

## Microreview

# CD8 T cell responses to viral infections in sequence

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### Summary

**Our current understanding of virus-specific T cell responses has been shaped by model systems with mice, where naive animals are infected with a single viral pathogen. Paradigms derived from such models, however, may not always be applicable to a natural setting, where a host is exposed to numerous pathogens over its lifetime. Accumulating data in animal models and with some human diseases indicate that a host's prior history of infections can impact the specificity of future CD8 T cell responses, even to unrelated viruses. This can have both beneficial and detrimental consequences for the host, including altered clearance of virus, distinct forms of immunopathology, and substantial changes in the pool of memory T cells. Here we will describe the characteristics of CD8 T cells and the dynamics of their response to heterologous viral infections in sequence.**

### Introduction

Disease severity during viral infections is a function of direct virus-induced cytopathology and of the non-specific inflammatory and antigen-specific immune responses of the host. An immune host encountering a virus for a second time usually resists any disease associated with re-infection, as the expanded pools of virus-specific memory B and T cells effectively restrict the replication of the pathogen. Individuals exposed to a virus for the first time, however, can vary dramatically in their symptoms and viral load. This variation is sometimes attributed to the initial viral dose or to the genetics or physiological state of the host, but we suggest here that another major factor lead-

ing to this variation is the contribution of the pools of memory cells specific to previously encountered and sometimes completely unrelated pathogens.

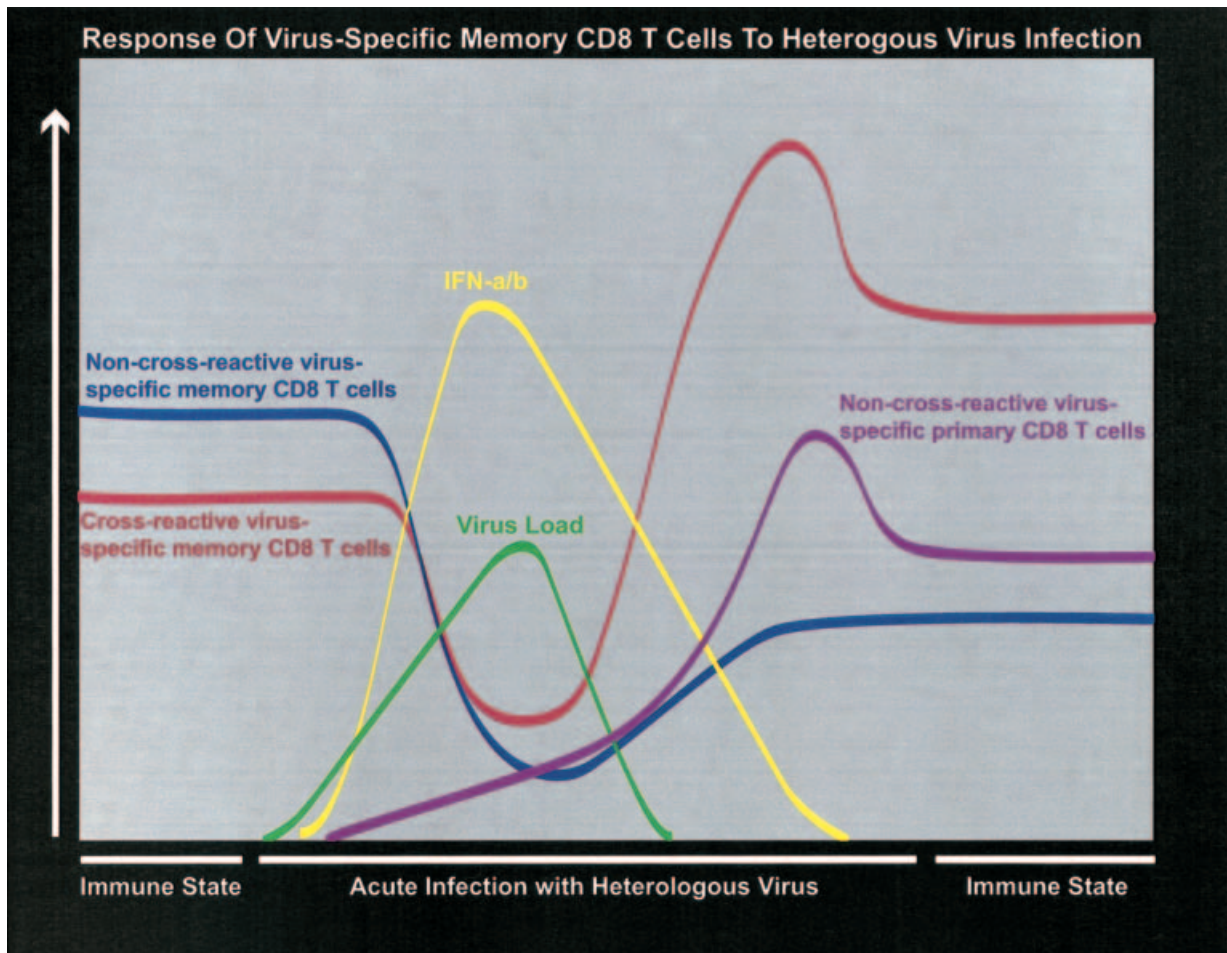
It has been recognized for some time that B cell responses generated by prior viral infections will influence both the production of virus-specific antibodies and the replication of virus during future infections with antigenically related but not identical viruses through phenomena such as 'original antigenic sin' (Francis, 1953; Fazekas de St and Webster, 1966) and antibody-dependent immune enhancement (Tirado and Yoon, 2003). Because of the profound involvement of CD8 T cells in viral immunity and immunopathology and of their propensity for cross-reactivity against a diverse array of epitopes, we have explored and documented their potential for being even more effective than cross-reactive antibody in altering the pathogenesis of virus infections in sequence. Here we will describe the properties of CD8 T cells and examine their modulation and function under conditions of infections with heterologous viral pathogens in sequence (Fig. 1).

