a promising approach for the development of new therapeutic interventions against CD, especially if they can be selectively targeted to the gut or lamina propria. Furthermore, the findings might also help to tackle a long-standing problem in the vaccine field. Oral vaccines have considerable attractions in terms of ease of administration but are constrained by poor immunogenicity and induction of tolerance by antigens delivered by this route. Suppression of Treg cell induction has been shown to enhance the efficacy of vaccines delivered by parenteral routes (Jarnicki et al., 2008). Therefore, suppression of Treg cell conversion by commensal DNA or synthetic TLR9 agonists has potential to reverse tolerance and improve the immunogenicity of vaccines delivered by the oral route.

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CMV and the Art of Memory Maintenance

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The CD8$^+$ T cell responses to CMV gradually increase in magnitude over time—so-called memory "inflation." In this issue of *Immunity*, Snyder et al. (2008) examine the dynamics of memory inflation and demonstrate continuous turnover of inflating T cells, drawing on both memory cells and naive cells to replace them.

Cytomegaloviruses are ubiquitous pathogens. Human CMV (HCMV) infects most of the human population, usually asymptomatically, and persists lifelong. The cellular immune responses to HCMV are vigorous and sustained—indeed they tend to increase over time and may come to dominate the peripheral blood of healthy elderly donors (Khan et al., 2002). They are also essential for viral control, given that immunosuppression for transplantation or as a result of human immunodeficiency virus (HIV) infection can lead to viral reactivation and severe disease.

This host-virus balance has been studied by Snyder and colleagues (Snyder et al., 2008, this issue of *Immunity*) with the murine (MCMV) model, in which a key role for specific CD8$^+$ T cell populations can be more precisely defined. MCMV-specific CD8$^+$ T cells may be protective against disease—for example in immunosuppressed mice, as shown by the group of Reddehase (Holtappels et al., 2000). In these experiments it was noted that some CD8$^+$ T cells populations may be sustained or even increase over time in the lung. In further experiments using MHC-peptide tetramers, a continuous accumulation of virus-specific CD8$^+$ T cells over time was noted in all organs—a feature termed "memory inflation" (Karrer et al., 2003). The expansion of CMV-specific T cells in the elderly, which appears to be rather similar, has been implicated in immunosenescence, and thus understanding how memory inflation is established may be important in understanding the negative effects of CMV infection on human longevity (Khan et al., 2002).

CMVs are large viruses, with a number of open reading frames, but memory inflation is only seen for a small subset of epitopes from CMV proteins. Previous work by the group of Hill, preceding the study of Snyder in this issue, has shown that the CD8$^+$ T cell response to acute CMV infection may be broad (Munks et al., 2006). As the virus establishes persistence, the T cell response narrows to focus on a smaller number of immunodominant epitopes, and these T cell populations tend to inflate. In contrast, T cells specific for many of the acute-phase epitopes show a classical memory response,

with a large early frequency peak that declines to a long-term low-frequency population. It is still not clear what drives the selection of the "inflationary" epitopes. In some cases, T cells responding to genes expressed under the immediate-early promotor may help provide checkpoints to limit viral replication (Simon et al., 2006). However, in general it has been difficult to link immunodominance with viral gene-expression patterns, especially in human CMVs (Sylwester et al., 2005), in which T cell responses to structural proteins that are not under the immediate-early promotor commonly dominate (Khan et al., 2002). Even in MCMV, the fact that only one of two epitopes from the same protein (M38) is inflationary suggests factors other than the kinetics of viral gene expression are involved (Munks et al., 2006).

CMV-specific responses that do not inflate have—despite the continuous presence of virus—a typical "central memory" profile of phenotype and function (Karrer et al., 2003). They exhibit strong capacity for lymph node homing, proliferation, and cytokine secretion and sustain expression of costimulatory molecules such as CD28 and CD27 and cytokine receptors such as the IL7 receptor (CD127). In stark contrast, "inflationary" CMV-specific responses show an extreme of the "effector-memory" phenotype. These cells are typically low in expression of molecules associated with lymph node homing, such as CCR7 and CD62L, and accumulate in many nonlymphoid organs. They reduce expression of both CD28 and CD27, lose expression of CD127, and acquire expression of inhibitory receptors such as CD85j and KLRG1. In humans, these cells are often characterized as re-expressing CD45RA, a marker normally associated with naive cells (Appay et al., 2002). Functionally, they are strong secretors of antiviral cytokines such as IFN-γ and TNF-α and they maintain lytic capacity. Importantly, this situation is quite different from the "exhaustion" seen in LCMV-specific $CD8^+$ T cells in the face of continuous viral replication.

Since the advent of MHC-peptide tetramer staining, these striking CMV-specific responses have attracted much attention in ex vivo human studies. Early descriptions of these cells, particularly those that re-express CD45RA (effector memory RA^+ T cells or "TEMRA") have described them as "late differentiated" or even "terminally differentiated." It is also known through a number of analyses of TCR usage that individual clonotypes may come to dominate the repertoire, and these may persist over time (reviewed in Waller et al., 2008). All of these pieces of data had originally led to the impression that the populations may represent an accumulation of highly differentiated cells, with limited capacity for self-renewal.

However, this concept of a "terminally differentiated" population has been challenged. In the MCMV model, analysis of lymph nodes in particular revealed a residual "central" core of $CD8^+$ T cells specific for inflationary epitopes, undergoing in vivo activation and turnover (Karrer et al., 2003). More recently, studies in humans have shown that the expanded TEMRA cell populations may regain proliferative capacity and indeed may re-express lymph node homing markers and costimulatory molecules upon appropriate antigen re-encounter (Waller et al., 2008).

