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Plasticity of T Cell Memory Responses to Viruses

Review

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Summary

Virus-specific memory T cell populations demonstrate plasticity in antigenic and functional phenotype, in recognition of antigen, and in their ability to accommodate new memory T cell populations. The adaptability of complex antigen-specific T cell repertoires allows the host to respond to a diverse array of pathogens and accommodate memory pools to many pathogens in a finite immune system. This is in part accounted for by crossreactive memory T cells, which can be employed in immune responses and mediate protective immunity or life-threatening immunopathology.

Introduction

On encountering viral antigens under conditions of appropriate costimulation, T cells proliferate and differentiate into IFNγ-producing cytotoxic CD8 T lymphocytes (CTL) and cytokine-producing Th1 (IFNγ, TNF) or Th2 (IL-4, IL-5) effector CD4 T cells (Kaech and Ahmed, 2001; van Stipdonk et al., 2001; Mercado et al., 2000; Swain, 1999; Dutton et al., 1998). This is a programmed event that can be initiated after only brief contact with the antigen-presenting cell (APC). Studies using limiting dilution assays and transfers of CFSE-labeled T cells indicate that CD8 T cells undergo as many as three divisions a day and divide at least 8 and as many as 15 times before reaching their peak (Selin et al., 1996; Kaech and Ahmed, 2001). Viral titers are greatly reduced during this T cell expansion phase, after which the T cells decline in number. This silencing of the T cell response is a consequence of T cell apoptosis in spleen and lymph nodes and of the dissemination of T cells into the peripheral tissue (Razvi et al., 1995a; Masopust et al., 2001; Marshall et al., 2001). Some of these T cells resist the apoptotic events and enter a memory pool, where they provide enhanced protection of the host on reexposure to a pathogen and act to prevent low-grade persisting viruses from reemerging.

Protective immunity to viruses is thought to be best mediated by B cell-secreted neutralizing antibody, but vaccines that induce T cell in addition to B cell memory responses may provide better protective immunity (Amara et al., 2001; Kaech et al., 2002). Memory T cells need to be antigen specific, easy to reactivate, and present at a high frequency for a substantial period of time to be fully effective in this role. Previous reviews have described these aspects of T cell memory (Dutton et al., 1998; Ahmed and Gray, 1996; Zinkernagel, 2002). We think it best, however, to view memory cells not simply as a cluster of individuals but, instead, as part of an interactive network, which is continually evolving as immune responses composed of some cells alter the frequencies, distributions, and activities of others. This network is composed of a diverse repertoire of naive and memory T cells, which compete with each other for niches in an ever-changing microcosm. With each virus infection, the adaptive immune response generates a diverse repertoire of antigen-specific memory T cells to a variety of immunodominant epitopes. These T cells need to be accommodated in many different local environments throughout the tissues of the host. During subsequent infections, the resident memory T cells must once again compete with the new T cells in the finite space of the immune system. These ever-changing networks of T cell populations provide the immune system with a resilient plasticity to combat infections.

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Plasticity in Functional Phenotypes

Memory T cells display extensive diversity in terms of antigenic phenotype, effector function, and anatomical distribution. Although memory CD8 T cells are consistently $CD44^{hi}$, they are heterogeneous in expression of other surface molecules, including lymph node homing receptors (CD62L, CCR7) (Razvi et al., 1995b; Sallusto et al., 1999; Tripp et al., 1995) and molecules associated with costimulation (CD27, CD28) (Appay et al., 2002; Tomiyama et al., 2002) or activation state (CD45RA and CD45RO) (Appay et al., 2002; Champagne et al., 2001). Viral antigen-specific CD8 T cells migrate to and reside at high frequencies throughout the organs of the host, including lung, kidney, gut, fat pads, and liver (Masopust et al., 2001; Chen et al., 2001; Hogan et al., 2001). In the absence of antigen, "resting" memory T cells are not quiescent. A portion of the memory CD8 T population undergoes a continuous but low-level homeostatic proliferation that must be offset by apoptosis, as their frequencies remain stable (Sprent and Tough, 1996; Zimmermann et al., 1996). Blast-size memory CD8 T cells in the spleens of lymphocytic choriomeningitis (LCMV)-immune mice are cytolytically active when exposed to sensitive targets, indicating that some memory cells exist in an effector state (Selin and Welsh, 1997). These cytolytically active "effector memory" cells are also present at relatively high levels in the peripheral tissues of mice immune to vesicular stomatitis virus (Masopust et al., 2001). Examination of memory cells by their physical or functional phenotype and anatomical location led some investigators to describe memory T cells in terms of two major subsets: central memory cells, which express CCR7 (or CD62L) and localize in secondary lymphoid tissue, and effector memory cells, which lack CCR7 (or CD62L) and remain in peripheral organs (Sallusto et al., 1999; Champagne et al., 2001).

There is controversy over whether this memory T cell heterogeneity is a function of different stages in a linear differentiation pathway or whether it reflects plasticity

in T cell antigenic and functional phenotype as a consequence of exposure to different types of antigenic stimuli within different microenvironments (Appay et al., 2002; Catalina et al., 2002; Baron et al., 2003). Three models have recently been proposed for lineage differentiation. The first model is based on expression of L-selectin (CD62L) and CCR7, which are receptors involved in homing to lymphoid tissue. These two receptors, along with the presence or absence of CD45RA, define three subsets of memory cells: central memory cells, effector memory cells, and terminally differentiated effector cells (Sallusto et al., 1999). Examinations of HIV- and human cytomegalovirus (HCMV)-specific CD8 T cells led investigators to propose a linear differentiation scheme whereby naive CD4 or CD8 T cells ($CD45RA^+CD62L^+CCR7^+$) on encountering antigen mature into central memory cells ($CD45^-CD62L^+CCR7^+$), effector memory cells ($CD45RA^-CD62L^-CCR7^-$), and finally, terminally differentiated effector cells ($CD45RA^+CD62L^-CCR7^-$) (Champagne et al., 2001). The second model for human T cell differentiation is based on downregulation of the costimulatory molecules CD27 and CD28, along with CD45RA in HCMV- and Epstein Barr virus (EBV)-specific T cell responses. In this pathway, naive cells ($CD45RA^+CD27^+CD28^+$) mature into early-differentiated cells ($CD45RA^-CD28^+CD27^+$), intermediate cells ($CD45RA^-CD28^-CD27^+$), and finally, fully differentiated cells ($CD45RA^-CD28^-CD27^-$) (Tomiyama et al., 2002; Wills et al., 2002; Hamaan et al., 1997). A third model using the murine LCMV infection utilized CD62L expression and the time course of virus clearance after infection. This third model demonstrated results that were the opposite of the first model, as naive cells ($CD62L^+$) upon antigen exposure matured into effectors ($CD62L^-$), then effector memory ($CD62^-$), and finally central memory ($CD62L^+$) (Wherry et al., 2003b).

