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12
Immunodominance in the T-Cell Response to Herpesviruses

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12.1
Introduction

Herpesviruses are ubiquitous companions of the adaptive immune system; most mammals are infected for life by at least one of these viruses. They are large, complex viruses with orchestrated gene expression cascades, and they encode a myriad of genes that enable them to manipulate and avoid host immune responses. Nevertheless, herpesvirus manipulation of the immune system is largely benign: rather than seeking short-term advantage, these viruses take the long view, and their evolutionary success can be attributed to the minimal cost they impose on host well-being. These viruses make use of the immune response to position themselves in the host: while the immunocompetent host remains an asymptomatic carrier of the virus, immunocompromise upsets the host–virus balance, and recrudescent herpesvirus infections can lead to death.

The T-cell response to herpesviruses is an essential part of the virus–host equilibrium: it controls but does not eradicate virus. In this chapter we review the immunodominance choices the immune system makes in responding to herpesvirus infections and consider how they fit into this scenario. Because the literature is much more extensive for CD8 than for CD4 T cells, we focus mostly on CD8 T cells. In addition, the CD8 T-cell response to herpesviruses provides two of the most extreme examples of immunodominance that have yet been described in the literature. While the basis of these is not yet understood, they are highlighted here because of the opportunity they provide to probe the finer decision-making processes that the immune system undertakes to determine immunodominance.

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12.2
General Considerations

12.2.1
Herpesviruses: A Brief Virological Primer

Herpesviruses are an ancient virus family with two broad lineages: one infecting birds and mammals and another that infects poikilothermic animals such as oysters [1]. Herpesviruses of birds and mammals are divided into three subfamilies: alpha, beta, and gamma. These three lineages were clearly established before the mammalian radiation 60–80 million years ago, and in general, herpesviruses are thought to have cospeciated with their hosts [1, 2]. Herpesvirus genomes contain genes that have clearly been acquired from their hosts, and it is apparent that there has been a close coevolution. Primordial herpesviruses may well have been with us as the adaptive immune system developed; at any rate, it is clear that this family of viruses has an intimate knowledge of the workings of our immune system and that it exploits that knowledge to maintain its impressive evolutionary stability.

Herpesviruses have very large genomes, encoding between 80 and 250 viral proteins. They have a conserved virion structure, and the mammalian and avian herpesviruses share a homologous set of core genes that control the basic program of virus replication [1]. These viruses also have a common lifestyle, establishing latent infection (Table 12.1) for the life of the host. The focus of this chapter is the nine human herpesviruses and their murine models (Table 12.2).

Table 12.1 Terms used to describe herpesvirus gene expression programs.

Productive infection/ lytic cycle	**Virus replication leading to production of new infectious virions; because this results in cell lysis, it is also referred to as the lytic cycle.**
Latency	Maintenance of the viral genome in a cell without production of infectious virions, with or without viral protein synthesis.
Reactivation	The conversion of latent virus infection to productive infection.
Abortive reactivation	Latent virus initiates the "reactivation" sequence of gene expression, but the process is aborted or the cell destroyed before infectious virions are produced.
Persistent infection	Continued virus infection with production of new virions after the acute phase of virus infection of the animal has passed.
Chronic infection	Many virologists use the term chronic infection interchangeably with persistent infection, implying continued lytic cycle virus replication. We use the term here to refer to an animal that continues to harbor the herpesvirus after the acute infection has been resolved. The infection may be latent or persistent; very likely both genetic programs occur simultaneously in the animal, with foci of virus replication alongside many cells and tissues in which the virus is maintained in a true latent state.

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Table 12.2 The nine known human herpesviruses and their mouse models.

Subfamily	Human virus	Disease	Mouse model	Comment on the mouse model
Alpha	Herpes simplex virus (HSV) 1	Herpes labialis	HSV	Human virus used to infect mice
	HSV-2	Genital herpes		
	Varicella-zoster virus (VZV)	Varicella (chicken pox) and herpes zoster (shingles)	None	
Beta	Human cyto megalovirus (HCMV)	Congenital malformations; disease in the immunocompromised	Murine CMV (MCMV)	Natural pathogen of laboratory mice (*Mus musculis* species)
	HHV-6A and HHV-6B	Roseola infantum	None	
	HHV-7		None	
Gamma-1	Esptein-Barr virus	Infectious mononucleosis, Burkitt's lymphoma, and nasopharyngeal lymphoma	MHV-68	Actually a gamma-2 herpesvirus, a natural pathogen of wood mice (genus *Apodemus*) that is able to infect mice of the *Mus* genus
Gamma-2	Kaposi's sarcoma–associated herpesvirus (KSHV)/ HHV-8	Kaposi's sarcoma	MHV-68	See above

The lytic cycle of herpesvirus infection leads to production of new infectious virions. For all herpesviruses, lytic cycle gene expression is characterized by a regulated, ordered sequence of gene expression (Figure 12.1), from immediate early (IE) to early (E) to late (L) genes. The programs of latency differ for the three herpesvirus families. The gammaherpesviruses encode sets of genes that are uniquely expressed during latency and are responsible for maintenance and propagation of the latent virus genome. Alphaherpesviruses express latency-specific transcripts, but no latent proteins are known. For betaherpesviruses, the program of gene expression during latency, if any, is not known.

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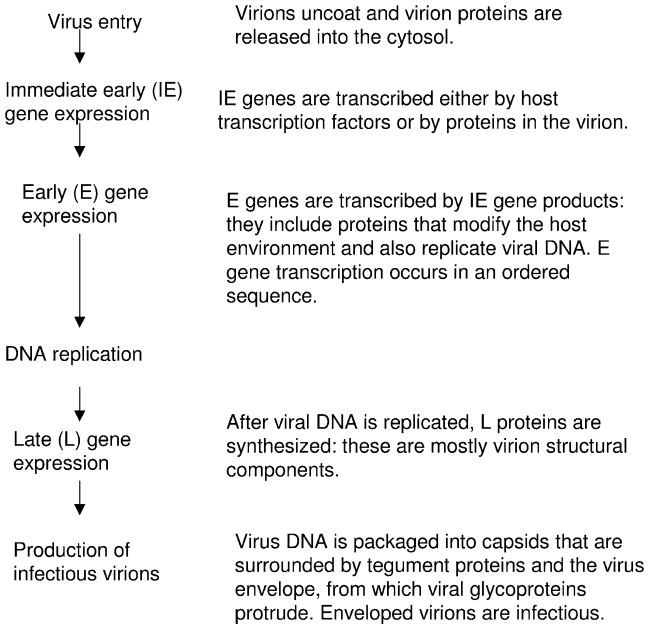

Figure 12.1 Scheme of herpesvirus replication showing the kinetic classes of viral gene transcription.

In choosing immunodominant epitopes for herpesvirus infections, the immune system operates within the parameters that the earlier chapters of this book attempt to elucidate. Overlying this, there are several herpesvirus-specific considerations that need to be taken into account. These considerations have led us to the following general framework, which will serve as a scaffold for the discussion of immunodominance patterns in the T-cell response to each of the three subfamilies of herpesvirus.

12.2.2 A General Framework for Thinking About Immunodominance in the T-Cell Response to Herpesvirus Infections

We can conceptualize three broad classes of explanation for immunodominance in herpesvirus infections, which we call virus centric, APC centric, and T cell centric.

Virus-centric explanations are based on the availability of viral antigen: the more abundant a parental antigen is, the more substrate there is available for processing and presentation, and the more likely an antigen is to be immunodominant. In herpesvirus infections, antigen abundance is affected by the program of viral gene expression.

During lytic cycle infection, herpesvirus gene expression occurs in a regulated cascade from immediate early (IE) to early (E) to late (L) proteins (Figure 12.1). Even before any viral gene activity occurs, virion structural proteins may be deliv-

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ered into the cytosol with the infective virus inoculum. If most virus-infected cells are destroyed (by the immune system) during the early phases of infection, then IE genes, or possibly virion structural proteins, will be more abundantly presented than genes expressed later during infection.

