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IMMUNODOMINANCE IN MAJOR HISTOCOMPATIBILITY COMPLEX CLASS I–RESTRICTED T LYMPHOCYTE RESPONSES[1]

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ABSTRACT

Of the many thousands of peptides encoded by a complex foreign antigen that can potentially be presented to CD8+ T cells (T_{CD8+}), only a small fraction induce measurable responses in association with any given major histocompatibility complex class I allele. To design vaccines that elicit optimal T_{CD8+} responses, a thorough understanding of this phenomenon, known as immunodominance, is imperative. Here we review recent progress in unraveling the molecular and cellular basis for immunodominance. Of foremost importance is peptide binding to class I molecules; only ~1/200 of potential determinants bind at greater than the threshold affinity ($K_d > 500$ nM) associated with immunogenicity. Limitations in the T_{CD8+} repertoire render approximately half of these peptides nonimmunogenic, and inefficient antigen processing further thins the ranks by approximately four fifths. As a result, only ~1/2000 of the peptides in a foreign antigen expressed by an appropriate antigen presenting cell achieve immunodominant status with a given class I allele. A roughly equal fraction of peptides have subdominant status, i.e. they induce weak-to-nondetectable primary T_{CD8+} responses in the context of their natural antigen. Subdominant determinants may be expressed at or above levels of immunodominant determinants, at least on antigen presenting cells in vitro. The immunogenicity of subdominant determinants is often limited by immunodomination: suppression mediated by T_{CD8+} specific for immunodominant

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determinants. Immunodomination is a central feature of T_{CD8+} responses, as it even occurs among clones responding to the same immunodominant determinant. Little is known about how immunodominant and subdominant determinants are distinguished by the T_{CD8+} repertoire, or how (and why) immunodomination occurs, but new tools are available to address these questions.

INTRODUCTION

Discovery of Major Histocompatibility Complex Class I–Restricted Immunodominance

In 1974, Zinkernagel & Doherty clearly established the biological importance of major histocompatibility complex class I molecules (hereafter referred to as class I molecules) by showing that cytotoxic T lymphocytes [now recognized as CD8+ T cells (T_{CD8+})] induced by viral infection recognize cells in a virus-specific, class I–restricted manner (1). Not long after, it was found that T_{CD8+} responses to even complex viruses expressing >100 gene products were often dominated by T_{CD8+} restricted to only one of the class I allomorphs expressed by the mice (allomorph refers to any of the alleles of the two to three class Ia genes expressed by a species) (2). When it became possible to examine responses to individual viral gene products with individual class I allomorphs, it was shown in mice (3) and humans (4) that few (if any) of the 10 influenza virus gene products were recognized in association with T_{CD8+} restricted by any given allomorph. Following the discovery that class I molecules bound only a small fragment of viral gene products (5), later appreciated to generally consist of 8–10 residues (6), it was shown that T_{CD8+} specific for individual gene products in association with a given allomorph often focused on a single peptide (6a).

These findings echoed prior reports that T_{CD4+} responses to proteins frequently focused on one or a few peptides, termed immunodominant determinants (7). Other peptides, subdominant determinants, could elicit T_{CD4+} that recognize antigen presenting cells (APCs) exposed to either peptides or intact proteins, but they were only weakly immunogenic in the context of the intact protein. A third group of peptides, cryptic determinants, were immunogenic and antigenic only as synthetic peptides or larger proteolytic fragments.

These terms apply equally to T_{CD8+} responses. Caution is in order, however. First, immunologists differ in their definitions of these terms and care must be taken to divine the usage in any given publication. Second, and crucially, these terms are defined strictly on a functional basis, and the classification of any given determinant depends entirely on the experimental conditions used to elicit T cells and gauge their numbers or activity.

Although immunodominance plays a role in T_{CD8+} responses to tumor and histocompatibility antigens, the determinants recognized by these responses

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are already severely limited by self-tolerance. Therefore, we focus on T_{CD8+} responses to utterly foreign antigens, in most cases viruses, because only a few industrious souls have ventured to the far more antigenically complex bacteria and parasites that, when present intracellularly, often induce T_{CD8+} responses. As most of the recent advances in understanding immunodominance have been in the area of antigen presentation, the bulk of the review deals with these findings: It is important to emphasize right from the beginning, however, that T_{CD8+} repertoire and regulation play large, if not as well explored, roles in immunodominance.

Antigen Processing and Presentation in a Nutshell

For a peptide to be immunogenic, it must do the following:

1. Be generated by "afferent" APCs from its precursor polypeptide and delivered to peptide-receptive class I molecules. (Afferent APCs trigger quiescent T_{CD8+} activation and proliferation. Under many circumstances, this task is accomplished by bone marrow–derived cells dedicated to the task, i.e. dendritic cells and macrophage/monocytes, referred collectively to as "professional" APCs).