Both human and mouse studies have shown some consistency in the functional phenotypes of memory cells, although there is still concern whether surface phenotype correlates with functional phenotypes (Catalina et al., 2002; Hislop et al., 2002). Central memory ($CCR7^+$, $CD62L^+$ [first and third models]) or early differentiated ($CD45^-$, $CD27^+$, $CD28^+$ [second model]) phenotypes appear to be more resistant to apoptosis and cycle more rapidly, and, upon antigen exposure, are better at producing IL-2 and proliferating when compared to the effector ($CCR7^-$, $CD62L^-$ [first and third models]) or late fully differentiated ($CD45^-$, $CD27^-$, $CD28^-$ [second model]) phenotype memory T cells (Sallusto et al., 1999; Champagne et al., 2001; Wherry et al., 2003b). The interchangeability of these populations remains unclear. As indicated in the third model, adoptive transfer studies in the mice have shown that effector memory T cells can convert back into central memory T cells, suggesting a reversible differentiation scheme (Wherry et al., 2003b). On the other hand, a human T cell receptor (TCR) repertoire study using CD45RA and CD62L to define effector and memory populations found that 90% of the clonotypes were not in common between the effector and central memory CD8 T cell populations (Baron et al., 2003). This surprising result would suggest that a differentiation pathway between those subsets was less likely or else that there was a very selective proliferation or apoptosis of T cells within those subtypes.

Memory CD8 T cells responding to different viruses may display a different phenotypic profile. For instance, the predominant memory CD8 T cell populations are $CD45RA^-CD27^+CD28^+$ against EBV and HCV, $CD45RA^-CD28^-CD27^+$ against HIV, and $CD45RA^+CD27^-CD28^-$ against CMV (Appay et al., 2002; Tomiyama et al., 2002; Champagne et al., 2001). The phenotype of T cells directed against different epitopes encoded by the same virus can also be different within the same individual (Catalina et al., 2002; Hislop et al., 2002). For example, T cells responding to the HLA-A2-restricted BMLF-1 lytic epitope of EBV, which is expressed on infected B cells, are $CCR7^-$ and equally mixed with $CD45RA^-$ or $CD45RA^+$ cells. In contrast, responses to the EBV latent epitopes, which are predominantly expressed in the tonsils, are associated with $CCR7^+$ $CD45RA^-$ cells.

The human infections listed above are all persistent to some degree, and T cells present during persistent infections are particularly heterogeneous at the functional level. Examples of this heterogeneity include cytokine-producing weakly cytotoxic cells low in perforin, as shown with HIV (Appay et al., 2000); cytokine-producing weakly cytotoxic cells high in perforin, as shown in murine polymavirus infections (Moser et al., 2001); and weakly cytotoxic cells that do not produce cytokines, as shown in SIV infection in monkeys (Xiong et al., 2001). Among the interesting factors now found to modulate CD8 T cell function are the acquisition of inhibitory natural killer cell receptors, such as CD94 (NKG2A) (Moser et al., 2001, 2002). Inhibitory receptors have now been reported to modulate the functions of T cells in HIV and EBV infections in humans and polyomavirus infections in mice (Moser et al., 2001, 2002; Vely et al., 2001; De Maria et al., 1997). Studies with persistent LCMV infections in mice, initiated as a consequence of high dose infection with a widely disseminating strain (clone 13), have illustrated how high viral loads can modulate T cell frequencies and functions in complicated fashions (Zajac et al., 1998; Moskophidis et al., 1993). In the LCMV system, some (e.g., NP396) specific T cells, which are probably most highly stimulated by antigen, are completely eliminated from the host. However, T cells with other specificities (e.g., GP33) undergo various states of anergy in which there is a loss in cytolytic ability, IL-2, TNFα, and then IFNγ production, in that order (Fuller and Zajac, 2003; Wherry et al., 2003a). In LCMV persistently infected hosts, the CD8 T cells seemed to be lost or anergized more in lymphoid organs than in the periphery (Wherry et al., 2003a). This may reflect recent evidence showing that LCMV-specific CD8 T cells during the acute infection are more resistant to apoptosis in peripheral than in lymphoid tissues (Wang et al., 2003). Thus, tissue-dependent factors may influence the fate of T cells.

This variation in phenotypes of memory T cell populations following different virus infections and between different epitopes of the same virus would favor the concept that memory subsets are defined by their initial and ongoing antigenic experience and cytokine environment. Similarly, there may be significant flexibility in converting from one phenotype to another, depending

on their local antigen or cytokine environment (Appay et al., 2002; Wherry et al., 2003b).

Plasticity in Recognition

Diversity of Antigen-Specific Memory TCR Repertoires

Antigen-specific TCR repertoires are highly diverse. The adaptive immune response needs to recognize a large number of foreign antigens and has evolved to generate a diverse $\alpha\beta$ TCR response by imprecise recombination of the variable, diversity, and junctional regions of the α and β chains, coupled with pairing of one β and one to two α chains per T cell. The development of MHC-tetramers has enhanced the study of viral antigen-specific T cell populations by allowing for the purification of epitope-specific T cell populations. Using this technique, Perlman and colleagues examined the Vβ13 TCR repertoire to a mouse hepatitis virus S510 epitope, sequenced 35 to 85 TCR per mouse in nine mice, and estimated a frequency of 300–500 unique clonotypes specific for that epitope (Pewe et al., 1999). Stern and colleagues demonstrated diversity in human memory CD4 T cell responses to influenza A using MHC class II HLA-DR1-restricted tetramers to an HA epitope with 35 different clonotypes out of 110 sequences (Camereon et al., 2002). Naumov et al. have shown in human studies that the CD8 memory response to the HLA-A2-restricted influenza A epitope M1-58-specific consisted of 141 unique clonotypes out of 500 Vβ17 sequences (Naumov et al., 2003).