### Degeneracy in antigen recognition and cross-reactivity

CD8 T cells recognize short, 8–11 amino acid residue peptide epitopes presented in the context of self-MHC class I molecules (Townsend *et al.*, 1986). The recognition of this peptide/MHC complex is mediated by the T cell receptor (TCR), which is a heterodimeric structure comprised of an  $\alpha$  and  $\beta$  chain (Davis *et al.*, 1998). Within each chain of the TCR are three variable sites, known as complementarity determining regions (CDRs) (van der Merwe and Davis, 2003), which protrude as loops from the TCR and directly contact sites on the peptide and the MHC molecule (Stewart-Jones *et al.*, 2003).

Theoretical considerations have suggested that the number of peptide-MHC complexes that could be generated by environmental antigens exceeds the number of mature T cells within an individual (Regner, 2001). Thus, the breadth of the T cell repertoire would not be sufficient unless T cells had the capacity to recognize more than one antigen. Otherwise, non-immunogenic pathogens would easily evolve to escape detection by T cells. T cells are, however, cross-reactive against numerous epitopes, and a mathematical modelling analysis predicted that a

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**Fig. 1.** Response of both non-cross-reactive and cross-reactive virus-specific memory CD8 T cells to heterologous virus infections. This figure depicts the events occurring after a secondary infection of a virus-immune mouse with a heterologous but cross-reactive virus such as PV. Shown on the figure is the stability of memory CD8 T cells before re-challenge, the virus-induced lymphopenia, the peak of type 1 IFN, the peak of virus load, the expansion of T cells, the apoptotic decline, and the return to an immune state. It shows the loss of non-cross-reactive memory CD8 T cells and the maintenance of cross-reactive memory.

single TCR may be able to interact with over one million different peptides (Mason, 1998). The CDR3 loops of the TCR undergo significant conformational changes in order to accommodate the 3-dimensional structure of the peptide-MHC surface (Garcia *et al.*, 1998; Reiser *et al.*, 2002; Wu *et al.*, 2002; Kjer-Nielsen *et al.*, 2003). This flexibility imparted to the TCR may allow a single TCR to be promiscuous in the recognition of peptides and thereby be inherently cross-reactive. This cross-reactive nature of T cells may allow memory CD8 T cells generated by prior viral infections to play roles in subsequent infections with unrelated viruses.

Cross-reactivity between virus-specific CD8 T cells has been identified in a variety of different forms in both human and murine models. An expected form of cross-reactivity would be against evolutionarily conserved sequences between very closely related viruses, where epitopes would either be identical or differ only slightly.

Such cross-reactivity has been observed in T cell responses between influenza virus A subtypes (Haanen *et al.*, 1999; Belz *et al.*, 2001), members of the Old World arenavirus family (Oldstone *et al.*, 2001), the four serotypes of dengue virus (Spaulding *et al.*, 1999; Mongkolsapaya *et al.*, 2003), very closely related hantaviruses (including Hantaan and Seoul) (Van Epps *et al.*, 1999), and serotypes of vesicular stomatitis virus (VSV-Indiana and New Jersey) (Puddington *et al.*, 1986; Yewdell *et al.*, 1986). Cross-reactivity does not have to be between homologous or highly conserved sequences, however. While mapping immunodominant epitopes cross-reactivity was occasionally found between two different and non-homologous sequences on the same protein or between different proteins derived from the same virus. Such cross-reactivity has been reported for T cells specific for peptides derived from influenza virus (Kuwano *et al.*, 1991; Anderson *et al.*, 1992; Haanen *et al.*, 1999), poly-

oma virus (Wilson *et al.*, 1999), and respiratory syncytial virus (RSV) (Kulkarni *et al.*, 1993).

