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9.1 Introduction

A hallmark of aging is a decline in immune function, resulting in increased susceptibility to infection and reduced vaccination efficacy. For example, elderly humans respond poorly to influenza virus infection and suffer increased morbidity and mortality. The elderly also respond poorly to vaccines, for example, for influenza virus, and fail to generate high titers of neutralizing antibodies (Vu et al. 2002) and efficient cytolytic CD8⁺ T cells (McElhaney et al. 2006, 2009). In addition, previously established memory can be disrupted during aging. Many factors impact immune dysfunction, including age-associated changes in innate cells, B cells, and T cells (Miller 1996; Solana et al. 2006; Agrawal et al. 2008; Gibson et al. 2009). These include age-associated increases in numbers of T-regulatory (Treg) cells, impaired T cell function, and repertoire perturbations in both mouse and human (Nishioka et al. 2006; Lages et al. 2008; Jiang et al. 2009; Pawelec et al. 2010; Goronzy et al. 2007; Cicin-Sain et al. 2010; Ahmed et al. 2009). As it is believed that a diverse T cell repertoire to allow a broad polyclonal response to pathogens

is essential for strong cellular immunity (Yewdell and Haeryfar 2005; Messaoudi et al. 2002; Kedzierska et al. 2005), declining repertoire diversity is strongly implicated in impaired immunity associated with aging, which is the focus of this chapter.

Much of our understanding of the impact of aging on immunity has come from the experimentally amenable mouse model. Mice display key characteristics of age-associated decline in immune function that have been described in human and other models. The mouse model allows longitudinal studies and direct determination of the impact of age on the ability to respond to infection with a variety of pathogens (Murasko and Jiang 2005; Ely et al. 2007b). For example, the mouse influenza virus model is frequently used to examine the impact of aging on vaccination and immunity (Po et al. 2002; Effros and Walford 1983). The basic observation is that aged mice are more difficult to vaccinate and are more susceptible to influenza infection, often succumbing to doses that are nonlethal for young mice. Aged mice are also impaired in their ability to clear infectious virus. Although effector cells in aged mice have been shown to be highly functional on a per cell basis, they are fewer in number as a consequence of impaired T cell proliferation (Effros et al. 2003). In addition, there are profound repertoire perturbations (Yager et al. 2008; Li et al. 2002; Jiang et al. 2011; Decman et al. 2012; Valkenburg et al. 2012).

Several factors affect the diversity of the T cell repertoire in mouse and human. First, there is

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progressive thymic involution with age, resulting in export of fewer naïve T cells. Second, with increasing antigen experience, the memory pool progressively expands, and there is a steady reversal of the naïve-memory ratio over time, with the memory pool eventually dominating the peripheral repertoire. Third, the memory compartment is further perturbed by the frequent appearance of T cell clonal expansions (TCEs) in the memory CD8⁺ T cells. In humans, chronic infection with cytomegalovirus (CMV) drives the development of TCE and is strongly associated with impaired immunity. Accumulating data from studies in mouse, primate, and human support the role of repertoire perturbation in immune dysfunction associated with aging, which have directed experimental approaches to reverse immunosenescence. While it is likely that there are many contributing factors to the decline of T cell function in aging, increasing evidence suggests that a major controlling factor is the reduction in the diversity of the antigen-specific repertoire of T cells, which will be the focus of this chapter.

9.2 Influence of Age on the T Cell Repertoire

As discussed above, aging is associated with decreased T cell repertoire and it has been hypothesized that decreased repertoire diversity is associated with impaired immune function. There are constraints in both the naïve and memory T cell repertoires with age.

9.2.1 Impact of Aging on the Naïve T Cell Repertoire

In young adults, T cells in the periphery are largely naïve, with a highly diverse repertoire. The mouse is estimated to have $\sim 1\text{--}2 \times 10^8$ T cells expressing $\sim 2 \times 10^6$ different specificities, suggesting that there are ~ 50 naïve T cells of each specificity (Casrouge et al. 2000). Humans are estimated to have $\sim 3 \times 10^{11}$ T cells with $\sim 10^8$ different specificities, suggesting that the clone size for each specificity in human is $\sim 1,000$ (Arstila et al. 1999; Goronzy and Weyand 2005).

T cells mature in the thymus and seed the periphery with naïve T cells. With age, the thymus undergoes atrophy, which results in loss of thymic epithelial cells and decreased production of new, naïve T cells (Taub and Longo 2005; Heng et al. 2010). Since maintenance of the naïve pool is dependent on homeostatic proliferation in the periphery, the absence of new T cells from the thymus results in a decline in numbers and loss of diversity of naïve T cells. Importantly, the homeostatic maintenance is not random, but becomes biased toward cells capable of higher rates of homeostatic proliferation and of higher T cell receptor (TCR) avidity (Rudd et al. 2011b). With aging, the naïve T cells become more “memory-like” and the repertoire becomes more focused. In addition, the proportion of naïve-memory T cells decreases with increasing antigen experience and increasing age.

