. 2004) and others.

Several types of age-related defects in the immune function can contribute to this increased susceptibility to infection, including defects in innate immunity, antigen uptake, processing and presentation, provision of second and third signals to the adaptive immune system and impaired humoral immunity, all of which are competently covered in other chapters of this handbook. However, T-cells have been known to exhibit some of the most pronounced age-related defects (Miller 1996), and intervention to correct these defects resulted in successful correction of the immune function in a number of cases (Effros et al. 1991; Haynes et al. 2004; Haynes et al. 1999; Messaoudi et al. 2006a). These defects can be grossly divided into cell-autonomous defects, which affect T-cells regardless of age-related or compensatory alterations that affect other components of the immune system and which can be detected in assays where T-cells are the only component of the immune system affected by aging; and age-related changes in the T-cell population balance, which mostly involve the initial loss of naïve T-cells and the compensatory, reactive changes aimed to maintain T-cell homeostasis in the face of this loss.

This chapter will focus upon the latter changes, given that other aspects of T-cell dysfunction will be covered in other chapters of this volume. Moreover, we will discuss the impact of a specific type of age-related T-cell disturbances, T-cell clonal expansions (TCE) (Callahan et al. 1993; Hingorani et al. 1993a; Posnett et al. 1994), upon immune defense and pathogen resistance, highlighting the extent and the limits of our current knowledge, and the tasks and problems that need to be solved before we can fully understand and treat these disturbances.

2 T-cell Homeostasis and Development of T-cell Clonal Expansions (TCE)

The current evidence strongly suggests that the involution of the thymus and the decline in production of new naïve T-cells are the initiating factors behind the generation of at least some TCE (Messaoudi et al. 2006b), whereas latent persistent viral infections may be the perpetrators driving other types of TCE (Pawelec et al. 2004). Moreover, homeostatic mechanisms that are activated as a consequence of naïve T-cell loss may themselves participate in the onset and/or maintenance of TCE (Messaoudi et al. 2006b, 2006c). Therefore, at the risk of being redundant, we will very briefly review thymic T-cell production, involution, latent persistent infections and T-cell homeostasis. For a more detailed review of these topics, the reader is encouraged to read sections of this handbook devoted to thymic involution, as well as the recent volume of Seminars in Immunology devoted to T-cell rejuvenation (Nikolich-Žugich 2007; Zuniga-Pflucker and van den Brink 2007).

2.1 Homeostatic Maintenance of T–cell Subsets

T-cell homeostasis is defined here as maintenance of naïve and memory T-cell pool numbers and diversity and the ability to restore these numbers and diversity following antigenic (Ag) challenge. T-cell homeostasis is regulated by the response of T-cells to environmental trophic and survival signals and by the presence and availability of such signals. The most important and best understood of these signals are the common γ-chain cytokines (most notably IL-7, IL-15 and IL-2) and self-peptide: MHC (pMHC) complexes. The contribution of each of these signals to homeostatic maintenance varies depending on the T-cell subset.

Following maturation and selection in the thymus, new T-cells are released into the periphery as recent thymic emigrants (RTEs) (Scollay et al. 1980). Release of RTEs bearing a variety of randomly rearranged TCRs ensures the diversity of the peripheral T-cell pool. Once released from the thymus, the RTE join the naïve T-cell pool. Naïve T-cells have no preset life spans and are maintained by IL-7 and trophic signals from interaction of their TCR with self-p:MHC complexes (rev. in (Lee and Surh 2005). When these two signals are present, naïve T-cells are believed to be

