Chapter 3

Memory T cell inflation: understanding cause and effect

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Abstract

Typically, during viral infections, T cells encounter antigen, undergo proliferative expansion and ultimately contract into a pool of memory cells. However, after infection with cytomegalovirus, a ubiquitous β-herpesvirus, T cell populations specific for certain epitopes do not contract but instead are maintained and/or accumulate at high frequencies with a characteristic effector-memory phenotype. This feature has also been noted after other infections, for example, by parvoviruses. We discuss this so-called memory T cell inflation and the factors involved in this phenomenon. Also, we consider the potential therapeutic use of memory T cell inflation as a vaccine strategy and the associated implications for immune senescence.

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MEMORY T CELL INFLATION AND CYTOMEGALOVIRUS INFECTION

The Herpesviridae are a large family of DNA viruses characterized by their ability to produce persistent infections. Human cytomegalovirus (HCMV) is a ubiquitous β-herpesvirus and 60–80% of people in the developed world display evidence of infection by the age of 80 years (1). HCMV infection in individuals with a healthy immune system is predominantly asymptomatic but on occasion can cause a mononucleosis-like disease similar to that caused by the γ-herpesvirus Epstein-Barr virus. In neonates and immunocompromised individuals, however, HCMV infection causes significant clinical problems. Currently, no vaccine is available and, although antiviral therapy exists, these drugs are both toxic and difficult to deliver and some viruses are resistant.

Of particular interest to immunologists and vaccinologists is the striking nature of the T cell response to HCMV, which is driven, in part, by the complex long-term, low-level persistence of the virus. Viral persistence and re-emergence are facilitated by the expression of numerous immune evasion genes encoded within the large viral genome. $CD8^+$ T lymphocytes play an important role in the control of intracellular pathogens, typified by large antigen-specific $CD8^+$ T cell populations after primary infection, which generally contract to form small pools of central memory T cells (2). Interestingly, comparison of HCMV-specific responses to other virus-specific T cell responses reveals the striking dominant impact of HCMV on the memory T cell compartment; a median of 9.1% and 10.2% of circulating $CD4^+$ and $CD8^+$ memory T cells are CMV-specific, respectively, which may increase even further in the elderly (3, 4).

The unusual nature of CMV-specific T cell responses has been dissected predominantly using mouse CMV (MCMV). Gradual accumulation of specific $CD8^+$ memory T cell populations was first noted in BALB/c mice (5) and subsequently described in longitudinal studies as memory 'inflation' (6). Memory T cell inflation is not restricted to CMVs, and arises also after infection with certain other persistent viruses (Box 1). Here, we focus on the factors that govern memory T cell inflation and the potential immune aging effects of memory T cell inflation in the elderly, and discuss recent research suggesting that inflationary T cell responses could be harnessed and used as a vaccine strategy.

CHARACTERISTICS OF MEMORY T CELL INFLATION

Infection of C57BL/6 mice with MCMV produces a $CD8^+$ T cell response to at least 24 epitopes derived from 18 viral proteins in the acute phase of infection; however, 50 days post-infection. When viral latency has developed, the immune response is dominated by five singular epitopes that elicit inflationary responses [including two epitopes derived from the immediate early (IE) gene products] (7). The MCMV-specific $CD8^+$ T cell responses that dominate during the acute phase are typified by classical expansion, contraction and formation of long-term central memory pools ($CD27^+$, $CD28^+$, $CD62L^+$, $CD127^+$ and IL-2^+), whereas inflationary responses display an effector-memory phenotype ($CD27^-$, $CD28^-$, $CD62L^-$, $CD127^-$ and IL-$2^{+/-}$) (7-9) (Figure 1). These inflationary T cells are found in diverse hematopoietic and non-hematopoietic organs but their specificity is restricted to a limited number of viral epitopes.

