

The Privacy of T Cell Memory to Viruses

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Abstract T cell responses to viral infections can mediate either protective immunity or damaging immunopathology. Viral infections induce the proliferation of T cells spe-

cific for viral antigens and cause a loss in the number of T cells with other specificities. In immunologically naïve hosts, viruses will induce T cell responses that, dependent on the MHC, recognize a distinct hierarchy of virus-encoded T cell epitopes. This hierarchy can change if the host has previously encountered another pathogen that elicited a memory pool of T cells specific to a cross-reactive epitope. This heterologous immunity can deviate the normal immune response and result in either beneficial or harmful effects on the host. Each host has a unique T cell repertoire caused by the random DNA rearrangement that created it, so the specific T cells that create the epitope hierarchy differ between individuals. This “private specificity” seems of little significance in the T cell response of a naïve host to infection, but it is of profound importance under conditions of heterologous immunity, where a small subset of a cross-reactive memory pool may expand and dominate a response. Examples are given of how the private specificities of immune responses under conditions of heterologous immunity influence the pathogenesis of murine and human viral infections.

Abbreviations

APC	Antigen-presenting cell
CDR	Complementary determining region
CMV	Cytomegalovirus
DC	Dendritic cell
EBV	Epstein–Barr virus
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
IFN	Interferon
J	Joining
LCMV	Lymphocytic choriomeningitis virus
MHC	Major histocompatibility antigen
NK	Natural killer
PKR	Protein kinase R
PV	Pichinde virus
TCR	T cell receptor
V	Variable
VV	Vaccinia virus

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Introduction

Immunological memory is a function of expanded clones of antigen-specific T and B cells. Its purpose is to protect a host from a second encounter with a pathogen, to keep low-grade persistent infections under control, and, by passive transfer of antibody, to protect a fetus or neonate from infection. The repertoire of T and B cells that constitutes a memory population is influenced by genetic and epigenetic factors, prior infection history, and innate response regulatory mechanisms (Welsh et al. 2004).

It has long been noted that an acute host response to viral infection comes in two waves, an early innate response associated with the induction of cytokines and activation of natural killer (NK) cells, dendritic cells (DCs), and macrophages, and a later response associated with the expanded clones of antigen-specific T and B cells (Welsh 1978; Biron 1995). A very important innate system cytokine characteristic of viral infections is type 1 interferon (IFN), which exerts many activities, including the direct inhibition of viral replication in infected cells (Welsh 1984; Biron and Sen 2001). Other important innate system cytokines are interleukin (IL)-12, IL-18, and inflammatory cytokines IL-1, IL-6, and tumor necrosis factor (TNF)- α (Biron 1995). Studies in the past 10 years have implicated toll receptors in the induction of many of these cytokines (Compton et al. 2003; Haynes et al. 2001). Viral proteins, RNA, and DNA can engage many of these receptors, as reviewed elsewhere in this volume. This receptor engagement triggers signal transduction events that release transcription factors such as interferon regulatory factor (IRF)-3 and nuclear factor (NF)- κ B to activate cytokine genes (Jiang et al. 2004). In addition, viral double-stranded RNA can activate protein kinase R (PKR), whose phosphorylated products release cytokine transcription factors into the nucleus (Biron and Sen 2001). The cytokines produced in response to these events can skew T cell responses into the type 1 (IL-2, IFN- γ) vs type 2 (IL-4, IL-5, IL-13) cytokine direction, and, as a consequence, influence the antibody isotype ultimately produced by the B cells. Viral infections tend to be strong inducers of type 1 cytokine responses, perhaps because of the early induction of IL-12 and IL-18, which themselves induce IFN- γ from NK cells and T cells.

The most important cellular interaction at the advent of a new immune response is the engagement of a naïve T cell with an antigen-presenting DC. DCs exposed to antigen in the periphery can become activated due to toll receptor engagement or to other “danger” signals, which induce the expression of co-stimulatory proteins such as CD80 (B7.1) and CD86 (B7.2) and of the CCR7 chemokine receptor, which directs their migration into the lymph nodes, where they encounter naïve antigen-specific T cells (Sallusto et al. 2000). CD80 and CD86 on the DCs engage CD28 on the T cells and induce CD154 (CD40L) expression on the T cells and the release of growth factors such as IL-2 (Harris and Ronchese 1999). This sets up a programmed proliferation of the T cells (Kaech and Ahmed 2001; Mercado et al. 2000; van Stipdonk et al. 2001), which may divide as many as 15 times before their peak in acute infection (Selin et al. 1994; Blattman et al. 2002; Welsh and Selin 2002). The CD4⁺ T cells through CD40L/CD40 interactions can provide help to B cells, which also proliferate (Liu et al. 1997). After clearance of viral antigens, the T cell response contracts by apoptosis (Razvi et al. 1995a) and

by dissemination into peripheral tissue (Marshall et al. 2001; Masopust et al. 2001; Reinhardt et al. 2001; Wiley et al. 2001), leaving the host with a stable population of memory T cells, which slowly divide but do not increase in number (Razvi et al. 1995b; Zimmermann et al. 1996). The B cell response is more complex, as some develop into long-lasting antibody-secreting plasma cells, and some become memory B cells, which do not secrete antibody but which can rapidly proliferate and produce antibody on antigen re-challenge (Han et al. 1997; Lin et al. 2003). Developing B cells triggered by viral antigens enter germinal centers and undergo somatic mutations and selection for higher affinity antibody responses (Han et al. 1997; Muramatsu et al. 2000). The high-affinity antibodies are more effective at the neutralization of viruses, though some viruses seem to induce high-affinity responses without affinity maturation (Clarke et al. 1990; Roost et al. 1995). This is likely because of germ line immunoglobulin sequences that encode antibody that is already high affinity.

This review will focus on the repertoire of cells constituting a T cell memory pool, how this repertoire is generated, how it is modulated by innate immune system cytokines, and how it is modulated in response to other infections. In particular, it will describe how properties of an immune system unique to an individual can alter the pathogenesis of infections. A more comprehensive though less focused review on immunological memory to viral infections can be found elsewhere (Welsh et al. 2004).

2 Diversity of Memory T Cell Repertoires

2.1 Immunodominance and TCR Repertoire Diversity

Generation of T cell repertoires in the immunologically naïve host is initially a stochastic process dependent on the randomness of DNA recombination events. There are about 10^{15} possibilities for the generation of T cell receptors (TCRs) with paired α - and β -TCR chains (Casrouge et al. 2000; Nikolich-Zugich et al. 2004). Since it is impossible to accommodate all possibilities within one body, only a subset of this repertoire is generated and present in any given host. The initial randomly generated repertoire is trimmed down as it passes through the positive and negative selection phases in the thymus. This has been elegantly shown in a transgenic mouse where a single β -TCR transgene is expressed along with an α -TCR artificial rearrangement substrate transgene that must undergo a recombination event to express an α -TCR that

would pair with the β -TCR and be expressed on a mature T cell. This approach, which provided a manageable number of α -TCR gene complementary determining regions 3 (CDR3) to sequence, showed a very high clonal diversity in CD4⁺CD8⁺ thymocytes (with few duplicates), which became less diverse in single positive thymocytes, with some sequences overrepresented 5–40 times, and even less diverse in peripheral T cells, perhaps as a consequence of homeostatic proliferation of some T cells but not others (Correia-Neves et al. 2001). In addition to the role of CDR3 in repertoire selection, the other TCR variable (V) regions, CDR1 and CDR2, may be involved in narrowing the repertoire due to their differences of affinity in binding to specific class I and class II MHC molecules. The consequence of this is an uneven V α and V β family distribution in the pre-immune TCR repertoires in the peripheral organs of mice or humans expressing different class I or II MHC antigens (Battaglia and Gorski 2002; Gulwani-Akolkar et al. 1991). Despite all of these trimming events, however, the naïve TCR repertoire in the immunologically naïve host remains quite diverse.