able to survive indefinitely, based upon the results of serial transfer experiments (Sprent et al. 1991). Murine RTE proliferate faster than naïve peripheral T-cells in the first three weeks after export, perhaps in order to maximize naïve T-cell diversity, before they equilibrate with other naïve T-cells (Berzins et al. 1998). Naïve T-cells display very low levels of spontaneous (or homeostatic) cycling in vivo. Homeostatic cycling is greatly increased in lymphopenia, where T-cells sense a signal, most likely provided by an excess of unused IL-7 and IL-15 (Surh and Sprent 2002). Under lymphopenic conditions T-cells undergo Ag-independent homeostatic proliferative expansion (HPE), in a seeming attempt to fill the empty compartment (Fry and Mackall 2005; Surh et al. 2006). Unlike naïve T-cells, memory T-cells do not require specific p: MHC contact for survival. Instead, their survival is dependent on continued homeostatic proliferation, driven mainly by IL-15, or by IL-7 in the absence of IL-15. Memory cells cycle and self-renew in vivo significantly (up to four times) faster than naïve T-cells and also exhibit faster proliferation during lymphopenia (Surh et al. 2006). It is likely that there may be other, presently unknown pathways regulating T-cell homeostasis, some of which could include energy metabolism regulation (Frauwirth and Thompson 2004).

The above described homeostatic mechanisms function to maintain a balanced and diverse T-cell pool. Over lifetime this means regulating the process of Ag-driven expansion of naïve T-cells, their contraction, and selection and maintenance of memory T-cells. The role of the homeostatic mechanisms is to balance the composition of the T-cell pool so that it contains both naïve precursors with diverse TCRs, as well as Ag-experienced memory T-cells, as both of these subsets are crucial for the health of the host. The homeostatic forces work very efficiently in adult mice housed under specific pathogen free (SPF) conditions, as evidenced by remarkably similar size and diversity of the T-cell pool among individual mice of the same strain. However, maintenance of homeostasis becomes more complicated in the face of constant encounters with new acute pathogens, long-term interactions with persistent pathogens and the aging-associated defects, all of which are discussed below.

2.2 Disruption of T-cell Homeostasis in Ageing

Thymic involution begins soon after birth in humans and quickly after puberty in mice, which results in decreased RTE output (Haynes et al. 2000; Hirokawa and Utsuyama 1984). Thus, 22-mo-old mice receive less than 10% of RTE compared to young adult mice (Hale et al. 2006; Heng et al. 2005). Even in old age the thymus continues to produce RTE proportionally to its overall cellularity, but as the cellularity itself decreases, so does the output (Gruver et al. 2007; Hale et al. 2006). The cause of thymic involution is discussed in more detail elsewhere in this volume. From the standpoint of this chapter, thymic involution presents a challenge for the homeostatic mechanisms, which strive to maintain the size and diversity of the peripheral T-cell pool in the face of decreased influx of diverse new T-cells.

Despite the fact that thymus involution begins early in life, it is only in old age that homeostatic mechanisms falter and allow dysbalance amongst T-cell subsets.

A marked difference between the adult and old lymphocyte T-cell compartment is an age-related decrease in representation of naïve phenotype T-cells and concomitant increase in frequency and numbers of memory phenotype T-cells. The exact mechanisms leading to this population shift were not formally dissected, but are believed to likely involve a combination of 1) decrease in naïve T-cell production, 2) their conversion into effector or memory cells as a result of encounters with pathogens, and 3) changes in the environment, including the availability of homeostatic cytokines (IL-7, IL-15, IL-2). For example, IL-2 production by CD4 T-cells is decreased in old mice(Gillis et al. 1981; Miller and Stutman 1981; Thoman and Weigle 1981). Less is known about age-related changes in IL-7 or IL-15 levels or the expression and function of their receptors on different T-cell subsets. In addition, the naïve T-cell pool could be indirectly affected by a growing pool of memory T-cells that may compete with naïve T-cells. Considering that there is some overlap in the use of survival and maintenance cytokines by these two pools, particularly in case of IL-7(Fry, Mackall 2005; Tan et al. 2002), it is possible that the two are not always independently regulated, particularly in aging where there is many fewer naïve T-cells. Thus, if naïve T-cells continue to decrease in number, this may lead to an excess of survival and maintenance cytokines which normally would have been consumed by naïve T-cells. This could trigger homeostatic proliferative expansion (HPE) of the remaining naïve T-cells and drive their conversion to memory-phenotype. This was demonstrated in mice under lymphopenic conditions (Cho et al. 2000; Goldrath et al. 2000), and strongly suggestive results were also obtained in aging monkeys (Cicin-Sain et al. 2007) and humans (Naylor et al. 2005).