It has been shown that the number of naïve peripheral T cells declines with age. The diversity of the T cell repertoire has been measured both by genetic approaches (spectratyping, which assesses T cell receptor CDR3 length) and by directly counting antigen-specific naïve T cells. Spectratyping and sequence analysis of naïve CD8⁺ T cells isolated from young and aged mice showed that aged mice had skewed spectratype profiles indicative of reduced diversity (Ahmed et al. 2009). In addition, unexpected sequence repeats in naïve T cells from aged mice suggested dysregulation in the normal homeostatic mechanisms that maintain diversity in young animals. This analysis was carried out in specific pathogen-free “naïve” mice and was not associated with the presence of clonal expansions in the CD8⁺ memory pool or with chronic infection.

More recently, the decline in the size of antigen-specific naïve pools has been measured directly using the tetramer pull-down assay (Moon et al. 2007; Obar et al. 2008). In one report, the number of naïve CD8⁺ T cells specific for a herpes simplex virus (HSV)-1 glycoprotein B epitope showed a decline in frequency from ~ 400 in adult, unprimed mice to ~ 125 in 22-month-old mice (Rudd et al. 2011b). Interestingly, the constriction with age was accompanied by the emergence of dominant clonotypes shared in individual

aged mice, indicative of selective rather than stochastic mechanisms (Rudd et al. 2011a). In another study, the naïve repertoire was analyzed in aged and young mice, as well as herpesvirus-infected aged mice. The data showed that there was a reduction in epitope-specific naïve precursors to ovalbumin (OVA) expressed in the context of *Listeria monocytogenes*, determined by tetramer pull-down experiments in aged compared to young mice, which was further reduced in mice infected with murine CMV (Smithey et al. 2012). The impact of CMV infection on repertoire will be discussed in detail below.

Studies in humans have shown that the decline in diversity of the naïve T cell repertoire with age is not linear. In the case of the CD4⁺ T cell repertoire, it has been shown that diversity was maintained relatively stably at $\sim 2 \times 10^7$ different T cell receptor β -chains until age 70, despite extensive loss of thymic function at that age. However, after age 70 the diversity plummeted to 2×10^5 specificities (Naylor et al. 2005). These data suggest that homeostatic proliferation, which maintains the T cell repertoire increasingly as thymic function declines, may have physiological limits, and the proliferative capacity of T cells may become greatly diminished by age 70 (Goronzy and Weyand 2005).

9.2.2 TCE Basic Parameters

One of the most profound changes in repertoire with age is the development of TCE, which are nonmalignant, monoclonal populations of CD8⁺ T cells, but not CD4⁺ T cells. These TCE were first detected in mice, using TCR V β antibodies (Callahan et al. 1993). The expansions varied in size and could be as great as 90 % of the population of peripheral CD8⁺ T cells in aged mice. TCE are found in about 60 % of mice over 2 years old (although the frequency probably varies in different animal facilities) (Clambey et al. 2007). In addition, clonal populations of CD8⁺ T cells in aged humans were identified in approximately one-third of individuals over 65 years of age (Posnett et al. 1994; Clambey et al. 2007). It has been hypothesized that such expansions cause

perturbation of the repertoire due to “crowding out” of naïve and/or memory cells of diverse specificities and thus contributing to impaired immunity associated with aging. Data supporting this possibility are presented below.

Characteristics of TCE have been described in detail (Clambey et al. 2007, 2008). One key characteristic is that most TCE do not require antigen for their generation or maintenance. This is supported by the observations that specific pathogen-free mice develop TCE and that TCEs transferred into β_2 microglobulin-deficient mice maintain their proliferative function. TCE sometimes arise as a consequence of chronic stimulation in mice (Lang et al. 2008). In humans, most TCE are thought to be triggered by infection with CMV, suggesting that they are driven by chronic antigen stimulation (Khan et al. 2002; Ouyang et al. 2003). TCE can also develop from the memory pool specific for viruses that are cleared following an acute infection (Ely et al. 2007a; Kohlmeier et al. 2010; Connor et al. 2012). It is hypothesized that these acute virus-specific TCE are formed as a consequence of slight variations in the rate of homeostatic proliferation of memory cells, in agreement with previous studies assessing rates of basal proliferation of TCE compared with normal memory homeostatic proliferation (Kohlmeier et al. 2010; Connor et al. 2012; Ku et al. 2001, 1997).