CD8 T cells recognize processed 8–9 amino acid viral peptides presented by class I MHC molecules (Townsend et al. 1986). For the most part these peptides are loaded into newly synthesized class I molecules in virus-infected cells, though there are examples of the “cross-presentation” of exogenous viral peptides on uninfected antigen-presenting cells (APC) (Sigal et al. 1999). Although hundreds of peptides in any viral infection have appropriate sequences to bind class I MHC, usually only a small number stimulate “immunodominant” T cell responses. What causes immunodominance is a function of (1) the number of T cells with receptors that recognize the peptide-MHC combination, (2) the expression level of the peptide and its affinity for binding the MHC, (3) how early during virus infection the epitope is expressed (earlier is better), and (4) whether there is “immunodomination” by T cells of other specificities (Yewdell and Bennink 1999). CD4 T cells recognize longer, usually 12–15 amino acid, viral peptides expressed by either endogenously or exogenously loaded class II MHC molecules. Similar issues are involved in CD4 T cell immunodominance as with CD8 T cells (Wang et al. 1992).

2.2

Public Vs Private Specificities

Despite the events that narrow the TCR repertoire, genetically identical individuals are still bequeathed with very different TCR repertoires because of the stochastic processes during T cell development. In the mouse, where it has been calculated that there are fewer than 10^7 T cell clones per host, there is substantial variability of the TCR repertoire from host to host (Cas-

rouge et al. 2000). Humans have about 3,000 times the body size of a mouse and will have many more clones of T cells, but this number will still be orders of magnitude below the theoretical possibility (Arstila et al. 1999). The repertoire selected in genetically identical environments, while being distinct between individuals, has similar potential specificities. When genetically identical, immunologically naïve mice are infected with a virus such as lymphocytic choriomeningitis virus (LCMV), they generate quantitatively similar responses to immunodominant epitopes, and their responses to these epitopes usually have similar hierarchies among individual mice. For example, most C57BL/6 mice will generate a range in T cell frequencies directed at a reproducible hierarchy of peptide epitopes, in the order of NP₃₉₆₋₄₀₄ > GP₃₄₋₄₁ > GP₃₃₋₄₁ > GP₂₇₆₋₂₈₆ > NP₂₀₅₋₂₁₂ > GP₉₂₋₁₀₁ (Kim et al. 2005; Fuller et al. 2004). This is a common “public specificity” (Cibotti et al. 1994) that can be predicted ahead of time in these genetically identical mice. Examination of TCR usage between mice will also show preference patterns for TCR V domains per epitope (Blattman et al. 2000). This is another manifestation of public specificity influenced in part by the importance of CDR1 and CDR2 regions in generating the pre-immune repertoire (Battaglia and Gorski 2002; Gulwani-Akolkar et al. 1991).

Sequencing of the CDR3 shows, however, that different TCRs are used by different hosts for similar types of antigen recognition. This is referred to as the “private specificity” of TCR repertoires (Cibotti et al. 1994; Maryanski et al. 2001). The CDR3 of antigen-specific TCR pools from different mice will have some similar CDR3 amino acids which establish a recognition “motif,” but many amino acids will be unshared. These private T cell responses are probably mostly accounted for by the diversity of T cells that emigrate from the thymus. In addition, there may be stochastic events involved with chance encounters between T cells and APC during early stages of infection. One might think that the T cell that is the first to encounter its antigen may become a dominant clone (Butz and Bevan 1998), whereas a T cell that first encounters antigen 2 days later may develop into a subdominant clone or else may never have a chance due to “immunodomination” by other clones competing for domains on APC.

2.3

Repertoire Selection During Infection

Events that shape the antigen-specific TCR repertoire occur very early in infection. T cell responses to *Listeria monocytogenes* epitopes occur rather normally if mice are treated with antibiotics 1 day after infection to prevent further antigen synthesis (Mercado et al. 2000). T cells that engage APC bind

to them for hours, but after disengagement the APC tend to die off, as has been elegantly visualized by in vivo videomicroscopy (Huang et al. 2004) and with APC infected with green fluorescent protein (GFP)-expressing recombinant viruses (Norbury et al. 2002). A question is how efficient this process is for stimulating potentially reactive T cells. This has been studied in mice with implanted 5(6)-carboxyfluorescein diacetate succinimidyl ester (CFSE)-labeled transgenic T cells, where LCMV or *L. monocytogenes* infections were shown to easily stimulate programmed expansions of nearly all of the cells (Mercado et al. 2000; Kaech and Ahmed 2001; van Stipdonk et al. 2001). These experiments would suggest that the stimulation of T cells, at least those of high affinity, can be quite efficient, and this probably means that the private specificity of the TCR repertoire is more a function of the pool of thymic emigrants than it is a function of inefficient random encounters with the APC. Nevertheless, limited titration of immunogen resulted in situations where only a subpopulation of the transgenic T cells responded (Kaech and Ahmed 2001). One should keep in mind that we are talking about systemic infections in mice, and the dynamics might be different with more limited localized infections or in humans. Due to their much larger thymic output, two MHC-matched humans may be 3,000 times more likely to have similar T cell clones than two mice and may be less likely to stimulate all of their antigen-specific clones during an infection. Perhaps in that case, stochasticity due to random encounters of T cells with APC may play a somewhat greater role in private specificities of T cell responses between humans.

The private specificity phenomenon complicates TCR studies between individuals but allows for longitudinal studies within an individual. Studies using CDR3 "spectratype" or "immunoscope" techniques, PCR-based techniques which analyze receptor diversity based on the CDR3 lengths of the expressed α and β TCRs derived from particular V families (Pannetier et al. 1993; Gorski et al. 1994), along with studies based on sequencing of the CDR3 region, all showed that genetically identical C57BL/6 mice infected with LCMV generated distinct TCR repertoires, as analyzed either by total leukocytes, total CD8 T cells, total CD4 T cells, or MHC tetramer or MHC-dimer defined and sorted antigen-specific T cells (Lin and Welsh 1998; Blattman et al. 2000; Wang et al. 2003). Sequential sampling of peripheral blood showed that the repertoire during an acute infection evolved until antigen was mostly cleared at about day 7 post-infection, after which it became relatively fixed as the T cell response contracted and entered the memory pool (Lin and Welsh 1998; Xiong et al. 2001). Similar spectratypes, with some minor variations, were noted when the memory pool was re-challenged with antigen.

2.4

The Complexity of Virus-Specific TCR Repertoires

Analyses of the TCR repertoires specific to viral peptides have been done in a number of systems, with perhaps the most extensive analyses being with human T cells recognizing an HLA-A2-restricted influenza M1₅₈₋₆₆ peptide (Lehner et al. 1995; Moss et al. 1991; Naumov et al. 1998). This is an invariant peptide present in all 35 influenza A virus strains that have infected humans since 1918 (Park et al. 1997). Over their lifetimes, people encounter many different influenza infections, which repeatedly should boost the response to the M1₅₈₋₆₆ peptide. During these repeated exposures the TCR repertoire changes. In young children only a small component of the repertoire is associated with V β 17 T cells, but by age 15 V β 17 T cells dominate the response (Lawson et al. 2001b). A detailed analysis of limiting dilution clones from 5 HLA-A2.1⁺ adult donors showed that 85% belonged to the V β 17 family, and a CDR3 sequencing analysis of 38 V β 17⁺ long-term clones revealed that 74% had a highly conserved XRSX motif (Lehner et al. 1995). Other M1₅₈₋₆₆-specific V β families (23, 13.6, 8.1) were found at lower frequencies and seemed of lower affinity, as they required high concentrations of epitope for cytotoxicity (Lawson et al. 2001a). This reflects a narrowing of a TCR repertoire that can occur on repeated exposure to antigen, and one might expect that this repeated exposure would select for a small number of dominant clones. However, a more extensive analysis of the full range of M1₅₈₋₆₆-restricted clones within individuals revealed a repertoire that is still quite diverse (Naumov et al. 1998, 2003). This showed that literally hundreds of clones constitute the M1₅₈₋₆₆-specific repertoire, but with no overwhelmingly dominant clones.