The second herpesvirus-specific aspect of abundance of gene expression that needs to be considered is the nature of viral latency. As described above, all herpesviruses establish latent infection, during which the viral genome is maintained without infectious virions being produced. For most of the life of the host, most herpesvirus infections are clinically latent, i.e., there is no evidence of productive infection. Therefore, if proteins are expressed during latency, these are likely to provoke immunodominant responses during the lifelong latent state. In order to maintain their genomes during latency, gammaherpesviruses express a unique set of latency-specific proteins that are not expressed during lytic infection. These genes can lead to transformation and uncontrolled replication of the host cell, presumably as a means of propagating the virus genome. Thus, viral antigen abundance for gammaherpesviruses will depend on whether latent- or lytic-phase infection is dominant. The alpha- and betaherpesviruses are not known to encode separate proteins that are expressed only during latency. For these viruses, the proteins that are first expressed upon reactivation from latency, such as the IE1 protein of cytomegalovirus (CMV), may have a viral protein abundance advantage during latency, especially if such reactivation is frequently abortive.

Another virus-centric explanation of particular relevance to herpesvirus infections is the impact of viral genes that interfere with antigen presentation (VIPRs) [3], which all herpesviruses seem to encode. VIPRs impair or prevent presentation in the infected cell. If directly infected cells are important for priming the CD8 T-cell response, VIPRs should have a profound impact on the immune system's choice of immunodominant CD8 T-cell epitopes.

APC-centric explanations are concerned with the processing and presentation of viral epitopes. Obviously, to be immunodominant a peptide must be able to bind to MHC, and the availability of peptides with high affinity for the available MHC types is likely to be the single most important factor influencing immunodominance [4, 5]. Similarly, the epitope must be processed and presented by the relevant antigen-presenting cell (APC) type. The relevant APC is presumed to be dendritic cells (DCs), either directly presenting or cross-presenting, for the initial priming of T cells. Whether other APC types can then go on to maintain (save from activation-induced cell death) or expand T-cell populations is not clear, although we note with interest that DCs seem uniquely able to promote activation even of memory CD8 T cells [6]. On the other hand, for the B cell–trophic gammaherpresviruses, antigen presentation by B cells seems likely to play a prominent role. (Interference with antigen presentation by VIPRs is, of course, another APC-centric consideration.)

T cell–centric explanations are based on the assumption that some T-cell receptors (TCRs) are better than others. All other things being equal, T cells bearing those receptors win out over other T cells. The concept of immunodomination, by which a dominant T-cell response actually suppresses other responses, is an

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extension of this explanation [4, 5]. One issue in T cell–centric explanations is that we don't really understand what makes one TCR better than another: it is not clear what the best relationship between TCR and MHC–peptide is, in terms of affinity, occupancy time, and conformational change induced by the interaction. Even within responses to some peptide–MHC complexes, certain T-cell clones come to dominate. We need to introduce the concept of TCR immunodominance (dominance of one TCR within the response to a given peptide–MHC ligand) to add to the more general concept of epitope immunodominance (dominance of a response to one peptide over others). The two most striking examples of TCR immunodominance in the literature are provided by herpesvirus immunology: one in the murine model of herpes simplex virus (HSV) infection and the other in human Epstein-Barr virus (EBV) infection. Although the basis for these is not understood, it can be hoped that the experimental clarity offered by this extreme immunodominance may lead in time to elucidation of the principles that underlie the more frequent examples of epitope immunodominance.

12.3 Immunodominance in the CD8 T-Cell Response to the Three Classes of Herpesvirus

12.3.1 Alphaherpesviruses

12.3.1.1 Human Studies: Immunodominance of Structural Virion Proteins that can be Presented in the Face of Immune Evasion

The alphaherpesviruses HSV and varicella-zoster virus (VZV) are neurotrophic viruses that cause recognizable clinical symptoms in both acute infection and upon reactivation (Table 12.1). VZV cannot be readily propagated *in vitro*, with the result that little is known about its immunobiology. HSV, on the other hand, grows easily in tissue culture and has been studied for some time in both mouse models and humans. HSV-2 causes genital herpes and is also the main cause of fatal HSV encephalitis in newborns. Because of its clinical importance, much of the best human HSV immunology has been performed for HSV-2. The clearance of virus from the lesions and the resolution of clinical symptoms in genital herpes correlates with infiltration of CD8 T cells into the lesions [7, 8]. This suggests that CD8 T cells are able to detect and control HSV infection *in vivo*.

Several HSV antigens that are recognized by CD8 T cells from infected people have been identified. Theses are mostly structural virion proteins: glycoproteins and tegument proteins [9, 10]. In addition, IE antigens are also targets for CD8 T cells [9, 11]. A careful analysis of a large panel of clones that could recognize HSV-2-infected cells confirmed the immunodominance of responses to structural virion proteins and to IE proteins [10].

Koelle et al. have used drug blockade and viral mutants to investigate when in the infectious cycle the structural viral proteins, which are all encoded by late genes, can

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be recognized by CD8 T cells [9]. If a high multiplicity of infection (MOI) is used, these structural virion proteins can be processed and presented without requiring synthesis of new viral proteins. Similarly, the immediate early protein ICP0 is presented very shortly after viral entry. HSV-2 encodes two powerful genes that inhibit the MHC class I antigen-processing pathway. ICP47 is an IE protein (the only one of HSV's five IE proteins that is not a transcription factor) that potently inhibits the TAP transporter [12, 13]. The virion host shutoff (vhs) protein encoded by UL41 is a potent inhibitor of host class I proteins, including HLA class I. vhs is a virion protein that begins to shut down host protein synthesis within the first hour after virion entry [14]. Antigens that are present in virions in large quantities, or that are synthesized in abundance from IE genes, would have the greatest chance of being presented before these two powerful immune evasion functions prohibit presentation of other epitopes. HSV can spread directly from an infected cell to its neighbor. When this happens, a large number of virions are delivered into the cell, analogous to infection *in vitro* with a high MOI. Hence, processing of antigens from input structural virions is physiologically feasible, and epitopes presented from this source may be the target of a protective CD8 T-cell response. Thus, Koelle et al. postulate that structural virion proteins and IE proteins are immunodominant in the CD8 T-cell response to HSV because of their ability to escape immune evasion.

However, are these responses truly immunodominant? Before accepting this conclusion, it is worthwhile to examine the way the data were generated. Koelle et al. generated CD8 T-cell clones from T cells that were infiltrating genital herpes lesions [9] or from peripheral blood CD8 T cells bearing a tissue-homing receptor [10]. Clones that could recognize HSV-2-infected cells were selected for antigen identification, and this may have introduced a major bias into their system. In the CMV and EBV systems, it is clear that CD8 T-cell responses are readily generated (we believe by cross-priming) that are unable to recognize infected cells (this is described later in the chapter). If that were also the case for HSV, these authors would not have detected them. The antigens identified by Koelle et al. are extremely important, because cytotoxic T lymphocytes (CTLs) recognizing them are actually able to recognize infected cells and hence would be useful vaccine candidates. However, we do not believe that studies have yet been performed that would enable us to determine whether or not these antigens are truly immunodominant.

12.3.1.2 **Mouse Studies of HSV**

Mice are not a natural host for HSV, and we are not aware of an alphaherpesvirus that is a natural mouse pathogen. Although mice can be efficiently infected by HSV, usually by footpad injection or corneal scarification, the natural course of infection is different from that seen in humans. In sublethal infection, after acute infection is resolved, virus does persist in a latent state. However, observable disease resulting from spontaneous reactivation does not occur. This may be due to virological reasons or to more efficient control of latent HSV by the adaptive immune system. HSV-specific CD8 T cells surround infected cells in the trigeminal ganglia [15]. The main HSV VIPR, ICP47, has only weak activity against

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mouse TAP [16] and has not been shown to inhibit antigen presentation. Mouse studies have mostly been carried out with HSV-1 strains, which have a weak ability to impair host protein synthesis that is not sufficient to impair antigen presentation. As a result, CD8 T cells are likely to be much more efficient in controlling HSV in mice. Thus, when the immune system "chooses" its immunodominant CD8 T-cell epitopes in HSV-infected mice, it is able to do so free from any constraints imposed by immune evasion of the class I pathway.

