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Review

Confronting Complexity: Real-World Immunodominance in Antiviral CD8+ T Cell Responses

Jonathan W. Yewdell[1,*]
[1] Laboratory of Viral Diseases
National Institute of Allergy and Infectious Diseases
Bethesda, Maryland 20892

Antiviral CD8+ T cells respond to only a minute fraction of the potential peptide determinants encoded by viral genomes. Immunogenic determinants can be ordered into highly reproducible hierarchies based on the magnitude of cognate CD8+ T cell responses. Until recently, this phenomenon, termed immunodominance, was largely defined and characterized in model systems utilizing a few strains of inbred mice infected with a handful of viruses with limited coding capacity. Here, I review work that has extended immunodominance studies to viruses of greater complexity and to the real world of human antiviral immunity.

Introduction

> Make everything as simple as possible, but not simpler.
>
> —Albert Einstein

CD8+ T cells play an increasingly well-documented role in clearing virus infections. There is mounting interest in designing preventive and therapeutic vaccines capable of eliciting or enhancing virus-specific CD8+ T cell responses. It is hoped that such vaccines will prove useful for infections with both persistent (e.g., human immunodeficiency virus [HIV]) and acute (e.g., influenza A virus [IAV]) infections that are poorly controlled by standard vaccine approaches.

Antiviral CD8+ T cells recognize virus-encoded peptides bound to major histocompatibility complex (MHC) class I molecules. Viral peptides, typically eight to ten residues in length, are predominantly generated from viral gene products through the initial action of proteasomes followed by trimming via aminopeptidases (Saveanu et al., 2005; Strehl et al., 2005). Proteasomes act either on antigen synthesized by the antigen-presenting cell (APC) (termed direct presentation) or on antigens acquired by the APC (cross-presentation). Viruses encode between a few thousand and tens of thousands of amino acids, and therefore potentially encode equivalent numbers of potentially immunogenic peptides. Remarkably, the bulk of responding CD8+ T cells recognize a tiny fraction of potential determinants, whose identities are governed by the MHC class I allomorphs expressed by the responding individual. (Allomorphs are the collection, present in any given vertebrate species, of class I alleles encoded by two or three class I loci; humans and mice have three such loci: HLA-A, -B, and -C, and H-2-K, -D, and -L, respectively.) This phenomenon, first described for CD4+ T cells, is termed immunodominance (Sercarz et al., 1993). "Immunodominant" determinants (IDDs) are recognized by the most abundant cognate T cell populations, whereas "subdominant" determinants are recognized by less abundant T cell populations. Antiviral responses to immunodominant and subdominant determinants form a hierarchy (α-, β-, etc.) that is remarkably reproducible between individuals of a given inbred mouse strain. It is important to note that immunogenicity is strictly an operational term and is limited by the technology available for detecting responses to potential determinants above background values (>1/2000 and >1/50,000 of total CD8+ T cells via flow-based and ELISPOT methods, respectively). It is (and always will be) impossible to definitively eliminate the possibility that a given determinant is immunogenic. Thus, any immunodominance hierarchy will be, to a greater or lesser extent, an oversimplification of reality.

Immunodominance reflects the final product of multitudinous positive and negative factors that govern antigen presentation and T cell activation (Yewdell and Bennink, 1999). In the broadest terms, this breaks down into two categories, each with numerous subcategories:

(1) Abundance of peptide class I complexes on afferent APCs (i.e., APCs that activate antiviral CD8+ T cells, typically dendritic cells, but probably other cell types as well [Pozzi et al., 2005]).
(2) Numbers of naive T cells with complementary T cell receptors (TCRs) that access afferent APCs and their capacity to proliferate and generate primary effector and memory CD8+ T cells.

More than 90% of immunodominance can be explained by the finding that only ~1% of peptides bind with sufficient affinity to a given class I allomorph to form a complex of sufficient stability to be presented in adequate numbers to activate naive CD8+ T cells. Bioinformatic analysis (Istrail et al., 2004) predicts that this low fraction of class I binding peptides applies to all organisms (including *Methanococcus jannaschii*, the extremophile that lives in deep ocean vents and is unlikely to have encountered the vertebrate MHC in its evolution) and does not reflect evolutionary pressure to avoid (or enhance) immune recognition. Rather, the class I binding groove simply appears to have evolved to bind a low fraction of peptides.

Although class I binding selectivity narrows the peptide repertoire considerably, it still leaves thousands of viral peptides for the immune system to potentially choose among (Figure 1). Studying how and why these choices are made and their consequences for immune function is the basis of current immunodominance studies. Understanding immunodominance is part and parcel of understanding the MHC class I-CD8+ T cell immunosurveillance system itself. Practically speaking, a thorough understanding of immunodominance is required to rationally design vaccines that elicit CD8+ T cell responses to defined determinants with sufficiently broad specificity to avoid selecting viral escape