Of significance is that virus-specific CD8 T cells have also been shown to recognize epitopes derived from heterologous or more distantly related viruses. CD8 T cell cross-reactivity amongst heterologous viruses has been defined between the two distantly related Arenaviruses (lymphocytic choriomeningitis virus (LCMV) and Pichinde virus (PV)) (Brehm *et al.*, 2002), between different flaviviruses (Murray Valley encephalitis virus, yellow fever virus, West Nile virus, and dengue virus) (Regner *et al.*, 2001), between influenza virus and EBV (Welsh *et al.*, 2004), between influenza virus and hepatitis-C virus (Wedemeyer *et al.*, 2001), between influenza virus and rotavirus (Shimojo *et al.*, 1989), between human papillomavirus-16 and a human coronavirus (Nilges *et al.*, 2003), and between LCMV and vaccinia virus (VV) (Welsh *et al.*, 2004). Cumulatively, these findings demonstrate the highly cross-reactive nature of virus-specific T cell responses, and it seems likely that with further investigation more examples will be discovered.

### Impact of viral infections on memory T cells

The T cell repertoire of a host that has been previously infected with a virus will be significantly altered relative to a naïve individual because a robust and stable population of memory T cells will have been established. New viral infections can have a profound impact on these pre-existing pools of memory cells as a consequence of inflammatory cytokines and chemokines and of antigen-dependent TCR stimulation (Fig. 1).

#### *Memory T cell apoptosis and compensatory division*

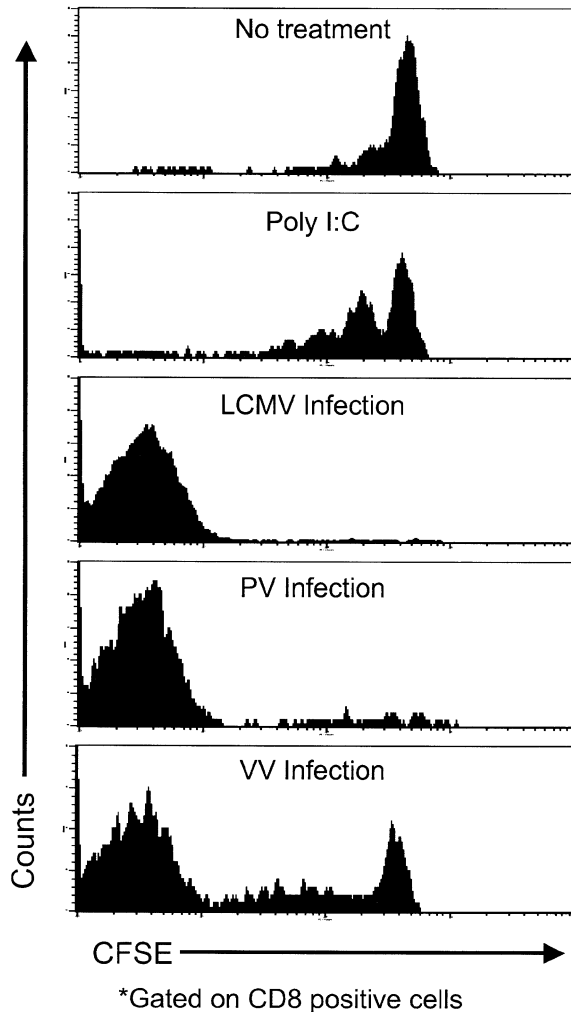
Initial stages of infection often involve a profound depletion of memory CD8 T cells throughout the body in both lymphoid and peripheral tissue (Fig. 1). This is a manifestation of a global virus-induced lymphopenia that occurs with many viral infections and that affects many cell types (Peacock *et al.*, 2003). This lymphopenia is at least partially dependent on type 1 IFN, as it parallels the type 1 IFN response, can be induced by the IFN stimulator poly I:C, and does not occur in type 1 IFN receptor knock-out mice (Binder *et al.*, 1997; McNally *et al.*, 2001). This cell loss is associated with high levels of apoptosis in the memory CD8 T cell subset and lower levels in the naïve CD8 T cell subset (McNally *et al.*, 2001). Whether IFN works alone or through another mechanism is not clear at present, but it should be noted that IFN can induce other pro-apoptotic proteins such as TRAIL (Barber, 2001; Chawla-Sarkar *et al.*, 2003) and that it can enhance the effects of other pro-apoptotic molecules such as TNF- $\alpha$  (Binder *et al.*, 1997).

This early loss of T cells is followed by antigen-specific and non-specific homeostatic events to fill the available space. Treatment with agents such as poly(I:C), IFN- $\gamma$ , LPS, and IL-15 can stimulate CD44<sup>hi</sup> memory-phenotype CD8 T cells to divide, as determined by uptake of bromodeoxyuridine (BrdU) into DNA (Tough *et al.*, 1996; 1997; Zhang *et al.*, 1998). However, this bystander division does not result in an overall increase in the number of T cells, suggesting that the division is balanced by cell death in order to maintain homeostasis (McNally *et al.*, 2001; Turner *et al.*, 2001). A careful examination of this division after poly(I:C) treatment shows first a profound apoptosis-driven loss of memory CD8 T cells (McNally *et al.*, 2001), followed by an IL-15-dependent recovery to steady-state levels (Zhang *et al.*, 1998; Kim and Welsh, 2004). Studies examining the recovery of memory cell populations after various forms of experimental lymphopenia showed that bona fide virus-specific memory CD8 T cells underwent fewer cell divisions than other homeostatically responding cells, such that there was a substantial permanent loss in virus-specific T cells under those conditions (McNally *et al.*, 2001; Peacock *et al.*, 2003).