2. Bind with sufficient affinity to class I molecules to produce enough cell-surface peptide–class I complexes to activate naïve T_{CD8+}.

3. Produce a complex with class I molecules on afferent APCs that is capable of triggering the activation and proliferation of a T_{CD8+} with a complementary T cell receptor (TCR).

The interplay of these interdependent factors determines the strength of the immune response to a peptide; deficiencies in one area can be offset by gains in the other. This latter point is critical because it precludes identifying a single factor that is "responsible" for immunodominance. Moreover, for the same peptide to be biologically relevant, similar criteria must be met in the recognition of "efferent" APCs by activated T_{CD8+}. (Efferent APCs trigger T_{CD8+} effector functions and are the raison d'être of the T_{CD8+}). Before discussing the relative contributions of these factors to immunodominance, it is necessary to recapitulate current understanding of antigen processing and presentation (to present the maximal amount of material in the space allotted, we refer to only those original research papers that directly impact immunodominance; for ancillary information we refer readers to recent reviews).

NATURE OF CLASS I–ASSOCIATED PEPTIDE LIGANDS The nature of class I–associated peptide ligands has been reviewed by several authors (8–10). The heart of major histocompatibility complex restriction is the interaction of the TCR with the peptide binding region of class I molecules. The free energy of

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the interaction derives from contacts between the TCR with the α-helices of class I molecules and those residues in the bound peptide oriented away from the class I molecule. Peptide binding to class I molecules is principally due to two types of interactions. First, the amino and carboxy peptide termini interact with groove residues highly conserved between different class I allomorphs. Extrusion of the peptide past these residues interferes with this interaction, which accounts for the observations that 90% or more of peptides recovered from class I molecules are between 8 and 11 residues in length, and that synthetic peptides of this length nearly always bind to class I molecules with the highest affinity and are optimally antigenic. For some allomorphs, upward of 70% of the peptide ligands are of uniform length (most often nine residues), and for the other allomorphs, $\sim$80% of the peptides can be accounted for by including an additional residue (e.g. both 9mers and 10mers). Second, the antigen binding groove has two (or less commonly three) pockets that display a marked preference for one to five (most often one or two) of the 20 possible amino side chains. One of these pockets always accommodates the COOH terminus of peptide; the residues accommodated by the other(s) varies, depending on the allomorph, but are nearly always the second, third, or fifth residue from the amino terminus. The residues that comprise the pockets are highly variable between allomorphs. This, with a less important but still significant contribution from other variable residues in the binding pocket, results in each allomorph binding a unique set of peptides. A given peptide may bind to more than one allomorph; the odds of this happening are proportional to the degree of similarity between the binding grooves of the allomorphs.

The influence of the pockets in peptide binding has an extremely important practical application: It enables the reasonably accurate prediction of peptides that may bind to a given class I allomorph based on the presence of the appropriate dominant anchor residues. As the number of known ligands for class I molecules grows, the more subtle effects of nonanchor residues on binding, and the cooperative (and noncooperative) effects of peptide residues on each other, can be computed by increasingly accurate algorithms that predict binding affinities. As described below, this has led to the ability to rapidly and reasonably inexpensively identify determinants present in proteins known to be recognized by T_{CD8+}.

GENERATION OF CLASS I–ASSOCIATED PEPTIDE LIGANDS As above, the generation of class I–associated peptide ligands has been reviewed by several authors (11–14). Most antigenic peptides presented by nonprofessional APCs (the exclusion of professional APCs is explained below) are derived from a cytosolic pool of proteins biosynthesized by the cells (endogenous antigens). The mechanism of targeting proteins to the cytosolic proteases that initiate

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the production of antigenic peptides is largely undefined. It is generally the case that increasing protein degradation enhances antigenic peptide production, but most antigenic peptides originate from gene products that exhibit very low rates of degradation. To what extent peptides are derived from native proteins versus defective forms that never achieve a native state remains in question.

The major cytosolic protease responsible for the production of antigenic peptides is the proteasome, but other cytosolic proteases probably contribute to antigen processing. Cytosolic peptides are delivered to the endoplasmic reticulum (ER) by the transporter associated with antigen processing (TAP). To be efficiently transported, peptides must be between 8 and 16 residues long and have the proper COOH-terminal residue. Mouse TAP prefers a hydrophobic residue, whereas human TAP prefers a hydrophobic or positively charged residue. These preferences match those exhibited by mouse and human class I molecules for COOH-terminal residues, which suggests that class I–binding peptides can either be produced in final form in the cytosol or possess amino terminal extensions of up to eight residues. In the latter case, trimming of NH_2-terminal extensions would be needed. It has been demonstrated that TAP-deficient cells trim NH_2-terminal residues from ER-targeted peptides, but whether TAP-transported peptides are similarly trimmed remains to be established.