2.3 T-cell Clonal Expansions (TCE)

One of the hallmarks of immune aging is loss of TCR repertoire diversity (rev. in (Nikolich-Žugich 2005)), due in large part to the dominance of memory T-cells over the naïve ones. However, on top of that reduction, the CD8 T-cell compartment often shows additional loss of diversity, in the form of large, often clonal expansions of T-cells bearing the same TCR, named T-cell clonal expansions (TCE) (Callahan et al. 1993; Hingorani et al. 1993b; Posnett et al. 1994). Development of TCEs has been documented across mammalian species, including rodents, nonhuman primates, and humans, with fractions between 30 and 60% of individuals surveyed exhibiting one or more age-associated TCE (rev. in (Nikolich-Žugich, Messaoudi 2005). More on the biology of TCE can be found in the excellent review by Clambey and Marrack elsewhere in this book. However, for the purpose of this chapter, it is most pertinent to classify TCE into at least two types with respect to the mechanism of their generation and/or maintenance. Large Ag-independent TCE (AI-TCE) are thought to arise and/or be maintained independently of antigenic stimulation, due to age-

related changes in perceiving homeostatic signals. This is based upon: (i) activation marker expression on these cells, which dominantly exhibit central memory phenotype, with no evidence of recent or repeated antigen-driven activation (Callahan et al. 1993; Ku et al. 2001; Messaoudi et al. 2006c); (ii) cytokine receptor, specifically IL-7R and IL-15R, expression, which is higher on these cells compared to other memory or naïve T-cells (Messaoudi et al. 2006c); (iii) the ability of these cells to proliferate upon adoptive transfer (Ku et al. 2001), with a constant rate regardless of whether the recipient is lymphopenic or not (Messaoudi et al. 2006c); and (iv) the ability of manipulations that induce lymphopenia to increase the incidence and accelerate the onset of development of AI-TCE (Messaoudi et al. 2006b). While these results have been obtained in mice, there is evidence that similar fundamental principles are at work in primates, including humans (Cicin-Sain et al. 2007; Naylor et al. 2005). In contrast, TCE that have general characteristics consistent with the response to antigen, also called Ag-reactive TCE (AR-TCE), were linked to latent persistent herpesviral infections in mice (Holtappels et al. 2000; Karrer et al. 2003; Podlech et al. 2000) and humans (Almanzar et al. 2005; Fletcher et al. 2005; Ouyang et al. 2003c; Pawelec et al. 2004). Broad discussion of these virus-related abnormalities is also presented in other chapters of this handbook.

TCE can occupy up to 90% of the total murine and up to 50% of the human memory CD8 T-cell pool. TCE themselves are not malignant and do not affect the overall size of the CD8 T-cell pool (there is no increase in total T-cell numbers in individuals carrying TCE). However, TCE do disturb T-cell homeostasis and diversity (Callahan et al. 1993; LeMaoult et al. 2000; Posnett et al. 1994) and a drastic disturbance of this type can be expected to impair the ability to mount T-cell responses. While T-cell responses are plastic, with a significant reserve that allows T-cells to respond to pathogens despite loss of much of the repertoire, this plasticity is not unlimited (rev. in (Nikolich-Žugich et al. 2004). However, we still do not have precise quantitative understanding of limits of T-cell diversity necessary to mount protective responses against pathogenic challenge, an issue highly relevant from the standpoint of evaluating the impact of TCE upon immune defense.