Viral infections resemble poly I:C in their induction of memory cell loss, but they also provide antigenic peptide epitopes to stimulate T cells. T cells stimulated by their cognate ligands are induced into many cycles of division and far out-compete the homeostatically dividing T cells (Fig. 2) (Kim *et al.*, 2002). Whether antigen ligation can prevent the initial apoptosis of memory T cells has been investigated. Studies using an adoptive transfer model to examine the attrition of memory T cells demonstrated that LCMV-specific memory CD8 T cells were significantly reduced in number 2 days after infection with the heterologous but cross-reactive PV, and T cells specific for either the cross-reactive or non-cross-reactive peptides underwent similar levels of attrition (Peacock *et al.*, 2003; Kim and Welsh, 2004). This suggests that the attrition of memory is a generalized phenomenon affecting all memory T cell specificities independent of TCR signalling. Cross-reactive T cells, however, selectively and rapidly expand in number following the early attrition process (Fig. 1) (Brehm *et al.*, 2002). A recent study examining the susceptibility of naïve T cells to attrition concluded that infection with *Listeria monocytogenes* selectively depleted naïve CD8 T cells that were not specific for antigens expressed by the bacteria by 3 days, while antigen-specific naïve T cells were not lost (Jiang *et al.*, 2003).

*Cytokine production.* Some viral and bacterial infections can induce a high proportion of memory CD8 T cells to synthesize IFN- $\gamma$  early in infection (Chen *et al.*, 2001; Lertmemongkolchai *et al.*, 2001; Berg *et al.*, 2003). This is an important observation relating to heterologous immunity, as many pathogens are controlled by IFN- $\gamma$ . Some work

### Division of CD8 T Cells\* from LCMV-Immune Donor Mice After Treatment with Poly(I:C) or Viral Infection (Day 6)



has suggested that cytokines alone may stimulate memory cells to produce IFN- $\gamma$  (Berg *et al.*, 2002; Kambayashi *et al.*, 2003). However, the mechanisms mediating this early production of IFN- $\gamma$  have not been satisfactorily elucidated, as viral and bacterial pathogens may encode numerous foreign epitopes having the capacity to trigger the TCR at low affinity, and the role of T cell cross-reactivity in these studies was not excluded. Results from our laboratory highlight the difficulties in distinguishing between TCR-dependent and TCR-independent stimulations. LCMV-immune CD8 T cells produced IFN- $\gamma$  3 days after vaccinia virus (VV) infection (Chen *et al.*, 2001). It could be argued that this was a non-specific phenomenon because T cells of multiple specificities were activated. However, IFN- $\gamma$  was made by higher proportions of some LCMV epitope-specific T cells than other epitope-specific populations. This diversity in the proportions of epitope-specific populations producing IFN- $\gamma$  suggested a level of

**Fig. 2.** CD8 T cells from LCMV-immune mice are stimulated to divide following infection with homologous and heterologous viruses. Splenocytes from LCMV-immune mice (Thy1.1) were labelled with CFSE and adoptively transferred into congenic naïve mice (Thy1.2). One day following transfer, recipient mice were left untreated, injected with poly(I:C), or infected with LCMV, PV, or VV. Six days later, splenocytes from recipient mice were stained with antibodies to CD8 and the congenic marker (Thy1.1) and then analysed by flow cytometry. The histograms showing the loss of CFSE were analysed by gating on cells positive for the donor marker and CD8. This figure was courtesy of S.K. Kim and based on previous studies (Kim *et al.*, 2002).

selectivity that may be supported by the recent identification of some cross-reactive epitopes between VV and LCMV (Welsh *et al.*, 2004). Regardless of the mechanism for IFN- $\gamma$  production, the IFN- $\gamma$  inhibited the replication of VV.