*Correspondence: jyewdell@nih.gov

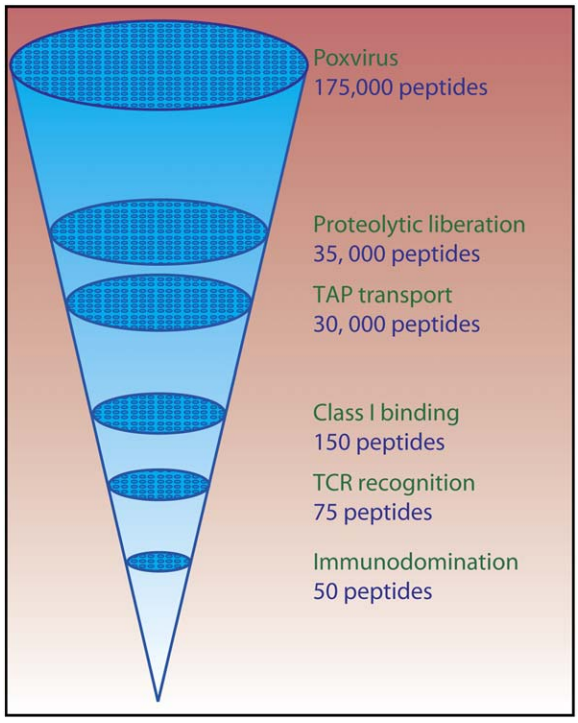

Figure 1. *E Pluribus Pauci*: Filters that Contribute to Immunodominance

Vaccinia virus, the prototypical poxvirus, encodes ~58,000 amino acids that can potentially generate greater than 175,000 8 mer, 9 mer, or 10 mer peptides presented by class I molecules ("greater than" because of potential alternative reading-frame peptides and peptides with posttranslational modifications). These are winnowed down into ~50 determinants that appear account for > 90% of the $CD8^+$ T cell response in B6 mice infected with vaccinia virus (Moutaftsi et al., 2006). Current knowledge (Yewdell and Bennink, 1999) suggests that only 20% of these peptides can be liberated in immunogenic quantities by proteasomes working in cahoots with other cytosolic and ER proteases. TAP functions to transport cytosolic peptides into the ER and exhibits a preference for transporting the types of peptides preferred by the allomorphs expressed by a given species. The net effect is that TAP has little selective effect on immunodominance, which is mainly governed by class I binding. Each class I allomorph binds on the order of 1% of potential peptides with sufficient stability to trigger ER export. The TCR repertoire is sufficiently diverse to recognize ~50% of the peptide class I complexes generated as a result of central and peripheral tolerance and perhaps innate holes in the repertoire as well. Immunodomination, the suppression of responses to subdominant determinants by immunodominant determinants, accentuates the hierarchy, but this effect varies greatly in magnitude on a system-specific basis.

variants. Readers are directed to a comprehensive review of immunodominance in antiviral $CD8^+$ T cell responses for a broad discussion of the phenomenon and contributing factors (Yewdell and Bennink, 1999). In addition, there are a number of excellent more recent reviews that provide detailed information on specific factors that contribute to immunodominance or focus on factors that contribute to immunodominance to specific viruses (Crowe and Woodland, 2006; Gaddis et al., 2006; Kedl et al., 2003; Landais et al., 2005; Lichterfeld et al., 2005; Maini and Bertoletti, 2006; Munks and Hill, 2006; Sette and Fikes, 2003; Sette and Sundaram, 2006; Tscharke and Suhrbier, 2005; Welsh, 2006; Whitton et al., 2004; Yewdell and Del Val, 2004; Yewdell and Haeryfar, 2005). The present review highlights recent advances in defining and understanding immunodominance in antiviral $CD8^+$ T cell responses to complex viruses in mice and in extending this knowledge to human antiviral responses.

Complexity of Responses to Complex Viruses

Until recently, studies on immunodominance largely focused on viruses with a relatively limited coding capacity: IAV and lymphocytic choriomeningitis virus (LCMV) infections in mouse model systems and HIV infections in humans. Exploiting technical advances, particularly those that have lowered the costs of peptide synthesis, viral immunologists are now tackling the problem of defining immunodominance in $CD8^+$ T cell responses to viruses with large genomes. Table 1 summarizes the strengths and weaknesses of the four major approaches that have been used for determinant discovery (the fifth approach is being developed by a number of laboratories).

Poxviruses

Poxviruses are among the largest viruses to infect humans, with a genome capable of encoding ~60,000 amino acids in 258 predicted open reading frames (ORFs). Tscharke et al. (2005, 2006) used a cDNA library encoding all of the annotated vaccinia virus (VV) ORFs to identify target-gene products, finding that in both $H\text{-}2^d$ and $H\text{-}2^b$ mice a handful of determinants account for about half of the total anti-VV response, with a single determinant accounting for approximately a quarter of the total response following intraperitoneal infection. The degree of dominance of the α-determinant was dependent on the route of immunization, with this determinant accounting for roughly half of the total response following intradermal infection, the route used for human vaccination. This appears to be the first published observation relating the route of viral immunization to the immunodominance hierarchy, which is surprising, given that this is potentially of great importance for vaccination.

Jing et al. (2005) used a random-expression library consisting of 3×10^4 unique colonies expressing ~300 bp fragments of VV genomic DNA (~50-fold over sampling of the VV genome) to characterize the specificity of $CD8^+$ T cells from VV-vaccinated patients. This approach confers the advantage of immediately confirming specificity by obtaining multiple independent hits with clones expressing overlapping fragments (it also provides a relatively short sequence for identifying the relevant antigenic peptide—like shooting peptides in a barrel). Including all clones and bulk cultures studied from eight individuals, determinants in 15 different VV gene products were identified. Interestingly, 10 of these gene products are known to be expressed early in the infectious cycle (i.e., prior to DNA replication), whereas only one is known to be a late gene product (the other four are undefined).