Another key characteristic is that TCE are phenotypically heterogeneous and their stability varies. There is considerable variability in the phenotype of TCE perhaps reflecting heterogeneity in the types or origin of TCE. In one study, microarray analysis was used to study integrin $\alpha 4$ -gene expression in TCE and polyclonal memory CD8⁺ T cells. There was variation in expression within individual TCE although levels were stable within a single TCE. Furthermore, clones with high levels of integrin $\alpha 4$ -gene expression were found to be unstable in vivo (Clambey et al. 2008). It has also been shown in the mouse that TCE are frequently unstable, with some clones disappearing in a 2–4-month time frame and new ones developing (LeMaoult et al. 2000). In some studies, TCE have been reported that appear to be a type of central memory cell, expressing higher

levels of CD122 and CD127, components of the interleukin (IL)-15R and IL-7R which regulate homeostasis (Messaudi et al. 2006b).

Are there perturbations in the CD4⁺ T cell repertoire? Although most of the attention has focused on CD8⁺ TCE in CMV-infected aged individuals, high levels of CD4⁺ CMV-specific T cells have been found in healthy seropositive individuals (Sester et al. 2002). It is unclear whether they increase with age and contribute to the immunosenescence (Pawelec et al. 2005). Infection with CMV has been shown to induce the accumulation of late-stage differentiated CD4⁺ T cells in addition to CD8⁺ T cells in middle-aged individuals, although in lower frequencies than CD8⁺ T cells (Derhovanessian et al. 2011). The late-stage differentiation phenotype was CD27⁻, CD28⁻, and with some cells CD57⁺, a putative senescence marker. Higher levels of anti-CMV antibody correlated exclusively with levels of late-differentiated CD4⁺ T cells, perhaps as a consequence of the helper role CD4⁺ T cells play in generating antibody (Derhovanessian et al. 2011). It has also been shown that accumulation of CMV-driven CD4⁺ T cells, rather than CD8⁺ T cells, correlated with poor humoral response to influenza vaccination (Derhovanessian et al. 2012).

There are conflicting reports of the functional capacity of TCE. Whereas *in vitro* studies have shown that TCE can respond in terms of cytokine secretion (Clambey et al. 2007; Ely et al. 2007a), *in vivo* studies of antigen-specific TCE generated from acute virus-specific memory CD8⁺ T cells show a reduced capacity to participate in recall responses. These studies directly compared the ability of antigen-specific TCE to respond to secondary infection in an *in vivo* model with the ability of young, non-expanded memory cells, using a dual adoptive transfer approach in which equal numbers of antigen-specific cells were cotransferred into a young naïve recipient and then challenged with virus. The data show that the TCE generally failed to compete equally with the young memory cells, although some TCE clones had comparable responsiveness (Kohlmeier et al. 2010; Connor et al. 2012).

9.2.3 Role of Chronic Infection in the Development of TCE

How do TCE arise? Early studies in the mouse were carried out in specific pathogen-free mice and were detected using T cell receptor V β antibodies, so that the antigen specificity was not known (Blackman and Woodland 2011). More recent evidence shows that TCE can develop from memory cells derived from previous encounters with viruses that cause acute infections, such as influenza virus and Sendai virus (Ely et al. 2007a; Kohlmeier et al. 2010; Connor et al. 2012). However, the bulk of data in humans show that chronic infections such as CMV are important drivers of TCEs.

CMV is a β -herpesvirus that infects a majority of the world's population. The seroprevalence increases with increasing age. Following acute infection, the virus goes dormant and persists for the life of the individual. Human CMV (HCMV) responses to the initial, acute infection occupy more than 20 % of the total CD8⁺ T cell pool (Sylwester et al. 2005). Initially there is a broad repertoire of CMV-specific cells, but with time the repertoire becomes focused on a restricted number of immunodominant epitopes (Munks et al. 2006; Day et al. 2007), and over time there is a preferential expansion of "inflationary" epitopes, and the human CD8⁺ T cell repertoire becomes dominated by CMV-specific cells (Khan et al. 2002, 2004; Karrer et al. 2003). The clonality of the populations increases with age, perhaps a result of exhaustion and dropping out of many specificities (Hadrup et al. 2006). It is unknown whether the strong repertoire effects are due to the persistence and periodic reactivation of CMV, as reactivation events are clinically silent. With age, large oligoclonal populations of CD28⁻CD8⁺ T cells accumulate in the periphery, with a corresponding decrease in naïve T cells. Over time, there is a further focusing, culminating in dominance by a single specificity with a higher avidity (Schwanninger et al. 2008). It is assumed, but not proven, that other clones disappear with age, due to exhaustion or superinfection (Trautmann et al. 2005; Hansen et al. 2010). CD8⁺CD28⁻ T cells dominate in CMV-infected

elderly individuals (Almanzar et al. 2005; Ge et al. 2002; Appay et al. 2002; Ouyang et al. 2004). CD8⁺CD28⁻ T cells are considered to be senescent T cells, with shorter telomeres, arising from chronic TCR stimulation (Effros et al. 1996; Monteiro et al. 1996). The noninflammatory epitopes maintain a functional, central memory phenotype, whereas the inflammatory epitopes develop an extreme effector memory phenotype. They are strong secretors of tumor necrosis factor alpha (TNF- α) and interferon gamma (IFN- γ), they maintain lytic capacity, and they are not exhausted, as has been described for LCMV (Klenerman and Dunbar 2008; Snyder et al. 2008; Waller et al. 2008). CMV not only acts through effects on T cells but also impacts proinflammatory status, as viral gene products upregulate production of IL-6, IL-1, TNF- α , IFN- γ , and a variety of chemokines (reviewed in (Varani et al. 2009)).