*Recruitment to sites of inflammation.* Memory CD8 T cells, surviving the early attrition process, can be non-specifically recruited to peripheral sites of infection early following a viral challenge (Zhang and Mak, 1986). This has been shown in transgenic and non-transgenic systems. Memory TCR-transgenic CD8 T cells specific for OVA and the LCMV-epitope GP33 migrate to the lung following intranasal infection with the unrelated influenza virus and RSV, respectively (Topham *et al.*, 2001; Ostler *et al.*, 2003). Migration of MHC-tetramer-defined memory T cell populations to peripheral sites of infection was also shown in studies demonstrating that memory CD8 T cells

specific to influenza virus, LCMV, murine  $\gamma$  herpes virus 68, and Sendai virus were recruited into the lung following infection with heterologous viruses (Topham *et al.*, 2001; Ely *et al.*, 2003; Ostler *et al.*, 2003). The mechanism mediating the recruitment of memory CD8 T cells to the lung following intranasal infection is not yet fully defined but would be consistent with a generalized migration to an inflammatory site driven by the production of chemokines (Stephens *et al.*, 2002), as this influx of T cells is independent of cell division (Ely *et al.*, 2003; Ostler *et al.*, 2003). The generalized recruitment of virus-specific CD8 T cells to the site of infection may be a normal characteristic of an inflammatory response that allows for efficient effector function in the periphery.

### Sequential infections with homologous and heterologous viruses

Memory CD8 T cells generated by prior viral infections will be reactivated upon re-challenge with the originally encountered virus and will contribute to the overall immunity against the second infection. Normally the presence of neutralizing antibody will mute a secondary T cell response by restricting viral replication and antigen presentation to T cells, but in the absence of neutralizing antibody sufficient antigen is expressed to induce an anamnestic T cell response. A well documented example is in the influenza system, where antigenic shift produces subtypes of influenza A virus that are not recognized by neutralizing antibodies generated by previous infections with different influenza A subtypes (Wright and Webster, 2001). In the absence of those protective antibodies, prior infection with one influenza subtype generally still provides substantial protection against a challenge with a different subtype. This is referred to as heterosubtypic immunity (Schulman and Kilbourne, 1965). The protection afforded against subsequent infections with heterologous influenza virus subtypes has been attributed, in some studies, to the massive cross-reactive CD8 T cell response at the site of infection following rechallenge (Yewdell *et al.*, 1985; Townsend *et al.*, 1986; Belz *et al.*, 2001). Similar types of responses have been seen between the New Jersey and Indiana strains of VSV, which do not share neutralizing antibody epitopes but have cross-reactive T cell epitopes that differ in only two amino acids (Puddington *et al.*, 1986; Yewdell *et al.*, 1986). All of these systems indicate that if virus replication is not restricted by neutralizing antibody, the response of epitope-stimulated T cells can be quite profound, and this feature could contribute to dramatic T cell responses under conditions of heterologous immunity.

The promiscuous nature of antigen recognition by the TCR allows memory CD8 T cells generated by prior viral infections to cross-react with heterologous viruses and to

impact the generation of immune responses against subsequently encountered pathogens. To characterize the response of virus-specific memory CD8 T cells against subsequent infections with heterologous but cross-reactive viruses, the proliferation of these memory cells was examined after a viral challenge using an adoptive transfer model that allows the division of CD8 T cells to be directly quantified (Lyons and Parish, 1994). Splenocytes from LCMV-immune mice were labelled with a fluorescent dye, CFSE, which divides equally between daughter cells during division and then transferred into a naive host. Infection of a recipient with the homologous virus, LCMV, induced substantial division and accumulation of the donor CD8 T cells (Fig. 2), with virtually all detectable T cells specific for LCMV undergoing at least seven divisions, the limit of detection with CFSE (Kim *et al.*, 2002). In contrast, treatment of recipient mice with poly (I:C) induced no increase in cell number of donor cells and stimulated only limited cell division, again demonstrating the requirement for TCR engagement for sustained proliferation. In this transfer model, infection with the heterologous but cross-reactive viruses PV and VV stimulated significant proliferation of a subset of the donor cells (Fig. 2), which were presumably those cross-reactive between the viruses. These findings show that cross-reactivity results in the proliferation of selected populations of virus-specific memory CD8 T cells and raises the question of what effect these expanded populations of memory T cells will have on the response against the current infection.

The stimulation of LCMV-specific CTL activity and proliferation of populations of virus-specific memory CD8 T cells by subsequent infections with heterologous viruses resemble the phenomenon of 'original antigenic sin' that has been described for B cell responses against influenza virus subtypes (Francis, 1953; Selin *et al.*, 1994; Kim *et al.*, 2002). In agreement, mice previously infected with a wild-type strain of LCMV and then challenged with variant strains of LCMV that encode altered T cell epitopes preferentially generated CD8 T cells that were specific for the epitope encoded for by the previously encountered wild-type virus (Klenerman and Zinkernagel, 1998). However, the recall T cell responses generated by secondary infection with the LCMV-variants did not efficiently recognize the variant epitopes encoded for by the second virus and resulted in the impaired clearance of the variant viruses. This impairment in immunity during sequential infections suggests that in some instances memory T cells generated by prior infections can have detrimental consequences for a host's defence against pathogens.