In the mouse system, in parallel to the human, attention has focused on structural viral glycoproteins and IE antigens, and evidence of responses to both has been found, with different immunodominance patterns found for different mouse strains. gB was identified as immunodominant in C57BL/6 mice, whereas gC [17] and gD [18] were implicated as antigens in mice that expressed the H-2^k MHC haplotype. The IE protein ICP4 is an antigen for H-2^k mice [19], whereas ICP27 is antigenic for H-2^d and H-2^b mice. In BALB/c (H-2^d), ICP27 is relatively immunodominant: limiting dilution assay indicated that 25% of clones that could recognize HSV were able to recognize a recombinant vaccinia encoding ICP27 [20].

12.3.1.3 The Remarkable Immunodominance of gB-SSIEFARL in B6 Mice

In C57BL/6 mice, a K^b-restricted epitope (SSIEFARL) from gB is immunodominant. Two other epitopes have been described, one from ICP6, the large subunit of the viral ribonucleotide reductase (RR) [21], and one from ICP27 [22]. The gB epitope is profoundly immunodominant: CD8 T cells recognizing gB comprise 60–90% of HSV-reactive CD8 T cells in draining lymph nodes at the height of the acute response [23]. This extraordinary immunodominance appears to be based on a very limited TCR usage and thus is likely to represent an extreme example of T cell–centric immunodominance. There is some disagreement in the literature about the extent of response to the other two epitopes. However, the massive immunodominance of gB has been confirmed by other groups, particularly when newer methodologies rather than limiting dilution analysis have been used.

The response to K^b-SSIEFARL in C57BL/6 mice is probably the most extreme example of immunodominance in the literature. Because gB is not particularly immunodominant in other mice or humans, its immunodominance in C57BL/6 mice is likely to be due to some unique features of the TCRs that interact with K^b-SSIEFARL. Despite its impressive immunodominance, the K^b-SSIEFARL response is not marked by high affinity, nor is it especially protective [24]. The Carbone group performed an interesting experiment that may shed some light on the question of how the immune system chooses which TCRs to use in response to a given determinant [25]. They generated a TCR β chain transgenic mouse bearing the TCR b chain from a K^b-SSIEFARL-specific T-cell clone. While a fairly diverse TCR repertoire was generated in these mice, the choice of TCR a chains was nevertheless constrained, and approximately 25% of all CD8 T cells in the spleen could bind K^b-SSIEFARL tetramers. When the mice were infected with HSV, only a subset of these tetramer-binding T cells expanded. The subset that expanded did not appear to be of higher affinity than those that did not expand,

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based rather roughly on tetramer binding. Thus, only a subset of TCRs that can bind a given ligand were able to transmit an activation signal to the T cell. While the authors propose that this select subset of TCRs included those that underwent a conformational change upon ligand binding, there is no experimental evidence to support this claim.

A crystal structure of the immunodominant TCR alone and complexed to K^b-SSIEFARL will be required to determine whether this TCR does undergo a marked conformational change upon ligand binding. To our knowledge, no other hypothesis has been proposed to explain this extraordinary TCR immunodominance, and at present it remains an unsolved mystery.

12.3.2 Betaherpesviruses

There are three known members of the betaherpesvirus family: the cytomegaloviruses, HHV-6, and HHV-7. As yet, no T-cell antigens have been described for HHV-6 or HHV-7, so these will be discussed only briefly in the section on T-cell cross-reactivity. However, a substantial body of literature on CD8 T-cell immunodominance to murine cytomegalovirus (MCMV) exists, and on both CD4 and CD8 T-cell immunodominance to human cytomegalovirus (HCMV), and these will be the focus of this section.

Each cytomegalovirus is specific for only one mammalian species. Both HCMV and MCMV are highly prevalent, with 60–90% of individuals infected. CMV infections are normally asymptomatic, with innate immunity (particularly NK cells) and all facets of adaptive immunity (CD4 T cells, CD8 T cells, and antibody) playing an important role in immune control.

12.3.2.1 The Acute CD8 T-Cell Response to MCMV

Because HCMV infection is normally asymptomatic and goes undetected, there is scant literature on acute T-cell responses to HCMV. In contrast, the acute CD8 T-cell response to MCMV has been characterized in great detail in both the BALB/c ($H\text{-}2^d$) and C57BL/6 ($H\text{-}2^b$) strains of mice.

Because more MCMV pathology is seen in the sensitive BALB/c mouse strain, many studies of MCMV immunity have focused on this model. The ability to restrict herpesvirus gene expression to the *IE* class was used to determine that an *IE* gene was a major CD8 T-cell antigen in BALB/c ($H\text{-}2^d$) mice [26], and in 1989 a peptide from IE1/pp89 became the first CD8 T-cell epitope from any herpesvirus infection to be identified [27]. Further epitopes from early genes and structural proteins were identified by motif-based epitope predictions or by analogy with the human response [28, 29].

Eight days after footpad infection, there is a codominant CD8 T-cell response to IE1 and to the E gene m164 in the popliteal lymph node, as measured by ELISPOT [30], with subdominant responses to m18 and M45 also detectable

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directly *ex vivo*. Responses to the other identified epitopes were detectable only after *in vitro* expansion of low-frequency memory CD8 T cells. Together, these epitopes account for most of the CD8 T cells that are capable of responding to anti-CD3 stimulation, which is used as an estimate of the total MCMV response.

The CD8 T-cell response in C57BL/6 mice is both larger and more diverse than that of BALB/c mice. Our laboratory first used an expression library of genomic MCMV DNA fragments to identify the E antigen M45-D^b as immunodominant in C57BL/6 mice [31]. In a separate venture, we cloned and expressed each open reading frame (ORF) from MCMV; by screening this "ORF library" with CD8 T cells taken directly from infected mice, we were able to identify a total of 27 *H-2*^{b}-restricted antigens that are recognized during the acute response, from which we have identified 24 peptide epitopes (M. Munks, unpublished data). These epitopes account for the majority of the CD8 T-cell response to MCMV. The M45 epitope still reigns as the most immunodominant, but it constitutes less than one-third of the virus-specific CD8 T-cell response. Most of these epitopes are encoded by E genes. Interestingly, no IE epitope was identified during the acute response to MCMV in C57BL/6 mice.

Thus, in BALB/c mice, the CD8 T-cell response is dominated by two epitopes, but responses against a total of four epitopes are detectable directly *ex vivo* and comprise most of the MCMV-specific response. The majority of CD8 T-cell epitopes are also identified for C57BL/6 mice, with 24 known epitopes, but no single epitope constitutes more than one-third of the total response.

12.3.2.2 The Memory CD8 T-Cell Response to MCMV Becomes More Focused Over Time

In MCMV, there is a growing body of evidence about how the CD8 T-cell response evolves from acute to chronic infection. In humans, where it is not possible to experimentally control the time of infection, information is more limited. But in both circumstances, signs of T-cell selection are evident.

In an MCMV bone marrow transplantation (BMT) model of infection, the latent viral load is highest in the lungs, and this also represents the organ where reactivation occurs most frequently [32]. Pulmonary CD8 T cells of many specificities peak during acute infection but then decline during the latent phase of infection. However, CD8 T cells specific for IE1 and m164 become enriched, in both relative and absolute terms, and are predominantly $CD62L^{lo}$, indicative of recent antigen exposure [30].