Peptides can associate with class I molecules in the ER in at least two distinct ways. First, peptides can bind to class I molecules associated with TAP. Class I molecules are recruited to TAP by binding to tapasin, a molecular chaperone apparently devoted to class I biosynthesis. The simple idea is that binding of class I molecules to TAP enhances the effective concentration of the peptide, thereby favoring loading. There may be additional complications, as tapasin has been reported to bind TAP-transported peptides (15). Second, as demonstrated by the ability of TAP-deficient cells to present peptides targeted to the ER by signal sequences, peptides can bind to class I molecules that are not bound to TAP. This latter process may also involve tapasin, which binds class I molecules prior to their association with TAP. Tapasin is not, however, required for class I assembly because tapasin-deficient cells demonstrate only a variable degree of impaired assembly of class I molecules, ranging from $\sim$20% to undetectable depending on the allomorph.

One or both of these peptide-loading pathways may involve the participation of general-purpose ER chaperones because TAP-transported peptides can be recovered from numerous ER chaperones. Cytosolic chaperones also bind antigenic peptides. Although there is no experimental evidence, molecular chaperones could potentially play a role in immunodominance, either by virtue of their specificity for peptides or by their ability to properly transfer the peptide

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to a relevant acceptor (16). A negative role can also be envisaged, if peptides are trapped in nonproductive association with chaperones.

Class I molecules with bound peptides are stable on the cell surface for many hours. The loss of peptide destabilizes the molecule, which denatures with a half time of ~15 min at 37°C. During this period, class I molecules can bind exogenous peptides. Such peptide-receptive molecules are stable for prolonged periods at 27C° or below. The existence of cell-surface peptide–receptive class I molecules has important practical applications. First, it enables sensitization of target cells by synthetic peptides (and probably accounts for the immunogenicity of synthetic peptides as well). Second, 37°C-induced dissociation of surface class I molecules (detected cytofluorographically by the loss of class I–specific mAb binding) accumulated by incubating cells at 27°C serves as the basis for the melting assay, a simple, highly informative method of determining peptide affinity for class I molecules.

T_{CD8+} ACTIVATION The immunogenicity of a peptide-class I complex depends on the presence of responsive T cells with a complementary TCR. For this to occur, the TCR repertoire must be capable of generating an appropriate receptor from the pool of variable Vα and Vβ genes with their corresponding D and J genes and the amino acids that can be added at the V-D-J junctions. T cells bearing a complementary receptor must then pass the thymic Goldilocks test, binding self class I molecule-peptide complexes with just the right affinity to enable positive selection and disable negative selection. The TCR must also avoid binding to complexes with self peptides in the periphery that result in deletion or anergy (although the latter could possibly be overcome during the course of an immune response and its attendant inflammation).

The triggering of T_{CD8+} activation by binding to peptide-class I complexes on the surface of an appropriate APC depends on the affinity of the TCR for the complex and the abundance of the peptide-class I complex, in principle according to the law of mass action (17, 18). T_{CD8+} activation is also greatly influenced by the interactions of accessory molecules (some expressed predominantly by professional APCs) that increase the avidity of cell-cell interaction and contribute in complex ways to signaling events in both cells. It is uncertain whether naïve T_{CD8+} clones (or members of a single clone, for that matter) behave uniformly regarding the amount of TCR ligation required for activation. If there is clonal variation (almost to be expected), then two clones expressing TCRs with identical affinities for their respective peptide-class I complexes will require different numbers of peptide-class I complexes for activation. Thus, lacking precise immunochemical information regarding the affinity of isolated TCRs for peptide-class I complexes (even this is subject to vagaries regarding the affinity of soluble, isolated TCR versus TCR in its natural state in a

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membrane teeming with other proteins, lipids, and saccharides), it is risky to rank relative affinities of the TCR–class I interactions based solely on the number of complexes required for stimulation.

Intimately related to the sensitivity of T cell clones is the phenomenon of peptide antagonism, in which peptide–class I complexes of one type block the agonistic effects of the nominal antigen (19, 20). To date, antagonism has been observed using nonself peptides, but given the existence of positive selection, it is certainly possible that for some TCRs, self peptides provide antagonistic signals (indeed, this may play a physiological role in maintaining tolerance to peripheral antigens). Of particular relevance to the preceding paragraph, the presence of antagonistic self peptides can increase the number of agonist complexes needed for stimulation, leading to a false impression of low TCR affinity.

Activation of naïve T_{CD8+} results in the generation of primary armed effector cells and memory cells. As described below, there is often not a simple, direct relationship in the primary and secondary T_{CD8+} responses to different determinants in complex antigen. The extent to which this reflects the independent generation of secondary and primary T_{CD8+} as opposed to alterations in activity during the differentiation of primary effector cells into memory cells is just now being sorted out.