3 Impact of TCE on Pathogen Resistance—the Mouse Model

The most important question related to the presence of TCE is related to their impact upon the health of the organism. One could envision several possibilities in that regard. First, TCE could be neutral and not impact the overall health or the immune defense of the old organism. While this possibility is intellectually unexciting, it is likely that many TCE coexist with the state of health based on their high incidence in asymptomatic individuals (Hingorani et al. 1993b; Posnett et al. 1994). Indeed, it is likely that a TCE needs to grow to a certain size before it becomes a problem for its bearer. Second, TCE could affect other components of the organism, without impacting immune defense. While this is possible, this scenario had not been

documented so far and will not be further discussed here. Third, TCE could have an active effect, whereby they would secrete cytokines and other short-acting mediators that could alter the function of other components of the immune (and other) systems in the body. This would be akin to the functional shift seen in replicatively senescent fibroblasts, which upon cessation of replication drastically change their secretory properties and have the potential to alter extracellular matrix, neovascularization and other microenvironmental properties (rev. in (Campisi 2002). At the present, there is some evidence in support of this possibility (Ortiz-Suarez and Miller 2002; Ortiz-Suarez and Miller 2003), but more precise studies at the level of isolated, highly purified TCE are needed. Moreover, the impact of the observed changes upon pathogen resistance remains untested.

Finally, the role of TCE could be passive, but nevertheless negative. Under that scenario, which was invoked by immunologists before (Callahan et al. 1993; Hingorani et al. 1993a; Posnett 1994 #1976), and which will be discussed in more detail as it currently appears the most likely, these accumulating T-cell clones would constrict the repertoire and reduce the useful T-cell repertoire that defends us against new infection. Mechanistically, this would most likely occur by these cells gaining a survival/maintenance advantage over other T-cells in the body. The fact that TCE which occur spontaneously in SPF mice express high levels of IL-7Rα and IL-2/15Rβ (Messaoudi et al. 2006c) is consistent with the possibility that TCE operate as IL-7 and/or IL-15 "cytokine sinks", taking them slowly away from other T-cells. Consistent with that, we (Lang et al. submitted) and others (Ely et al., 2007) have recently found that often TCE can arise from the pool of cells that respond(ed) to prior acute or latent infection. Of interest, once these cells begin to significantly expand in old age, they tend to acquire high levels of IL-7 and IL-15 receptors (Lang et al. submitted), raising the possibility that the "cytokine sink" may be the unifying mechanism by which both "spontaneous" and antigen-specific large TCE constrict the remainder of useful T-cell repertoire. In fact, it is likely that the "spontaneous" TCE designation simply covers up the fact that we don't know the original antigen that was recognized by these cells, and that may be irrelevant if indeed these cells primarily respond to cytokines once they become TCE.

In order for a TCE to have a demonstrably negative effect upon immune defense via TCR repertoire constriction, such a TCE needs to sufficiently erode the numbers and diversity of other T cells needed to respond to a new pathogen. Numerous studies have shown that manipulations which take away up to half or more of TCR diversity are reasonably compatible with T-cell responsiveness (rev. in (Nikolich-Žugich et al. 2004). However, in other models losses of this or greater magnitude have been shown to impair responsiveness to certain antigens (rev in. (Nikolich-Žugich et al. 2004) and references therein). In terms of the impact of TCE upon the residual diversity of aged naïve T- cells in relationship to immune defense against infectious diseases, it is important to consider the overall diversity and overall numbers of T-cells involved in a typical response to a pathogen. Exciting new studies with direct measuring of precursor T-cell frequencies concur that on the average a hundred, and in some cases as few as 15-20 CD4 or CD8 T-cells may