TCE can also arise as a consequence of chronic infection of the mouse. For example, HSV-1 was shown to elicit an acute response, which contracted but then exhibited memory inflation and an accumulation of antigen-specific cells. However, continuous treatment with an antiviral drug that prevented viral replication did not prevent the inflation, showing that continual viral replication was not required for the development of TCE (Lang et al. 2008). There is such a strong association between CMV and senescence that it has been proposed that immunosenescence is “infectious” (Pawelec et al. 2005) (reviewed in (Pawelec et al. 2009)). CMV drives the development of CD8⁺CD28⁻ dysfunctional T cells and is strongly implicated in the immune risk phenotype (Wikby et al. 2002).

9.2.4 Is CMV Unique, or Will Other Chronic Infections Induce TCE?

The strong association between CMV, TCE, and immunosenescence has raised the question as to whether there is something unique about CMV infection or whether any persistent virus can induce the same effect. Although Epstein-Barr

virus (EBV) is also a persistent herpes virus, it is not as strongly associated as CMV with accumulations of memory cells and development of TCE (Khan et al. 2004). Although the frequency of CMV-negative EBV-positive individuals is low, making an analysis of the impact of EBV in the absence of CMV is difficult, EBV memory cells have been shown to accumulate in some CMV seronegative individuals, and TCE to EBV have been demonstrated at reduced frequencies compared with CMV-specific TCE (Colonna-Romano et al. 2007; Ouyang et al. 2003; Vescovini et al. 2004). In addition, important phenotypic differences between CMV- and EBV-induced T cells have been identified. CMV drives differentiation of CD8⁺ T cells to a highly differentiated phenotype-effector memory (CCR7^{null}CD45RA^{low}) or effector (CCR7^{null}CD45RA⁺)⁻ and to become CD28⁻, a phenotype associated with dysfunction and immunosenescence, whereas this was not the case of EBV-specific T cells (Derhovansian et al. 2011), which maintained expression of CD28 (Pawelec et al. 2005). Although CMV and EBV elicit TCE, it appears that not all chronic viruses can do this. For example, infection with HSV, another persistent virus, does not result in clonal expansions in CMV-negative aged humans (Derhovansian et al. 2011). In contrast, the virus does cause TCE in mice (Lang et al. 2009). One possible reason for the disparate results is that the mouse model employs systemic infection, so is different from naturally occurring infections in humans and may be more similar to CMV in terms of dissemination and latency reservoirs.

The reason that CMV apparently preferentially induces TCE is not entirely understood. One key feature of CMV is that, although infections with the three herpes viruses studied in detail (CMV, EBV, and HSV) are all persistent, the site of latency establishment differs. CMV establishes a latent infection in myeloid progenitor cells and monocytes that can serve as antigen-presenting cells and, in addition, there is a low-level chronic infection in epithelial cells of the salivary gland of the liver that may serve to continually present antigen and drive CD8⁺ T cells. In contrast, HSV

resides in neurons, hidden from the immune system, reappearing only occasionally during reactivation events. Another possibility is that CMV has higher rates of reactivation, but this is difficult to assess since reactivation is asymptomatic (Derhovanessian et al. 2011). Yet another explanation may be that there is a greater degree of cross-reactivity in CMV compared to EBV-specific cells. Finally, it may be due to the nature of the primary infection, which has been understudied due to its asymptomatic nature.

9.3 Impact of Age-Associated Changes in T Cell Repertoire on Immunity

The T cell repertoire changes discussed above constitute a key contributing factor to age-associated immune dysfunction. Constriction of the naïve T cell repertoire results in impaired immunity to new infections in the elderly. In addition, perturbation over time in the memory T cell repertoire can impact protective immunity and the ability to generate recall responses to previously encountered infections.