Sette and colleagues used a 2,256 member library of peptides predicted by computer algorithms to bind with high affinity to K^b or D^b to characterize the C57BL/6 mouse immunodominance hierarchy to VV (Moutaftsi et al., 2006). Forty-nine peptides were antigenic, including all of the five identified by Tscharke et al. (2005). All 49 antigenic peptides demonstrated

Table 1. Determinant-Mapping Technology

Method for Determinant Discovery	Advantages	Disadvantages	Complex Virus Application to Date
cDNA library of complete gene products	Identifies naturally processed peptides, including many posttranslational modifications Less expensive	Determinants must still be found in full-length gene Less sensitive Limits based on APC transfectability	VV-mouse MCMV mouse
cDNA library of viral genome fragments	Identifies naturally processed antigens Potentially more sensitive due to rapid degradation of many fragments Less expensive Overlapping positive fragments help locate determinant Possbily higher potential to detect alternative reading frame peptides	Will miss posttranslational modifications that require intact protein Limits based on APC transfection Potential limits in expressibility of some genomic fragments	VV-human
Optimally sized synthetic-peptide library predicted in silico	Immediate identification of determinants More sensitive	More expensive Must confirm natural processing and presentation Misses peptides that don't conform to algorithms MHC allomorph specific	VV-mouse VV-human
Overlapping 15 mer peptides	Less thinking Covers all MHC class I and II allomorphs	Less thinking Low sensitivity Many spurious cross-reactions Must define peptide and confirm natural processing and presentation Generation of determinants depends on processing by serum proteases	HCMV-human HSV-human
Mass-spectroscopy identification of class I bound peptides	Only method to precisely define viral peptides, essential knowledge for understanding cellular immune response	Technically highly demanding Labor intensive per peptide discovered Difficult to detect low-abundance peptides	In development

a dissociation constant (K_D) for class I of less than 2.6 μM (note that a lower dissociation constant means *higher*-affinity binding), with 66% of the peptides binding with very high affinity (K_D < 20 nM). Nearly 80% of the antigenic peptides scored in the top 300 in terms of in silico predicted binding, with all antigenic peptides present in the top 500 (welcome news, surely, for laboratories with more limited budgets for purchasing peptides). Altogether, the 49 peptides appeared to account for nearly the entire anti-VV response measured using VV-infected APCs as global VV-specific $CD8^+$ T cell activators. The α-determinant identified by Tscharke et al. (2005) accounted for 25% of the total response, with 50% and 75% of the total response accounted for by the top three and six peptides, respectively. Determinants were preferentially derived from viral proteins of greater than 100 residues and from those synthesized early in the infectious cycle.

Team Sette used an even larger synthetic-peptide library, composed of more than 6000 VV-encoded sequences predicted in silico to bind to the five most prevalent HLA-A and -B supertypes to measure $CD8^+$ T cell specificity induced by standard smallpox vaccination (i.e., live VV introduced intradermally) of 58 volunteers with disparate HLA types (Oseroff et al., 2005). Of the 53 antigenic peptides identified, 52 demonstrated a K_D of < 500 nM with the relevant HLA allomorph. Thirty-five different ORFs were recognized. Consistent with the findings of Jing et al. (2005), early gene products were overrepresented by more than 5-fold. The bias toward early gene products may be even greater than appears, because "late" genes may actually be expressed in small quantities prior to DNA replication. Many of the late proteins are viral structural proteins. Of 30 known virion proteins, only one was antigenic, suggesting that virions themselves are weak immunogens. The preferential immunogenicity of early viral proteins suggests that priming of VV responses is largely based on dendritic cell (DC) presentation of endogenous viral proteins (Norbury et al., 2002). This would be consistent with the reported block in late gene expression in VV-infected DCs (Bronte et al., 1997).

Contrasting with mouse systems, Oseroff et al. (2005) found that the human anti-VV response was relatively "immunodemocratic," i.e., responses were equally distributed among determinants. Further, individual determinants recognized by individuals could not be predicted simply on the basis of their HLA genotype, echoing previous findings with HIV specific $CD8^+$ T cells (Betts et al., 2000). Insight into this phenomenon comes from VV-specific $CD8^+$ T cell responses in HLA transgenic mice (Pasquetto et al., 2005), where the HLA-restricted immunodominance hierarchy was influenced by the presence or absence of endogenous mouse class I genes. Thus, in humans, the antiviral repertoire restricted by a given class I allomorph may be heavily influenced in some circumstances by the other class I allomorphs expressed by a given individual.

Studies in mouse model systems have demonstrated such interallomorphic influences on immunodominance hierarchies (Belz et al., [2000] and see below), but this is not a universal finding. Immunodominance hierarchies to IAV meld fairly smoothly when responses in F1 and parental mice are compared (Belz et al., 2000; Chen et al., 2002), and knocking out single class I genes was found to have little effect on the acute immunodominance

hierarchy to LCMV (van der Most et al., 2003). In humans, immunodominant responses to individual viral determinants can also be largely controlled by expression of the restricting HLA allomorph (Yu et al., 2002). This phenomenon is well known to human cellular immunologists who use an immunodominant HLA-A2-restricted peptide from IAV or cytomegalovirus (CMV) as positive controls in A2-positive volunteers (but, as discussed below, these determinants may be exceptional in being recognized by highly conserved TCRs).

Herpesviruses

Unlike VV, which causes an acute infection and is cleared relatively rapidly, herpesviruses, which include CMV, herpes simplex virus (HSV), and Epstein-Barr virus (EBV), generally cause life-long infections. During their long coevolution with their vertebrate hosts, herpesviruses devised numerous stealth strategies for avoiding immune detection, including limiting their gene expression to the bare minimum (latency) and directly impeding $CD8^+$ T cell surveillance by expressing viral genes that interfere with antigen presentation (VIPRs). The contribution of these virus life-style strategies to the composition of immunodominance hierarchies is one of the more interesting aspects of the $CD8^+$ T cell responses to herpesviruses.