NATURE OF THE APC A crucial question for immunodominance is the nature of the afferent APC after infection with different agents. Current dogma dictates that naïve T_{CD8+} require multiple signaling events for activation: one transmitted through the TCR-CD3-CD8 complex as a result of binding to peptide-class I complexes, the other(s) transmitted by costimulatory T_{CD8+} cell-surface proteins, most often CD28. As the activating ligand for CD28, B7-1 (CD82) is expressed predominantly by professional APCs, it is thought that these cells (particularly dendritic cells) do most of the heavy lifting in stimulating naïve T_{CD8+}. Expression of costimulatory molecules can be induced in numerous cell types by the types of cytokines secreted early in the inflammatory process, however, raising the possibility of nonprofessional APC-induced T_{CD8+} activation in some immune responses. The "quality" of the APC involved in T_{CD8+} activation may be related to the variation in the requirement for T_{CD4+} in generating T_{CD8+} to different viruses. Possibly, viruses that utilize nonoptimal APCs (due to the effects of the virus on APC function or inability to infect professional APCs) are those that require more T_{CD4+}-mediated help.

A related, equally important question is the nature of antigens presented to naïve T cells. In most situations in which T_{CD8+} are stimulated by virus-infected cells, the antigen is presumably endogenously synthesized by the APC. There must be instances, however, in which viruses of limited host cell range

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are incapable of infecting effective APCs. In these circumstances, professional APCs must present exogenous antigens. This was initially discovered as the cross-priming phenomenon, viz mice mounted self class I–restricted T_{CD8+} responses to minor histocompatibility antigens presented by cells lacking the appropriate self-class I molecules (21). It has been repeatedly demonstrated that such cross-priming is dependent on histocompatible bone marrow–derived cells, presumably the professional APCs.

Precisely what form of exogenous antigen is processed by these professional APCs is uncertain. The major issue is whether the APCs acquire antigen in a form requiring major proteolysis (e.g. a full-length protein) or whether a preprocessed (or nearly so) peptide is provided. The latter is suggested by findings that naïve T_{CD8+} can be activated by immunization of mice with molecular chaperones containing viral or tumor peptides (22). In situations in which proteolysis is required, it is uncertain whether proteins are delivered to the cytosol or whether the peptides are generated by endosomal proteases. Although there is evidence that supports the loading of class I molecules from endosomally generated peptides (23), the biological relevance of these findings awaits confirmation.

The importance to immunodominance of understanding how peptides are generated from exogenous antigens stems from the requirement that under most circumstances, efferent APCs present peptides derived from endogenous antigens. If endosome-generated peptides provide a significant source of ligands for stimulating naïve T_{CD8+}, then a safe prediction is that T_{CD8+} specific for a subset of these peptides will be of little use in the immune response because the rules for generating peptides in the cytosol and endosome cannot be identical. Conversely, the absence of such T_{CD8+} would suggest that the endosome is not a physiological source of class I ligands.

ANTIGENIC SUBTERFUGE In response to ability of T_{CD8+} to interfere with their propagation, replicating antigens such as tumors or viruses have the potential to evolve mechanisms to interfere with T_{CD8+} activation or effector functions. To cite some known examples, tumor cells may secrete cytokines that interfere with T_{CD8+} activation or function, and viruses can produce proteins that interfere with peptide generation or class I biosynthesis. If the interference is selective for a subset of T_{CD8+} clones, antigenic peptides, or class I molecules, it can contribute to immunodominance in a given system.

GETTING TECHNICAL Understanding natural phenomena depends entirely on the means used to observe and measure the phenomena. Until relatively recently, T_{CD8+} responses were assessed almost exclusively by their capacity to lyse target cells (as measured by ^{51}Cr release), using T_{CD8+} populations ex vivo

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(i.e. assessing lytic activity of cells without in vitro culturing) or following short-term stimulation in bulk or, more quantitatively, under limiting dilution conditions. In the past few years, three novel methods of measuring T_{CD8+} responses were introduced that will greatly expand understanding of T_{CD8+} responses: ELISPOT, which quantitates individual armed effector T_{CD8+} based on cytokine release; intracellular cytokine staining, which cytofluorographically identifies and quantitates armed effector T_{CD8}; and tetrameric peptide–class I–avidin/streptavidin complexes, which cytofluorographically identify and quantitate resting or active T_{CD8+} bearing TCRs specific for a given peptide–class I complex.

A number of recent studies utilizing these methods have revealed that the ^{51}Cr release assay grossly underestimates the numbers of T_{CD8+} that respond to viral or bacterial antigens (24). These new data will not, however, negate immunodominance-related findings made using the ^{51}Cr release assay, which—limited as they may be to a subset of responding T_{CD8+}—are probably reasonably representative of the entire response.