Molecular analyses of the V β 17 receptor repertoire from tetramer-identified M1₅₈₋₆₆-specific T cells or from M1₅₈₋₆₆-stimulated T cell lines revealed a power-law-like distribution in clonal composition, where a small number of clones were present at high frequency and larger numbers of clones were present at ever decreasing frequencies. Why this distribution occurs is unclear, though a power law distribution of the clonal ranks and rank frequencies can be mathematically generated simply by taking virtual clones of comparable affinity and allowing for proliferation after chance encounter with antigen (E. N. Naumova, in preparation). This might suggest that there are many clones of comparable affinities in the highly evolved M1₅₈₋₆₆-specific T cell response, but this is not definitive, as power law distributions can be generated on the basis of other paradigms. Similar power law distributions have been noted for the mouse repertoire specific to a mouse hepatitis virus epitope (Pewe et al. 2004). Mathematical analyses have also shown that the distribution of M1₅₈₋₆₆-specific TCR β regions in humans can be described in terms of fractals (Naumov et al. 2003, 2006). The reason for this is not clear, though influenza virus

hemagglutinin-specific B cell repertoires based on immunoglobulin structural diversity can similarly be mathematically described in terms of fractals (Burgos 1996). All of these analyses imply a repertoire that is quite complex.

Despite the high numbers of antigen-specific clones, the influenza M1₅₈₋₆₆-specific response conforms to distinct structural features, even when examined between different healthy individuals (Fig. 1; Clute et al. 2005). Within the very dominant antigen-specific V β 17 population lies a hierarchy of joining region (J) β usage, with J β 2.7 > J β 2.3 > J β 2.1 (Fig. 1). This percentage distribution of J β usage is remarkably similar between individuals and within samples from the same individual over a number of years. The amino acid sequences of the CDR3 within most of the J β -defined subpopulations share the IRSS amino acid sequence, with amino acid substitutions frequently occurring in the first and fourth positions. Thus, the specific clones of T cells are different between individuals, but the structure of the T cell repertoire is very similar, and apparently obeying the same rules. The similarity between individuals is likely due to the structural properties of HLA-A2.1-positively selected T cells that emigrate from the human thymus.

A major question is whether all T cell repertoires distribute themselves in patterns similar to or different from the influenza M1₅₈₋₆₆-specific repertoire, but insufficient analyses have been made to clearly answer this question. What is known is that the T cell repertoire can have different levels of complexity. During viral infections, T cell responses are sometimes directed against a small number of immunodominant epitopes, but in other cases directed against a large number of epitopes. The CD8 T cell response to the LCMV infection in the C57BL/6 mouse is directed against at least seven epitopes, whereas the vesicular stomatitis virus infection and Sendai virus infections in mice are each directed mostly at one epitope (Oldstone 1991). An epitope-specific response may involve several V β families, such as V β 7, V β 8, V β 13, and others for the HLA-A2-restricted HTLV-1 Tax₁₁₋₁₉ epitope (Lim et al. 2000b), and V β 2, V β 4, V β 16, and V β 22 for the HLA-A2-restricted Epstein-Barr virus (EBV) immunodominant epitope BMLF1₂₈₀₋₂₈₈ epitope (Annels et al. 2000; Lim et al. 2000a), or there may be a predilection for the use of one V β family, such as the dominance seen for V β 17 directed against the M1₅₈₋₆₆ epitope (Lehner et al. 1995). Within a V β family the repertoire may be composed of literally hundreds of clones, such as that seen with influenza M1₅₈₋₆₆ (Naumov et al. 2003, 2006), or it may be "oligoclonal," indicating a predominance of a smaller number of clones. Examples of oligoclonal responses have been reported, particularly in persistent viral infections, such as with human immunodeficiency virus (HIV), hepatitis C virus (HCV), cytomegalovirus (CMV), and EBV (Lim et al. 2000a; Annels et al. 2000; Meyer-Olson et al. 2004; Wilson et al. 1998; Manfras et al. 2004; Pantaleo et al. 1994; Khan et al. 2002).

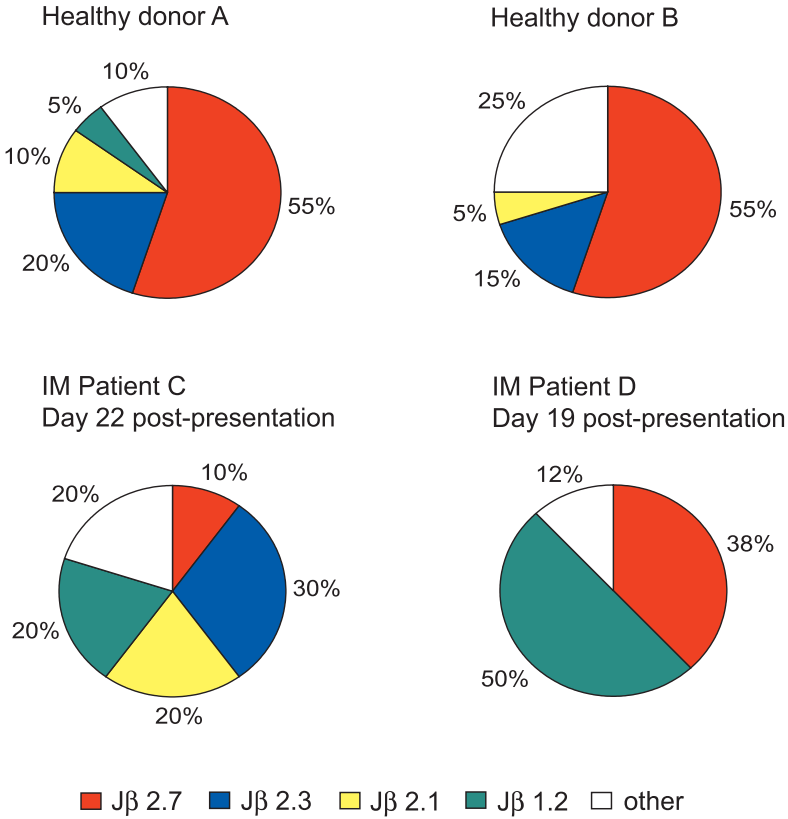
J β hierarchy of influenza M1₅₈₋₆₆-specific V β 17 T cell receptor repertoire

Fig.1 CD8 T cells isolated from two healthy donors, with previous exposure to Epstein-Barr virus (EBV) and influenza A virus, and from two patients, presenting with infectious mononucleosis (IM) during an acute EBV infection, were cultured for 3–4 weeks in the presence of 1 μ M M1₅₈₋₆₆ peptide-pulsed T2 cells transfected with and expressing HLA-A2.1. Following RNA isolation and cDNA synthesis of those M1-specific T cell lines, the CDR3 regions of V β 17 clones were sequenced. Each V β clone was defined by its unique nucleotide sequence, and the pie charts illustrate the percentage of unique clones using each J β family (A 88 clones out of 152 sequences; B 20 clones out of 104 sequences; C 20 clones out of 31 sequences; D 8 clones out of 17 sequences). Based on Clute et al. (2005)

Narrowing of the diversity of TCR repertoires for a viral epitope may occur by evolution for the most perfect fit during persistent virus infections, such as those with HIV, HCV, CMV, and EBV, or with repeated antigenic challenges, such as with influenza virus. A repertoire could theoretically also be narrowed because of clonal exhaustion and activation-induced cell death that may occur under conditions of antigen excess (Zhou et al. 2002). The repertoire may be restricted if the host is partially tolerant to the epitope. Transgenic expression of LCMV proteins can cause complete or partial tolerance to the epitope when the host is later challenged with virus (von Herrath et al. 1994). The repertoire may also be restricted for structural reasons. The TCR repertoire generated in response to the mouse influenza virus epitope PA₂₂₄₋₂₃₃ tends to be far more diverse than to the influenza epitope NP₃₆₆₋₃₇₄. Crystal structures showed that the PA₂₂₄₋₂₃₃ epitope had an arginine in position four sticking out of the MHC groove, whereas the NP₃₆₆₋₃₇₄ epitope was more buried into the MHC (Turner et al. 2005). “Flattening” the PA₂₂₄₋₂₃₃ epitope by way of alanine substitutions resulted in a more restricted repertoire, leading to the suggestion that epitopes that structurally blend into the MHC may induce a narrower repertoire. Further examples are needed to confirm this hypothesis.