Cytomegalovirus. Munks et al. (2006) used a DNA library encoding all 170 predicted murine CMV (MCMV) ORFs (encoding ~70,000 amino acids) to identify determinants recognized by C57BL/6 mice. Herpesviruses are, in general, highly host specific, but MCMV provides an excellent model for human CMV (HCMV) infections. During a primary infection, $CD8^+$ T cell responses to 27 distinct gene products were detected. Responses to a single determinant accounted for ~25% of the overall response, with the top four peptides in the hierarchy accounting for ~50% of the response. Using $H\text{-}2^b$ or F1 mice with different background genes, Munks et al. (2006) found that although the most immunodominant determinants retained their spots atop hierarchy, some shuffling was evident. Importantly, this indicates that non-MHC genes can influence the immunodominance hierarchy, and it represents another factor contributing to variation in human antiviral immunodominance hierarchies among individuals sharing identical HLA alleles.

It will be of great interest to determine the influence of MCMV VIPRs on this complex immunodominance hierarchy, particularly in light of previous evidence that although deletion of three VIPRs from the viral genome greatly impacts antigen presentation in vitro, it has little affect on the magnitude of overall anti-MCMV $CD8^+$ T cell responses in vivo (Gold et al., 2002). The effect of VIPRs on viral immunogenicity has been used as a means of gauging the contributions of direct versus cross-priming of antiviral $CD8^+$ T cell responses, the assumption being that VIPRs should act only in cells that express them, and therefore should exclusively inhibit direct priming (Basta et al., 2002; Shen et al., 2002). If this assumption is correct, it implies that VIPRs function to conceal virus-infected cells from efferent $CD8^+$ T cells, thereby facilitating virus replication and ultimately transmission between hosts. Lu et al. (2006) provide the initial evidence that the MCVM VIPRs indeed serve this function. The capacity of MCMV to serve as a persistent recombinant vaccine that induces strong and gradually expanding $CD8^+$ T cell responses to inserted genes (Karrer et al., 2004) is presumably based on persistent cross-presentation enabled by this VIPR-mediated concealment of infected cells. This exceptionally promising finding suggests a novel vaccine strategy for maintaining strong $CD8^+$ T cell responses, a strategy that could be an essential feature for a prophylactic HIV vaccine or for vaccines that maintain consistently high numbers of anti-influenza $CD8^+$ T cells.

To understand immunodominance to HCMV, Sylwester et al. (2005) performed the tour de force of determinant-mapping studies. Using a 13,687-strong panel of synthetic peptides representing overlapping 15 mer peptides covering the entire HCMV genome encoding 213 predicted ORFs, they determined the anti-HCMV specificities of $CD8^+$ T cells (and $CD4^+$ T cells) in peripheral blood mononuclear cells (PBMCs) from 33 seropositive patients. Remarkably ~5% of total circulating $CD8^+$ T cells recognize HCMV determinants. From 33 individuals examined, $CD8^+$ T cells recognized determinants present in 107 ORFs, representing all known functional and kinetic classes of viral proteins (i.e., early versus late expression). Individual seropositive patients responded to an average of eight ORFs, with a high of 32 and a low of a single ORF recognized, and an average magnitude of 0.5% of total $CD8^+$ T cells responding to each ORF recognized.

Though individual immediate early (IE) proteins (the very first proteins expressed in herpesvirus-infected cells) had a moderately greater statistical chance of immunogenicity per residue of protein encoded, nearly 90% of the response was directed to proteins synthesized later in infection. Because HCMV VIPRs are expressed after IE genes, this suggests that the VIPRs have at most only a minor impact on immunogenicity of HCMV gene products, consistent with previous findings made at the level of overall virus-specific responses (Manley et al., 2004). Ironically, for VV, which lacks VIPRs, human (but not mouse) responses appear to focus on early viral-gene products, pointing out how quirks in each virus-host relationship can influence the nature of $CD8^+$ T cell targets.

Sylwester et al. (2005) did not determine the HLA restriction of individual determinants, so interallomorphic and extra class I genetic effects on the immunodominance hierarchy await further detailed investigation. Interestingly, the one pair of seropositive twins studied responded nearly identically to the panel of peptides, extending similar findings with anti-HIV responses observed in two sets of twins (Draenert et al., 2006; Yang et al., 2005). Twins are nearly the human genetic equivalent of A × B F1 mice (but not truly equivalent because, unlike inbred mice, all humans have some recessive lethal genes, in addition to lacking other genetic alterations associated with inbreeding), so these findings (though limited in scope) support the validity of the mouse model for studies of immunodominance in human antiviral responses.

HSV. Hosken et al. (2006) used a panel of 4968 overlapping 15 mer peptides to study $CD8^+$ T cell responses to 48 herpes simplex virus 2 (HSV2) ORFs in 37 seropositive individuals. Each ORF examined was recognized by at least one patient in the study. Individuals responded to determinants from as few as three ORFs or as many as

46 ORFs, with a median response to 11 ORFs. Although IE proteins were preferred targets, late proteins were also recognized.