9.3.1 Impact of Reduced Naïve Repertoire Diversity on Immunity to New Infections

As discussed, there is a reduced naïve repertoire with age. Several studies have defined an approximate 10-fold decline in precursor frequency of epitope-specific T cells in aged compared with young mice (Rudd et al. 2011b; Smithey et al. 2012). Constriction in the naïve repertoire with age is due to diminished thymic function and the generation of fewer naïve T cells, coupled with increasing antigenic experience, and/or the development of TCE resulting in a shift in the ratio of naïve to memory T cells in the periphery. Reduction in diversity of the naïve T cell repertoire profoundly impairs the ability of aged individuals to respond to new infections. This has been extensively documented in both mouse and human. In some, but not all, cases, the immune

dysfunction has been directly linked to the development of TCEs, which can dilute out the diversity of the naïve repertoire. Below we will discuss the impact of reduced naïve repertoire diversity, with or without a direct link to TCEs, on immunity to new infections.

Examination of the age-associated constriction of the naïve CD8⁺ T cell repertoire in the absence of large TCEs and the impact on immune function to a new virus infection was carried out in the mouse influenza virus model. Although comparable numbers of CD8⁺ T cells were elicited in the lung airways following primary influenza virus infection in young and aged mice, most, but not all of the aged mice showed perturbations in the repertoire of responding cells (Yager et al. 2008). There was a preferential decline in the response to the immunodominant epitope from the influenza virus nucleoprotein (NP), which varied in individual mice. In some cases there was a failure to respond to the NP epitope, with a shift to other epitopes, indicating a “hole” in the repertoire. In addition, in aged mice that retained the ability to respond to NP, the prototypical T cell receptor V β usage was perturbed. Development of the repertoire perturbations were accelerated in thymectomized mice, consistent with a decline in naïve T cell repertoire diversity with aging. Importantly, the decline in response to NP correlated with impaired protective immunity to challenge with heterosubtypic influenza virus that bypasses humoral immunity and is dependent on CD8⁺ T cell protection. In this study, efforts were made to eliminate mice that exhibited large TCE from the study.

It has also been shown that CMV-infected individuals have impaired immunity to other infections. For example, CMV infection impairs immunity to a coresident EBV infection in old age (Khan et al. 2004, 2002). Although CMV is associated with the development of TCEs, the immune dysfunction has not always been directly correlated with the presence of TCEs. Because of difficulties studying the impact of a chronic infection longitudinally in humans, many experiments have been carried out with the mouse virus, murine CMV (MCMV). The MCMV virus is well characterized and is considered an

appropriate model for human CMV infection (Cicin-Sain et al. 2012). MCMV-infected mice showed inflation of CMV-specific memory cells, reduced numbers of naïve CD8⁺ T cells in the periphery and a perturbed repertoire assessed by T cell receptor V β analysis. Importantly, there was also impaired immunity to new infections with influenza virus, HSV-1, and West Nile virus in the CMV-infected, aged animals. This effect was specific for CMV, as it was not seen in aged mice chronically infected with HSV-1. Further experiments with mutant CMV viruses that lacked key immune evasion genes still caused the impairment in immune function. This impaired immunity to new infections corresponded to an observed reduction in diversity of the naïve repertoire (Smithey et al. 2012). In a related study, Mekker et al. showed that challenge infection with LCMV was more profoundly impaired in MCMV-infected mice compared with aged or thymectomized mice (Mekker et al. 2012). However, in contrast to the Smithey study mentioned above, this did not correlate with reduced numbers of naïve T cells specific for CMV (although the authors did not specifically examine the repertoire of naïve T cells), as there were similar naïve T cell numbers in MCMV-infected and noninfected mice (Mekker et al. 2012). In the Mekker study, the authors concluded that rather than reduction in the naïve repertoire, MCMV infection impairs immunity due to the increased competition between MCMV-specific memory and a “de novo” immune response in aged individuals (Mekker et al. 2012).

In other studies, the presence of TCE has been directly correlated with declining immunity to new infections. They have been shown to dilute out the repertoire diversity of naïve and other memory T cells, which results in impaired immune function. In addition, TCE may be dysfunctional and have a direct effect on immune responses. Early correlative data in humans showed that individuals with TCE have an impaired ability to respond to influenza vaccination, in terms of both humoral immunity and cellular immunity (Saurwein-Teissl et al. 2002; Trzonkowski et al. 2003; Goronzy et al. 2001; Xie and McElhaney 2007). Also, the presence of

TCE predicted poor control of HIV, resulting in increased progression to AIDS (Sinicco et al. 1997).

In mice, it was directly demonstrated that TCE impair the ability to respond to a new pathogen (Messaoudi et al. 2004). Specifically, the response to HSV is known to be focused exclusively on a single epitope and dominated by T cells bearing V β 8 or V β 10. The study showed that a TCE in either of those two V β families corresponded with a poor response to HSV infection (Messaoudi et al. 2004). Development of immunity to *Listeria monocytogenes* in young and old mice was comparable in terms of numbers, but yet aged mice were inferior in terms of protection (Smithey et al. 2011).