A similar phenomenon has been demonstrated for dengue virus-specific CD8 T cell responses in humans (Mongkolsapaya *et al.*, 2003). Dengue viruses are arthropod-borne flaviviruses occurring as four serotypes defined in part by neutralizing antibody (Rothman and

Ennis, 1999). Initial infection with one dengue serotype provides protective immunity against re-infection with the identical virus, but subsequent infection with other serotypes can be associated with the development of life-threatening disease such as dengue haemorrhagic fever and dengue shock syndrome. Interestingly, secondary infections with dengue virus elicited CD8 T cells with a higher affinity for different dengue serotypes than for the current infecting virus, suggesting the reactivation of memory CD8 T cells specific to previously encountered dengue virus serotypes.

### T cell immunodominance and sequential viral infections

Epitope-specific CD8 T cells generated by viral infections have defined hierarchies, with some dominating the response and others being weak to barely detectable (Mylin *et al.*, 1995). These immunodominance hierarchies are extremely reproducible between individual hosts of the same haplotype and are maintained in the memory population (Belz *et al.*, 2000). Multiple parameters, including the efficiency of peptide processing and presentation, the affinity of the peptide for the presenting MHC molecule, the quantity of MHC-peptide complexes, the available TCR repertoire, and competition between T cells (immunodominance) are thought to contribute to these immunodominance profiles (Yewdell and Bennink, 1999).

The activation of virus-specific memory CD8 T cells following infections with heterologous viruses is a newly described factor influencing the immunodominance of T cells specific for the challenge virus (Brehm *et al.*, 2002). Cross-reactive T cells are not always readily apparent following an infection with a single virus because the frequencies of cross-reactive cells may be very low. Cross-reactivity is more easily detectable following multiple infections, because it is those cross-reactive T cells that are selectively amplified after infection (Selin *et al.*, 1994; Haanen *et al.*, 1999). The expansion of cross-reactive CD8 T cell responses by sequential infection with natural variants of influenza virus has been elegantly demonstrated (Haanen *et al.*, 1999). The two influenza variants differ by two amino acid residues in the dominant epitope NP366. Sequential infection with the two influenza variants preferentially stimulated the expansion of the cross-reactive CD8 T cells that represent only a minor population during infection with a single variant. These findings show that the potential for cross-reactivity between heterologous viruses may be completely obscured unless the cross-reactive T cells are amplified by infections in sequence.

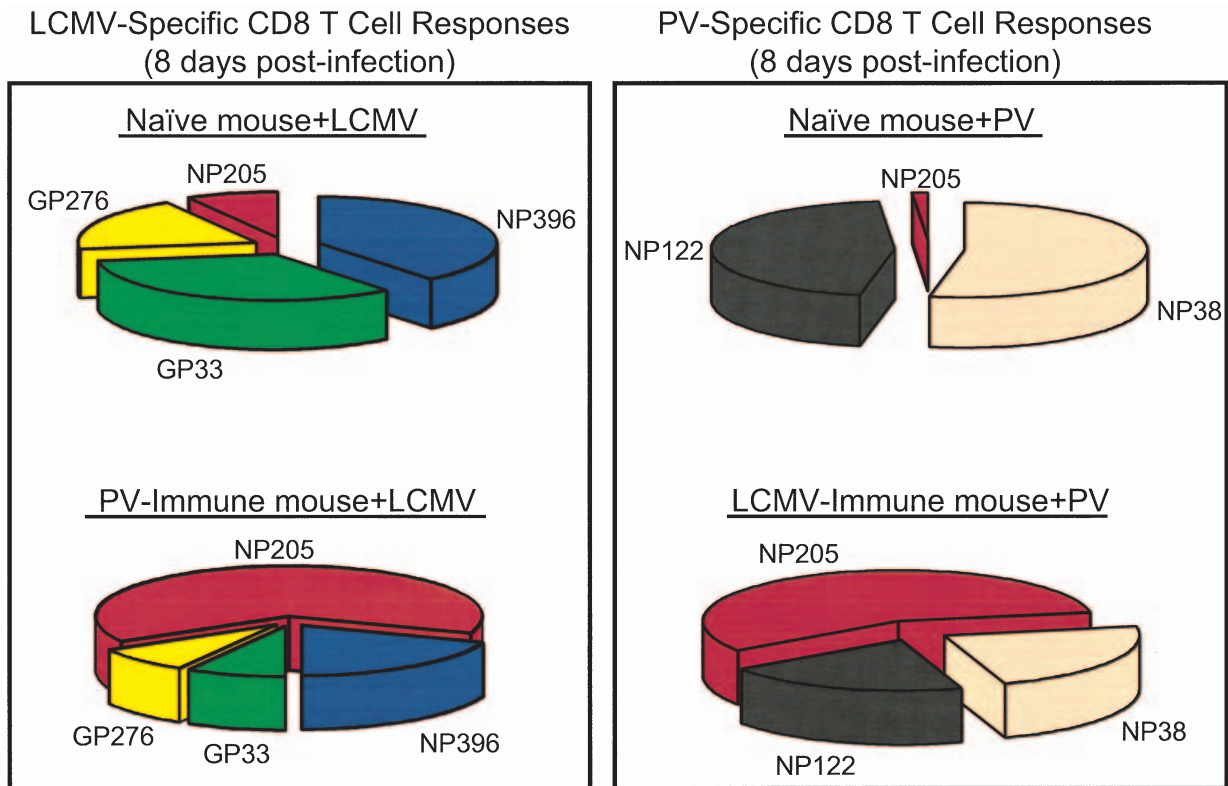
The immunodominance hierarchies for H-2<sup>b</sup>-restricted CD8 T cells specific for LCMV are dramatically altered in mice previously infected with heterologous but cross-reactive