Of greater concern to interpreting immunodominance-related findings are methodological differences in stimulating T_{CD8+} whose activation is assessed by ^{51}Cr release assay. There is a particularly wide gulf in the methods used for studying mouse and human T_{CD8+}. Because of ethical/medical constraints and the 1000-fold difference in body mass, human T_{CD8+} are almost always derived from peripheral blood lymphocytes (PBLs), whereas mouse T_{CD8+} are derived from lymphatic organs (routinely spleen, occasionally lymph nodes). Additionally, exposure of mice to antigens can be rigorously controlled whereas the antigenic history of humans is always subject to some uncertainty.

It is important to recognize that even within a single system, seemingly minor variations in methods used to induce T_{CD8+} can produce major variations in apparent immunodominance. This is particularly true in the numerous studies in which memory T_{CD8+} (both mouse and human) are expanded in vitro prior to assay. If the conditions are suboptimal (which to some extent they will always be), there is a good chance that T_{CD8+} specific for "weaker" determinants will not be activated sufficiently to drive cell division to the point of distinguishing lytic activity from background values. Although it remains important to determine how the T_{CD8+} against the weaker determinants differ from T_{CD8+} specific for the immunodominant determinant (IDD) (whether simply in quantity or quality), it is never safe to conclude that the failure to detect a response against a given determinant means that such a response is completely absent.

Extreme caution must be exercised in the assignation of cryptic to a determinant, as this is strictly dependent on the assay conditions utilized. For example, even an IDD may appear to be cryptic if virus-infected APCs fail to express sufficient levels of peptide–class I complexes because of low levels of viral

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protein synthesis, or if the T_{CD8+} used for detection require an excessive amount of peptide–class I complexes. The solution to this problem is obvious but not simple, as it entails quantitation of peptide–class I complexes expressed on the cell surface. This is an arduous biochemical task, but the development of mAbs specific for peptide–class I complexes offers some hope for the future (25, 26).

Finally, a cautionary note regarding the use of synthetic peptides. Only in few instances have the structures of naturally processed determinants been definitively established by structural methods, i.e. mass spectroscopy. More frequently, although still relatively uncommonly, the naturally processed peptide is shown to co-elute with a synthetic peptide in high-pressure liquid chromatography (HPLC). Most commonly, the identity of the natural peptide is inferred by identifying a synthetic peptide that activates T_{CD8+} optimally in vitro. In the latter two cases, it must always be considered that the natural peptide is not identical to the "optimal" synthetic peptide. The natural peptide may possess an extension or may be posttranslationally modified. Cys-containing peptides may cause considerable difficulties because Cys can dimerize the peptide or react with either sulfhydryl groups in serum or cellular proteins or with heavy metals (27). The bottom line is that one may be led astray by qualitative differences between optimal peptides and the genuine article that result in very large errors in quantitation of peptide–class I complexes expressed on APCs.

IMMUNODOMINANCE: CONTRIBUTION OF ANTIGEN PRESENTATION

Affinity for Class I Molecules: The Highest Hurdle

The discovery that cellular peptides recovered from a given class I allomorph exhibit highly conserved residues in two or three positions was a major advance in the study of immunodominance because it enabled the identification of upward of ~80% of potentially antigenic peptides in a given antigen (9). Importantly, antigenic peptides identified independently of such motifs exhibit the same bias as pooled cellular peptides bound to the same allomorph, affirming the validity of using the motifs for prediction of antigenic determinants.

The predictive value of peptide motifs and the role of peptide affinity for class I molecules in immunodominance have been most thoroughly examined by Sette and colleagues (28, 29). Initially, they synthesized a series of viral peptides conforming to the HLA-A*0201 motif and correlated the affinity of the peptides for A*0201 with their immunogenicity in mice expressing a chimeric K^b transgene in which the $\alpha 1\alpha 2$ domains are replaced by those of HLA-A*0201 [enabling partial (30, 31) CD8 interaction with A*0201 via the $\alpha 3$ domain].

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Affinity was determined by the ability of the peptide to compete with the binding of a radiolabeled standard peptide to purified A*0201 molecules in solution. Immunogenicity was assessed by the ability of the peptide to stimulate in vitro splenocytes derived from mice immunized with the same peptide (in adjuvant with a peptide that induces a T_{CD4+} response). Peptide-specific T_{CD8+} were induced by five out of five of the highest-affinity peptides (K_d < 50 nM), three out of five of the intermediate affinity peptides (K_d 50–500 nM), and none out of 13 of the lowest-affinity peptides (K_d > 500 nM). Measuring the affinities of 11 defined A*0201-restricted viral IDDs and 30 sequenced cellular peptides recovered from HLA-A2 indicated that 90% were high affinity, 7% intermediate affinity, and 4% low affinity, affirming similar findings by Parker and colleagues (32).