There are many examples of T cell cross-reactive peptides encoded by different viruses (Welsh et al. 2004), and another major cause of TCR repertoire restriction could be due to cross-reactive T cell responses. Here, a rather narrow subset of an epitope-specific T cell memory pool is selectively stimulated to proliferate on exposure to a cross-reactive pathogen (Haanan et al. 1999). On infection with a heterologous virus, these high-frequency, but not very diverse set of clones, may immunodominant an emerging T cell response from naïve precursors and cause a further restriction of the repertoire. This will be discussed in Sect. 6.

3

Distribution of Repertoire in Different Tissues

T cells can freely circulate throughout the body and can migrate into peripheral tissues. Probably because of different levels of viral antigen expression in different tissues, T cells of some specificities may be at relatively higher frequencies than T cells of other specificities (Wang et al. 2003). Tissue-dependent differences in the TCR repertoire of antigen-specific T cells, at least to the degree in which they have been studied, are minor at best (Wang et al. 2003; Turner et al. 2003). Perhaps of greater importance are the biological properties of these antigen-specific T cells. T cells in lymphoid organs tend to proliferate faster, express higher levels of Fas (CD95) and Fas L, and are more prone to

apoptosis than T cells in, for example, lungs, adipose tissue, or peritoneal cavity (Wang et al. 2003). Lung T cells appear to be protected from apoptosis by signals derived from the engagement of very late antigen (VLA)-1 on T cells with collagen in the lung parenchyma (Ray et al. 2004). T cells from peripheral organs also express more IL-7 receptor α , or CD127 (Wang et al. 2004). These tissue differences can occur independently of the TCRs, as transgenic T cells distributed into these areas have comparable phenotypic differences (Wang et al. 2003, 2004). Some gene array data have suggested that T cells responding to antigen in peripheral tissues may have enhanced expression of genes regulating cytotoxic and cytokine effector functions (Marshall et al. 2005).

Nevertheless, T cells of different specificities have different “personalities,” in that they may have quantitative differences in their expression of apoptotic properties, such as mitochondrial electron transport potential, annexin V-reactive phosphatidyl serine, an early indication of apoptosis, Fas and Fas L, and the expression of IL-7R (Grayson et al. 2003; Wang et al. 2004). The reason for this remains unclear, as these personality differences are found during acute infection, the resting memory state, and the recall response (Wang et al. 2004). They occur under conditions of various forms of immunization—leading to the suggestion that they may relate to the inherent properties of the epitope—that are perhaps influenced by the genetic background of the host, either in selecting the repertoire in the first place or else in interacting with T cells in the periphery.

After the contraction phase of the immune response, memory T cells can be found in lymphoid organs, including bone marrow, and in peripheral tissues. There are some tissue-dependent phenotypic differences, in that “central memory” cells, which are CCR7^{high} CD62L^{high}, are preferentially found in lymphoid organs and bone marrow and gradually undergo homeostatic division (Wherry et al. 2003; Sallusto et al. 2000; Razvi et al. 1995b; Tough and Sprent 1994). CCR7 is the chemokine receptor that directs lymphocytes into lymphoid tissue (Sallusto et al. 2000). “Effector memory” cells, which are CCR7^{low}, CD62L^{low}, tend to be more in peripheral tissues and are thought to be less proliferative and have a higher level of effector function (Sallusto et al. 2000). Adoptive transfer studies have indicated that these populations can be somewhat interchangeable (Wherry et al. 2003).

4 Homeostasis of Memory T Cells

At any given moment, a small subpopulation of memory CD8 T cells is undergoing division and is cytolytically active (Razvi et al. 1995b; Selin and Welsh

1997; Zimmermann et al. 1996). In vivo studies using bromodeoxyuridine to label dividing cells indicate that after a few weeks most of the memory T cells have divided at least once, but they do not increase in number (Zimmermann et al. 1996; Selin et al. 1996). The limits in their number could be imposed by the available space in the lymphoid organs. Alternatively, with each division there could be one surviving and one dying daughter cell. This steady state homeostatic division appears to be mediated by IL-15 and IL-7 (Prlic et al. 2002; Tan et al. 2002; Kieper et al. 2002). CD8 T cell memory tends to wane in IL-15 knockout (KO) mice (Becker et al. 2002) and is poorly generated in the first place in mice lacking IL-7 (Bradley et al. 2005). Of note is that cells from the acute infection that survive into the memory state are those with the highest expression of IL-7 receptors (Kaech et al. 2003). Recent work has indicated that there may be a higher turnover of memory CD8 T cells in the bone marrow than in other organs (Becker et al. 2005).

So far as anyone can tell, the steady state turnover of CD8 T cells in a replete and unchallenged immune system is generally across-the-board, affecting all CD44^{high} CD8 T cells somewhat equally, but in a non-synchronous manner. Though not extensively studied, there is little evidence for TCR repertoire changes. There appears to be quite a different dynamic if a host is rendered lymphopenic and if the immune system needs to replenish itself, as some of the T cells undergo several cycles of division and appear to compete with each other. In fact, bona fide virus-specific memory cells do relatively poorly in this competition, and it is thought that T cells that are either self-reactive or else reactive with foreign environmental antigens may proliferate the best (Peacock et al. 2003). This is clearly different from a typical foreign antigen-stimulated response, which is driven by IL-2, and where there is a transient enlargement of the lymph nodes and a considerable expansion and then apoptosis of the T cells. Instead, the proliferation is more IL-7 and IL-15-dependent, and it occurs without a lymph node expansion phase or a discrete apoptotic deletion phase. One is left with many CD44^{high} CD8 cells that are not true memory cells, but instead are something else that is derived from a CD44^{low} naïve cell (Kieper and Jameson 1999) but is neither a naïve cell nor a bona fide memory cell.

The most significant point about the above findings in the context of this review is the depletion of bona fide virus-specific memory cells under conditions of homeostatic proliferation in the lymphopenic host. CFSE-labeled LCMV-immune spleen leukocytes were transferred into lymphopenic environments caused by genetics (severe combined immunodeficient or T cell-deficient mice), irradiation, or toll receptor stimulation by the type 1 IFN-inducer poly I:C, and the proliferation of the LCMV antigen-specific memory cell population was monitored in comparison with the rest of the donor cells. In each

case there was substantially less proliferation of the bona fide memory cells; in some cases their frequency within the donor population dropped from about 20% to as little as 3% after 2 weeks (Peacock et al. 2003). Irradiation studies were particularly intriguing. Because of their enhanced expression of Bcl-2 (Grayson et al. 2000), bona fide memory cells were initially more resistant to irradiation than the rest of the T cell population and were enriched in number (Grayson et al. 2002), but with the passage of time even they were significantly diluted out (Peacock et al. 2003). A second interesting point is that lymphopenia and a subsequent loss of bona fide memory T cells occurs with toll-like receptor (TLR) agonists, and many viral infections can stimulate TLR and simulate the effects of their agonists (McNally et al. 2001; Jiang et al. 2003b; Kim and Welsh 2004; Peacock et al. 2003). Under these conditions, the TCR repertoire of the host most certainly changes, due to the failure of bona fide memory cells to recover from the deletion (Peacock et al. 2003).

5

Virus-Induced Lymphopenia and Loss of Memory T Cells

Although new thymic emigrants continually cause repertoire shifts in the naïve T cell compartment, the memory cell repertoire remains relatively constant, providing that there are no antigenic challenges or events that cause lymphopenia. When these antigenic challenges occur there are substantial reductions and alterations in memory T cell populations (Selin et al. 1996, 1999; Brehm et al. 2002). In general, an infection with an unrelated virus will induce the formation of new memory cells specific to the second virus and will delete the frequencies of memory cells specific to the previously encountered virus (Brehm et al. 2002). This is a permanent change that remains for the lifetime of the mouse, though it has never been demonstrated or sufficiently studied in the human. We have proposed two models to explain this loss in memory T cell frequency: the passive attrition model, whereby old memory cells are lost simply by their competition with newly formed memory cells for survival niches in the immune system after immune response silencing, and the active attrition model, whereby there is a directed apoptosis of the pre-existing memory cells (Selin and Welsh 2004). Most of our data support the active attrition model (Kim and Welsh 2004).