Both the influenza A M1-58-specific response and the MHV S510-specific response included a small number of high-frequency clonotypes and a large number of low-frequency clonotypes, which could be described by a power law-like distribution (Naumov et al., 2003; Pewe et al., 1999). This means that a small number of clones were present at high frequencies and ever-increasing numbers of clones were present at lower and lower frequencies. Furthermore, when the structure of the M1-specific repertoire was analyzed by focusing on many different subsets of the repertoire, such as clonotypes using Jβ2.7 or those whose CDR3 region encodes the amino acid sequence IRSS, the clonotype frequencies still maintained a power law-like distribution. This indicates a self similarity to the repertoire in which smaller subsections of the repertoire form a distribution similar to that of the larger whole repertoire. The power law-like distribution and the self similarity, which described this influenza A M1-58-specific response, suggested that this repertoire was organized in the form of a fractal system. Fractal systems occur throughout nature, in such common forms as snowflakes, trees, and blood vessels, where there is a self similarity of structure. We do not understand the mechanisms which drive the memory T cell repertoire to develop as a fractal system, but it is notable as it is also seen in B cell systems. This type of organization may be another reflection of the flexibility of the adaptive T cell immune response. The high number of low-frequency clones means there is a great breadth to an epitope-specific memory response. Thus, if antigenic variants were to elicit the recall response, then some of the many diverse low-frequency clones expressing TCRs capable of recognizing the mutant Ag might be selectively expanded as new major clones. This flexibility of repertoire selection would be an advantage in both antiviral and antitumor responses where antigenic variants can be quite common.

Despite heterogeneity in TCR usage, many epitope-specific responses have in the CDR3 region distinct amino acid motifs that are maintained between clonotypes and between different individuals. For example, in the human HLA-A2-restricted influenza A M1-58 Vβ17 response, the amino acid motif IRSS is common (Naumov et al., 2003), and in the H2-K^d-restricted HLA-CW3 Vβ10 response in DBA/2 mice, SxG in the first three positions of the CDR3 region was a common motif (Maryanski et al., 1999). Other CDR3 binding motifs have been identified, including the murine H2-D^b-restricted NP396-specific Vβ8.1 response (GxxN) in LCMV infection (Wang et al., 2003) and the HLA-B14-restricted HIV Env EL9 response (GQG) (Cohen et al., 2002). Conservation of CDR3 amino acid motifs suggests that they are required for the TCR to bind to the MHC-ligand structure. These similarities in Vβ usage and amino acid motifs can be thought of as the *public specificities* of epitope-specific T cell responses that are similar between individuals.

Private Specificity and Diversity between Individuals. Despite the public specificities in T cell responses, there can be tremendous diversity in the TCR repertoire between individuals. The TCR usage per epitope differs between individual hosts, even though there might be general similarities in preferred TCR Vβ usage or specific CDR3 amino acid motifs (Maryanski et al., 1999; Lin and Welsh, 1998; Blattman et al., 2000; Camereon et al., 2002). Thus, the TCRs on the antigen-specific T cell clones are unique to the individual, and these unique regions have been referred to as the "private specificity" for that epitope-specific response. This variation is probably a consequence of the random stochastic process of TCR rearrangement in the thymus, which results in variations in the naive peripheral TCR repertoire, and of the random stochastic process whereby a T cell encounters an APC presenting its cognate ligand (Bousso et al., 1998). T cell clones that are stimulated early may dominate the response by interfering with the stimulation of other T cells (Yewdell and Bennink, 1999).

Crossreactive Memory T Cells

Each of the T cells in this diverse pool of memory cells is degenerate in the number of antigens it can recognize. It has been calculated, on the basis of positional analysis of various amino acid substitutions at different residues of a peptide, that a given TCR has the potential to recognize a million different peptide-MHC combinations (Mason, 1998). Reports of CD8 T cells recognizing epitopes encoded by apparently unrelated viruses are increasing. For example, crossreactive T cells have been reported between influenza and hepatitis C viruses, human papillomavirus and human coronavirus, LCMV and Pichinde virus (PV), LCMV and vaccinia virus (VV), influenza and rotavirus, and influenza and EBV (Wedemeyer et al., 2001; Nilges et al., 2003; Brehm et al., 2002; Shimojo et al., 1989; Welsh et al., 2004; Selin et al., 1994). Crossreactive CD8 T cell responses are also observed within different strains of influenza virus and Dengue virus (Haanen et al., 1999; Spaulding et al., 1999).

Models for Crossreactivity. There are several mecha-

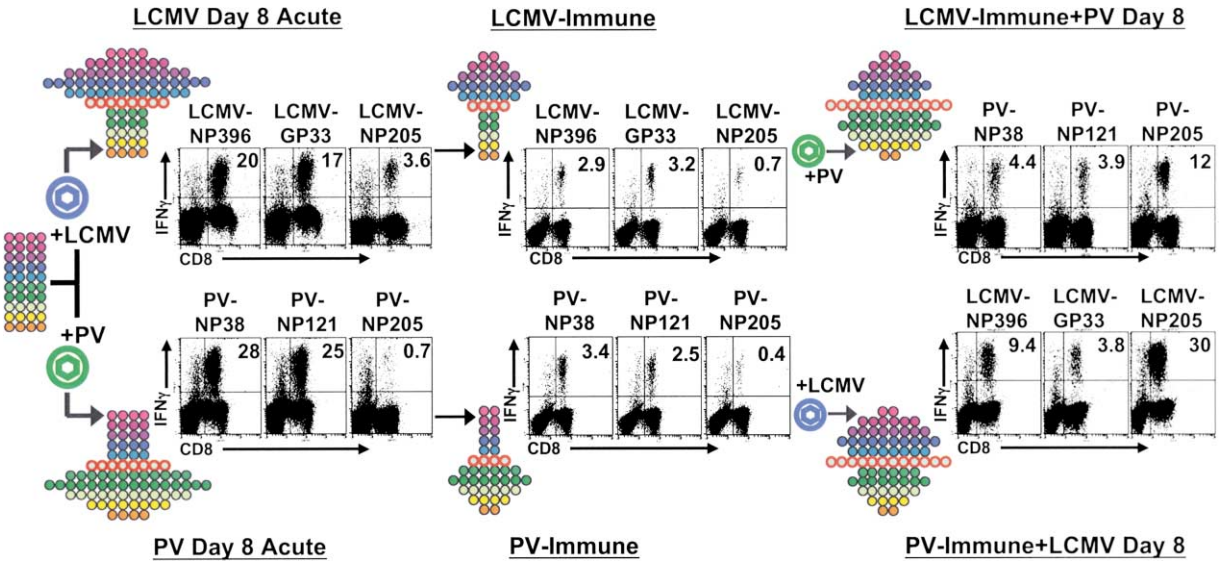

Figure 1. Plasticity of T Cell Repertoire during Viral Infections

The colored dots represent T cell populations that have different specificities. The intracellular IFNγ staining for epitope-specific responses during particular viral infections are also depicted. A naive immune system is challenged with either of two heterologous viruses, LCMV or PV, and generates a T cell response to the immunodominant epitopes. These acute responses then decline but maintain the same hierarchy of immunodominant responses in the memory state. If an immune system that has been conditioned with one virus is exposed to the other heterologous virus, T cell populations that are crossreactive with the two viruses (red outlined) will expand preferentially, dominate the response, and go onto memory.