viruses (Brehm *et al.*, 2002). Following sequential infections with LCMV and PV in either order, CD8 T cells specific for the normally subdominant but cross-reactive NP205 epitope become dominant, while CD8 T cells specific for the normally dominant epitopes are reduced in frequency (Fig. 3). Alterations in the immunodominance hierarchies of LCMV-specific memory CD8 T cells have also been observed following infection with VV (Chen *et al.*, 2001; Kim *et al.*, 2002). These findings all lead to the conclusion that the nature of CD8 T cell immunodominance in a 'real world' scenario where a host is continuously exposed to viral pathogens may be very different when compared to a naive host.

### Heterologous immunity and immunopathology

A host's history of viral infections may also influence how well the new infection is controlled, and this is a function of heterologous immunity. Mice previously infected with a panel of heterologous viruses (LCMV, PV, MCMV and VV) were often protected against challenges with the unrelated viruses (Selin *et al.*, 1998). For example, LCMV-immune, PV-immune and MCMV-immune mice were afforded a significant level of protection against challenge with VV (greater than a 100-fold decrease in titres relative to naive mice by four days post infection). Both CD4 and CD8 T cells from LCMV-immune mice were necessary to provide protection against PV and VV, and immunity against VV was mediated at least in part by the production of IFN- $\gamma$ . Of note is that this immunity was not reciprocal, as VV-immune mice were not protected against infection with any of the heterologous viruses. This lack of reciprocity for heterologous immunity in VV-immune mice correlated with the functional activity of T cells, as VV-specific memory T cells were not stimulated by LCMV infection to proliferate or to be cytolytic (Selin *et al.*, 1994; Kim *et al.*, 2002).

Heterologous immunity has been studied extensively with respiratory viral infections (Chen *et al.*, 2001; 2003). LCMV-immune mice were protected against a lethal dose of VV delivered intranasally, with significant reductions in VV titres in the lung compared to naive mice. Mice previously infected with MCMV or influenza also were protected against intranasal challenge with VV. Following VV challenge of mice that were previously infected with either LCMV or influenza, a shift in cytokine production towards a Th1 phenotype (such as IFN- $\gamma$  production) was observed in the lungs 3 days after infection in comparison to the cytokines produced in VV-infected naive mice. Heterologous immunity in the lung was not always protective, however, as clearance of LCMV and MCMV was impaired in influenza-immune mice, and a history of an LCMV infection impaired the ability of mice to clear RSV from the lung following an intranasal inoculation (Ostler *et al.*,



**Fig. 3.** The immunodominance hierarchies of virus-specific CD8 T cell responses are altered in mice that have been previously infected with a heterologous virus. The sections of the pie charts reflect the proportion of each epitope-specific response relative to the T cell repertoire shown. The LCMV-specific response is shown on the left and the PV-specific response is shown on the right. The cross-reactive T cells specific for the NP205 epitope are shown in red for both responses. The figure is based on previous studies (Brehm *et al.*, 2002).

2003). The mechanisms of diminished protective immunity in mice previously infected with heterologous virus are uncertain and may correlate with altered cytokine profiles. Alternatively, the reduced levels of protection may be attributed to the stimulation of memory T cells that are not effective at controlling the infection and to the suppressed development of T cells that would be effective, as shown in other studies on original antigenic sin (Klenerman and Zinkernagel, 1998; Mongkolsapaya *et al.*, 2003). Overall these results indicate that prior viral infections can either augment or inhibit viral clearance during subsequent infections with unrelated viruses.

Heterologous immunity may also be associated with the development of severe immunopathology. LCMV-immune mice infected intraperitoneally with VV developed an IFN- $\gamma$ -dependent acute fatty necrosis associated with a cellular infiltrate in the visceral fat pads (Selin *et al.*, 1998). In an intranasal model, LCMV-immune mice infected with VV developed a severe lung pathology that was characterized by a strong lymphocytic response, and the induction of bronchiolitis obliterans, which involves the obstruction of bronchioles by fibrin and inflammatory cells (Chen *et al.*, 2001). This development of immunopathology during sequential viral infections correlates with the activation of

pre-existing memory LCMV-specific CD8 T cells, but additional factors may also contribute to the pathology, including altered cytokine profiles. Other studies have shown that prior infections with MCMV, influenza, and Sendai can dramatically alter the histopathology of the lung during subsequent infections with unrelated viruses (Jakab, 1990; Chen *et al.*, 2003). Some of the immunopathologies developed following sequential viral infections resemble forms of immune-mediated human diseases of unknown aetiology, with acute fatty necrosis being similar to erythema nodosum, the most common form of human panniculitis, and the induction of bronchiolitis obliterans in humans being associated with viral and bacterial infections and transplant rejections (Bologna and Braverman, 1992; Schlesinger *et al.*, 1998).