The early phases of many acute human and experimental animal viral infections is characterized by a profound lymphopenia, occurring throughout the body's organs, and particularly affecting the memory-phenotype (CD44^{high}) CD8 T cell population (McNally et al. 2001). These T cells appear to be undergoing apoptosis, as reflected by their reactivity with annexin V, their activation

of caspases, and their staining with TdT-mediated dUTP-X nick end labeling (TUNEL) assay (McNally et al. 2001; Jiang et al. 2003b). This apoptosis seems at least in part due to type 1 IFN, as it correlates with the IFN response and does not occur in type 1 IFN receptor-deficient mice (McNally et al. 2001). There also can be concomitant severe effects of type 1 IFN on DC, preventing their expansion and development (Hahm et al. 2005). The significance of this lymphopenia remains unresolved. Clearly, extremely severe lymphopenia may be an indicator of an overwhelming infection that may lead to death of the host. A more moderate lymphopenia does not seem to impair the development of the T cell response. Given that pathogens which are some of the strongest inducers of lymphopenia stimulate some of the strongest CD8 T cell responses, it is possible that, by making room in the immune system, the lymphopenia serves to stimulate the new T cell response. Creation of lymphopenic environments by irradiation or cytotoxic drug treatment can enhance immune responses to antigens (Oehen and Brduscha-Riem 1999; Pfizenmaier et al. 1977), possibly either by making space or reducing the number of regulatory T cells. It is noteworthy that infections cause less lymphopenia in older mice (Jiang et al. 2003a), and influenza virus and LCMV inducer weaker CD8 T cell responses in older mice (Po et al. 2002; Kapasi et al. 2002).

A second function of the lymphopenia may be to kill off some memory cells to allow more naïve T cells to participate in a new immune response. When a host immune to one pathogen is infected with a second pathogen, any memory T cells cross-reactive with the second virus will dominate the new immune response, by virtue of their higher starting frequency (Klenerman and Zinkernagel 1998; Haanan et al. 1999; Brehm et al. 2002). Reducing the numbers of these memory cells would result in less immunodomination and allow for more naïve T cells to participate in the response (Bahl et al. 2006). Thus, lymphopenia may create conditions allowing for a more diverse immune response to a pathogen, and studies have linked better prognosis with more diverse responses (Meyer-Olson et al. 2004; Borrow et al. 1997).

The third important effect of lymphopenia is the ultimate loss of pre-existing memory. Mouse kinetic studies have shown that the memory T cells depleted during the early lymphopenia stage of infection never recover to their original frequencies as the infection progresses (Kim and Welsh 2004). This argues on behalf of the active deletion model of memory cell loss during infections.

6 Heterologous Immunity and Memory TCR Repertoire Shift

Experimental viral immunologists go to great lengths to assure themselves that their animal colonies are free of endogenous pathogens in order to design reproducible experiments. Results from those experiments are then thought to provide the basis for human immune responses to viruses. Indeed sometimes they are, but humans are not immunologically naïve, and they often have memory T cells that can cross-react with and respond to a new infectious agent. Cross-reactivity is a common property of the TCRs. Crystal structural studies reveal the general principles of TCR engagement with peptide-presenting MHC molecules (Ding et al. 1999; Kjer-Nielsen et al. 2003; Rudolph and Wilson 2002; Reiser et al. 2002, 2003), but thermodynamic studies of TCR peptide–MHC interactions have provided new insights into the kinetics of T cell recognition (Boniface et al. 1999; Borg et al. 2005; Willcox et al. 1999; Wu et al. 2002). Using surface plasmon resonance and calorimetry assays to define energy consumption during TCR binding to peptide-MHC, several groups have reported that the TCR undergoes significant conformational changes for proper accommodation to cognate antigen. These conformational modifications involve the TCR CDR3, as shown for human T cells binding flu-M1_{58–66}/HLA-A2.1 and EBV-EBNA3_{339–347}/HLA-B8 epitopes (Willcox et al. 1999; Borg et al. 2005) and mouse T cells binding a cytochrome C epitope MCC_{88–103}/H2-E^k (Boniface et al. 1999). An “induced fit” model has been proposed, where $\alpha\beta$ -TCRs with low conformational complementarity to peptide-MHC initially contact the MHC molecule using the more rigid CDR1 and CDR2 loops and then readjust the flexible CDR3 loops for particular shapes and charges created by the peptide-MHC complex (Wu et al. 2002). This wobbling effect of the CDR3 may enable it to accommodate structurally diverse peptides.

T cell cross-reactivity can be seen between closely related viruses, such as different strains of influenza virus (Haanan et al. 1999; Effros et al. 1977; Boon et al. 2004) or dengue virus (Mongkolsapaya et al. 2003; Zivny et al. 1999), and between different members of the same virus group, such as hantaviruses (Maeda et al. 2004), arenaviruses (Brehm et al. 2002), and flaviviruses (Spaulding et al. 1999). Cross-reactivity between evolutionarily conserved sites within virus groups may not be surprising, but examples of cross-reactivity between completely unrelated viruses such as LCMV and vaccinia virus (VV) (Kim et al. 2005), influenza virus and HCV (Wedemeyer et al. 2001), influenza virus and EBV (Welsh et al. 2004), influenza virus and HIV (Acierno et al. 2003), and human papillomavirus and coronavirus (Nilges et al. 2003), have now been shown. When cross-reactive immune responses are present, they can

Table 1 Potential pathological aspects of heterologous immunity

-
1. Alterations in immunodominance and amplification of an ineffective response
 - a. Deviation of a response toward non-protective epitopes
 - Weakly expressed epitopes
 - Epitopes expressed late in infection
 - Epitopes cross-presented on uninfected cells
 - b. Deviation toward a low-affinity response
 - Less effective at cytotoxicity and viral clearance
 - Less likely to have a full complement of cytokine production
 2. Cytokine deviation—replacement of a type 1 with a type 2 cytokine response
 - a. Reduced clearance of virus
 - b. Altered immunopathology (e.g., eosinophilia)
 3. TCR repertoire narrowing
 - a. Increased probability of T cell-escape variants
-

alter the pathogenesis of infection and either inhibit or enhance the replication of a newly encountered heterologous virus (Selin et al. 1998; Chen et al. 2001, 2003; Ostler et al. 2003). This alteration in T cell dynamics can have considerable pathogenic consequences (Table 1).

6.1

Heterologous Immunity Between LCMV and VV

The most explored experimental model of heterologous immunity has been between LCMV and VV in the mouse (Selin et al. 1998; Yang et al. 1985; Chen et al. 2001). Immunity to LCMV can provide resistance to an otherwise lethal VV infection, and cause a substantial 10- to 100-fold lowering of viral titers early during infection. However, there often are marked changes in immunopathology. On intraperitoneal challenge, VV-infected mice develop panniculitis, presenting as severe inflammation and fatty necrosis of visceral fat pads. In humans, panniculitis can occur in Weber-Christian disease but more commonly presents as erythema nodosum, a disease of unknown etiology sometimes occurring after viral infections or vaccinations and involving painful lesions on the shins (Di Giusto and Bernhard 1986; Bologna and Braverman 1992). On intranasal challenge, VV-infected mice may develop a blockage of the airways with cells and fibroid tissue in a pathology known as bronchiolitis obliterans, another human condition of unknown etiology occurring in association with viral infections or during lung transplant rejection (Schlesinger et al. 1998). In these models both the protective immunity, i.e., en-

hanced clearance of virus, and the immunopathology are mediated by T cells producing IFN- γ (Selin et al. 1998; Chen et al. 2001). Altered pathogenesis of VV infection also occurs in mice previously exposed to influenza virus, murine cytomegalovirus, and Pichinde virus (PV) (Selin et al. 1998; Chen et al. 2003).