In humans and in primate models of CMV, inflationary CMV-specific $CD8^+$ T cells accumulate at high frequencies and have a similar characteristic effector-memory phenotype (e.g. $CD27^-$, $CD28^-$ etc.)(10-12). Inflationary T cells often show restricted T cell receptor (TCR) usage, with many carrying T cell receptors

Box 1. *Other persistent viruses that promote memory T cell inflation.*
Memory inflation is not confined to cytomegaloviruses; it occurs also after systemic, but not corneal, infection with herpes simplex virus-1 (HSV-1), an α-herpesvirus, during which a subset of CD8⁺ T cells specific for a structural glycoprotein B of the virus (gB-8p) gradually accumulates. Inflation begins as early as 3 months post-infection and these cells are maintained until old age. Ex vivo antigen-specific IFN–γ production, and in vivo recall response to HSV-1 between adult and 'aged' memory T cells show that inflating cells can maintain their functionality (18,72).
In a murine polyomavirus (PyV) model, a 'memory inflating' immune response can be elicited by a PyV peptide presented by Q9, an MHC class Ib molecule. This peptide, from the VP2 capsid protein, elicits a CD8⁺ T cell response that expands over 3 months and then reaches a plateau (73). Inflating CD8⁺ T cells in MCMV infection are mostly effector memory cells displaying little or no expression of costimulatory molecules such as CD27 and CD28 or receptors for the homeostatic cytokines IL-7 and IL-15 (8,9). By contrast, CD8⁺ T cells specific for PyV reach stable high frequencies, express receptors for both IL-7 and IL-2 and are maintained in the absence of homeostatic proliferation (74). It is not clear if the different phenotypes are related to differences in presentation by MHC 1a and MHC 1b molecules or in host–virus interaction or in different levels of antigen persistence.
Memory inflation has been noted after acute parvovirus B19 infection in humans, with expansion of effector memory CD8⁺ T cells many months after exposure (75,76). This small DNA virus is not a classical latent virus but the T cell responses appear phenotypically very similar to those directed against CMV and similar findings have been made in studies of the related parvovirus PARV4 (77). Thus, persistent viruses from different taxonomic families can elicit inflationary responses, indicating that memory T cell inflation is not a stranger in our midst but a more habitual feature of viral specific T cell responses.

specific for immunodominant CMV antigens (13). Inflation of memory CD4⁺ T cells has also been observed but, at least in the mouse model of CMV, is less exaggerated (14) and outside the scope of this review. Despite the persistent nature of CMVs, inflationary T cells remain functional and do not show features of exhaustion as observed with certain chronic viral infections (Box 2) (9,10,15,16).

FACTORS INVOLVED IN MEMORY T CELL INFLATION

Antigenic stimulation: antigen, tissues and cells

The underlying mechanisms of memory T cell inflation are beginning to be understood (Figure 2). One factor driving this phenomenon must relate to repetitive antigen exposure (17,18). Support for the hypothesis that antigen persistence and re-exposure are required for memory T cell inflation comes from a series of observations and experiments. Firstly, inflationary memory T cells have phenotypically signs of activation such as low expression of CD62L, CD127, CD27 and CD28 (6,8). Moreover, adoptive transfer of inflationary T cells into naive recipients results in a failure to divide and survive, whereas the same cells are maintained when transferred into chronically infected mice (9). Also, when an epitope that, in the context of MCMV or HSV-1 infection, induces T cell inflation is expressed in a recombinant vaccinia virus, memory inflation is not elicited (6). This implies that long-term presentation of viral antigen is required and T cell intrinsic