A similar phenomenon occurs in immunocompetent BALB/c mice, and in both situations IE1- and m164-specific CD8 T cells accounted for only ~20–30% of MCMV-specific CD8 T cells during acute infection but accounted for ~70–80% of MCMV-specific CD8 T cells during chronic infection [30]. Interestingly, IE1-specific CD8 T cells do not simply persist after infection is cleared. Exponential expansion until 10 days post-infection is followed by rapid contraction by day 14, reminiscent of well-characterized CD8 T-cell responses to lymphocytic choriomeningitis (LCMV) and *Listeria monocytogenes*, but then a phase of continuous inflation of the memory CD8 T-cell response occurs for presumably the remainder of the lifetime of the mouse [33].

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Interestingly, memory inflation in C57BL/6 mice does not depend on initial immunodominance. The CD8 T-cell response to most epitopes, including the immunodominant M45 epitope, contracts between days 7 and 14 and remains constant throughout the life of the mouse [34, 35] (M. Munks, in preparation). However, CD8 T-cell responses to m139 and M38 epitopes, which rank second and fifth in the acute immunodominance hierarchy, become more prominent during chronic infection, and responses to two epitopes in IE3 that are barely detectable during acute infection increase dramatically during the next several months. Similarly, the CD8 T-cell response to a recombinant LCMV epitope expressed behind the IE1 promoter is just above the threshold of detection at day 7 but undergoes an impressive 10-fold increase in less than 4 months [34]. Together, these results indicate that some feature of these epitopes/antigens and/or the CD8 T cells that recognize them, other than numerical superiority, is responsible for their dominance during chronic infection.

In addition to numerical differences, there are phenotypic differences between CD8 T cells of different specificities. CD8 T cells specific for "inflationary" antigens are $CD62L^{lo}$ [34], reminiscent of "effector memory" T cells [36]. They also lack two receptors for cytokines involved in homeostatic proliferation, CD127 (IL-7Ra) and CD122 (IL-15Ra), and are $CD27^{-}$ and $CD28^{-}$. In contrast, CD8 T cells specific for "non-inflationary" antigens are $CD62L^{hi}$, $CD127^{+}$, $CD122^{+}$, $CD27^{+}$, and $CD28^{+}$.

12.3.2.3 The Impact of Interference With Antigen Presentation by MCMV's Viprs on the CD8 T-Cell Response

Both HCMV and MCMV encode proteins that interfere with the MHC class I pathway of antigen presentation [3, 28] It is often presumed that an important function of these viral genes that interfere with antigen presentation (VIPRs) is to prevent or diminish priming of some T-cell specificities. It logically follows then that the T-cell response will focus on those epitopes least affected by the VIPRs. *In vitro* data have mostly supported this assumption; until recently, virtually all memory CD8 T-cell responses described from HCMV were derived from IE1 or from the structural protein UL83/pp65, which is brought into the cell as a part of the virion and can thus be processed and presented before any VIPRs are expressed [37]. In MCMV, the only CD8 T-cell epitope known for more than a decade was derived from IE1/pp89, which is expressed before the VIPRs. Furthermore, the dominance of the m164 epitope, encoded by an E gene, could be explained by its ability to be presented by infected cells despite the effects of the VIPRs [38].

Excellent tools are now available for studying the effect of MCMV VIPRs on CD8 T-cell priming *in vivo*. Surprisingly, this work has not revealed any significant effect of the VIPRs on the size, specificity, or surface phenotype of the responding CD8 T-cell population. Infection with wild-type MCMV or an MCMV mutant lacking *m152* results in equivalent priming of CD8 T cells specific for M45 [31], even though *m152* completely abrogates recognition of infected cells by M45-specific CD8 T cells. This lack of effect of *m152* on CD8 T-cell priming was later confirmed in BALB/c mice. [37]. Even more definitively, the CD8 T-cell response to a mutant

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virus lacking all three VIPRs (*Dm4+m6+m152)* has now been compared to wild-type MCMV infection. In both BALB/c mice, where seven epitopes were examined [29], and C57BL/6 mice, where 24 epitopes were tested (M. Munks, in preparation), the CD8 T-cell response was virtually identical for these two viruses. The VIPRs failed to influence the order of the immunodominance hierarchy in both the acute and chronic phases of infection.

Is it possible that (1) there are many antigens whose presentation is not affected by these genes, (2) that these genes do not affect antigen presentation *in vivo*, or (3) that they do not function in the cell type responsible for T-cell priming *in vivo*, namely DCs? If none of these statements is true (discussed below), then the simplest explanation is that *in vivo*, CD8 T cells are primed by cross-presentation, not by infected DCs.

1.Of the known BALB/c (H-2^d) epitopes, five of seven are not presented by wild-type MCMV-infected cells *in vitro*, with IE1 and m164 representing the exceptions. Additionally, wild-type MCMV prevents presentation of all 15 C57BL/6 (H-2^b) epitopes tested to date [39] (A. Pinto, unpublished data). Thus, only a minority of epitopes are able to escape the effects of the VIPRs *in vitro*, and the first explanation can be ruled out.

2.In an elegantly designed study using a bone marrow transplantation model of MCMV infection, it was demonstrated that CD8 T cells specific for the immunodominant C57BL/6 M45 epitope did not restrict replication of wild-type MCMV *in vivo* [40]. However, these same CD8 T cells did restrict replication of MCMV lacking the VIPR *m152*. Thus, the VIPRs do indeed affect antigen presentation *in vivo*, and the second explanation can be ruled out as a blanket phenomenon. It has not been excluded that there may be an important antigen-presenting cell *in vivo* in which the VIPRs do not function, but at present there is no positive evidence to support that contention. It should be noted that CD8 T cells of some other specificities do offer some protection against wild-type MCMV *in vivo*, and hence they must be able to recognize infected cells to some degree. However, all available evidence suggests that the VIPRs cause a large quantitative difference in the presentation of most epitopes, especially those from E genes that are expressed later in the replication cycle than the VIPRs.

3.Most studies of VIPR function and biochemistry have been performed in fibroblasts. Although an earlier report suggested that VIPRs might not function in macrophages, this has been disputed [41, 42]. Recent studies from our laboratory also indicate that VIPRs function effectively in bone marrow–derived DCs. Because DCs are the most relevant cell type for priming the CD8 T-cell response, there is no reason to believe that priming *in vivo* is performed by a cell type immune from the impact of VIPRs.

The cumulative data suggest that VIPRs (1) are effective against most antigens, (2) function *in vivo*, and (3) function in bone marrow–derived DCs. Yet there is no evidence that VIPRs can affect CD8 T-cell priming *in vivo*. The most plausible explanation for this apparent paradox is that *in vivo*, MCMV-infected DCs do not prime CD8 T cells. Rather, infected cells are most likely phagocytosed by DCs, and their antigens are cross-presented to naïve CD8 T cells.

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12.3.2.4 The Memory T-Cell Response to HCMV

Primary HCMV infection of immunocompetent individuals is normally asymptomatic. Thus, most of the literature on HCMV-specific T-cell responses describes memory, rather than acute effector, T-cell responses. From 1987 to 1996, the prevailing paradigm was that most CD8 T-cell responses were directed against the tegument protein pp65 [43]. Development of tetramer, ELISPOT, and intracellular cytokine staining assays simplified the analysis and led to more powerful quantitation. Reevaluation revealed that IE-1, previously thought to be very subdominant, was codominant with pp65 in many individuals [43].

A study by Elkington et al. [44] shifted the paradigm yet again. Fourteen potential HCMV antigens, with a variety of cellular functions, were selected for study. From these, more than 200 peptides were synthesized that matched HLA-binding motifs. CD8 T-cell responses were detectable for 9 of 14 antigens, including 31% of all peptides tested. Importantly, nearly half of the HCMV-specific T-cell responses detected were directed against antigens other than pp65 and IE-1. Another study soon confirmed this general finding. Manley et al. [45] derived CD8 T-cell clones from five HCMV-infected subjects. The clones were screened with an HCMV mutant lacking VIPRs, and 385 virus-specific clones were identified. Of these, 46% were pp65 specific or IE1 specific. The remainder were pp150 specific (5%), gB specific (2%), or of unknown specificity (47%). These studies demonstrated that while CD8 T-cell responses to pp65 and IE-1 are numerically important, many other antigens are also recognized that in combination account for a large percentage of the total HCMV response.