6.1.1

Lack of Reciprocity

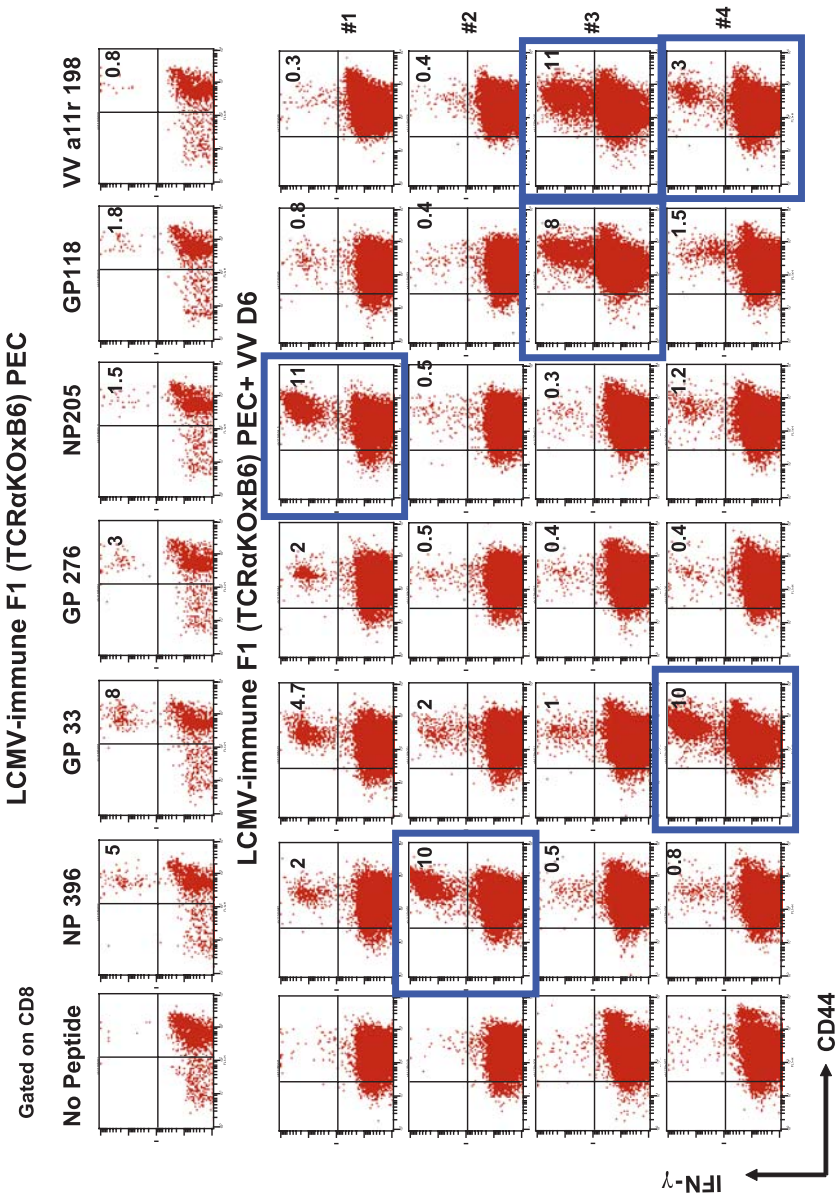
The LCMV+VV model shows that heterologous immunity is not necessarily reciprocal. LCMV protects against VV but VV does not protect against LCMV (Selin et al. 1998). Also, VV elicits the proliferation of subpopulations of a CFSE-labeled adoptively transferred LCMV-specific memory cell population, but LCMV stimulates very little proliferation of a VV-immune population (Kim et al. 2002). A possible explanation for this lack of reciprocity is that VV, encoding over 200 proteins and perhaps thousands of potential epitopes, is probably much more likely to encode an epitope that would activate some cells from an LCMV-immune T cell population, whereas LCMV, which encodes only four proteins and a far more limited number of epitopes, may be less likely to encounter a VV-immune T cell to stimulate. This may in fact be why so many large DNA viruses have evolved to encode gene products that interfere with class I antigen presentation (Ploegh 1998). Other factors may also be involved. For instance, VV might be a better inducer of IL-12 than LCMV, and this might augment the ability of any cross-reactive T cells to produce IFN- γ (Chen et al. 2001).

6.1.2

Private Specificity of Heterologous Immunity Between LCMV and VV

Studies on the heterologous immunity between LCMV and VV can be flawed by high variability among the VV-challenged mice in regards to

Fig. 2 Private specificities of the VV-induced T cell response in LCMV-immune F1 (TCR α KO \times B6) mice. *Top row:* Hierarchy of CD8 T cell responses to LCMV-encoded and a VV-encoded (a11r_{198–205}) epitope in the peritoneal cavity (PEC) of LCMV-immune mice, as shown by a peptide-induced intracellular IFN- γ assay. The hierarchy is very similar among individual LCMV-immune mice (Kim et al. 2005). *Bottom four rows:* CD8 T cell responses in four LCMV-immune mice challenged with VV for 6 days intraperitoneally. This shows the differences in the specificities of the LCMV-immune T cells amplified in the individual mice. It also shows that the response to a cross-reactive VV-encoded epitope (a11r_{198–205}) is amplified in two of the four mice, indicating that private specificities can dictate immunodominance of T cells specific to the challenge virus. The use of the F1 (TCR α KO \times B6) mice ensures that cross-reactivity is not mediated by T cells bearing two α -TCRs



immunopathology and immune response (Chen et al. 2001). In a study to predict which LCMV-encoded epitopes might be driving cross-reactive responses to VV, substantial variability was noted when individual mice were tested. For instance, in 50% of mice the VV infection stimulated strong expansion of T cells specific to the LCMV NP₂₀₅₋₂₁₂ epitope, in 23% of mice they were specific to either the GP₃₃ or 34 overlapping epitopes, and in 15% of mice specific to the GP₁₁₈₋₁₂₅ epitope. Often there was expansion of T cells specific to only one LCMV epitope, but sometimes T cells specific to more than one epitope were expanded (Kim et al. 2005). In other cases there was no expansion at all.

Figure 2 shows the strong but very different specificities of expansions of LCMV-specific memory T cells in individual mice challenged with VV. This experiment was also performed with F1 progeny of wildtype C57BL/6 mice crossed with α -TCR-deficient mice, to rule out the presence of two α -TCRs as accounting for cross-reactivity.

The question was whether these variations in expansion represent random stochastic events in an LCMV-immune mouse challenged with VV, where only a limited number of the cross-reactive T cells actually engage antigen, or whether each mouse had a unique T cell repertoire in regards to its potential cross-reactivity with VV. To address this point, CFSE-labeled splenocytes from different donor LCMV-immune mice were adoptively transferred into three recipients, which were each then challenged with VV. The pattern of epitope-specific T cell expansion was virtually identical among the recipients of a single donor, but different in recipients from different donors. This showed that these variations in T cell responses were reflections of the private specificities of the individual immune host (Kim et al. 2005).

6.1.3

Matrix of Cross-Reactivity Between LCMV and VV

How then could this cross-reactivity pattern between LCMV and VV be explained? In a quest for cross-reactive epitopes based on searching for VV sequence homology with the LCMV NP₂₀₅₋₂₁₂ epitope, an epitope (VV a11r₁₉₈₋₂₀₅) was found that cross-reacted with three LCMV epitopes: NP₂₀₅₋₂₁₂, GP₃₄₋₄₁, and GP₁₁₈₋₁₂₅ (which, incidentally, showed no cross-reactivity with each other) (Kim et al. 2005; Cornberg et al. 2006). VV a11r₁₉₈₋₂₀₅ also cross-reacted with a PV epitope (PV NP₂₀₅₋₂₁₂) and an immunodominant VV epitope (e7r₁₃₀₋₁₃₈). Hence, a whole matrix of cross-reactivity was revealed, and this was directed at only one of possibly many cross-reactive VV epitopes. Of significance, however, was when a11r₁₉₈₋₂₀₅-stimulated cell lines were derived from individual LCMV-immune donors, each line had different patterns of

cross-reactivity, with some high for one epitope and others high for a different epitope, again reflecting the private specificity of cross-reactivity (Cornberg et al. 2006).