be responding to a single epitope (Badovinac et al. 2007; Moon et al. 2007). Even if this is an underestimation, reducing that number by 90%, or even by half, due to the presence of a TCE, certainly has the potential to diminish and cripple the response to epitopes where few T-cell precursors exist. This low responsiveness would be further compounded by an already diminished overall reserve of naïve T-cells in aging, as well as by the blunted T-cell signaling (Tamir et al. 2000). On the other hand, most pathogens present multiple epitopes to the immune system, and even if one accounts for immunodominance, usually a handful of epitopes are available for T-cell stimulation. Moreover, in many cases other arms of the immune system will synergize to provide protection even if T-cell responses are diminished. Thus, for a TCE to impact pathogen resistance, T-cells have to provide primary and nonredundant protection against that pathogen, the pathogen should have few, rather than many, immunodominant and protective epitopes and frequency of T-cells specific for these epitopes should be low. In the one case where the impact of TCE upon immune defense was tested (Messaoudi et al. 2004), most, if not all, of the above conditions were met. In that study, resistance to herpes simplex virus (HSV-1) was studied in B6 mice, where an octamer derived from the glycoprotein B accounts for > 90% of the total CD8 T-cell response (Dyall et al. 2000; Messaoudi 2001 #1644; Wallace et al. 1999). Moreover, the response itself is highly restricted with regard to TCRV region utilization (with Vβ10 and 8 contributing >80% of the response (Cose et al. 1995)). Old animals with and without TCE were challenged with HSV and magnitude and functional characteristics of the response measured. It was found that TCE could impair the generation of productive responses in a selective manner. So, when an animal contained a large TCE which expressed Vβ10 and 8, it was unable to mount a response to HSV gB, whereas TCE expressing other TCR Vβ segments did not impair responsiveness beyond the reduction seen due to age in a littermate control group (Messaoudi et al. 2004). These results were somewhat puzzling and suggested that TCE preferentially competed out against the T-cells bearing the same TCRVβ segment. This could be explained, for example, if TCRVβ residues conserved within the Vβ family but differing between Vβ families (e.g. CDR1 & 2 and "framework" parts of CDR3) were important in contacting self-pMHC complexes in the course of trophic interactions needed for T-cell maintenance, so that a TCE would compete out naïve T-cells of the same TCRVβ family. Such a mechanism remains to be substantiated. Nevertheless, the above study (Messaoudi et al. 2004) does show that TCE can potentially impair protective immunity.

While the above experiments were performed with spontaneously arising TCE, which were most likely AI-TCE, there is no reason to believe that a similar situation may not exist with AR-TCE as well. Our group is in the process of testing this possibility. Another unaddressed question relates to the impact of TCE upon memory responses. Memory T-cells are more difficult to compete out than naïve T-cells, possibly due to their ability for self-renewal and relative resistance to apoptosis. Perhaps the most pertinent question is whether TCE can affect the response to latent and/or chronic persistent pathogens, where a large fraction of the immune system is periodically or continuously stimulated by these pathogens. At the present, this issue remains unresolved.

4 Impact of TCE on Pathogen Resistance—Evidence from Humans

In reviewing the known impact of TCEs on pathogen resistance, one needs to distinguish between two parameters: 1) correlation of presence of TCE with presence of other immunological factors known to impair immune responses, and 2) direct evidence for impact of TCE on pathogen resistance. The occurrence of TCEs has been well documented in patients and in a variety of animal models, so we shall first review that scenario. One should bear in mind, however, that it is often difficult to distinguish the specific effect of TCE from the effects of old age-associated defects in antipathogen immunity, since in most cases TCEs are detected only in advanced age. It is therefore most appropriate to evaluate TCE as a superimposing, possibly aggravating factor that may, or may not, further impair protective immunity in an already suboptimal setting of an old organism.