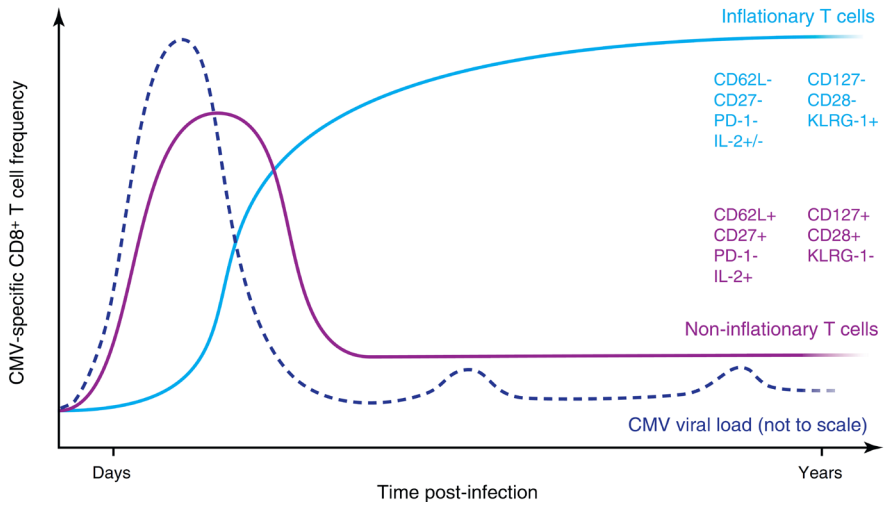

Figure 1. *Development of CMV-specific memory T cell populations.*
During CMV infection, non-inflationary T cells undergo expansion, contraction and formation of stable memory pools. By contrast, inflationary T cell pools are characterized by lack of contraction and gradually accumulate in frequency over time aided by low levels of viral reactivation. Characteristic phenotypic features of inflationary versus non-inflationary T cells are shown.

factors such as TCR affinity or specificity do not drive memory inflation. In fact, the functional avidity for peptide–MHC complexes does not predict whether epitopes elicit inflationary or non-inflationary T cell responses, nor do they change during the course of a chronic infection (7,19). Furthermore, it was shown recently that an MCMV mutant unable to spread between cells by deletion of the essential glycoprotein L does not impair the development of memory T cell inflation (20). This suggests that once infection is established, systemic viral production is not a prerequisite and merely the presence of the virus in a latent state is sufficient to drive inflation.

The genes containing epitopes that induce inflationary responses can be expressed early or late in CMV infection (6,7). Moreover, both inflationary and non-inflationary epitopes may be found within the same protein, suggesting that differential antigen processing events may be involved as well (7). It is interesting to note that the immunoproteasome, which is constitutively expressed in professional antigen-presenting cells and particularly important for processing of MHC class I proteins, is less important for memory inflating than non-inflating responses (21). Thus, epitopes inducing memory inflation might be selected because of this difference in immunoproteasome dependence.

During acute infection, MCMV replicates in diverse tissues such as spleen, liver and lung, whereas the salivary glands are the primary site where viral replication is detected during the ensuing persistent phase (22,23). However, removal of salivary glands does not influence memory inflation (24), supporting the idea that tissues where the virus becomes latent are important. Although active virus production is not measurable during latency, viral peptide epitopes are most likely to be present. The ability of CMV to sporadically re-activate in certain tissues, such as the lung, and thereby produce relevant viral transcripts possibly contributes (5,25-27). The antiviral $CD8^+$ T cell pool during memory inflation is also not affected by thymectomy, suggesting that the contribution from the naive pool to 'top-up' such large populations is not crucial (24).

Box 2. *Inflation versus exhaustion.*
Chronic viral infections can produce a number of outcomes in man and mouse, and much attention has been paid to the impact of high levels of viremia on T cell function. First noted in the LCMV model, one key outcome is loss of T cell functionality, such as autocrine cytokine production, leading ultimately to lack of proliferation and deletion of responses, a process termed exhaustion (78). Related observations have been made in human infections such as HIV, and in both mouse and man T cell exhaustion is associated with high expression of inhibitory receptors, notably PD-1 (15,79). Although CMVs are persistent and the inflationary T cell responses show features of repetitive antigen exposure, they do not show features of exhaustion along the lines of the LCMV model. Instead, inflationary T cells retain their functionality, including proliferative capacity, cytotoxicity and cytokine secretion, and are low in PD-1 expression (9,10,15,16). A number of transcriptional features, such as high expression of T-Bet, EOMES and BLIMP1, have recently been revealed for HCMV-specific $CD8^+$ T cells (16) and it will be of interest to explore the role of these key transcriptional regulators in vivo using experimental CMV models.