#### Maintenance of T cell memory during sequential viral infections

Virus-specific memory CD8 T cells generated by a single virus are maintained at relatively stable frequencies over time (Selin *et al.*, 1996; Homann *et al.*, 2001). However, following a subsequent viral infection these populations of memory CD8 T cells are dramatically altered, with the

permanent loss of the non-cross-reactive T cells from the memory repertoire and an enrichment of T cells that are cross-reactive with the challenge virus (Fig. 1) (Selin *et al.*, 1996; 1999; Brehm *et al.*, 2002). Challenge of virus-immune mice with heterologous viruses that establish either acute sterilizing infections (LCMV strain Armstrong, PV, MCMV, and VV) or persistent infections (murine herpes virus 68 and LCMV clone 13), can trigger the attrition of virus-specific CD8 T cells from the memory repertoire (Liu *et al.*, 2003; Kim and Welsh, 2004). Infection with the clone 13 variant of LCMV is associated with the continuous production of cytokines and T cell activation and resulted in an even more dramatic attrition of memory CD8 T cells relative to LCMV strain Armstrong, with greater than 10-fold drops in CD8 memory T cell frequencies specific to VV, VSV, and PV-encoded antigens (Kim and Welsh, 2004).

This depletion of CD8 T cell memory could be a consequence of competition between newly generated memory T cells and previously formed memory cells for protective niches in lymphoid tissue. Alternatively it could be a by-product of the virus-induced lymphopenia that leads to memory T cell apoptosis early in infection. Recent kinetic studies indicate that once memory cells are lost early in infection, they never return to their original frequencies, arguing for the importance of the virus-induced lymphopenia in this process (Kim and Welsh, 2004). Thus, pre-existing memory populations can play substantial roles in the host response to new virus infections, and those virus infections permanently alter the memory T cell pool that was present before infection.

### Sequential viral infections and transplantation

Virus-specific CD8 T cells can cross-react with allogeneic MHC molecules presenting endogenous non-viral peptides (Braciale *et al.*, 1981; Yang and Welsh, 1986; Nahill and Welsh, 1993; Burrows *et al.*, 1994; Brehm *et al.*, 2003), and have the potential to hinder transplantation of allogeneic tissues. Acute infections with viruses such as LCMV and PV interfere with the induction of tolerance using costimulatory blockade against allogeneic skin grafts (Welsh *et al.*, 2000). Recent studies have also shown that LCMV-immune mice are refractory to the induction of tolerance against allogeneic skin resulting in graft rejection (Brehm *et al.*, 2003), and mice that have been sequentially infected with heterologous viruses become even more difficult to tolerate to allo-antigens (Adams *et al.*, 2003). Sequential infections with heterologous viruses can increase the frequency of T cells specific for allo-antigens, and create a larger pool of memory cells to tolerate before engraftment. In H-2<sup>b</sup> mice that had previously been infected with PV and then infected with

LCMV, the induction of allo-antigen-specific CD8 T cells was altered, with higher frequencies of T cells specific for H-2<sup>k</sup> antigens and slightly reduced populations specific for H-2<sup>d</sup> antigens in comparison to naïve mice infected with LCMV (Brehm *et al.*, 2003). The alterations in the allo-antigen-specific T cell response generated in PV-immune mice infected with LCMV may reflect the changes in the hierarchies of the LCMV epitope-specific (Fig. 3) CD8 T cells, which cross-react with allo-antigens. These findings suggest that the generation of allo-antigen-specific memory CD8 T cells by viral infections presents a unique impediment for transplantation.

### Concluding remarks

We demonstrated here that a host's history of viral infections, by changing the repertoire of the T cell memory pool, may influence T cell immunodominance, protective immunity, and immunopathology occurring in response to subsequent infections. Although parameters of this heterologous immunity have been studied most definitively in murine systems, the identification of T cells cross-reactive between many common human viruses suggests that the imprint left on the human immune system by previous viral infections may alter the course of human diseases. Many viral infections present more severely in teenagers and young adults than in children (Weinstein and Meade, 1956; Simonsen *et al.*, 1998), and one questions whether prior infections encountered earlier in life have altered the immune responses to these pathogens. Similarly, because of T cell cross-reactivity, vaccination might unexpectedly impact responses to pathogens unrelated to the vaccine. The full impact of heterologous T cell-dependent immunity in human infections should become clear as more is learned about the activation and fate of cross-reactive T cells in human viral infections.

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