This work was extended by Sylwester et al. [46], who definitively examined the immunogenicity of all HCMV proteins across a large number of HLA types. Overlapping 15-mer peptides from each HCMV protein were synthesized and then pooled by protein. Peripheral blood mononuclear cells (PBMC) were isolated from 33 HCMV seropositive subjects, who were selected for their HLA diversity. Each individual peptide pool was tested by cytokine flow cytometry for its ability to stimulate CD4 and CD8 T-cell responses from each subject's PBMC. The most significant finding was that 151 of the 213 HCMV ORFs tested elicited a CD4 and/or CD8 T-cell response in at least one individual, and responses were detected against all functional categories of proteins. The CD8 T-cell antigen most frequently responded to was UL48, which had not been previously described as an antigen, followed, not surprisingly, by pp65 and IE-1. CD4 T cells most frequently responded to gB, pp65, and UL86. A typical subject had an HCMV response that was astonishingly large and complex. The median subject had 4.0% HCMV-specific CD4 T cells recognizing 12 antigens and 4.6% HCMV-specific CD8 T cells recognizing eight antigens. Not surprisingly, all functional classes of ORFs were represented. Interestingly, a significant bias towards highly conserved ORFs was found. Although all kinetic classes were represented, IE antigens were highly over-represented, a finding that has important implications for how memory T-cell "inflation" might be occurring (described below).

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12.3.2.5 Increasing Size and Oligoclonality of the CD8 T-cell Response to HCMV With Age

There is substantial evidence that the HCMV T-cell response increases in size and becomes more clonal over time. In a study by Khan et al. [47], CD8 T-cell responses were compared between HLA-A2$^+$ and/or HLA-B7$^+$ HCMV-infected adults that were either old (age 60–95) or young (age 20–55). The old group had more than three times as many A2-pp65 tetramer$^+$ CD8 T cells as the young group and more than five times as many B7-IE-1 tetramer$^+$ cells as the young group, on average.

The increased size of T-cell responses to HCMV coincides with increased clonality. Wills et al. [48] initially demonstrated that in some individuals, up to 75% of CD8 T cells specific for pp65 utilized a common TCR Vβ chain. Bitmansour et al. [49] have described a similar phenomenon in CD4 T cells, using a more refined analysis of CDR3 lengths and TCR sequencing. Here, the pp65-specific response in four healthy subjects was limited to 1–3 dominant clonotypes, which accounted for up to 50% of the total CMV-specific CD4 T-cell response. These powerful molecular analyses have recently been reapplied to HCMV-specific CD8 T cells, quite remarkably showing that CD8 T cells frequently share TCR Vβ chains, even when those CD8 T cells are derived from different individuals [43].

Given that the number of HCMV-specific CD8 T cells increases over time, and that both CD4 and CD8 T cells have restricted Vβ usage indicative of massive clonal expansion, it is not surprising that HCMV-specific T cells have a surface phenotype and functional properties characteristic of being highly differentiated [43]. They are usually CD45RA$^+$, CCR7$^-$, lack CD27 and CD28, and are positive for perforin and bcl-2. CD57, a marker of replicative senescence, is also commonly found on HCMV-specific T cells. Taken together, these markers are indicative of an effector memory rather than central memory phenotype.

12.3.2.6 What Accounts for Memory "Inflation" and TCR Vb Focusing?

Although the time scale is very different, we assume that the CD8 T-cell inflation observed in MCMV infection is related to the memory T-cell response described in HCMV-infected humans. But if MCMV inflation is independent of the acute immunodominance hierarchy, a very important question arises: What factors determine which CD8 T-cell specificities will become dominant during chronic MCMV and HCMV infection? Some of the likely explanations will be discussed in the context of the factors discussed in Section 12.1 of this chapter: (1) virus-centric models, (2) APC-centric models, and (3) T cell–centric models.

Virus-centric Explanation 1: Does MHC Class I Immune Evasion Allow Presentation of a Subset of CD8 T-Cell Epitopes?

Despite the instinctive assumption that CMV VIPRs would have a profound impact on immunodominance, the available evidence in both human and mice suggests that this is not the case. As previously stated, Manley et al. [45] isolated 385 HCMV-specific CD8 T-cell clones of varying specificities. Of these, 39% were pp65- or pp150 specific and all of these could recognize wild-type HCMV (AD169) or a mutant HCMV lacking

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its VIPRs. However, of the 14% that were IE-1- or gB specific, or of the 47% that were of an unknown specificity, none could recognize wild-type HCMV. This demonstrates that the majority of HCMV-specific memory CD8 T cells are specific for antigens that are not presented by HCMV strains with intact VIPRs.

The effect of VIPRs on immune focusing was studied more directly in MCMV infection. No major difference has been observed in the CD8 T-cell response when mice are infected chronically with either wild-type MCMV or a mutant lacking its VIPRs [35] (M. Munks, in preparation). Thus, there is convincing evidence that VIPRs do not have a major effect on either CD8 T-cell priming or the subsequent inflation that occurs during chronic CMV infection.

Virus-centric Explanation 2: Does Abortive Reactivation Lead to Selective Expression of Proteins?

Unlike the gammaherpesviruses, which have well-defined "lytic" and "latent" gene expression programs, there is no evidence in CMV infections of different genetic programs for lytic replication and reactivation from latency. This has been examined in some detail in a BMT model in the lungs of mice latently infected with MCMV [32]. It was found that after a sublethal dose of γ-irradiation, MCMV expressed the transcription factors *IE1* and *IE3* and the virion component *gB* in a sequential manner, with unknown "checkpoints" existing between each of these genes. Yet infectious virus was produced infrequently, indicating that even in an experimental system designed to reactivate MCMV, the vast majority of reactivation events are abortive.

Is abortive reactivation the norm in latent infection? If so, it logically follows that T cells specific for those proteins expressed earliest during reactivation, and therefore most frequently, will have a selective advantage in terms of antigen "hits" and proliferation. If correct, this model predicts an "inflation advantage" for CD8 T cells that recognize IE antigens, then E antigens, with L antigen–specific CD8 T cells trailing behind.

Data from HCMV are largely consistent with this model. When Sylwester et al. [46] examined the expression kinetics of ORFs that elicited the largest and most frequent memory CD4 and CD8 T-cell responses, they indeed found that IE antigens were most favored in both cases, particularly for CD8 T cells. While IE ORFs represent less than 5% of the HCMV coding capacity, they represent more than 10% of both CD4 and CD8 T-cell responses. Additionally, CD8 T-cell responses to IE antigens are more than four times larger than E antigens, on average. However, while memory T-cell responses to E antigens and E-L antigens are least favored, L antigens are slightly favored over E and E-L antigens.

This latter finding is difficult to reconcile with the proposed model. But perhaps this occurs only because some L antigens are strongly favored during initial T-cell priming following primary HCMV infection (e.g., because they are expressed at much higher levels than other genes). We are aware of only one published study of the T-cell response during primary HCMV infection, but it may support this explanation. Using HLA-A2-restricted CD8 T-cell epitopes, previously defined from chronically infected adults, Gibson et al. examined the kinetics and antigen

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specificity of CD8 T-cell responses in infants [50]. Notably, in infants who responded to both pp65 and IE1, the relative immunodominance of pp65 compared to IE1 evolved as well. Regardless of whether initial CD8 T-cell responses to pp65 were larger or smaller than IE1-specific responses, by one year of age the IE1-specific response predominated over the pp65-specific response in all infants.

In MCMV infection, there is also considerable support for the model that IE antigens undergo preferential inflation. The two BALB/c antigens that predominate in chronic infection (IE1 and m164) are indeed transcribed under IE conditions, although m164 protein has not been demonstrated to appear until E times [38]. In C57BL/6 mice, responses to IE3 are barely detectable during acute infection but then inflate. Finally, recombinant epitopes expressed behind the IE1 promoter also undergo inflation [34]. In contrast, the other epitopes (M38, m139) that inflate in chronically infected C57BL/6 mice are not known to be expressed at IE times. However, by analogy with HCMV, there is some reason to believe that these may be IE genes. Clearly, this needs to be tested.