It is clear that antigen persistence is important for memory inflation, but it remains unclear which cells present the viral antigens and whether both direct and cross-priming are involved. Despite the induction of CMV-encoded immunomodulatory proteins that interfere with MHC class I presentation, strong $CD8^+$ T cell responses are elicited, suggesting that cross-presentation plays a dominant role (28-30). Expression of MHC class I molecules by infected antigen-presenting cells may not be inhibited completely, and thus direct presentation cannot be excluded from contributing to priming. In *Batf3*$^{-/-}$ mice, which have impaired cross-priming owing to the lack of $CD8\alpha^+$ and $CD103^+$ DCs, memory inflation still occurs (31) but cross-presentation by other cells in these mice is still possible (32). Using bone marrow chimera models, it was shown that inflationary response (e.g. to IE-1 protein) were dependent on antigen presentation by non-hematopoietic cells (33,34). However, it was not shown in these models whether direct presentation or cross-presentation (or potentially the recently described cross-dressing) (35) of MHC-loaded viral antigens (derived from infected non-hematopoietic cells) is important to drive memory inflation. Thus, further evidence is required to establish whether both direct and cross-presentation during persistent MCMV infection are operational and contribute to memory inflation.

Costimulation

In addition to TCR triggering, costimulatory molecules and inflammatory cytokines provide important signals for programming of effector and memory $CD8^+$ T cell responses (36). The main costimulatory receptor for T cells, CD28 (binding to CD80 or CD86), plays a dominant role during the acute phase because $CD8^+$ T cell responses in mice lacking this costimulatory interaction are strongly diminished at early time points. Remarkably, during the persistent phase, the inflationary T cells are able to 'catch up' to wild-type levels, indicating no CD28 requirement (37). By contrast, the costimulatory molecules 4-1BB and OX40, which are upregulated on T cells upon TCR triggering, clearly contribute to the development of inflationary MCMV specific T cell responses because interference with such triggering has a major impact on inflation (38,39).