Moving to T_{CD8+} responses in human PBLs, Team Sette examined the ability of 91 hepatitis B virus (HBV)-derived, A*0201 conforming nonamers (affinity breakdown: 22 high, 21 intermediate, 48 low) to restimulate PBLs derived from A2-positive individuals acutely infected with HBV (29). Responses were induced by 45% of the high-affinity peptides, 14% of the intermediate-affinity peptides and 6% of the low-affinity peptides. A similar analysis was performed using synthetic peptides (from mostly viral sources) conforming to the HLA-A11 binding motif (33). Of the 45 motif-containing peptides synthesized from viral and cellular peptides, 41 bound with intermediate or high affinity. This is a much higher percentage than was observed with A2 motif peptides, making the point that the predictive values of available peptide motifs can vary considerably between allomorphs. All the known viral IDDs bound to A11 with high affinity. When the immunogenicity of motif-containing peptides was examined in primary human PBL cultures (i.e. from virus seronegative donors), responses were elicited by 21 of 28 peptides with high affinity, 7 of 13 with intermediate affinity, and 1 of 4 with low affinity.

In collaboration with the Ahmed laboratory, Sette and coworkers applied this approach to mouse T_{CD8+} responses to lymphocytic choriomeningitis virus (LCMV) (K^d-, D^d-, K^b-, and D^b-restricted) (34–36), and influenza virus (IV) (K^b-and D^b-restricted) (37). Of four previously defined LCMV IDDs, two bound to restricting molecules with high affinity and two with intermediate affinity. Searching for new determinants in two LCMV proteins using defined motifs, 2.2% of the potential number of peptides conformed to the peptide binding motif for any given allomorph. Approximately one quarter of these peptides bound with high or intermediate affinity to their respective allomorph (0.5% of all possible peptides in the two proteins). In contrast to the defined IDDs, none of the 21 intermediate or high-affinity binding peptides consistently sensitized target cells for ex vivo lysis by T_{CD8+} derived from LCMV-infected mice. Six peptides (all of intermediate affinity) were subdominant determinants (SDDs),

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however, as they induced in vitro secondary responses in splenocytes from infected animals. SDD-specific T_{CD8+} were detected by limiting dilution assay (LDA) at ~2%–5% the frequency of IDD-specific T_{CD8+}.

The even more extensive analysis with IV studied 47 D^b and 151 K^b motif-bearing peptides (1% and 3% of the potential number of IV-encoded peptides), of which 7 and 16 bound with high or intermediate affinities (0.15% and 0.35% of IV-encoded peptides, respectively). Following peptide immunization and restimulation, 12 of 14 high-affinity peptides and 4 of 9 intermediate-affinity peptides induced peptide-specific responses. Of the 16 peptide-specific T_{CD8+} populations generated, only two were capable of lysing IV-infected cells. Taking a crucial experimental step forward, T_{CD8+} specific for 13 of the peptides were tested for their ability to lyse cells exposed to decreasing amounts of peptide. Among the 10 high-affinity peptides tested, there was a ~10,000-fold difference in the amount of peptide required to achieve an arbitrary level of lysis. T_{CD8+} raised to the previously defined IDD required the least amount of peptide, but T_{CD8+} specific for two other high-affinity determinants demonstrated a similar sensitivity (one of these was able to lyse IV-infected cells). Factoring in T_{CD8+} sensitivity, the two newly defined SDDs appeared to be expressed at levels at or above the IDD on IV-infected cells. Only one of these peptides could induce secondary in vitro responses in splenocytes from IV-primed animals, pointing to a possible difference between in vitro and in vivo presentation of the determinant.

We have made similar findings regarding the K^d-restricted response to IV (38). Of the 27 nonameric peptides that conformed to the K^d binding motif, 10 (including the two known IDD) bound to K^d, as detected by the melting assay. The IDDs were not the most avid binders, ranking second and even fifth for the most dominant determinant [but both are of high affinity according to the classification of Sette et al (29)]. Of the eight novel peptides with low to high affinity, only the three high-affinity peptides stimulated T_{CD8+} from IV-primed mice in vitro. Genes encoding each of the 10 peptides were inserted into vaccinia virus (VV) and expressed as ER-targeted peptides. When TAP-deficient cells were infected with the rVVs, only rVVs encoding high- or intermediate-affinity peptides enhanced K^d cell-surface expression. This provides a direct correlation between endogenous and exogenous peptide binding to class I molecules and offers an explanation for the sharp cutoff between immunogenicity of intermediate- and low-affinity peptides. Immunization with the rVVs followed by restimulation with homologous peptide in vitro revealed that only the six most avid binders (including the two IDDs) were able to prime for peptide-specific T_{CD8+} responses. T_{CD8+} raised against the four novel determinants were able to lyse IV-infected cells, but at lower levels than IDD-specific T_{CD8+}, demonstrating that antigen processing from viral gene products is more

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limiting for the SDDs. Similarly, when IV-infected cells were used to stimulate splenocytes from IV- or rVV-infected mice, responses to the SDDs were at low levels (rVV-primed mice) or undetected (IV-primed mice).