This broad human anti-HSV2 response stands in stark contrast to the anti-HSV1 response in C57BL/6 mice, where up to 90% of primary $CD8^+$ T cells recognize a single determinant in glycoprotein B (gB) (Wallace et al., 1999). Compromising the $CD8^+$ T cell response to this one determinant increases viral pathogenicity, indicating that functional responses to other determinants cannot replace the antiviral activity exerted by gB-specific $CD8^+$ T cells (Messaoudi et al., 2002). This is reminiscent of the observation by Rosenthal and Zinkernagel (1981) that despite encoding 3536 amino acids, vesicular stomatitis fails to elicit a detectable $H\text{-}2^k$ restricted cytotoxic response in mice (the virus is highly immunogenic in $H\text{-}2^b$ or $H\text{-}2^d$ mice). Certain combinations of individual MHC allomorphs and viruses seem to result in highly limited $CD8^+$ T cell responses that beg explanation, exposing a significant gap in our understanding of immunodominance.

EBV. Brander and colleagues studied $CD8^+$ T cell responses to EBV in 40 acutely or chronically infected individuals by using a panel of 80 optimally sized peptides with previously defined antigenicity and restriction elements (Woodberry et al., 2005). Notably, they found "unexpected" responses in no less than 72% of individuals who lacked the expected HLA allele (the extent to which this reflects "promiscuity" as concluded versus spurious cross-reactivity due to the use of peptides at 10 μM for $CD8^+$ T cell activation [at least 4 logs higher than should be necessary] remains to be determined). Acutely infected individuals responded on average to three determinants increasing to five determinants in chronic infections. These are minimal estimates because of the limited size of the peptide pools used, which covers a relatively small fraction of the EBV proteome. The study included four siblings with identical HLA types. Response to individual determinants between siblings was highly similar, but not identical.

Brander's laboratory extended this approach to include a panel of 184 HIV determinants and examined the influence of EBV coinfection on HIV responses and HIV coinfection on EBV responses (Bihl et al., 2006). They used three patient sets: patients infected with HIV only, patients infected with EBV only, and patients infected with both viruses. This analysis provides a number of important insights into immunodominance in humans:

(1) HIV coinfection (whether treated with antiretroviral drugs or not) did not greatly influence the magnitude or composition of the EBV response.
(2) There was a good correlation between the magnitude of responses to individual HIV or EBV determinants and the frequency of responses to determinants in individuals with the appropriate restricting HLA allomorph. As a corollary, responses to HLA-B-restricted determinants were the most robust and the most prevalent. The dominance of HLA-B in antiviral responses over HLA-A and particularly HLA-C (which appears to be used only infrequently) may extend to other viruses. In a more limited study, HLA-B was preferentially recognized by $CD8^+$ T cell populations specific to polyclonal influenza A or B virus (Boon et al., 2004). The how and, particularly, the why of these HLA-locus-specific effects in viral immunity remain an important mystery (see dirty little secret #5 in [Yewdell, 2005]).
(3) Among defined EBV or HIV determinants, the affinity of synthetic peptides for class I with a given class I allomorph did not closely correlate with the magnitude or frequency of responses to the determinant. This is somewhat surprising, because all things being equal, peptide affinity must affect the number of peptide-class I complexes available for activation of naive and memory $CD8^+$ T cells. Although the processes of peptide generation and delivery also are major variables in controlling complex number, this should average out with a large enough data set to reveal a contribution of peptide affinity. The data set may simply be too small for the relationship between affinity and immunogenicity to be clear. Alternatively, the measured affinity of synthetic peptides for purified class I molecules or peptide-receptive cell-surface class I molecules may not accurately reflect the natural interaction of peptides with class I molecules in APCs.
(4) There was no clear correlation between $CD8^+$ T cell sensitivity (i.e., the number of synthetic-peptide-generated complexes needed for activation) and the magnitude of responses to given HIV or EBV determinants. This finding is consistent with previous studies that have failed to closely correlate the immunodominance hierarchy or the sensitivity of responding $CD8^+$ T cells with numbers of complexes generated via the endogenous pathway (Chen et al., 2000; Crotzer et al., 2000), although exceptions have been reported (Fu et al., 1998; Restifo et al., 1995; Wherry et al., 1999). It seems that for many, though not all, determinants, endogenous presentation in vivo reaches sufficient levels to achieve near-maximal activation of their cognate $CD8^+$ T cell.

Man versus Mouse

The patient studies described above demonstrate that immunodominance hierarchies exist in human antiviral responses but, compared to mouse model systems, appear to be more immunodemocratic and less predictable. Although the mouse systems utilized may not be perfect models for human antiviral responses, clearly they provide a useful knowledge base that enables formulation of hypotheses of great value in understanding human responses.

A number of factors probably contribute to the greater variability of real-world immunodominance hierarchies in human antiviral responses.

First, mice are infected in a given experiment with the same virus, at the same dose, and via the same route. Even DNA viruses, which exhibit much less variability than RNA viruses (particularly HIV), exhibit significant variation between strains that circulate in human populations. Amino acid substitutions potentially affect immunogenicity by altering either determinants themselves

or flanking residues that influence antigen processing. Other substitutions could affect viral tropism or other parameters of replication that alter antigen presentation to $CD8^+$ T cells. Viral dose does not seem to have a significant influence on immunodominance hierarchies, but probably only because this has not been studied with sufficient zeal. Likewise, little is known about the influence of the route of infection, although, as cited above, it does affect the immunodominance hierarchy in the mouse anti-VV response (Tscharke et al., 2005).