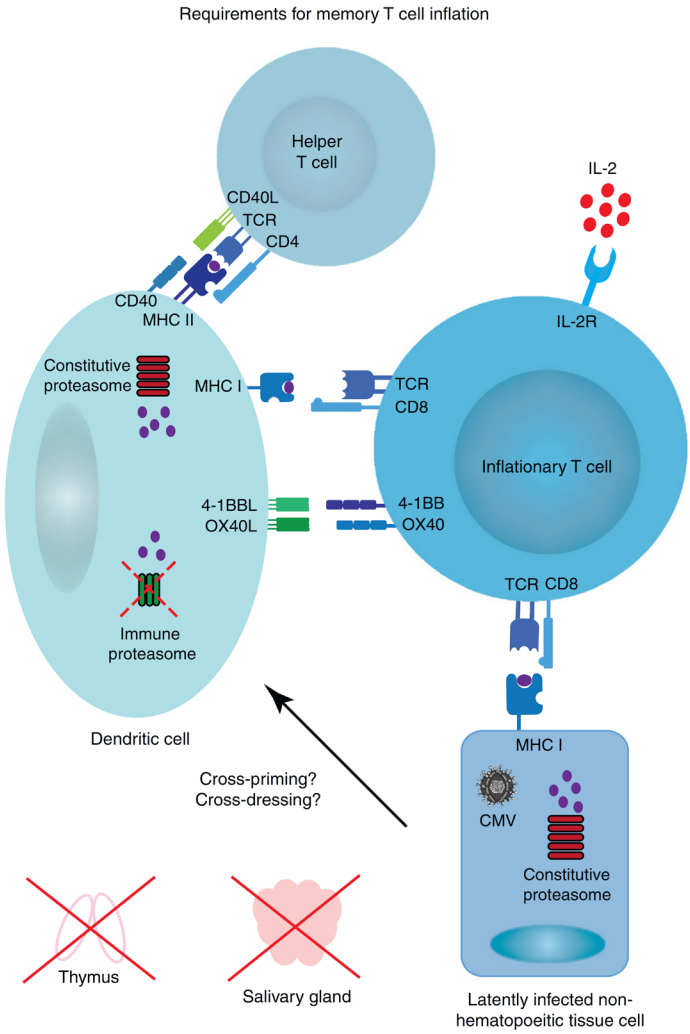

Figure 2. *Factors that contribute to memory inflation in CMV infection.*
In persistent CMV infection, the virus can sporadically reactivate from latency upon which viral proteins are processed by the constitutive proteasome and viral peptides are presented in MHC class I molecules. Viral antigens can either be presented by latently infected non-hematopoietic tissue cells or by cross-primed/cross-dressed dendritic cells. The presentation of the viral peptides persistently restimulates the inflationary T cell populations. Additional signals via the costimulatory molecules 4-1BB and OX40 contribute to the size of the inflationary T cell pool and IL-2 production is needed for maintenance of the inflationary T cells. $CD4^+$ T cell help is important for memory inflation but new thymic immigrants and viral replication in the salivary glands are not.

Cytokines

Using a bone marrow chimeric mouse model with wild-type and *Il2ra*$^{-/-}$ cells, it was shown that an inflationary CMV-specific T cell response is dependent on IL-2, whereas a non-inflationary response was not (40). In an acute infectious model, it was shown recently that autocrine but not $CD4^+$ T cell or DC-derived IL-2 is important for optimal secondary expansion of memory $CD8^+$ T cell populations (41). Inflationary $CD8^+$ T cells produce lower levels of IL-2 than those produced by non-inflationary T cells (7,37) thus, the question is whether autocrine IL-2 is similarly crucial for inflationary $CD8^+$ T cells or if other sources supply this. The

cytokines IL-7 and IL-15, which are important for homeostatic proliferation of memory $CD8^+$ T cells, have potent proliferative properties for HCMV-specific T cells *in vitro* (42), but their precise role for memory T cell inflation in an *in vivo* setting remains to be elucidated. Another important cytokine is IL-10, which clearly restricts inflation of T cells in the chronic phase of CMV infection by unknown mechanisms (43).

$CD4^+$ T cell help

$CD4^+$ T cells are crucial for controlling MCMV replication (44,45), especially in the salivary gland during chronic infection, and it was shown recently that these cells operate via direct antiviral actions involving IFN-γ (46). $CD4^+$ T cells are generally important for the development of memory $CD8^+$ T cell responses and even more so for inflationary responses (47,48). The mechanisms for this apparently increased helper-dependence are, however, unidentified.

We conclude that for the maintenance of the effector-memory-like inflationary T cell populations, the presence of latent virus in non-hematopoietic cells is essential and that additional cytokine and costimulatory signals (e.g. IL-2, OX40 and 4-1BB) are required to preserve the high frequency of this population.

IMPORTANCE AND THERAPEUTIC EXPLOITATION OF MEMORY T CELL INFLATION

The development of CMV-related disease in the immunocompromised, such as post-transplant and AIDS patients, indicates that T cells are important in controlling CMV (49). Severe disease can develop in immunologically immature children after congenital infection or after infection of premature infants (50). The specific importance of T cells is underlined by the successful usage of adoptive immunotherapy, whereby donor T cells are stimulated *in vitro* using viral lysate or CMV-specific peptides and subsequently are transfused into the patient (51). CMV replication has been shown to be controlled in the majority of cases in a clinical trial of bone marrow transplant recipients (52) and there is similar, although more limited evidence for solid organ transplantation (53).