Altogether, these findings provide crucial insight into the relative contributions of the factors that contribute to immunodominance. First and foremost is the role of peptide binding to class I molecules. Based on the body of work by Sette and colleagues, it appears that 90+% of peptides recognized by T_{CD8+} bind to their respective class I molecules with an affinity constant of 500 nM or better. What are the odds of a given 8- to 11-residue stretch of a protein binding to any given allomorph with this affinity? Using the published peptide binding motifs for 17 human and 6 mouse class I allomorphs, the odds of a peptide of a given length randomly possessing anchor residues for a given allomorph can be calculated to be $\sim$1/132 on average for the different allomorphs. This calculation is based on the overall frequency of individual residues in proteins and assumes a random distribution of amino acids in anchor positions. The simple motifs are, of course, imperfect predictors of peptide binding. Accounting for the flexibility that class I molecules demonstrate in accommodating extended peptides would increase the odds to $\sim$1/100 and, accounting for those peptides that do not possess the canonical dominant anchor motif, to $\sim$1/70 (since approximately one third of defined IDDs do not fit their respective motif). The results of Sette et al suggest that approximately one third of motif-conforming peptides bind to class I molecules with a K_d of 500 nM or better, making the odds $\sim$1/200 for the binding of random peptide binding to a given class I allomorph with an immunologically significant affinity. This estimate is supported by studies that have examined the binding of randomly generated peptides to class I molecules (39–41).

Thus, the possession of the proper sequence accounts (literally) for 99.5% for the immunodominance phenomenon. Of the 0.5% of peptides that bind to class I molecules with biologically significant affinity, evidence suggests that approximately half or more of these can induce T_{CD8+} responses as synthetic peptides (or virus-encoded minigenes), and that of these, approximately four fifths are expressed in quantities that relegate them to subdominant or cryptic status, because of low sensitivity of the T_{CD8+}, poor antigen processing, or both. Multiplying these odds ($1/200 \times 1/2 \times 1/5$) results in the estimate that $\sim$1/2000 peptides in foreign antigens achieve IDD status in association with a given class I allomorph, with perhaps twice as many SDDs—at least in mice, where much of the evidence has been accumulated [or more accurately, in two inbred mouse strains maintained under germ-free (more or less) conditions].

The evidence for the degree of immunodominance in human anti-viral responses varies among viruses. The number of A*0201-restricted determinants defined in responses to HBV, human immunodeficiency virus (HIV), and

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hepatitis C virus is clearly greater than 1/2000. This discrepancy may be related to the chronic nature of these infections, or to the outbred nature of the population, because response to determinants can vary greatly among individuals (see below). Moreover, for many of these determinants, their IDD versus SDD status has not been established. In the case of HIV, where a very large number of determinants have been identified, evidence that T_{CD8+} exert strong selective pressure for determinant loss variants argues strongly for immunodominance, at least in some individuals (42), as does the oligoclonal expansion of T_{CD8+} (43). In the other relatively well-characterized human T_{CD8+} anti-viral responses [Epstein-Barr virus (EBV) (44) and cytomegalovirus (CMV) (45)], the frequency of IDDs seems similar to that observed in mice.

There are a number of other important points to made from these findings:

1. T_{CD8+} responses to viruses encompass more SDDs than has generally been appreciated. This is potentially of great practical importance because T_{CD8+} specific for viral SDDs can afford protection to subsequent infection (36, 46–49) and enhance protection afforded by IDD-specific T_{CD8+} (48). T_{CD8+} specific for tumor SDDs can prevent tumor growth when induced by tumor cell (50, 51) or synthetic peptide (51) immunization.

2. The striking correlation between peptide affinity (measured by the binding of optimally sized synthetic peptides to either purified soluble class I molecules or class I molecules on the surface of TAP-deficient cells) and the immunogenicity of the peptide in the context of its natural antigen demonstrates that the association of TAP-transported peptides with class I molecules in the chaperone-rich ER must largely recapitulate the hierarchy in binding as measured in the absence of any facilitating factors. "Largely" is used advisedly, as the immunogenic peptides that score low in affinity measurements may actually bind with higher affinity in the ER. Two lines of evidence support this conclusion. First, it has been reported that immunogenic peptides of low or intermediate measured affinity are more likely to exhibit K_{off} values characteristic of high-affinity peptides than are nonimmunogenic peptides of similar affinity (52). This implies that the decreased affinity of the peptides measured reflects a diminished K_{on} value. It is not difficult to imagine mechanisms operative in the ER that could enhance the K_{on} values of a subset of peptides. Second, and more directly, there is at least one example in the literature of a peptide that is more antigenic as a biosynthesized minigene product than as a synthetic peptide (53).