In conclusion, a circumstantial but strong case can be made that (1) most viral attempts at reactivation are abortive and (2) CD8 T cells specific for antigens expressed most frequently by the virus during abortive reactivation will tend to dominate the T-cell response in chronic infections.

Even if this model is true, it is not the sole explanation for chronic immunodominance. Two MCMV antigens, M38 and m164, contain multiple CD8 T-cell epitopes that are discordant in their ability to undergo inflation. In the case of M38, CD8 T cells specific for M38 316–325 ■AQ1■ inflate during chronic infection of C57BL/6 mice, but CD8 T cells specific for M38 38–45 do not (M. Munks, unpublished data). Similarly, BALB/c CD8 T cells specific for m164 257–265 undergo inflation, but C57BL/6 CD8 T cells specific for m164 267–275 and m164 283–290 do not. Perhaps early expression of antigens during reactivation is required, but is not sufficient, for inducing CD8 T-cell inflation. In this case, one or more of the APC-centric and/or T cell–centric models (described below) may provide an additional mechanistic layer on the process. Clearly, significant work is required to further test this model.

APC-centric Explanation: Does the Predominant APC Cell Type Differ Between Acute and Chronic MCMV Infection?

In the influenza model in C57BL/6 mice, a change in immunodominance in secondary infection has been attributed to differential presentation of two epitopes by different APCs [51]. Responses to an epitope that could only be presented by DCs dominate the primary response, whereas responses to an epitope that could also be presented by macrophages, B cells, and epithelial cells dominate the secondary response. Thus, chronic immunodominance could reflect better presentation by a broader array of APCs. As a variant of this explanation, epitopes that are dependant on immunoproteasome (constitutively expressed by DCs) for presentation may dominate the acute response, whereas those that can be processed by normal proteasomes may be advantaged in the chronic response.

While it may be tempting to speculate that a similar phenomenon occurs after MCMV infection, there is currently no data to support this model. First, most of

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the epitopes for C57BL/6 mice can be presented from a transgene by fibroblasts without IFN-γ treatment and thus are not dependent on the immunoproteasome. Second, as described, available evidence indicates that because of the function of VIPRs, directly infected cells of all types are impaired in their ability to present most of these epitopes. Hence, the role of directly infected cells in stimulating MCMV-specific CD8 T-cell responses remains to be demonstrated.

T Cell–centric Model: Is There Selection for T Cells With "Optimal" TCRs During Chronic Infection?

In MCMV infection, it is clear that CD8 T-cell "inflation" occurs for only some epitopes. However, even among T cells of the same specificity, there is evidence that some TCRs are "optimal" compared to others. For example, TCR Vβ chain usage by IE1-specific CD8 T cells is more diverse during acute MCMV infection and more focused during chronic infection [33]. In HCMV infection, both CD4 and CD8 T-cell responses can be extremely focused in clonality, as described above, presumably the endpoint of the same phenomenon that has been clearly documented in mice.

So far there are no data to indicate that IE1-, m164-, or m139-specific CD8 T cells from chronically infected mice are significantly more sensitive to low doses of peptide than are CD8 T cells from acutely infected mice (S. Sierro, personal communication, and M. Munks, unpublished observation). While it remains unknown which features of a TCR make it optimal, it is clear that some form of selection is occurring *in vivo* among T cells of a shared specificity, adding an additional layer of complexity to unravel.

12.3.3
HCMV and Immunosenescence

What are the health implications of being HCMV seropositive and having large virus-specific oligoclonal T-cell expansion? It is becoming increasingly clear that HCMV infection impairs vaccination and antiviral immunity and is associated with higher mortality rates in the elderly (for a review, see Ref. [52]).

For example, it is known that HCMV seropositivity and the high frequency of $CD8^+CD28^-$ T cells that are associated with HCMV infection are correlated with poor antibody responses following influenza vaccination [52]. Additionally, CMV-specific immune responses appear to suppress the expansion of EBV-specific T cells that occurs in HCMV seronegative individuals, suggesting that HCMV may also impair EBV immunity [53]. In two aging studies of elderly Swedish, an "immune risk phenotype" (IRP) has been defined that accurately predicts mortality. CMV seropositivity is an important indicator of the IRP, and individuals with expanded populations of $CD8^+CD45RA^+$ $CD27^-CD28^-CD57^+$ T cells are most at risk [52].

It is unknown whether the effect of HCMV on other immune responses is due to active suppression or is simply a result of "overcrowding" of the T-cell compartment. However, evidence is mounting that HCMV is an important player in the immunosenescence process.

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12.3.4
T-Cell Cross-reactivity Between Betaherpesviruses

As mentioned in the overview of the betaherpesvirus section, no T-cell antigens have been reported for HHV-6 or HHV-7. While this precludes an analysis of T-cell immunodominance in individuals infected with these viruses, some interesting and relevant data on CD4 T-cell responses do exist.

CD4 T-cell clones, stimulated with antigen from HHV-6, HHV-7, or HCMV, were isolated from seropositive subjects and used to analyze epitope cross-reactivity between the viruses [54]. Of the T-cell clones driven with either HHV-6 or HHV-7 antigen, 28% reacted to both HHV-6 and HHV-7 antigen. In other words, a large percentage of HHV-6- and HHV-7-specific CD4 T-cell clones recognized an antigen shared by the two viruses. In contrast, only 4% of T-cell clones were cross-reactive between HHV-6 and HCMV, and only 2% of HHV-7-specific and HCMV-specific T-cell clones were cross-reactive. Not surprisingly, an even smaller percentage (1%) of all CD4 T-cell clones recognized all three viruses.

In retrospect these results are not completely surprising, given that genomic sequencing efforts later revealed that HHV-6 and HHV-7 are more closely related to each other than either virus is to HCMV. Additionally, the limiting dilution analysis (LDA) data should be considered semi-quantitative compared to contemporary techniques such as peptide–MHC tetramers, ELISPOT, and ICS. Yet these data are instructive in a more conceptual sense. Whereas different HCMV strains could be thought of as siblings, HHV-6 and HHV-7 are analogous to cousins. Within this conceptual framework, it is less surprising that T cells will cross-react between these "different" viruses. Instead, T-cell cross-reactivity between viruses sharing a common ancestor can be interpreted as an indication of the amino acid relatedness of any two viruses.

This may help to explain the observation that the HCMV-specific T-cell response is stronger against conserved genes than against non-conserved genes [46]. Herpesvirus infections are highly prevalent, and co-infection of the same individual is frequent. Thus, it is likely that some HCMV seropositive individuals are infected with multiple HCMV strains, or also with HHV-6 and/or HHV-7. In these individuals, T cells that were initially primed against one strain of HCMV may receive an additional stimulus each time they are super-infected with another HCMV strain or another close viral relative. As the individual becomes infected with more strains, or more viruses, the largest T-cell responses will be towards the genes that are most conserved among all viruses present.

12.3.5
Gammaherpresviruses

The third subfamily of mammalian herpesviruses is the gammaherpesviruses. This subfamily has two genera: gamma-1 and gamma-2. The prototypical member is Epstein-Barr virus (EBV), a gamma-1 herpesvirus [55]. More recently described are Kaposi's sarcoma herpesvirus (KSHV) [56] and the rodent virus MHV-68, both

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classified as gamma-2 herpesviruses. The orchestration of latency by gammaherpesviruses differs from that of the alpha- and betaherpesviruses in that they encode a separate set of genes that are expressed only during latent infection. They are also distinguished by their ability to cause malignancies. These two features are related: the propagation of viral genome in latently infected cells is achieved in part by driving the cells into proliferative cycle, which can lead to tumors in the host. Latency is established in B cells for each of these viruses. Lytic infection primarily occurs in epithelial cells—in the oropharynx in the case of EBV—although B cells can also undergo lytic infection with production of infectious virus progeny.