Similar results have been observed in old rhesus monkeys. Old primates have been shown to develop TCEs with corresponding reductions in naïve T cells (Jankovic et al. 2003; Pitcher et al. 2002; Cicin-Sain et al. 2007). It was shown that aged monkeys with reduced proportions of naïve T cells (assessed by phenotyping) and expressing TCEs (determined by spectratyping), responded poorly to vaccination with the modified vaccinia strain Ankara (Cicin-Sain et al. 2010). Thus, the correlation between age-associated reduction in naïve repertoire diversity and poor immunity to new infections observed in mouse studies was confirmed in primate studies. These data in experimental models support the correlation between reduced repertoire diversity and impaired response to new infections and vaccination observed in elderly humans.

9.3.2 Impact of Reduced Memory Repertoire Diversity on Memory Maintenance and Recall Responses

Perturbation of the memory repertoire with age can also have profound effects on protective immunity generated to previous infections. The repertoire of memory cells is reduced during aging due to the focusing of certain clones and presumable loss of others and/or the development of TCE. In addition, memory cells may become dysfunctional over time, resulting in impaired recall function.

Previous studies in the mouse have shown that memory generated in young mice retains function, whereas memory generated in aged mice is poor, for both CD8⁺ and CD4⁺ T cell memory (Kapasi et al. 2002; Haynes et al. 2003). These data suggest that function depends more on the age of the naïve cell when it first encounters antigen than on the age of the memory cell when it is restimulated by antigen. This observation potentially explains one of the difficulties associated with successful vaccination of the elderly against new pathogens. Additional studies examined the functional capacity of memory cells generated in young mice with time into old age and showed that memory generated when young actually improved with age, whereas memory generated in aged mice was poorly functional. The data showed a progressive increase in the recall response to secondary pathogen challenge over time that was especially apparent in the central memory population (Roberts et al. 2005).

Recently, it has been shown that memory in aged mice was generated at a comparable magnitude to memory in young mice, although the memory in the aged mice was biased toward an effector memory/senescent phenotype, and protective immunity against viral challenge was impaired (Decman et al. 2010). The aged mice experienced morbidity and mortality after the same challenge dose from which the young mice were protected. This feature of the memory cells was “cell intrinsic,” because the same defects were observed after transfer of memory cells into naïve congenic recipients and challenged. Several possible explanations for the impaired memory discussed by the authors include extended exposure to antigen during the development of memory because of delayed clearance of the initial infection, differences in the inflammatory environment during memory T cell development, and/or differences in the repertoire of T cells that develop into memory in the aged versus young mice.

Despite these data, it has been shown in both mice and humans that previously established memory can deteriorate with age, as a result of clonal expansions and reduced diversity as a consequence of “focusing” of the repertoire. In

the mouse, it has been shown that TCE can develop from the memory population. As previously discussed, in addition to TCE arising as a result of chronic infections, it has been shown that TCE can arise from memory cells established to acute (nonpersistent) viral infections (Ely et al. 2007b; Kohlmeier et al. 2010). Sendai virus- or influenza virus-infected mice developed clonal expansions in their memory pool. The cells retained function as assessed by cytokine secretion, maintained proliferative capacity, and proliferated *in vitro* in the absence of antigen. In addition, TCE transferred into naïve recipients were maintained for several weeks and had high rates of homeostatic proliferation. Further functional analysis, however, showed that the TCEs varied in their ability to mediate a recall response to antigen challenge, three out of the four TCE examined were functionally impaired compared with their non-expanded counterparts in responding to secondary viral challenge (Kohlmeier et al. 2010). The TCE appeared progressively with time, between 320 and 700 days postinfection. More recently it was shown using a more sensitive detection assay that the memory repertoire perturbations first started to develop within months of infection and increased over time into large TCE (Connor et al. 2012). These data suggest that the development of TCEs is a natural outcome of long-term maintenance of memory and is independent of the aging process.

Also, in the mouse LCMV model, it has been shown that the memory T cell response to LCMV is diverse and maintained for long periods of time. However, analysis over time showed that although the magnitude of the virus-specific CD8⁺ T cells was maintained, the distribution of clones within the population changed dramatically. In one of two mice examined, the percentage of a single clone reached 100%! This supports the contention that TCE can arise from memory cells (Bunzmann et al. 2012), as discussed above (Ely et al. 2007a; Kohlmeier et al. 2010; Connor et al. 2012).

A similar focusing of the influenza-specific memory CD8⁺ repertoire was shown in healthy elderly people (Lee et al. 2011). Using a sensitive assay to expand the population of memory

T cells, it was possible to assess responses to both dominant epitopes and subdominant influenza virus epitopes. The data showed that young individuals had a response to dominant epitopes as well as a strong response to subdominant epitopes, which was statistically significantly reduced in healthy elderly individuals, resulting in greatly diminished diversity in the influenza-specific recall response. It was speculated that perturbation of the memory repertoire in T cells specific for a virus that is not a persistent virus could be driven by multiple exposures to influenza virus.