nisms by which a single T cell can interact with multiple antigens. Structural studies examining T cell crossreactivity against a peptide-modified syngeneic target and an allogeneic target have shown that different regions of the same TCR can bind to two different targets (Daniel et al., 1998; Speir et al., 1998). This type of crossreactivity would be difficult to predict. However, a crossreaction involving the same determinants on the TCR would be easier to predict and identify by searching databases for peptide sequences with similar amino acids accessible to the TCR; this "molecular mimicry" method was used to identify some of the crossreactive epitopes identified above (Mason, 1998; Wedemeyer et al., 2001). A third mechanism for T cell crossreactivity occurs when a T cell, due to incomplete allelic exclusion of the TCR α chain, expresses two different TCRs (Alam and Gascoigne, 1998).

Crossreactive T cells may have widely different affinities to two different targets, but certain aspects of a viral infection may augment the significance of a low-affinity crossreaction. Studies on T cell recognition using amino acid substitutions in target peptide epitopes have indicated that highly activated T cells during an acute response in LCMV infection can lyse a broader range of targets than can less activated T cells (Bachmann et al., 1997). This suggests that lower-affinity altered peptide ligands might more easily activate effectors or effector memory cells in comparison to resting memory CD8 T cells. The virus-induced cytokine environment may also enhance crossreactive effector responses; for instance, since cytokines such as IL-12 are known to synergize with antigen to enhance IFNγ production by T cells it is easy to envision IL-12 enhancing IFNγ production in low-affinity TCR interactions (Gately et al., 1998).

Consequences of Plasticity in Memory T Cell Recognition

Plasticity of Immunodominance Hierarchies. The mobilization of crossreactive memory cells into a primary immune response can alter the immunodominance of subsequent T cell responses. In genetically identical animals with a naive immune system, the hierarchy of T cells specific to immunodominant epitopes is consistent and predictable (Brehm et al., 2002). However, studies in humans have shown that there is variability in the hierarchies of T cells responding to different HLA-A2-restricted HIV epitopes in individual patients (Betts et al., 2000). This disparity in epitope hierarchies could be caused either by genetics or by the environment, where humans have a lifetime history of earlier infections that may have altered the T cell repertoire and influence the immunodominance hierarchy. In fact, studies in the mouse have shown that T cell immunodominance can be greatly affected by previous antigenic exposures that might elicit crossreactive responses. Brehm et al. showed that LCMV and PV encoded crossreactive epitopes, with six of eight amino acids in common (Figure 1) (Brehm et al., 2002). Responses to these epitopes were subdominant in each infection, accounting for less than 3% and 1% of the acute and memory CD8 responses, respectively, for each infection. However, if LCMV-immune mice were infected with PV, or if PV-immune mice were infected with LCMV, the T cell responses to these epitopes became dominant, reaching levels as high as 20% of the CD8 T cells. In contrast, T cell responses to the normally dominant epitopes were much lower. Thus, infections with heterologous viruses can alter immunodominance when crossreactive responses are present. This phenomenon is reminiscent of the concept of clonal imprinting or original antigenic

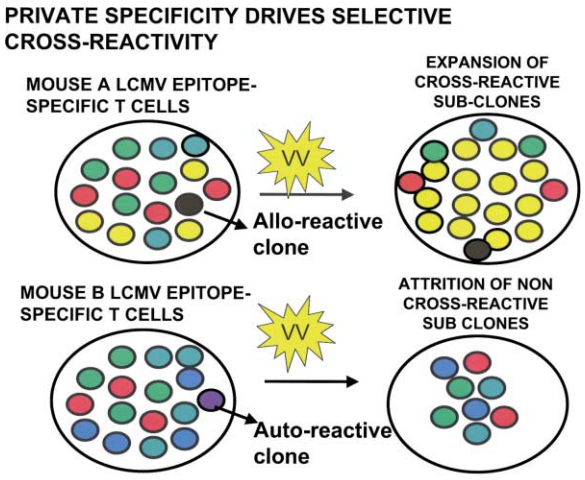

Figure 2. Private Specificity Drives Selective Expansion of Cross-reactive Clones

The colored dots represent unique T cell clones that all recognize the same antigen. In a naive host, each individual host develops a unique repertoire of antigen-specific cells to the same immunodominant epitope during LCMV infection. Depending on the private specificity of the TCR repertoire in each host, this repertoire may contain memory T cells crossreactive with allo-antigens (mouse A) or with autoantigens (mouse B). If there are LCMV-specific memory T cells crossreactive with a heterologous virus, such as VV, as in mouse A (yellow dots), these would preferentially expand upon infection with VV. A portion of the LCMV-specific memory T cells that are not crossreactive with the second virus VV (mouse B) are lost due to bystander attrition as the host accommodates the new memory T cells.

sin that was proposed initially to explain the anamnestic antibody response to crossreactive B cell epitopes of related influenza virus strains (Fazekas de St. Groth and Webster, 1966).