From the perspective of immunodominance, the gammaherpesviruses are both interesting and instructive in that the T-cell responses to the two classes of proteins (lytic and latent) follow quite distinct courses. There is a much greater body of literature describing the CD8 T-cell response to EBV than to the gamma-2 herpesviruses; consequently, most of this section will deal with EBV.

12.3.6
Epstein-Barr Virus

EBV infects a majority of people worldwide, although the seroprevalence is lower in industrialized countries [57]. It is spread by saliva. Primary infection is usually asymptomatic, especially when it occurs in the first decade of life, as is the norm in non-industrialized countries. Primary infection later in life, typically during adolescence, can lead to the syndrome of infectious mononucleosis (IM). EBV is the cause of Burkitt's lymphoma, a B-cell malignancy, and of nasopharyngeal carcinoma.

EBV infection of B cells *in vitro* leads to their transformation into lymphoblastoid cell lines (LCLs). LCLs express all six of EBV's latent genes—the leader protein (LP); the Epstein-Barr nuclear antigens (EBNA) 1, 2, 3A, 3B, and 3C—as well as two latent membrane proteins (LMP1 and LMP2). The easy ability to generate autologous LCLs from seropositive individuals means that immunity to these six proteins has been extensively studied for two decades. In contrast, there is no easy model for lytic EBV infection. In contrast to HSV or CMV, EBV does not infect fibroblasts or epithelial cell lines *in vitro*; therefore, data on immune responses to lytic antigens are much more scarce. There are some 60+ lytic cycle genes. Two IE genes are known: BZLF1 and BRLF1, which are transcription factors. There are 30+ early genes: these can be subdivided into transcription factors expressed in the earliest times points of E gene expression, such as BMLF1 and BMRF1, and later E genes. There are also approximately 30 late genes. A proportion of LCLs undergo spontaneous lytic cycle infection, and this is the main model for studying lytic infection.

12.3.6.1 Interference with the MHC Class I Pathway by EBV

Both alpha- and betaherpesviruses encode genes expressed during lytic cycle infection that impair presentation of antigens via the MHC class I antigen presentation pathway (VIPRs). Although such genes have not yet been identified for lytic

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cycle EBV infection, it seems very likely that they exist: as lytic infection progresses, MHC class I is downregulated on the cell surface [58]. Latent cycle genes do not downregulate MHC class I. However, one latent cycle protein, EBNA 1, interferes with its own degradation by the proteasome [59]; this diminishes, but does not abolish, its presentation by MHC class I molecules [60, 61]. For many years, this was thought to completely prevent the generation of EBNA 1–specific responses. However, it has become clear that such responses are in fact generated.

12.3.6.2 **Overview of the Response to Lytic and Latent Proteins**

The CD8 T-cell response to EBV has been studied for more than 20 years by several major groups: this extensive literature has recently been reviewed [62]. Acute infection by EBV is generally identified only if the patient experiences infectious mononucleosis (IM); thus, studies of acute infection are largely limited to IM patients. During this phase of infection, massive CD8 T-cell responses are elicited. These are predominantly directed towards lytic cycle proteins, although responses to latent proteins are also made. As IM resolves, the responses to lytic cycle proteins contract to a small memory population. Responses to latent cycle proteins dominate the repertoire of EBV-specific T cells during latent infection [63].

12.3.6.3 **Immunodominance Hierarchy for the Acute Response to Lytic Cycle Proteins**

The first lytic cycle epitopes identified were from IE genes. Recently, Hislop and colleagues more systematically addressed the question of which classes of EBV lytic proteins are most immunodominant during the primary CD8 T-cell response to EBV [64]. CD8 T-cell clones were derived by limiting dilution from IM patients. To drive the clones, PBMC were exposed to either autologous LCLs, some of which express lytic antigens, or anti-CD3 and IL2. The latter methodology was an attempt to avoid skewing the repertoire of clones by the nature of the *in vitro* restimulation, and it was reassuring that the antigenic specificities of the derived clones were similar by both methods. The antigens recognized by the clones were then identified using recombinant vaccinia viruses expressing a panel of candidate antigens. Because the clones were screened directly on recombinant vaccinia viruses, the method avoided bias towards clones that would be able to detect lytically infected cells. Unfortunately, the authors did not indicate what percentage of the CD8 T-cell clones they derived were able to recognize one of these antigens. In other words, they did not gain a clear picture of how much of the acute response they had detected. However, this study provides the most systematic description to date of the immunodominance hierarchy to EBV.

The panel of candidate antigens included the two known IE genes. Eleven E genes out of the expected 30+ E genes were chosen. These covered a range of functions: transcriptional activators, which would be expressed early during the E phase; components of viral DNA replication machinery, which are expressed later

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during the E phase; and several others. Ten L genes, out of the 30+ L genes, were chosen. These included virus capsid and tegument components, glycoproteins, and a secreted IL10 homologue. This panel of 23 antigens was chosen in an attempt to randomly sample EBV lytic cycle genes. Eleven patients were studied, covering a range of HLA types. As for the CMV study by Picker and colleagues [46], the power of this human study with multiple HLA alleles allows genuine virological determinants of immunodominance to emerge. These authors found that all classes of protein were recognized. However, when the authors asked what percentage of their positive clones recognized different classes of antigens, they found a marked skewing towards IE genes and those E genes that are earliest expressed—namely, those encoding transcriptional activators. This skewing was not based on affinity: when peptides were identified, those expressed from L genes were generally recognized at lower concentrations than those from IE or E genes.

Why are IE and the earliest E genes immunodominant? As has been discussed above, there are two main (virus-centric) explanations for this phenomenon. (1) Antigens from this kinetic class are likely to be least affected by the action of VIPRs. (2) If over time most infected cells are destroyed by host defenses early during the infectious cycle, the earliest expressed antigens will gain an incremental numerical advantage. Using LCLs that spontaneously entered the lytic cycle, these authors determined that IE and the dominant E antigens were generally more efficiently presented by the infected cells. As described above, as the lytic cycle continues, MHC class I expression decreases, presumably because of the action of as yet unidentified EBV VIPRs. This likely explains the much poorer presentation of L genes. These authors therefore concluded that the skewing of the CD8 T-cell repertoire towards IE and E genes is explained by their being better presented by infected cells: explanation 1 above. However, as they acknowledge in their discussion, explanation 2 is also consistent with their data. Given that the same skewing towards IE antigens occurs in CMV infection whether or not the VIPRs are functional, we tend to favor explanation 2. We note, however, that EBV and CMV are two very different viruses, and extrapolation from CMV to EBV requires a high degree of caution.

12.3.6.4 **Kinetics of the Response to Lytic Proteins**

The syndrome of IM is accompanied by massive EBV-specific CD8 T-cell expansions. Interestingly, primary asymptomatic EBV infection, which in global terms is probably the norm, does not elicit such massive responses, despite equivalent levels of virus load [62]. The factors that trigger the massive T-cell response in IM are not known. To date, no studies of immunodominance in asymptomatic infection have been reported, and it will be interesting to see whether the same immunodominance hierarchy is reached under those conditions.