Second, humans exhibit a great number of genetic differences outside of the MHC that potentially affect antigen presentation and T cell activation. Genes encoding transporter associated with antigen processing 1 (TAP1) and TAP2 exhibit polymorphism that may alter the presented peptide repertoire (Kjer-Nielsen et al., 2004). Class I allomorphs can affect each other's function by immunodomination, in which $CD8^+$ T cells specific for IDDs suppress responses to subdominant determinants (reviewed in (Kedl et al., 2003) or by altering the TCR repertoire via tolerance mechanisms (Burrows et al., 1995). Tolerance to polymorphic self-minor histocompatibility antigens can also sculpt the TCR repertoire. Polymorphic differences in genes involved in T cell-APC interactions or controlling viral replication could influence the immunodominance hierarchy. Detailed mapping in $H\text{-}2^b$ mice of the genetic differences in background genes leading to the alterations in the immunodominance hierarchy to MCMV (Munks et al., 2006) might shed considerable light on the contribution of non-MHC genes to immunodominance, an area of woeful ignorance.

Third, whereas humans experience frequent encounters with extraneous viral and other infectious agents and antigens, no efforts are spared to protect mice from exposure to microbial agents save the normal commensal epithelial organisms. On the basis of the sizable ratio of the universe of possible foreign antigens versus the number of TCRs that constitute the $CD8^+$ T cell repertoire, it has been argued that TCR recognition should be highly degenerate (Mason, 1998). On the other hand, a compelling case has been made that the probability of cross-reaction of a given TCR with any given peptide determinant is miniscule, on the order of 10^{-7} (Borghans and De Boer, 1998). Still, with an antiviral response composed of 10^4 to 10^5 specificities, and 10^1 to 10^3 viral determinants presented by class I molecules for potential cross-reactive recognition, some cross-reactive recognition appears to be possible, if not likely.

Heterologous Immunity: A Critical Appraisal

Ballpark numbers generated by theory are interesting (and fun), but ultimately, reality counts. Welsh, Selin, and colleagues have extensively studied the influence of prior viral exposure on subsequent mouse responses to heterologous infection (Welsh, 2006; Welsh and Selin, 2002; Welsh et al., 2004). They have shown that responses to a determinant in LCMV NP are enhanced by prior infection of mice by Pinchinde virus, a different arenavirus (Brehm et al., 2002; Kim et al., 2005). The homologous determinants in the two viruses are identical at six of eight positions. Moreover, the source proteins are 69% homologous, so this cross-reaction is not a bolt out of the blue. It has been shown in numerous systems that memory T cells suppress responses by naive T cells (Jamieson and Ahmed, 1989) (one form of immunodomination), providing a general mechanistic explanation for the Pichinde-virus-induced alteration in the LCMV ID hierarchy.

More interesting are Welsh and Selin's examples of cross-reactivity between peptides from unrelated viruses (Urbani et al., 2005; Wedemeyer et al., 2001). The most thorough mechanistic exploration of the phenomenon comes from Kim et al. (2005), who showed that VV infection of C57BL/6 mice previously infected with LCMV resulted in the selective expansion of $CD8^+$ T cells specific for distinct LCMV determinants. Despite being inbred, individual mice demonstrated great variation in the specificities of memory LCMV $CD8^+$ T cells expanded by VV infection. Adoptive transfer of $CD8^+$ T cell populations from individual LCMV-primed mice into recipients then infected with VV provided an elegant demonstration that this was due to differences in the anti-LCMV repertoires of individual mice and not to stochastic factors associated with VV infection. A missing piece of this puzzle, however, is the identity of the cross-reactive antigens in VV that expand the corresponding LCMV populations. A candidate VV peptide proposed by Kim et al. (2005) to account for expansion of one of the LCMV populations was tested by Moutaftsi et al. (2006) and was not antigenic (the antigenicity observed by Kim et al. may be an artifact of using the VV peptide at high concentrations [5 μg/ml] for stimulation). Importantly, the antigenicity of synthetic viral peptides is not equivalent to the immunogenicity of the actual determinant in the context of a viral infection. Nonimmunogenic "mimotopes" have been described (Belz et al., 2001) that cross-react with true determinants in the same virus, even though the determinants are remarkably divergent in sequence (ISPLMVAYM versus SSYRRPVGI; a result that should give pause to those attempting to identify cross-reactions on the basis of sequence homology!). Ultimately, demonstrating true heterologous immunity will entail knocking out putative cross-reactive determinants in priming and challenge viruses and showing that this eliminates enhancement attributed to heterologous immunity.

Despite these caveats, Welsh and Selin's findings provide important insight into the phenomenon of heterologous immunity, particularly in demonstrating that cross-reactions are likely to vary on the basis of private specificities that arise via stochastic events in the generation of TCR repertoires in individuals (Welsh, 2006). With the additional genetic differences present in outbred human populations in MHC and non-MHC genes, heterologous immunity in human responses is predicted to vary considerably between individuals.

With the constant exposure of humans to viruses (typically a total of ~10 subclinical and clinical infections per year per individual), it would be expected that individuals would possess memory $CD8^+$ T cells that cross-recognize determinants from previously unencountered viruses, particularly viruses with large coding capacity. Yet Oseroff et al. (2005) failed to detect recognition of any of the >6000 VV-encoded peptides by PBLs from patients prior to immunization. Likewise, Sylwester et al. (2005) found that PMBC from six of ten patients seronegative for HCMV failed to recognize any of the

13,000 HCMV peptides tested. Of the other seronegative patients, two recognized peptides from a single gene product, and two patients recognized two or three gene products. Similarly, Hosken et al. (2006) found very limited recognition of HSV-2 peptides by two seronegative patients.

Thus, although the jury is still out, it appears that cross-recognition in humans may occur less frequently than predicted by mouse studies.