6.2

Heterologous Immunity Between LCMV and PV

LCMV and PV are arenaviruses which encode nucleoprotein NP₂₀₅₋₂₁₂ epitopes that share 7 of 9 amino acids in an evolutionarily conserved site (Brehm et al. 2002). T cell responses to these epitopes are normally subdominant in either infection, but when mice immune to one virus are infected with the second, the T cell responses to the NP₂₀₅₋₂₁₂ epitope become dominant and T cell responses to the normally dominant epitopes are much subdued (Brehm et al. 2002). Protective heterologous immunity occurs between these viruses, with LCMV protecting against PV more than PV protects against LCMV, perhaps in part due to higher frequencies of NP₂₀₅₋₂₁₂-specific memory cells induced by LCMV (Selin et al. 1998; Brehm et al. 2002). There is a high level of T cell cross-reactivity between the two epitopes in regards to peptide induced IFN- γ production, but double tetramer staining and peptide dilution experiments suggest many affinity differences. Of note is that a heterologous virus challenge selects for a very small subset of the cross-reactive T cells, leading to a substantial narrowing of the TCR repertoire (Fig. 3). This narrowing of the repertoire has different patterns between individuals, and adoptive transfer studies have indicated that this variation is again a reflection of the private specificities of the immune system that developed after the primary infection (Cornberg et al. 2006). It is noteworthy that, with a recent exception in the HIV system (Dong et al. 2004), most studies have linked narrow TCR repertoires to poor clearance of virus and to the enhanced probability of selecting for epitope escape variants (Wilson et al. 1998; Pantaleo et al. 1994; Meyer-Olson et al. 2004; Borrow et al. 1997).

6.3

Immune Deviation

A byproduct of heterologous immunity may be immune deviation caused by shifts in cytokine production. Three days after acute infection of naïve mice with VV, there are high levels of IL-6 and low levels of IFN- γ produced. This contrasts to VV infection of LCMV-immune mice, where there are much higher levels of IFN- γ and lower levels of IL-6 (Chen et al. 2001). In general, studies in murine models with several virus infections have shown alterations in cytokine responses to a virus caused by previous virus infections (Chen et al. 2003).

PV+LCMV mice have highly skewed and variable cross-reactive NP₂₀₅₋₂₁₂-specific TCR V β repertoires

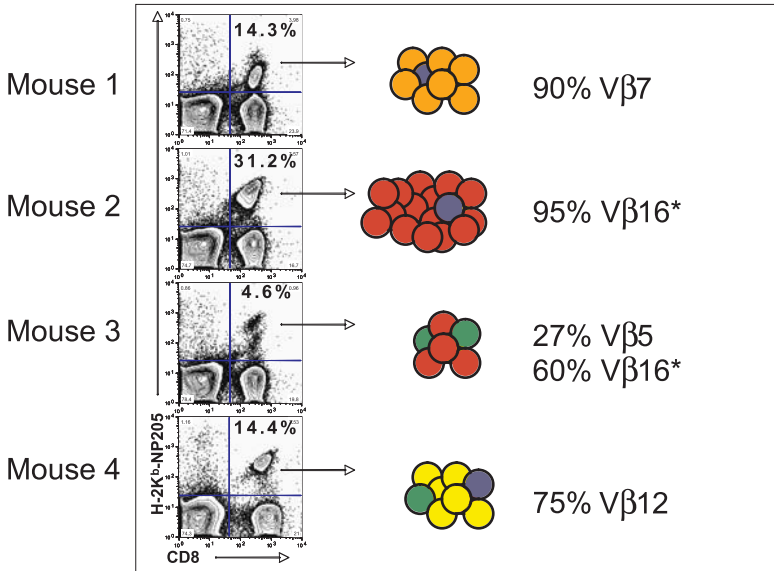


Fig. 3 TCR repertoire narrowing during heterologous immunity between PV and LCMV. T cells specific to the subdominant LCMV epitope NP₂₀₅₋₂₁₂ are mostly in the V β 16 family and seldom represent more than 5% of the acute CD8 T cell response to LCMV in naïve C57BL/6 mice. This figure shows that acute LCMV infections of PV-immune mice elicit responses that can be immunodominant yet highly variable between mice, reflecting the private specificities, and that they can result in a narrowing of the repertoire by stimulating expansions of T cell V β families that would never be prevalent in an acute response in naïve mice. *The percentage of V β ⁺ cells was calculated by staining tetramer-defined T cells with V β -specific antibodies. An exception was V β 16, for which no antibodies are available, and which was detected less quantitatively by PCR amplification. Based on a manuscript submitted by Cornberg et al. (2006)

Immune deviation away from type 1 responses is a problem in respiratory syncytial virus (RSV) infection and may have been what occurred when children in the 1960s contracted RSV infections after being immunized by an ineffective vaccine (Kapikian et al. 1969). Mice immunized with a VV recombinant expressing the RSV G protein and later challenged with live RSV developed a type 2 response and severe eosinophilia in the lung. However, if mice had been immune to influenza virus prior to the recombinant VV im-

munization, no such deviation occurred on RSV challenge, and the pathology was much less severe (Walzl et al. 2000). It is also interesting to note that prior immunization with RSV G led to an extreme repertoire narrowing of V β 14 CD4 T cells specific to the dominant epitope G₁₈₅₋₁₉₃ on live RSV challenge (Varga et al. 2001).

6.4

Heterologous Immunity in Human Infections

Evidence is accumulating for heterologous immunity in humans between commonly occurring viruses.

6.4.1

Influenza Virus Strains and Variants

It has been noted for some time that a prior history of an influenza virus infection can lead to an altered immune response to a different but related strain.

The original observation involved assessment of antibody responses, where antibodies cross-reactive between the strains dominated the immune response and squelched new immune responses to non-cross-reactive antigens. These alterations of B cell repertoires were referred to as “original antigenic sin,” (Fazekas de St. Groth and Webster 1966) and a similar phenomenon can happen with T cell responses (Klenerman and Zinkernagel 1998; Mongkol-sapaya et al. 2003). Different strains and variants of influenza are commonly cross-reactive at the human and mouse T cell level, leading to speculations that these cross-reactive cells may be involved in the pathogenesis of influenza virus infections (Effros et al. 1977; Haanan et al. 1999; Boon et al. 2004). Perhaps of even greater conceptual interest is the observation of T cell cross-reactivity between influenza and other viruses, as discussed in the following sections.

6.4.2

Hepatitis C Virus

The pathogenesis of HCV in humans is remarkably variable, ranging from asymptomatic to fulminant, with most patients undergoing long-term persistent infections and others clearing the virus (Farci et al. 1996). The reasons for this extreme variability are unknown, and it has been questioned whether heterologous immunity may play a role (Urbani et al. 2005; Rehmann and Shin 2005). HCV encodes an HLA-A2-restricted epitope (NS3₁₀₇₃₋₁₀₈₁) that shares 7 of 9 amino acids with the influenza epitope (NA₂₃₁₋₂₃₉), and T cells from influenza-immune individuals with no evidence of a past HCV infection

can often respond to the HCV epitope in vitro (Wedemeyer et al. 2001). Hence, the human population may be partially immune to HCV as a consequence of this cross-reactivity. We know that LCMV and PV similarly share 6 of 8 amino acids in their cross-reactive peptides and that sequential infections can lead to immunodomination of the normally protective epitope-specific T cell responses, as well as to a substantial narrowing of the repertoire, and that the degree of this is influenced by the private specificity phenomenon (Brehm et al. 2002; Fig. 3). In 2 of 8 patients examined, a very pronounced cross-reactive T cell response between influenza and HCV was noted; and in these same individuals there was a narrowing of the repertoire, in that T cell responses to other epitopes were minimal (Urbani et al. 2005). These same patients experienced a hepatitis far more severe than patients who mounted a more diverse T cell response against a variety of epitopes. The fact that these patients were all immune to the ubiquitous influenza virus suggests that private specificities may have dictated the altered immune responses.