With resolution of acute viral loads, the reactive T-cell compartment contracts. Margaret Callan's group has described a fascinating phenomenon that so far appears to be unique to EBV [65]. The specificities that dominate the acute response contract most severely, whereas specificities that are subdominant during IM are less severely culled during the contraction phase. The result is that

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there is more pronounced focusing of the response to lytic antigens during IM than there is during the chronic phase of infection. These authors suggest that those clones of highest affinity are most expanded during acute IM, see antigen more frequently, and consequently are more activated than subdominant clones. However, as a consequence of their activation, they are more susceptible to activation-induced cell death (AICD) and hence "crash" more profoundly upon antigen withdrawal. Studies of other non-persisting infections in mice have generally found that the hierarchy of the acute response predicts the hierarchy of memory. The reasons for the discrepancy are not yet clear. The situation in IM differs from the model systems in mice in that the acute phase lasts much longer; therefore, the clones that dominate the acute response may have reached the limit of the number of possible divisions. These authors described a mathematical model of T-cell expansion and contraction, which takes cellular senescence into account, and found that this can explain their data. This is thus an entirely T cell–centric explanation for chronic immunodominance, with the interesting twist that over time it is moderate rather than high-affinity responders that become immunodominant. However, the fact that acute EBV that does not elicit the syndrome of IM does not drive these very large T-cell responses, despite equivalent virus loads, suggests that there is something unusual about the nature of the acute CD8 T-cell response in IM. If so, the rules that govern the expansion and contraction of this response may well differ from other model infections.

12.3.6.5 **Immunodominance Hierarchy of the Response to Latent Proteins**

All six of the latent proteins are known to stimulate responses. However, in most individuals, responses to the EBNA 3 proteins dominate. HLA-A2-positive individuals make a strong response to an epitope in LMP2, an example of the impact of MHC polymorphism on immunodominance. All six latent proteins are expressed in EBV-transformed lymphoblastoid cells lines (LCLs). However, only EBNA 1 is needed to maintain the latent virus episomal DNA, and many latently infected B cells *in vivo* express only EBNA 1. The response to EBNA 1 is usually of very low frequency. EBNA 1 contains a Gly–Ala repeat sequence that impairs the efficiency of its degradation by proteasome to generate CD8 T-cell epitopes. For a long time, it was thought that no EBNA 1–specific responses were generated. However, it has recently been appreciated that such responses are generated. Cross-presentation has been proposed as the means by which these responses are generated [66], although, of course, this has not been proven *in vivo*.

12.3.6.6 **Kinetics of the Response to Latent Proteins**

The kinetics of the response to latent proteins is quite different from that of the response to lytic proteins [63]. During IM, responses to latent antigens are usually detectable but are much less prominent than responses to lytic antigens. However, responses to latent antigens do not contract markedly in transition to chronic infection. Thus, during chronic infection, responses to latent proteins are more

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prominent than to lytic antigens. The responses to the two classes of antigens also differ phenotypically. In the memory phase, many lytic antigen–specific CD8 T cells downregulate CD45 RO and re-express CD45 RA; these cells are mostly $CCR7^{-}CD62L^{-}$ "effector memory" cells. In contrast, latency antigen–specific T cells remain CD45 RO^{+} and CD45 RA^{-}, and are $CCR7^{+}CD62L^{+}$ "central memory" in phenotype. Two factors are likely to impact both the size of the memory CD8 T-cell response and its phenotype: antigen abundance and the nature of the antigen-presenting cell. During acute infection and IM, there is abundant expression of lytic cycle proteins in the oropharynx, predominantly in epithelial cells. The immunodominant latent antigens are expressed exclusively in B cells. As is the case for all herpesviruses, the amount of antigen (of both classes) expressed during chronic infection is difficult to ascertain.

The murine gammaherpesvirus MHV-68 is used to model EBV infection, and there are intriguing similarities in the two systems. As with EBV, in MHV-68 infection, lytic cycle epitopes dominate the acute response, and latent specific responses ■AQ2■ arise a little later and are more prominent during the later (latent) phase of infection [67]. Also as with EBV, the lytic cycle–specific CD8 T cells are largely effector memory ($CD62L^{-}$), whereas latent antigen specific CD8 T cells are largely central memory ($CD62L^{+}$). However, it was the latent antigen–specific central memory T cells that displayed the greatest *in vivo* cytolytic potential, at odds with the normal relationship between lymph node homing potential and effector capability. Most explanations of memory phenotype relate phenotype to frequency of antigen encounter, more frequent TCR triggering being associated with the effector memory phenotype, and less frequent encounters leading to the central memory phenotype. The picture in EBV and MHV-68 infection is at odds with this: when compared to lytic antigen–specific memory CD8 T cells, latent antigen–specific CD8 T cells are maintained in greater numbers and have greater immediate effector potential, both features that suggest more frequent antigen encounter. However, these latent antigen–specific CD8 T cells are uniformly central memory in phenotype. Because latent antigen–specific CD8 T cells encounter their antigen primarily on B cells, it seems possible that this type of APC influences the memory phenotype of the responding T cells.

Understanding the complex pattern of immunodominance and T-cell phenotype in chronic EBV infection is bedeviled by the same issue that occurs for all the chronic herpesvirus infections that have been discussed in this chapter: despite abundant evidence of T-cell reactivity, viral antigen is rarely directly detectable. We are left making a circular argument, in which a phenomenon that we are trying to understand (T-cell specificity and phenotype) is itself the main evidence for the causative factor we would like to ascribe it to (virus activity).

12.3.6.7 Immunodominance of the LC13 TCR in Responding to HLA-B8/FLRGRAYGL: An Extreme Example of TCR Immunodominance

The CD8 T-cell response to latent EBV antigens includes one of the most fascinating immunodominance stories in the literature [68, 69]. Individuals who express

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the MHC class I allele HLA-B8 make an immunodominant response to an epitope from the EBNA-3A protein FLRGRAYL. Essentially all the T cells responding to this epitope from disparate individuals use an identical, "public" TCR known as LC13. The selective pressure on LC13 is underscored by the fact that different nucleotide sequences are used by different T-cell clones to arrive at the identical LC13 TCR sequence. This is therefore an extreme example of TCR immunodominance. What makes this particular TCR so overwhelmingly preferred? One possibility would be that because of holes in the repertoire induced by negative selection, LC13 is the only TCR sequence capable of recognizing HLA-B8/ FLRGRAYL. However, it is known that this is not the case. Fortuitously, the LC13 TCR recognizes HLA-B44 as an alloantigen. Thus, individuals who are heterozygous for HLA-B8 and HLA-B44 do not express LC13, as this TCR is deleted in the thymus. These individuals make a response to HLA-B8/FLRGRAYL that is polyclonal and utilizes a variety of TCRs. Hence, there appears to be something special about LC13 that makes it the best possible of all TCRs for its cognate ligand.

LC13's advantage does not appear to lie in high affinity: the rough estimate of its K_d is about 50 μm. The LC13 TCR has been crystallized alone and in complex with HLA-B8/FLRGRAYL [69]. The TCR undergoes considerable conformational change upon ligand binding: a proline in the CDR3 loop that is not involved in interaction with the peptide–MHC complex acts as a critical "crumple point" that enables CDR3 to reshape itself to fit the ligand. It is a matter of considerable controversy whether conformational change in the TCR alpha and beta subunits contributes to signal transduction or is merely a means of accommodating the TCR to its diverse ligands. While proponents of the importance of conformational change use the LC13 data to support their model, the argument is not yet resolved. However, the extreme immunodominance of the LC13 receptor should provide important information as to just what the immune system prefers when it is making its immunodominance choices at the TCR level.

12.4 Concluding Remarks

Herpesviruses are large, ancient, complex viruses. It is impossible to study the herpesvirus–host interaction for long without developing a deep respect for the multiple layers of redundancy that underpin this very stable relationship. Given the complexity of immunodominance even to simple model agents, it should not be surprising that we are not yet in a position to explain the basis of immunodominance in most herpesvirus infections.

The seemingly safe prediction—that genes that interfere with antigen presentation would have a major impact on immunodominance—turns out not to be correct, at least for MCMV. This highlights some of the major holes in our knowledge. We do not know, for example, whether direct or cross-presentation accounts for most of the priming of CD8 T cells or for maintaining the response during chronic infection. Furthermore, in the chronic phase of infection, which lasts for

the life of the host and is the most studied, we have little knowledge of the nature or frequency of viral gene expression. Nevertheless, mammalian immune systems devote a large amount of their resources to herpesviruses, which have been their companions through 80+ million years of evolution. Understanding the basis of these responses would seem to be an important part of understanding normal immunobiology.

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