So, if it occurs, how active is such turnover in vivo? To address this, Snyder et al. (2008) have performed an elegant set of experiments using adoptive transfer of memory populations from different stages of disease, coupled with analyses of proliferation in vivo. They transferred "inflationary" memory T cell populations into infected recipients and controls and analyzed the fate of these cells. First, transferred memory cells can proliferate in vivo within the new recipient—particularly if these are transferred from recently infected mice. Even splenocytes transferred from mice infected >3 months previously were still able to proliferate when transferred into chronically infected recipients. However, they did not accumulate. Because the overall size of the memory population changes little, these data indicated that the population must turn over rapidly (estimated half life of approximately 50 days).

If memory populations are relatively dynamic, to what extent might naive cells be recruited in persistently infected mice to maintain this pool? Snyder et al. (2008) performed experiments in which the recipient mice were treated with busulphan, a cytotoxic drug used in man in hematologic disorders, to induce bone marrow suppression without killing established memory T cells. Interestingly, upon adoptive transfer of naive congenic cells, it was apparent that naive cells can be primed and expanded in vivo, even in the presence of established memory T cells specific for the same epitopes. Although this experimental result is clear, it was also noted that thymectomized mice infected with MCMV (and without immunosuppression) may also develop and sustain "inflationary" memory pools. Thus, although naive cells can contribute to the maintenance of memory in certain settings, the exact requirement for this is not fully defined. Overall, Snyder et al. (2008) conclude that a combination of naive cells and memory populations laid down early in infection may sustain memory inflation over time through continuous renewal (Figure 1).

So is this the end of the road for the terminal-differentiation hypothesis? A number of questions remain unanswered. In particular, how might such dynamics play out in man, in which infection extends over decades rather than weeks? One implication of Snyder and colleagues' data is that there might be different phases of memory maintenance with a particular impact on dynamics of a thymic-output decline in later life. One of the related lessons from this study for those analyzing human infection is that it is still very difficult to draw precise conclusions about the function and fate of cells on the basis of their surface phenotypes, and current linear-differentiation models need some reassessment.

Other questions are more general. In particular, how is the function of these "inflationary" cells maintained, in comparison to those of the intensively studied LCMV model, in which chronic infection is associated with progressive dysfunction of $CD8^+$ T cells? The dose of antigen and the interval between re-encounters, as well as the site of re-encounter, inflammatory environment and functionality of $CD4^+$ T cell populations may all differ, but clearly over time, quite distinct patterns of T cell development may emerge, some of which are beginning to be dissected in molecular terms. The extremely long-term coevolution of mammals and CMVs suggest that these pathogens could have had a profound impact on the programming of T cell behavior.

Finally, this study demonstrates that the populations induced by CMV are large, functional, dynamic and sustainable. This is exactly what may be required in a T cell-inducing vaccine for cancer or

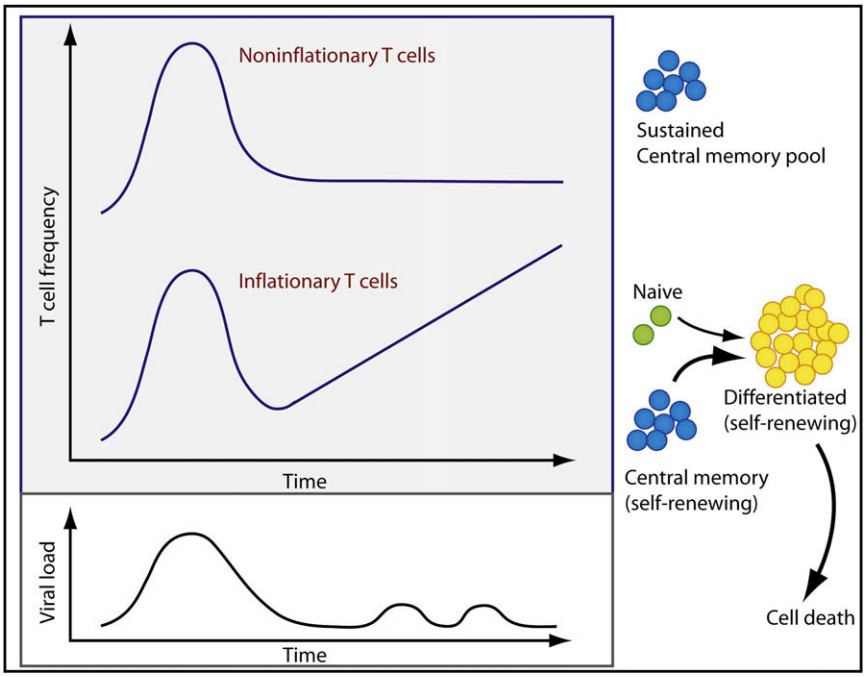

Figure 1. Evolution and Maintenance of Memory T Cell Memory after CMV Infection
The left-hand panels indicate two potential outcomes of CMV infection: The upper panel indicates conventional memory responses, and the lower panel indicates "inflationary" responses. Over time, viral reactivation events serve to restimulate the inflationary responses but not the classical memory responses. On the right-hand side, for the noninflating response, the central memory pool (blue) is sustained at low levels and enriched within lymphoid tissue. In the case of the inflating response, the situation is much more dynamic. The large pools of differentiated cells found in blood and tissues (yellow) are turning over continuously, with some cells progressing to death, but with rapid resupply from self-renewing differentiated cells, central memory pools, and naive (green) precursors.

infection. Previous studies using recombinant MCMVs have indicated that such vectors can induce protective T cell responses against heterologous challenges with vaccinia and LCMV (Karrer et al., 2004). Even if the mechanisms that allow this expansion of protective memory require further definition, there exists an opportunity to harness this unique behavior. Inflation may represent a threat for economists, but immunologists may yet learn to profit from it.

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