Some TCEs have known antigenic specificity. Two types of conclusions on the effects of these TCEs on pathogen resistance can be drawn: 1) effect upon resistance to the pathogen the TCE is specific for, and 2) effect upon resistance to unrelated pathogens. In humans, the most commonly documented cases of TCEs of known specificity involve memory CD8 T-cells specific for CMV (rev. in (Pawelec et al. 2004)) and, to a lesser extent, EBV (Ouyang et al. 2003b). Original studies documented the presence of $CD28^{-}$ $CD8^{+}$ TCEs in elderly patients (Hingorani et al. 1993b; Posnett et al. 1994). With the advent of tetramers and intracellular cytokine staining techniques that allowed enumeration of Ag-specific T-cells, it was shown that the CD28- CD8 T-cell expansions were frequently specific for CMV and were clonal or oligoclonal in nature (Ouyang et al. 2002). Moreover, longitudinal studies in the Swedish elderly cohorts concluded that CMV seropositivity, together with an array of additional immune characteristics such as the inverted CD4:CD8 ratio and poor proliferative responses of T-cells to mitogens, constitute an immune risk phenotype (IRP, discussed in detail elsewhere in this book) (Wikby et al. 2005), which predicted mortality within 2 years in octogenerians of the Swedish cohort (Hadrup et al. 2006). It will be important to reproduce these results in genetically diverse populations of the elderly, particularly in light of early reports that the elderly from West Sicily may not show the same effect (Colonna-Romano et al. 2007). Moreover, it is not clear exactly how the presence of CMV-specific TCE might affect pathogen resistance, in isolation from the other IRP-associated defects, highlighting one of the problems inherent to the otherwise highly informative human longitudinal studies.

At the present, there is some evidence that CMV-specific T-cells may themselves be compromised as a direct result of development of TCE. Several studies demonstrated accumulation of dysfunctional CMV-specific memory CD8 T-cells in the elderly (Ouyang et al. 2003a; Ouyang et al. 2003c; Ouyang et al. 2004). In addition, the large CMV-specific memory cell population expressed a marker of replicative senescence, KLRG-1, and its expression correlated with decreased production of IFNγ upon antigenic stimulation (Ouyang et al. 2004). The key question is whether this leads to inability to mount an adequate functional response to viral reactivation, permitting viral replication above the subclinical level normally associated with

CMV seropositivity. In that regard, one study (Stowe et al. 2007) demonstrated the presence of CMV and EBV DNA in urine (CMV) and blood (EBV) of elderly patients, as opposed to the seropositive adults, implying some loss of control of viral reactivation in the elderly. Consistent with that explanation, these authors also found elevated expression of lytic and latent EBV genes in blood of elderly but not adult seropositive patients (Stowe et al. 2007). It is possible that accumulation of dysfunctional CMV- or EBV-specific TCE, which were unable to control the virus, may be the reason for increase in viral reactivation in aging. However, in that study, the elderly actually had an elevated frequency of IFNγ-producing CMV- and EBV-specific memory CD8 T-cells, making the hypothesis unlikely. Moreover, CMV-mediated disease does not seem to be associated with aging in the absence of iatrogenic or acquired immune suppression, suggesting that a manifest loss of CMV control does not occur in the elderly. Further studies are needed to decisively address the role of accumulation of dysfunctional CMV-specific TCEs on the persistent latent Herpes virus control in old age.

A separate issue is whether CMV-specific TCE affect immunity to other infections in humans, and how. There is some evidence that presence of CMV-specific TCE is associated with lower frequency of memory CD8 T-cells specific for coresident EBV infection (Khan et al. 2004). This study did not examine whether control of latent EBV in patients with large CMV-specific TCE is impaired. While one could speculate that the T-cell response, and therefore immunity to EBV will be compromised in patients with large CMV-specific TCE similar to the results seen in mice with the effect of spontaneous TCE upon HSV immunity (Messaoudi et al. 2004), the mechanism by which these TCE affect the size of the EBV memory CD8 T-cell pool is currently unknown.