In murine models, depletion of multiple cell subsets (including B cells, $CD4^+$ T cells and/or NK cells) is required for full viral reactivation to occur (54). However, even in otherwise immunocompetent mice, there is evidence that inflating memory T cell pools specific for an epitope from an IE gene product provide a 'checkpoint' limiting the stochastic viral reactivation that occurs (5).

Experimental T cell-inducing vaccines typically use non-persistent vectors. In the initial phase post-vaccination, when antigen is readily available, an effector memory response develops. However, as the vector is eliminated, the immune response becomes centered on secondary lymphoid tissue and central memory cells become dominant. It is possible to theorize that these central-memory cells, which require re-exposure to antigen to drive proliferation, differentiation and trafficking, may not be the ideal subpopulation for rapid control of all proliferating pathogens in tissues. Instead, effector-memory T cells respond more rapidly and are located in different tissues, including mucosal sites. Thus, placing target antigens in a virus that induces effector-memory T cells could provide the holy grail of vaccinology for certain chronic viral infections and perhaps cancer. The property of CMV to induce such strong effector-memory T cell responses together with the relatively easy transmission (despite pre-existing immunity) and lack of pathogenicity in healthy animals has led to consideration of CMV-based vaccines in both human and animal populations.

CMV as a vaccine vector

A recombinant MCMV expressing foreign viral epitopes either from influenza A virus nucleoprotein ($NP_{366–374}$) or lymphocytic choriomeningitis virus (LCMV) glycoprotein ($GP_{33–41}$) induces 'inflationary', antigen-specific $CD8^+$ T cells at a high frequency. These cells protect against subsequent challenge with a recombinant vaccinia virus expressing either the nucleoprotein or the glycoprotein, respectively, as well as LCMV challenge with maintenance of protection up to 200 days post-infection (55). More recently, CMV was assessed as a vaccine against simian immunodeficiency virus (SIV), in rhesus macaques (*Macaca mulatta*) using rhesus CMV (RhCMV) expressing SIV Gag, Rev-Tat-Nef and Env (RhCMV-SIV). Regardless of RhCMV serostatus, macaques could be infected and developed transgene-specific $CD4^+$ and $CD8^+$ T cell responses that were predominantly effector-memory in nature and were very long-lived; antigen-specific responses could be detected up to 4 years post-vaccination (56). A subsequent study compared the phenotype of SIV-specific T cell responses in RhCMV seropositive macaques using persistent (RhCMV) vectors and traditional DNA prime /Ad5 boost. Macaques in this study also underwent mucosal SIVMAC239 infection. Vigorous $CD4^+$ and $CD8^+$ T cell responses to all vector-encoded SIV antigens were detectable up to 400 days post-vaccination in all groups and, although the kinetics of the SIV-specific T cell responses developed differently between groups, the magnitude of the total SIV-specific T cell response for both $CD4^+$ and $CD8^+$ T cells were similar by day 400. As expected, RhCMV induced an effector-memory phenotype (characterized as $CD28^-/CCR7^-$) contrasting with the central-memory phenotype induced by DNA/Ad5. At 400 days after initial vaccination, animals were challenged with SIVMAC239; macaques immunized with RhCMV-SIV displayed rapid complete control of SIV, and ~50% of the animals were protected for >1 year, which correlated with the magnitude of the SIV-specific $CD8^+$ T cell responses in the vaccine phase. By contrast, all of the DNA/Ad5-vaccinated macaques developed progressive infection, with reduced mean plasma viral load compared to controls (57). The recently reported development of spread-deficient CMV vectors (20) and attenuated CMV vectors expressing activating NK cell ligands (58) allays some of the safety and bioethical concerns about using persistent vectors with potential for person-to-person transmission, which could generate a potentially pathological immune response. Although hurdles still exist, the prospect of using CMV as a vaccine vector for infectious diseases or cancer is great.