Specificity and Function

One of the most important, yet least studied aspects of the relationship between immunodominance and immunity is the influence of $CD8^+$ T cell specificity for individual viral determinants on their antiviral activity in vivo. This information is critical to designing defined determinant-based vaccines. Specificity could potentially influence $CD8^+$ T cell function by either quantitative or qualitative effects. Obviously, one important factor in the specificity-function relationship is the magnitude of responses to individual determinants: Up to a certain point, more responding $CD8^+$ T cells should exert more antiviral activity. At one extreme of the response spectrum are naturally processed and presented determinants that are unable to elicit detectable $CD8^+$ T cells because of deficiencies in the $CD8^+$ T cell repertoire, i.e., there are no suitable clones capable of responding (Yewdell and Bennink, 1999). No doubt, more subtle differences in precursor numbers to naturally processed determinants will lead to parallel differences in responding $CD8^+$ T cell numbers and antiviral activity. Although this has yet to be demonstrated experimentally, Choi et al. (2002) and La Gruta et al. (2006) established, respectively, a direct relationship between precursor frequency and immunodominance in minor histocompatibility antigen responses and secondary anti-IAV responses.

It is also predictable that determinant-specific differences in antigen presentation by infected target cells will result in differences in antiviral activity of the cognate $CD8^+$ T cell clones. For example, $CD8^+$ T cells specific for late viral antigens should, on average, exert less antiviral activity against infected cells than early-antigen-specific $CD8^+$ T cells, given a virus that produces infectious progeny within a few hours of turning on late viral genes (typically, viral structural proteins are encoded by late genes). Even among $CD8^+$ T cells specific for early antigens, $CD8^+$ T cells specific for determinants that are more abundant should, on average, recognize target cells earlier and exert more potent antiviral activity. These factors probably contribute to the determinant-dependent differences in antiviral activity of HIV-specific $CD8^+$ T cells (Ali et al., 2004; Yang et al., 2003). Herpesviruses provide an extreme example of kinetic differences in target-antigen expression. Latently infected cells that serve as a viral reservoir express only a few viral proteins compared to lytically infected cells (i.e., cells supporting a full-blown infectious cycle). Evidence suggests that $CD8^+$ T cells specific for latent antigens exert more effective anti-CMV activity (Bunde et al., 2005; Sacre et al., 2005).

Discrepancies in the determinant-generating abilities of afferent APCs in activating $CD8^+$ T cells versus efferent APCs in serving as $CD8^+$ T cell targets will also result in determinant-based differences in $CD8^+$ T cell effector function (Crowe et al., 2005; Crowe et al., 2006; Crowe et al., 2003). Such differences can arise from cross-priming by DCs versus direct presentation by target cells (Chen et al., 2004), or from differences in proteases in DCs versus target cells (Chen et al., 2001; Ito et al., 2006; York et al., 2006), particularly prior to target-cell exposure to IFN-γ, which induces many of the proteases constitutively expressed by DCs.

In principle, peptide specificity can also influence the *qualities* of responding $CD8^+$ T cells. This could result from the particular circumstances of the duration or anatomical location of antigen priming, or by the nature of costimulatory signals delivered by the afferent APC. Intrinsic features associated with the specific class I-peptide complexes may result in consistent signaling differences among heterogeneous $CD8^+$ T cell clones. This might particularly apply to determinants recognized by $CD8^+$ T cell populations with limited TCR heterogeneity, because TCR conservation should favor functional uniformity. Such less diverse $CD8^+$ T cell populations may typically recognize "plain vanilla" peptides (Davis, 2003), determinants whose residues available for TCR interaction lack prominent side chains (Lehner et al., 1995; Meijers et al., 2005; Moss et al., 1991; Stewart-Jones et al., 2003; Trautmann et al., 2005; Turner et al., 2005). Indeed, Kjer-Nielsen et al. (2003) reported that interaction of one such highly conserved TCR with its cognate EBV IDD resulted in conformational alterations transmitted to the TCR constant region that are potentially capable of modifying TCR-mediated signaling. Such determinant-specific alterations in signaling need not be evident from structural studies using isolated TCRs and class I-peptide complexes (Ely et al., 2006), however, particularly if multiple TCRs engaging cognate ligands interact with each other on the surface of a responding T cell. As an alternative approach, determinant-specific structural alterations in TCRs might be inferred from differences in downstream signaling events associated with activation of $CD8^+$ T cell populations specific for different determinants.

Presumably, such differences in signaling would enable determinant-specific differences in $CD8^+$ T cell function, a topic ripe for detailed investigation. $CD8^+$ T cells are known to be capable of independently modulating cytotoxicity and secretion of IFN-γ and TNF-α (Guidotti et al., 1996; Snyder-Cappione et al., 2006). Determinant-based differences in the abilities of $CD8^+$ T cells to modulate immunodomination, apparently independently of differential antigen presentation, have been reported in mouse responses to IAV and LCMV (Chen et al., 2000; Rodriguez et al., 2001) and may play a key role in establishing immunodominance hierarchies. Whitton, Slifka, and colleagues also demonstrated determinant-specific differences in the ratio of IFN-γ secretion to cytolytic activity in LCMV infections (Rodriguez et al., 2001). Extending these findings, Liu et al. (2004) reported that IDD-specific $CD8^+$ T cells secrete IFN-γ faster upon specific activation than $CD8^+$ T cells specific for subdominant determinants. This is a key finding, because it could well account for immunodomination by IDD-specific $CD8^+$ T cells (Rodriguez et al., 2002). It would be of great interest to broaden these findings to other virus systems in mice and to human responses. mRNA expression profiling of $CD8^+$

T cells sorted on the basis of determinant specificity (with MHC-tetramer technology) could provide a number of further leads into potential determinant-specific differences in antiviral $CD8^+$ T cell functions.