Since many of the TCEs identified in humans are specific for CMV, it was proposed that CMV is the main driver behind generation of TCEs (Pawelec et al. 2004). While this may be the case, evidence from murine studies suggests that virus-specific TCE can also develop independently from ongoing antigenic stimulation. Ely et al. (Ely et al. 2007) detected presence of TCE specific for Sendai virus and flu in old mice that had been infected as adults. Similarly, we have found that old mice infected with WNV at a young age developed expansions of virus-specific memory CD8 T-cells in old age (A Lang et al. submitted). In a different infection model, we found that following localized (ocular) HSV-1 infection, mice develop expansions of HSV-specific memory CD8 T-cells once they reach old age. This process was unlikely to be caused by viral reactivation, as mice treated continuously with antiviral drugs also developed these age-associated T-cell expansions. At present, only a small number of these antigen-independent age-associated expansions were confirmed to be clonal, with oligoclonality being seen more often (A Lang et al. submitted). Unlike is the case with CMV-specific TCEs, the T-cell expansions that developed independently from ongoing antigenic stimulation were fully functional, showing excellent correlation of percentage of tetramer$^+$ and IFNγ$^+$ cells (A Lang et al. submitted). Therefore, it is not likely that development of TCEs by this mechanism will affect immunity to the cognate pathogen. Additional studies will be required to determine whether TCEs can develop from

preexisting memory CD8 T-cells specific for nonpersisting pathogens in elderly humans, as they do in old mice.

Are these TCE impairing productive immunity in humans? Of interest, the number of influenza-specific memory CD8 T-cells was shown to decline with age in humans (Goronzy et al. 2001). This phenomenon was independent of the patients' CMV status. In another study of success of flu vaccination in CMV-seropositive patients, CMV seropositivity correlated with impaired response to vaccination (Saurwein-Teissl et al. 2002). However, the authors did not delineate whether this correlates best to the presence of TCE, to the overall decrease in number of naïve cells or to proliferative/replicative senescence, and, as with most clinical studies, the mechanism responsible for this outcome has not been resolved. Therefore, the presence of TCE could be one of the useful biomarkers predicting poor outcome of flu vaccination (Goronzy et al. 2001; Saurwein-Teissl et al. 2002), or perhaps even general immunological vulnerability, but that requires further and rigorous verification in larger and heterogeneous populations of human subjects.

5 Concluding Remarks, Challenges and Questions

It follows from the above discussion that much remains to be learned about the biology of TCE and their precise impact upon resistance to infectious diseases. Drawing generalized conclusions about the impact of TCEs on pathogen resistance from the available data is often difficult, since they come from a number of different experimental models. At present we do not know how closely the mechanisms of generation of TCEs and their subsequent effects on pathogen resistance compare between them. However, the models and the reagents that are currently available provide good tools to systematically address the questions that still remain regarding the impact of TCEs on immunity. In particular, new quantitative tools are becoming available allowing us to precisely dissect the breadth and the reserve of T-cell receptor repertoire and the size of precursor populations specific for immunodominant epitopes of various pathogens, and that should allow us to quantitatively evaluate to what extent is TCR repertoire constricted by different types of TCE, and to determine what type of intervention (many of which are now in clinical trials (Zuniga-Pflucker, van den Brink 2007)) could be applied in individual situations.

Overall, the most important practical issues related to TCE and the infectious diseases of the elderly are:

1. Which groups of elderly are at an increased risk of infection and which are not? Are TCE a risk factor in that regard?
2. For those groups that are at risk, can they be helped with the existing vaccines or do they need alternate ways of immunostimulation? Can TCE be removed or shrunken?
3. If immunostimulation is to be attempted in a targeted manner, which modes of immunostimulation are the most efficacious? Different vaccination regimens, additional costimulation or cytokine treatments?

4. For those where immunostimulation may be insufficient, is T-cell rejuvenation the best option?

Answering these questions will undoubtedly be rewarding for scientists and physicians,as well as to the growing populations of elderly around the world.

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