Heterologous Immunity. The mobilization of cross-reactive memory cells into a primary immune response can alter not only immunodominance profiles but also disease outcome by influencing both protective immunity and immunopathology. Protective heterologous immunity has been shown with a number of viruses (Selin et al., 1998, 2000; Welsh and Selin, 2002). For instance, LCMV-immune mice challenged with VV (LCMV + VV) demonstrated partial protective immunity at 4 days postinfection, with altered immunopathology in both systemic (i.p.) and respiratory (i.n.) infection models (Selin et al., 1998; Chen et al., 2001). Protective immunity was transferred by LCMV-immune CD8 and CD4 T cell populations and was dependent on IFNγ. Notably, at 3 days after VV infection, 15%–30% of lung memory CD8 T cells specific to each of six LCMV epitopes produced IFNγ in vivo. It is not clear whether this early IFNγ production was caused by crossreactivity or by a nonselective cytokine-dependent activation, but the IFNγ contributed to the clearance of VV. Later in infection there was a selective expansion LCMV-specific T cells with some but not other epitope specificities, consistent with a crossreactive antigen-driven expansion (Welsh et al., 2004).

Our studies with VV infection of LCMV immune mice showed that VV sometimes elicited the expansion of T cells with one LCMV epitope specificity but other times elicited the expansion of T cells with a different specificity. This could be explained by the stochastic nature of clonal dominance and by the private specificities in antigen-specific TCR repertoires in each immune host. This would predict that the proportion of epitope-specific memory T cells crossreactive with another antigen would differ from host to host. This is indeed what we have observed in LCMV-immune hosts challenged with VV (Kim et al., 2002; S.K. Kim and R.M.W., unpublished data) (Figure 2).

Prior immunity to LCMV also had a dramatic impact on immunopathology upon VV challenge. In the systemic (i.p.) model, the mice developed severe mononuclear infiltration and acute necrosis of the visceral fat pads (Selin et al., 1998). This type of pathology is known as panniculitis, and it can occur in humans with lupus erythematosis and Weber-Christian syndrome (Welsh and Selin, 2002). The most common form of panniculitis in humans is erythema nodosum, a pathology associated with viral and intracellular bacterial infections and sometimes observed after vaccination of humans with VV or hepatitis B antigen (Di Giusto and Bernhard, 1986; Bolognia and Braverman, 1992). The respiratory infection with VV resulted in dramatic differences in pathology, associated with accumulation of LCMV-specific CD8 T cells in a greatly enhanced bronchus-associated lymphocyte tissue (BALT) surrounding the airways and with the induction of bronchiolitis obliterans (Chen et al., 2001), which can be observed in humans sporadically during viral and intracellular bacterial infections and during lung transplant rejection (Schlesinger et al., 1998). Human panniculitis and bronchiolitis obliterans are diseases of unknown etiology and are thought to be mediated by cellular immune responses.

Because of the tenuous balance between T cell immunodominance, protective immunity, and immunopathology, heterologous immunity is not always beneficial. Although immunity to LCMV protected against respiratory VV infection, it inhibited the clearance of RSV (Chen et al., 2001; Ostler et al., 2003). Similarly, a history of influenza A infection protected against VV but inhibited clearance of MCMV and LCMV (Chen et al., 2003). During MCMV infection, prior immunity to influenza A also dramatically altered early cytokine profiles, enhancing pro-inflammatory cytokines, such as IL-6, IL-12, and IL-1β (Figure 3). Instead of the usual mild mononuclear infiltrate observed in acute MCMV infection of naive mice, influenza-immune mice infected with MCMV developed a severe consolidating mononuclear pneumonia with evidence of bronchiolization (Figure 3). During bronchiolization, alveolar epithelium is replaced by bronchiolar-like cells, and this is thought to be an indicator of lung repair (Nettesheim and Szakal, 1972). Patterns of heterologous immunity can therefore be complicated and difficult to predict, although they are quite reproducible in experimental models.

We suggest that heterologous immunity may underlie variabilities in pathology observed in some human viral infections. It is noteworthy that many viruses, including EBV, VZV, and measles, cause more severe pathology in young adults than in children (Weinstein and Meade, 1956; Rickinson and Kieff, 1996). The much larger repertoire of memory T cells from earlier infections present