6.4.3

Epstein–Barr Virus

EBV-associated mononucleosis is one of several viral diseases that are more severe in teenagers and young adults than in children. Others that come to mind are mumps, chicken pox, polio, and measles. What is unique about mononucleosis is that the characteristic diagnostic feature of the disease is an overzealous CD8 T cell response (Silins et al. 2001). Could this relate to the reactivation of memory T cells? It may, as a subset of T cells directed against a major HLA-A2.1 restricted immunodominant EBV peptide, BMLF-1_{280–288}, cross-react with the invariant HLA-A2.1-restricted influenza A virus epitope M1_{58–66}, even though they share only 3 of 9 amino acids (Welsh et al. 2004). Our recent studies have shown activation of these cross-reactive T cells in some but not all acute mononucleosis patients, perhaps again reflecting private specificities in the host response (Clute et al. 2005). Of note, as mentioned above in Sect. 2.4, is that the TCR repertoire to influenza M1_{58–66} normally has a consistent structure in regards to the dominance of V β 17 and its hierarchy of utilization of J β genes (Fig. 1). Analyses of the M1_{58–66} repertoire from two individuals experiencing EBV-associated acute infectious mononucleosis, however, revealed a substantially different hierarchy of J β usage, suggesting that a skewed subset of the M1_{58–66}-specific TCR repertoire, probably those cross-reactive with EBV, were being stimulated to proliferate (Fig. 1).

6.4.4

Dengue Virus

Perhaps the most recognized human examples of heterologous immunity come from dengue virus infections. Dengue viruses occur as four distinct but cross-reactive serotypes which fail to elicit neutralizing antibody effective against each other (Morens 1994). A host immune to one serotype when challenged with a second serotype may, instead of having protective immunity, develop a much more severe disease known as dengue hemorrhagic fever and shock syndrome. One theory for its occurrence is that non-neutralizing cross-reactive antibodies can cause “immune enhancement” and increase the uptake of virus into target cells via Fc receptors, resulting in a higher frequency of cells infected (Morens 1994). These could also inhibit the formation of effective neutralizing antibodies to the second virus. A second theory is that original antigenic sin of T cells may be the cause of disease (Mongkolsapaya et al. 2003). A recent study with cases of dengue hemorrhagic fever has shown that some T cell responses were of a higher avidity to a strain of dengue virus other than the one causing the disease. The interpretation is that weakly effective T cells dominated the repertoire to a second dengue virus infection because of their high frequency in the memory pool after an infection with a different serotype.

7

Why Private Specificities Are Important in Pathogenesis

Studies have shown that immunologically naïve mice, while using different TCRs to mount an immune response, generate responses with similar epitope hierarchies, similar effector functions, and similar outcomes. This is because primary responses are likely to select for T cells with suitable CDR1 and CDR2 and which have CDR3 amino acid motifs at positions important for engagement of the peptide MHC complex. The other CDR3 amino acids reflected in the private specificities of the response may be less relevant for those epitopes, which will select the best T cell fits from the highly diverse naïve TCR repertoire. The relevance of the private specificities may increase when a cross-reactive epitope generated by a second virus engages the TCRs of expanded pools of memory cells (Fig. 4). Expanded clones harboring these non-motif amino acids would vary from host to host, a result of the private specificity of the repertoire, but these other amino acids could have great impact on a cross-reactive read-out. Cross-reactive stimulations may result in repertoires not ideal to fight infection, but they are generated nevertheless because of the high frequency of those cells in the memory pool.

TCR PRIVATE SPECIFICITIES AND CROSS-REACTIVE VIRUS CHALLENGE

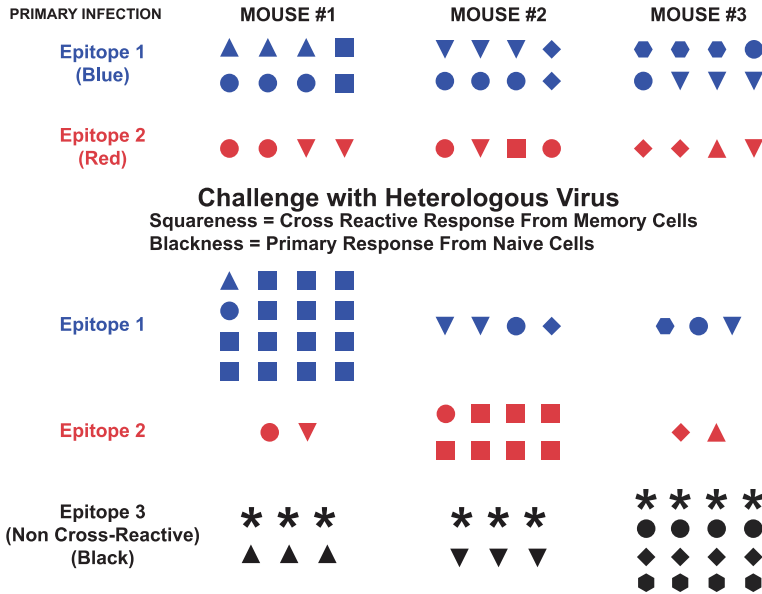


Fig. 4 Model demonstrating how private specificities can alter and narrow the repertoire in a cross-reactive memory response but not in a primary naïve response. Here the color and shape of the epitope determine its specificity. Three naïve mice generate quantitatively similar but qualitatively different repertoires against an immunodominant (*blue*) and subdominant (*red*) epitope. When challenged with a cross-reactive heterologous virus, which responds to either “square” or “black,” the private specificities of the memory response dictate immunodominance and epitope specificity and repertoire diversity. T cells specific to non-cross-reactive epitopes are deleted, while cross-reactive T cells can become dominating and oligoclonal

The potential consequences of this stimulation, depicted in Fig. 4, are summarized in Table 1. Exposure of a memory T cell population to a cross-reactive epitope from a heterologous virus may cause a suppression or immunodomination of T cell responses to normally protective epitopes (an effect subdued somewhat by the early lymphopenia). Thus, an immune response to a poorly presented epitope might dominate and be ineffective at eliminating the virus. The T cell response may have a very narrow repertoire, possibly leading to the selection of escape variants. That subset of T cells that is selected may be skewed in terms of its functional capacities and may be ineffective at controlling infection. An immune deviation of type 1 or type 2 cytokines could also ensue. Depending on the private specificities of the host’s memory pool, it is

possible that either a strong protective response or a response more likely to cause immunopathology would develop (Table 1).

8

Revisiting the Interactions Between Innate and Adaptive Immunity

Innate immunity is thought to provide the host with time for the differentiation and proliferation of low-frequency antigen-specific clones of naïve T and B cells to reach sufficient numbers to attack and clear the pathogen. Cytokines produced by innate effector mechanisms may retard the replication of the pathogen and influence the deviation of an immune response into a type 1 or type 2 direction. These distinctions between the timing and the roles of innate vs adaptive immunity are not so clearly delineated in the context of heterologous immunity, a phenomenon that we would argue is quite common. A cross-reactive memory cell population may be constitutively effective and not need the time for clonal expansion that a naïve population does. If the cross-reactive memory population is deviated in a type 1 or type 2 direction, it, rather than innate mechanisms, may dictate the deviation of the immune response in the newly developing T and B cells. The laws of innate immune system effects on antigen-presenting cells for the generation of immunodominance hierarchies become perverted in the presence of high frequencies of pre-existing cross-reactive T cell clones. One might also suspect that the rapid production of memory T cell cytokines, such as IFN- γ , might curb the replication of the pathogen, thereby reducing the induction of type 1 IFN and its subsequent effects on lymphopenia, memory cell loss, DC suppression, and NK cell activation. Superimposed on the uncertainty of these events is the variation due to the memory pool's private specificities that are unique to an individual host.

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