9.4 Therapeutic Approaches

As the human population ages and anticipates an extended lifespan, there has been much emphasis on how to overcome or reverse the impact of aging on immune function. Some strategies are discussed below.

9.4.1 Vaccination Strategies

Aging is correlated with poor vaccination efficacy. Consequently, a major emphasis has been placed on developing better adjuvants or vaccine delivery systems for the elderly (Haynes and Swain 2006; Maue et al. 2009; Coler et al. 2010; Zhu et al. 2010). In addition, it has been shown that vaccination early in life when immune cells are functional and there is a highly diverse repertoire of T cells elicits strong immunity that can last into old age. Thus, early priming leads to long-term maintenance of memory T cells and preserves optimal responses in old age (Valkenburg et al. 2012). For example, in studies where mice were vaccinated at 6 weeks and then challenged at 22 months strong functional T cell responses developed that expressed the repertoire characteristic of a young mouse, whereas vaccination at 22 months had responses of the same magnitude as young mice but with reduced receptor diversity. Also, infection of aged mice with LCMV or influenza resulted in poor protective immunity, whereas if memory was established

when young, protective memory cells persisted (Kapasi et al. 2002), and memory generated in young mice against respiratory viruses actually improved with age (Roberts et al. 2005). These data have supported the idea of increased vaccinations in middle age to establish memory before immune dysfunction limits the ability to respond to new infections. However, there were concerns that there were limitations of “space” for memory T cells, and that previously established memory would be replaced with new memory cells upon new infection. These concerns were somewhat allayed by the report suggested that there is no limit to the size of the memory compartment (Vezyz et al. 2008). However, it was later reported that although the memory compartment can grow in size, function can be lost (Huster et al. 2009), so limitations to memory “storage space” remain a concern.

9.4.2 Thymic Rejuvenation

The fundamental importance of the loss of new naïve T cells as a consequence of thymic involution to impaired immunity in the elderly has prompted attempts to rejuvenate the thymus. Several treatments have been tried with varying degrees of success, including modulation with sex hormones, administration of growth hormone, anti-transforming growth factor beta (TGF- β), IL-7, Flt3L, or keratinocyte growth factor (KGF) (Holland and van den Brink 2009; Lynch et al. 2009; Aspinall and Mitchell 2008; Heng et al. 2010). Recently it has been shown that IL-22 promotes thymic regeneration in mice after stress, infection, or immunodepletion (Dudakov et al. 2012). Maintenance of the naïve T cell pool in mice was shown to be almost exclusively due to thymic output, whereas homeostatic peripheral T cell proliferation was shown to play a key role in maintenance of the naïve human pool (den Braber et al. 2012), suggesting that thymectomized mice may serve as a better experimental model for humans than aged mice (Mackall and Gress 1997). The report showed that peripheral T cell division was responsible for maintaining numbers of peripheral naïve cells, even in young

children with a healthy thymus. However, it was pointed that maintenance of the peripheral repertoire by homeostatic proliferation does not introduce T cells of new specificity, but merely amplifies preexisting specificities. However, with age, the loss of repertoire diversity suggests that homeostatic proliferation is unable to maintain adequate numbers of naïve T cells. Therefore, the thymus may still make an important contribution to maintaining a diverse peripheral repertoire in humans, consistent with the loss of repertoire diversity with age. It has also been shown that thymocyte development relies on a source of progenitors from the bone marrow, which also have been shown to exhibit age-related defects (Waterstrat and Van Zant 2009). New approaches, exploiting new tissue engineering and stem cell technologies and still under development in the mouse and dependent on recently-discovered thymic epithelial progenitor cells, include de novo thymus generation (Heng et al. 2010).

9.4.3 Rejuvenation of Peripheral T Cells

With increasing age, the periphery fills up with exhausted CD28⁻ CD8⁺ T cells. It has been suggested that a possible approach to enhancing immune function in the elderly would be to deplete CD28⁻ CD8⁺ T cells from the periphery to create space for new naïve T cells and functional memory cells. This approach has been successful with systemic lupus erythematosus (SLE) patients (Alexander et al. 2009; Brunner et al. 2010). In the mouse it has been shown that deletion of aged CD4⁺ T cells allowed the repopulation with cells that were functional, even in the aged host (Haynes 2005). These strategies depend on residual thymus function in the elderly.

9.4.4 Calorie Restriction

Studies in rhesus monkey studies showed that calorie restriction (CR) in CMV⁺ animals enhanced maintenance of naïve T cells, which were proliferatively “younger” (excision

circles), had lower levels of proinflammatory cytokines IFN- γ and TNF- β , and had fewer effector memory cells (Messaudi et al. 2006a). Although previous studies in mice and monkeys correlated CR with longer life, a recent 25-year study in rhesus monkeys fed 30 % less than control animals, failed to show a simple correlation, and instead suggested that genetics and composition of the diet predict longevity rather than simply numbers of calories (Mattison et al. 2012).