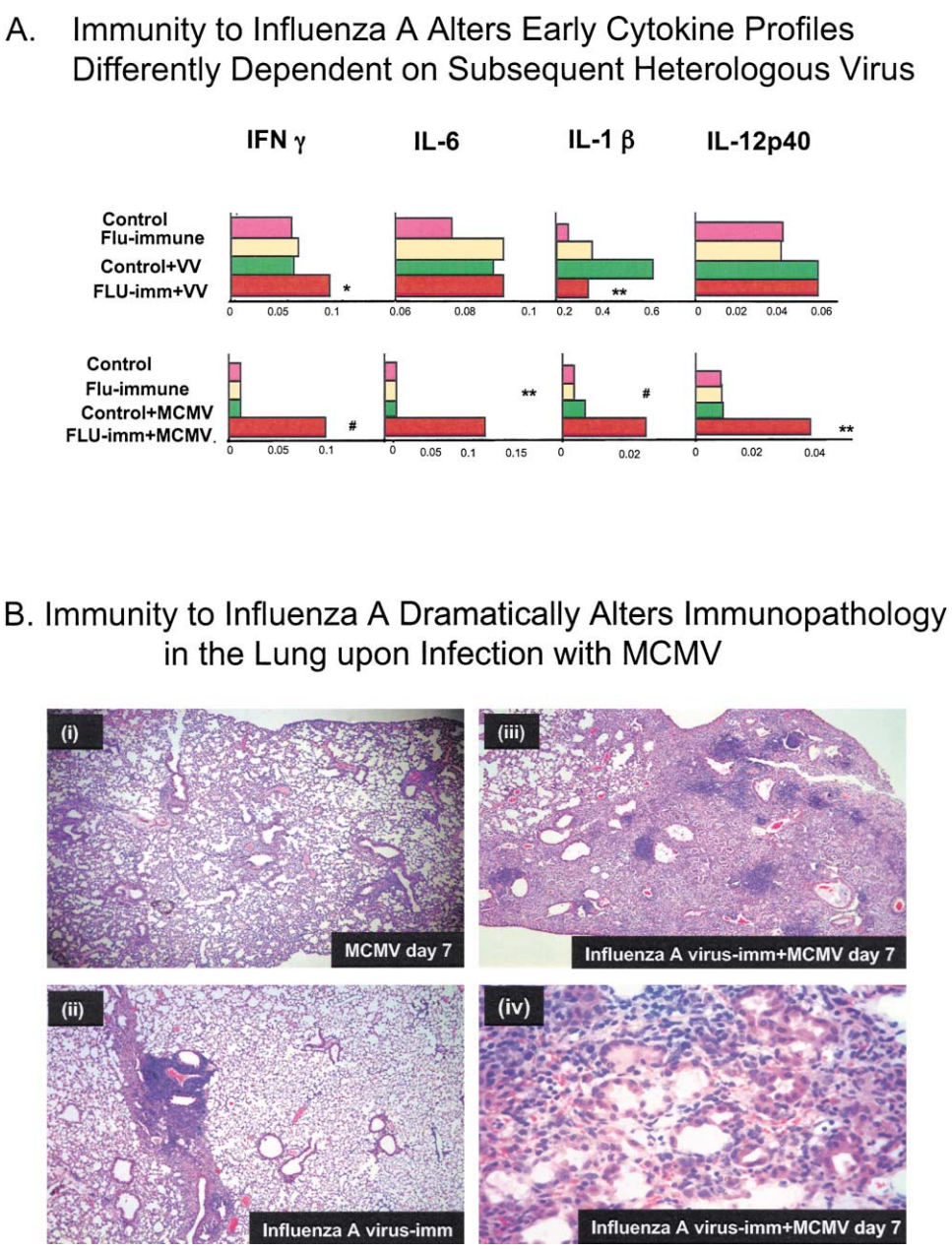

Figure 3. Previous Immunity to Influenza A Alters Early Cytokine Profiles and Immunopathology in the Lung on MCMV Infection

During MCMV infection of influenza A-immune mice there is a dramatic alteration of early cytokine profiles in the lung, as measured by Rnase protection assay, with enhanced proinflammatory cytokines IL-6, IL-12, and IL-1, as well as IFN-γ. Influenza A-immune mice have essentially normal lung architecture, with only mild residual scaring (ii). MCMV infection of naive mice results in mild mononuclear infiltrates (i), but MCMV infection in influenza A-immune mice results in a severe mononuclear pneumonia (iii) with evidence of bronchiolization (iv).

in the young adult might lead to recruitment of cross-reactive T cells and altered disease pathology.

Heterologous Immunity and Transplantation. Cross-reactive T cell responses may play a role in organ transplant rejection. Many virus infections in mice and man induce epitope-specific T cell responses to viruses that crossreact with allogeneic MHC molecules (Nahill and Welsh, 1993; Brehm et al., 2003; Burrows et al., 1999), and viral infections have often been noted to precede allograft rejection in humans (Gaston and Waer, 1985). Naive mice have very few allospecific T cells of the memory phenotype, but, as a consequence of cross-reactivity, viral infections leave mice with much higher frequencies of allo-specific memory T cells. For example, about 1% of the CD8 T cells in LCMV-immune C57BL/6 mice ($H2^b$) are memory cells specific to $H2^d$ alloantigens (Brehm et al., 2003). Crossreactivity with alloantigens is extremely diverse, in that some of the T cells specific to each of four tested LCMV-encoded epitopes reacted against allogeneic targets expressing $H2^d$. Considerable effort has gone into developing tolerization protocols to enable hosts to accept and maintain

allogeneic tissue engraftment. However, mice with a history of viral infections are more difficult to tolerize to accept grafts (Welsh et al., 2000), and acute viral infections can break tolerance and stimulate the rejections of allografts in tolerized mice (Brehm et al., 2003; Adams et al., 2003).

Heterologous Immunity and Autoimmunity. Reactivation of crossreactive memory T cells may play a role in mediating autoimmune diseases, such as multiple sclerosis, diabetes mellitus, Crohn's disease, or rheumatoid arthritis. Virus-induced autoimmunity may be a consequence of many factors, including disregulation of regulatory T cells or providing helper factors for self-reactive T cells. However, there may be conditions where there is a direct crossreactivity between viral and self antigens, and such T cells might get activated again by another viral infection. An HSV-specific CD8 T cell response in mice is directed against a viral epitope that is sufficiently similar to a corneal self antigen to induce autoimmune herpes stromal keratitis (Zhao et al., 1998). Some EBV-specific CD8 T cell clones, which recognize crossreactive self peptides, have been found in joints of humans afflicted with rheumatoid arthritis (Edinger et al., 1999; Misko et al., 1999). Mice expressing an LCMV NP transgene in the brain develop transient encephalitis after infection with LCMV but not with heterologous viruses PV or VV (Evans et al., 1996). However, after LCMV has broken tolerance and elicited a memory CD8 T cell response that is specific to the self "NP" antigen expressed in the brain, subsequent infections with these heterologous viruses are now able to reactivate these memory T cells and reelicit the disease. This may be a mechanism for autoimmune diseases that undergo exacerbations and remissions.

Crossreactive CD4 T cell responses between pathogens and self antigens have also been associated with autoimmune disease. For instance, infection of SJL/J mice with the neurotropic picornavirus Theiler's murine encephalomyelitis virus (TMEV) leads to a late onset CD4 T cell-mediated demyelinating disease similar to multiple sclerosis (Olson et al., 2001; Theil et al., 2001). When a TMEV variant was engineered to encode a peptide containing the encephalitogenic myelin proteolipid protein (PLP139-151) epitope, mice infected with this virus developed a rapid onset paralytic demyelinating disease. Furthermore, mice infected with TMEV encoding a variant peptide, which shared only 6 of 13 amino acids with PLP139-151, also displayed rapid-onset disease and developed Th1-type CD4 T cells crossreactive with PLP139-151.

Heterologous Immunity and Immune Deviation. Reactivation of memory T cells may be one of many factors contributing to immune deviation. If a memory pool is heavily populated with Th1 or with Th2 memory CD4 T cells, the stimulation of them by a crossreactive antigen may alter the cytokine milieu of a new immune response and influence the Th1/Th2 polarity. Individuals who have been immunized with Bacillus Calmette-Guerin (BCG), a strong Th1 inducer, have a decreased frequency of atopy, which is Th2 dependent, as compared to those who did not receive this immunization (Shirakawa et al., 1997). Also, mice immunized with BCG suppressed a Th2-type response and the associated eosinophilia in the lung when exposed to an allergen (Erb et al., 1998). Mice infected with influenza virus before vaccination with VV-RSV G protein developed a Th1-type response on RSV challenge instead of the expected Th2 response and cleared RSV without developing severe eosinophilia (Walzl et al., 2000). Thus, memory T cells specific to heterologous agents may affect the Th1 or Th2 bias on subsequent exposure to allergens or new infections.