Understanding the relationship between $CD8^+$ T cell specificity and function is particularly important in HIV, where there is intriguing evidence that $CD8^+$ T cells of the "right" specificity can control HIV infection for decades in "elite suppressors," individuals able to maintain normal $CD4^+$ T cell numbers and low levels of virus in plasma without antiretroviral chemotherapy. A pioneering study showed that eleven of 13 such patients expressed HLA-B57, or ~10-fold higher than expected from its overall frequency in the relevant population (Migueles et al., 2000). This cannot be attributed to the inability of targeted determinants to vary because of structural constraints of the source proteins, given that escape mutants clearly are selected (Bailey et al., 2006). Still, it seems likely that the anti-HIV activity of HLA-B57 is based on the properties of its peptide-binding domain, because individuals with other HLA-B alleles with similar peptide specificity demonstrate resistance to HIV (though typically to a lesser extent than elite suppressors) (Frahm et al., 2005). Attaining a detailed understanding of this phenomenon should provide considerable insight into manipulating $CD8^+$ T cell responses to treat HIV infections and, more generally, illuminate that connection between $CD8^+$ T cell specificity and antiviral function

Future Prospects

The leap to mapping human $CD8^+$ T cell responses to large genome viruses represents an important milestone in understanding immunodominance in the real world. A bump, however, on the road to immunodominance enlightenment is the wholesale use of synthetic peptides as antigenic mimics without supporting evidence for the immunogenicity of the corresponding sequence in the context of translated viral proteins (see dirty little secret #1 in [Yewdell, 2005]). This problem becomes acute when high-affinity peptides are used at high concentrations, resulting in the generation of tens of thousands of complexes per APC, which is 10- to 10,000-fold greater than naturally generated complexes (Yewdell and Bennink, 1999). The use of overlapping 15 mer peptides to measure antigenicity may circumvent this problem (though inadvertently), because the actual antigenic peptides are generated only through the action of cell-surface, secreted, and fetal bovine serum (FBS) proteases in what is typically an inefficient process. On the other hand, the generation of such a heterogeneous mixture of peptides can only hamper reproducibility. Admittedly, demonstrating the immunogenicity of putative determinants by genetic approaches entails a considerable effort for what will typically result in confirmation and not discovery. Without this information, however, it is possible to be seriously misled by putative determinants that actually represent nonimmunogenic sequences that cross-react with $CD8^+$ T cells specific for other determinants in the same virus, or in a completely different antigen. Such cross-reactions could result in partial agonism or antagonism and might be largely based on private specificities and therefore result in the erroneous conclusion of individual-based differences in immunodominance hierarchies. Inasmuch as we will have to deal with a core group of medically important viruses for as long as *Homo sapiens* exists, surely it is worth the effort to define determinants from these viruses with a reasonable degree of rigor. Ultimately, it will require mass spectroscopy to identify the full repertoire—which likely includes peptides of atypical lengths, usual and unusual posttranslational modifications (e.g., peptide splicing), and unexpected translation products—of viral peptides presented to the immune system.

Indeed, at this point, there are no real remaining technical hurdles to generating torrents of precise data regarding the composition and complexity of human T cell responses to medically relevant viruses. In combination with continued advances in mouse immunodominance model systems and structural studies on the nature of TCR-class I interactions, these data should provide insight into a number of important issues including the following:

(1) The properties of viral proteins (size, stability, time of expression in the virus life cycle, intracellular localization, function) that influence their immunogenicity and effectiveness as $CD8^+$ T cell targets.
(2) The influence of the route of infection or immunization on immunodominance hierarchies.
(3) The contribution of past exposure to other viruses in generating primary $CD8^+$ T cell immunodominance hierarchies.
(4) The contributions of cross-recognition and allelism in non-MHC genes (including TAP and TCR genes) in generating immunodominance hierarchies.
(5) The relationship between peptide-MHC structure and the repertoire of responding $CD8^+$ T cell populations, and more specifically, whether plain vanilla and "chocolate chip cookie dough" peptides are consistently recognized in a fundamentally different manner.
(6) The relationship between $CD8^+$ T cell specificity and antiviral functionality, and particularly the contribution of MHC class I locus- and allele-related differences to the process.

Indeed, a thorough understanding of immunodominance is ultimately essential to understanding the evolution of the MHC-$CD8^+$ T cell immunosurveillance system. Since Zinkernagel and Doherty's discovery of MHC-restricted recognition of virus by T cells, it has been widely assumed that pathogen immunity is by far the most powerful selection factor in MHC evolution and is responsible for the remarkable polymorphism of MHC class I (and class II) genes. Recent findings, however, raise the possibility that tumor surveillance and mate selection exert powerful selection pressure on MHC class I genes, perhaps even surpassing selection exerted by pathogen immunity (Murgia et al., 2006; Pearse and Swift, 2006; Slev et al., 2006). The practical point is that functional compromises may have been reached in evolution to optimize MHC functions in multiple tasks. This cautions against seeking perfection in explaining every feature of immunodominance in $CD8^+$

T cell responses to viruses, whose quirks may be real faults that cannot be improved without a concomitant decrease in other tasks that MHC class I genes have tackled in evolution.

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