POTENTIAL DOWNSIDES OF MEMORY T CELL INFLATION

In contrast to the potential utility of a CMV-driven immune response in vaccinology is the consideration that CMV may be deleterious to health. CMV is recognized to be associated with several vascular diseases, including atherosclerosis, restenosis, transplant vascular sclerosis (TVS) and chronic rejection of solid organ transplants (59). Recently, the Sacramento Area Latino Study on Aging showed that individuals with CMV IgG antibody titers in the highest quartile compared with lower quartiles had an all-cause mortality 1.43-fold greater, with a higher risk or cardiovascular mortality (hazard ratio = 1.35) (60).

CMV and immunosenescence

Definitions, mechanisms and markers are controversial but, broadly speaking, immunosenescence is used to describe the deleterious phenotypic and functional changes observed in the immune system as it ages. In the mid-1970s it was shown that 80-year-olds with anergic T cells had a 2-year mortality rate of 80%

compared to 35% in non-anergic age-matched controls (61). In addition, age-associated poor response to T cell mitogens has been associated with increased mortality (62). Two major longitudinal studies, OCTO and NONA, focused on groups of healthy elderly volunteers and yielded a set of immune parameters that were capable of predicting mortality. Measurements such as a reduction in the number of B cells, poor T cell proliferative capacity and increased numbers of $CD8^+$ $CD28^-$ T cells, thus altering the CD4:8 ratio, were part of an immune risk profile (IRP) associated with increased all-cause mortality (63,64). Notably, evidence of CMV infection was part of this IRP cluster(65).

Infection with CMV substantially modifies the distribution of cellular subsets in peripheral blood, with greater numbers of $CD8^+$ T cells because of the increase in memory T cells and consequent reduction in size of the naive T cell pool. These changes are apparent in CMV seropositive individuals at all ages, and this might accelerate immunosenescence (66).

The increasing frequency of CMV-specific cells over a lifetime, especially at extremes of old age, suggests immune control of CMV is of paramount importance. Although some studies have associated CMV infection with increased risk of all-cause mortality, it is unclear at the molecular level why this is so. It has been reported that CMV-seropositive elderly do not respond as well as uninfected age-matched controls to influenza vaccine (67) but others have found no association of CMV with the poor responsiveness to influenza vaccination (68). Likewise, it was reported that CMV seropositivity results in reduced frequency of EBV-specific T cells in the elderly, suggesting CMV infection could affect responses to other viruses (69), but others found that the magnitude and phenotype of EBV- or influenza-specific $CD8^+$ T cells were not changed by primary CMV infection (70). The latter finding was confirmed in experimental models in which virus-based vaccines elicited an increase in the size of the memory $CD8^+$ T cell compartment with no impact on other cell populations, suggesting that the immune system retains plasticity and can accommodate development of new memory T cells against other pathogens (71). Overall, we conclude that the accumulation of clonal expansions of $CD8^+$ T cells specific for CMV antigens, excessive 'memory T cell inflation', in the very elderly might be a characteristic of immunosenescence, but further work is required to define a causal relationship.

CONCLUDING REMARKS

Memory T cell inflation has emerged over the past few years as a prominent feature of CMV immunobiology. Like the definition of immune exhaustion that was observed initially in the LCMV mouse model and has now proven its importance for active chronic viral infections in humans, memory inflation could have important implications well beyond CMV persistence. In particular, exploiting the influence of the inflated populations to protect against infection and possibly cancer is an exciting prospect. To do this well we will need to understand the molecular signals that govern the maintenance and function of such T cell pools and develop CMV-based vaccine vectors that induce and sustain these. Finding the crucial signals that could underpin safe vaccine development is an important, practical and attainable goal for the future.

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