Plasticity in Accommodation

Epidemiological data have shown that resistance to reinfection is lost after some types of infections or vaccinations but not others. Attenuated viral vaccines tend to be stronger and longer-lasting immunogens than inactivated viral vaccines, and this has been linked to their ability to replicate in antigen-presenting cells, to stimulate CD8 T cell immunity, and perhaps to maintain a low-grade persistence after the immunization. Protective immunity against small pox as a consequence of vaccinia virus immunization wanes after a few years, but VV does not induce any type of detectable persistence (Hammarlund et al., 2003). These observations had led to the speculation that persisting antigen is required to maintain immunological memory, but numerous experiments in mouse models have indicated that this certainly is not the case with CD8 T cells. Memory CD8 T cell frequencies remain very stable after resolution of infections with LCMV, PV, VV, and influenza (Selin et al., 1996; Mullbacher, 1994; Lau et al., 1994), and adoptively transferred virus-specific memory T cells rapidly reach a steady state in a recipient host (Kim et al., 2002; Lau et al., 1994). This CD8 T cell stability is maintained by an IL-15-dependent continuous low-grade division of all of the memory CD8 T cells (Zhang et al., 1998).

These seemingly disparate observations are explained by the fact that the memory CD8 T cell stability seen in the murine studies occurs in hosts that do not encounter other infections or strong antigenic challenges. The stability of memory CD8 T cell frequencies is greatly disrupted by other infections, which lead to a loss in memory to previously encountered antigens (Selin et al., 1996, 1999; Smith et al., 2002). Thus, the apparent need for antigen persistence to maintain long-term memory in humans may have more to do with restimulating memory populations reduced after other infections than with an absolute need of memory cells to receive an antigenic stimulus to survive. Several recent publications have stressed the long-term stability of CD8 T cells specific to nonpersistent human viruses but there still is greater than a 10-fold loss with time (Hammarlund et al., 2003).

Part of the plasticity of the memory CD8 T cell response is its volatility in the wake of infections. Deletions in memory T cells have been observed in the very early stages of viral and bacterial infections and have been associated with the phenomenon of virus-induced lymphopenia (Peacock et al., 2003; McNally et al., 2001; Jiang et al., 2003a). Permanent losses of memory T cells specific to previously encountered viruses have also been shown to occur in the long-term memory state after subsequent viral or bacterial infections (Selin et al., 1999; Smith et al., 2002). A pertinent question is whether these events are interrelated or distinct.

Attrition of Memory Early in Acute Viral Infection

Infections with many viruses, including measles, influenza, West Nile, Ebola, varicella zoster, and LCMV, induce a dramatic lymphopenia early in infection (Peacock et al., 2003). Mechanisms for this lymphopenia are likely to be diverse, but in the LCMV system it is dependent on, though not necessarily directly mediated by, type 1 IFN and does not require IFNγ or Fas/FasL interactions (McNally et al., 2001). The lymphopenia, which occurs throughout the body's lymphoid and peripheral organs, is particularly devastating to CD8 T cells of the memory phenotype, i.e., those coexpressing CD44 or else reacting with tetramers to previously encountered viruses (McNally et al., 2001; Jiang et al., 2003a; Peacock et al., 2003). This can be mimicked in mice with inoculations of the double-stranded RNA analog poly I:C, and it does not occur in mice lacking type 1 IFN receptors. Memory CD8 T cell loss can be as high as 80% after inoculation of mice with poly I:C. The memory T cell loss is by apoptosis, as the residual T cells react with annexin V and stain with markers for caspase activation.

A question that arises is whether the virus-induced lymphopenia actually aids in the vigorous induction of the virus-specific T cell response by making room for the development and proliferation of more T cells. It has long been known that T cell responses to diverse antigens improve in hosts rendered slightly lymphopenic by irradiation or cytotoxic drug treatment (Oehen and Brduscha-Riem, 1999). For, example, T cell responses to HSV are more profound in hosts treated with low doses of cyclophosphamide (Pfizenmaier et al., 1977). The relationship between these events remains unclear, but it has recently been shown that the virus-induced lymphopenia is more dramatic in young than in old mice, and young mice tend to generate a stronger T cell response to a virus than old mice (Jiang et al., 2003b).

Bona Fide Memory Cells versus Homeostatically Dividing Cells with a Memory Phenotype. The recovery of bona fide memory CD8 T cells from their lymphopenia-associated loss would be dependent on their ability to compete with the antigen-specific T cells responding to the ongoing infection and with T cells homeostatically expanding to fill the lymphopenic environment. Interestingly, lymphopenic environments induce the homeostatic proliferation of CD8 T cells, which expand in number until the environment is replete with cells that phenotypically resemble bona fide memory cells (Freitas and Rocha, 2000). These naive CD44lo CD8 T cells, which upregulate CD44 and proliferate in response to signals from IL-7 and IL-15, represent another example of the plasticity in functional phenotypes of memory T cell populations (Tan et al., 2002; Goldrath et al., 2002). Not all of these cells proliferate comparably, and it is thought that those undergoing the greatest degree of homeostatic division may be self reactive with host antigens. The net effect is that there are considerable numbers of these CD44hi CD8 "pseudomemory" cells that have not gone through the differentiation scheme of bona fide memory cells.

CD8 T cells responding to the new viral antigens proliferate vigorously at a rate of about three divisions a day and rapidly dilute out preexisting bona fide memory T cells unless those T cells are crossreactive with the new virus (Selin et al., 1996; Brehm et al., 2002). Bona fide antigen-specific memory T cells also compete poorly with the homeostatically proliferating pseudomemory T cells not specific for the virus (Peacock et al., 2003). When CSFE-labeled LCMV- or PV-immune splenocytes were transferred into environments rendered lymphopenic by irradiation, genetic deficiencies, or viral infections, the bona fide virus-specific T cells competed poorly with other T cells in the donor population, underwent fewer cell divisions, and were diluted about 10-fold as other pseudomemory T cells expanded to replete the host. Thus, there appear to be strong obstacles inhibiting the recovery of bona fide memory cells that have been depleted by virus-induced lymphopenia.