9.4.5 Targeting CMV

CMV infection strongly impacts human immunosenescence, and is a key characteristic defining the “immune risk phenotype,” which describes progressive changes in the immune system associated with predicted mortality in the elderly. The immune risk phenotype includes, in addition to CMV seropositivity, an inverted CD4⁺/CD8⁺ ratio and a preponderance of CD28⁻ CD8⁺ T cells (Wikby et al. 1998, 2002; Hadrup et al. 2006). Senescent CD28⁻ CD8⁺ T cells are found almost exclusively in CMV seropositive individuals, and 80–90 % of the elderly population are CMV seropositive, making it difficult to separate CMV as the culprit or “an innocent bystander” (Tatum and Hill 2010). Also, elderly individuals with very high levels of CMV-specific serum immunoglobulin had the highest risk of mortality (Roberts et al. 2010). A study of individuals who attained 100 years of age showed that they did not have the hallmarks of the immune risk phenotype, in that they maintained a high CD4⁺ to CD8⁺ ratio and had low numbers of CD28⁻ CD8⁺ T cells. However, most of them were positive for CMV, raising the question of what factors influence the response of individuals to CMV. Is it due to the duration of infection, the frequency of reactivation events, the genetics of the individual, or variation in the proinflammatory profile (Finch and Crimmins 2004)? It is important to note that the immune risk profile predicts mortality independently of health status (Strindhall et al. 2007).

9.4.6 Vaccination Against CMV

The strong association between CMV infection, impaired immunity, and the immune risk phenotype has raised the suggestion that approaches to prevent CMV infection or to control reactivation would improve the life expectancy of the elderly. One approach would be to develop a vaccine to prevent infection. There are two difficulties with this approach. First, it would be difficult to develop a sterilizing vaccine to prevent infection, as these viruses are masters of immune evasion. A more feasible approach would be a therapeutic vaccine that would prevent reactivation events that may be responsible for driving the development of TCEs and immunosenescence, but such a vaccine is currently not on the horizon. Second, it has been suggested that chronic herpes virus infections are beneficial (Barton et al. 2007), and it would be detrimental to vaccinate against them. Taking this possibility into account, it might be better to focus on therapeutic vaccines to be administered in healthy adults to allow the putative beneficial effects of CMV infection early in life, yet prevent the onset of dysfunction associated with CMV infection in the elderly.

9.4.7 Antiviral Therapy

It was previously shown that latent infection and viral reactivation are necessary for memory inflation (Lang et al. 2009), suggesting that antiviral therapy may be effective in controlling the development of CMV-driven immunosenescence. A recent study showed that extended antiviral therapy (for as long as 12 months) can reverse the development of CMV-associated immunosenescence in aged mice (Beswick et al. 2013). These studies supported the possibility that antiviral therapy to suppress CMV reactivation events can prevent/reverse the inflation of CMV infection. Not only was the magnitude of the CMV-specific CD8⁺ T cells reduced, but the remaining virus-specific T cells displayed a less-differentiated phenotype. In addition, drug-treated aged mice showed improvement in their response to influenza virus infection. It had previously been

shown that transferred inflated memory cells had a short lifespan (Klenerman and Dunbar 2008) and that memory inflation was maintained by continuous stimulation of naïve T cells (Snyder et al. 2008), which probably explains the recovery. Regarding the possibility that CMV has advantages against other acute infections mentioned above (Barton et al. 2007), the authors noted both clinical and immunological differences between the drug-treated aged animals and uninfected animals. This raises the possibility that the drug treatment “may have unmasked a potential benefit of underlying MCMV infection that is otherwise lost in elderly animals due to uncontrolled memory inflation.” These studies suggest that memory inflation is reversible.

9.4.8 T Cell Therapy

As it is thought that periodic viral reactivation events may be driving CMV-mediated memory inflation, a possible therapy might be adoptive T cell therapy, the transfer of CMV-specific T cell clones (Schmitt et al. 2011; Feuchtinger et al. 2010) or ex vivo-generated CMV-specific CD8⁺ T cells, to control reactivation events, similar to the approaches that have been successful in controlling EBV in bone marrow transplant recipients (Pawelec 2005).

Conclusion

People are living longer, and the proportion of the population considered elderly is increasing. The experimental mouse model, despite its limitations, has allowed great advances in our understanding of the impact of aging on immune function. The mouse allows longitudinal studies and in vivo functional studies not possible in human. Therapeutic strategies to prevent or reverse immune dysfunction associated with aging can be tested in the mouse model, and the fundamental principals learned then translated to primate and human studies. Although difficult, more longitudinal studies of humans are needed and CMV-negative populations need to be studied alongside the majority of the CMV-positive elderly.

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