Attrition of Memory T Cells over Time Following Subsequent Heterologous Infections

A separate observation possibly related to the above mentioned lymphopenia is the finding that viral infections cause a permanent loss in memory to previously encountered viruses. This was initially shown by limiting dilution assays in mice infected with LCMV, PV, VV, and MCMV in sequence (Selin et al., 1996), but it has since been established in other viral and bacterial infections by more sensitive assay techniques (Selin et al., 1999; Smith et al., 2002). The question is, what frequencies can be maintained in the memory state and with LCMV over 20% of the CD8 T cells and over 30% of the CD44hi CD8 T cells can be shown to be LCMV specific long after resolution of infection (Peacock et al., 2003). These high frequencies mean that the capacity of a host to accommodate T cells specific to a wide variety of pathogens is limited, instead of being a proverbial bottomless pit. Here, the immune system demonstrates plasticity by deleting some memory T cells in order to accommodate others.

One can envision two models to account for the long-term reduction in memory CD8 T cells specific to previous pathogens after a host encounters a second pathogen. A *passive*, or competition model, would predict that there are a finite number of survival niches for memory cells in the lymphoid organs, and the large numbers of newly arising CD44hi CD8 T cells simply compete with the previously residing cells for these niches. An *active* model would predict that there is a mechanism that selectively kills off the preexisting memory cells. The obvious candidate for such an active model would be the lymphopenia that occurs early during infection. Recent kinetic studies on the survival of PV-specific T cells after LCMV infection in mice have indicated that the memory cells, once depleted, fail to recover and remain depleted in long-term memory (Kim and Welsh, 2004). This argues on behalf of the active model, though it certainly seems that under some conditions competition between the old and new memory cells must be a factor.

Attrition during Persistent Virus Infections

The depleted populations of virus-specific memory T cells stay at reduced frequencies after resolution of infection but then remain stable thereafter, unless the host receives another infection (Selin et al., 1996, 1999). These dynamics change under conditions of persistent infections. Persistent infections with mouse γ herpes virus or with LCMV-clone 13 can cause a dramatic attrition of preexisting memory (Liu et al., 2003; S.K. Kim and R.M.W., submitted). Adoptive transfer studies of

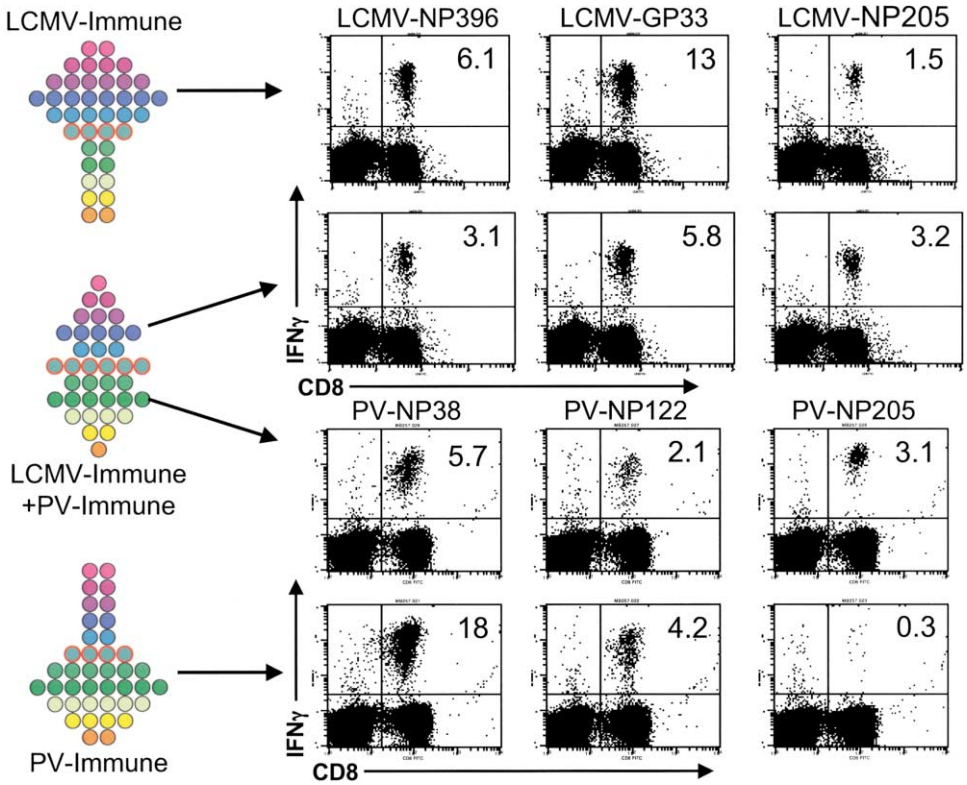

Figure 4. Accommodation of New Memory T Cells on Heterologous Virus Infection

The colored dots represent T cell populations that have different specificities. The intracellular IFNγ staining for epitope-specific responses during particular viral infections are also depicted. The immunodominant hierarchy of antigen-specific responses is established during the peak of the CD8 T cell response to virus and remains the same during the silencing phase into memory for both LCMV and PV infection. After a heterologous virus infection, such as PV challenge of an LCMV-immune host, the LCMV-specific hierarchy is modified, the crossreactive-epitope responses (NP205) are preserved and expanded in response to PV infection, and the noncrossreactive epitope responses are reduced in number. In an LCMV-immune host, the PV-specific immunodominant hierarchy is different from that of a naive host, with the crossreactive epitope response (NP205) being immunodominant.

CFSE-labeled PV-immune splenocytes into mice persistently infected with LCMV as adults and mounting a low-grade antiviral T cell response led to a substantial deletion of the PV-specific T cells, in comparison to control or LCMV-immune recipients (S.K. Kim and R.M.W., submitted). Thus, persistent infections may enact a severe and continuous toll on preexisting memory cells, and this may be reflected in the immune suppression to other antigens so often seen in persisting infections (Rouse and Horohov, 1986; Finkel et al., 1995).

Attrition and Crossreactivity Modulate Memory Responses

An important factor that influences the deletion of memory T cells and contributes to the plasticity of the T cell repertoire is when memory T cells specific to previously encountered pathogens crossreact with the newly encountered pathogen. Indeed, after an across-the-board deletion as a consequence of lymphopenia, crossreactive T cells will extensively proliferate and influence T cell immunodominance in the next infection (Brehm et al., 2002) (Figure 4). Hence, a history of a previous infection can influence the T cell response to a subsequent infection, and a second infection can activate memory T cells specific for previously encountered pathogens. The T cell repertoire specific to a pathogen can thus be permanently changed by subsequent infections with putatively unrelated viruses. Significantly, these continuously evolving memory T cell responses participate in and influence disease outcome of each new infection, whether it be harmful or beneficial to the host.

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