3. The generation of natural anti-viral T_{CD8+} responses to determinants that score as cryptic on virus-infected APCs in vitro appears to be an infrequent event (see 54, 55 for a possible exception). This strongly implies that afferent

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APCs in vivo present viral determinants in a manner quantitatively similar to virus-infected APCs used in vitro. As mentioned above, this argues against the endosome as a significant source of immunogenic peptides in vivo. The extent to which this applies to tumor or minor histocompatibility antigens (or infections with other viruses) remains to be established.

4. Although peptide binding to class I molecules is the major factor in immunodominance, IDDs are frequently not simply the most avid binding peptides encoded by the virus. In some cases, IDD-specific T_{CD8+} are clearly very sensitive, requiring low levels of peptide–class I complexes for target cell lysis. In other cases, however, IDD-specific T_{CD8+} may require more complexes than SDD-specific T_{CD8}. Only in a fraction of the latter cases is the determinant clearly present in sub-limiting amounts.

In the subsequent sections, we discuss the three factors that combine to cause the poor immunogenicity of non-IDD class I–binding determinants: production of insufficient amounts of peptide–class I complexes, low numbers or sensitivity of T_{CD8+}, and interference by IDD-specific T_{CD8+}. It is important to recognize that these first two factors can be considered only in combination. Thus, for a peptide–class I complex expressed at a given level by APCs, this level may or may not be limiting, depending on the number of complexes required by T_{CD8+} that recognize the complex. Given the ability of T cells to recognize vanishingly small numbers of peptide–class I complexes [there is even a description of a T cell that recognizes cells calculated to express a single complex (56)], antigen processing can only safely be said to be absolutely limiting in cases in which APCs cannot produce a single determinant (also the definition of true crypticity).

Generation of Peptide–Class I Complexes

QUANTITY OR QUALITY? Assessing the contribution of antigen processing to immunodominance requires quantitation of the levels of peptide–class I complexes expressed on the surface of APCs. Ideally, the APC would be the cell that actually presents the antigen to primary T_{CD8+} in vivo. Even if the identity of this cell were established (it is not), obtaining sufficient quantities of representative cells for analysis would be a considerable technical achievement. In practice, studies have been limited to tissue culture cells infected with viruses or bacteria.

Quantitation of peptide–class I complexes can be performed in three ways. The first two methods described depend heavily on the assumption that the naturally processed peptide is identical to the synthetic peptide thought to represent the determinant.

The simplest method is to determine the amount of synthetic peptides required to obtain a similar degree of lysis obtained with infected cells expressing levels

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of complexes that do not saturate the T_{CD8+} used. If different peptides are restricted by the same class I molecule and bind with a similar affinity, the relative ratios of complexes can be estimated from peptide titration curves. Better, but more difficult, real numbers of complexes can be estimated by quantitating peptide binding; this also enables comparisons between peptides of different affinities or peptides that bind different allomorphs.

More rigorously, peptides are HPLC purified from acid extracts of cells or isolated class I molecules. The amount of acid-soluble peptide present in HPLC fractions is determined by target cell sensitization using a synthetic peptide standard curve. This method is both arduous and expensive when dealing with peptides from infectious organisms, and it also suffers from uncertainties regarding the efficiency of peptide recovery and the presence of co-eluting peptides that compete for binding to class I molecules. It also cannot distinguish whether peptides were derived from intracellular or cell-surface class I molecules.

The most elegant method for quantitation is the use of T-AGs, mAbs specific for individual peptide–class I complexes (25, 26). This method is both simple and precise, but it suffers from relatively low sensitivity (at least several hundred complexes are needed for detection) and requires the production of the T-AG, which to date has been a hit or (mostly) miss proposition. One possible solution to the difficulty in producing T-AGs is the use of soluble TCRs. Although the affinities of monovalent TCRs are usually too low for use in standard sandwich assays, the avidities of TCRs can be increased to useful levels by chemical or genetic cross-linking (57). Given the availability of a T_{CD8+} clone for a given determinant, this strategy offers a good chance of obtaining a reagent suitable for quantitating the complex on the APC surface.

Only a limited number of studies have examined the number of foreign peptide–class I complexes generated by APCs. Rammensee and colleagues first showed that IV K^d- and D^b-restricted nucleoprotein (NP) IDDs $NP_{147–155}$ and $NP_{366–374}$ were present in HPLC fractions of acid extracts at ~300 copies per IV-infected cell (6). Using this method, we found that only ~30 copies of $NP_{147–155}$ are recovered per cell following infection with a rVV-expressing IV NP (58). The same cells expressed 1800 copies of the K^k-restricted IDD $NP_{50–57}$. Following infection of cells with a rVV-encoding $NP_{1–168}$ [this 168-residue fragment is degraded with a $t_{1/2}$ of 30 min; full-length NP (498 residues) is essentially stable], 105 copies of $NP_{147–155}$ and 9300 copies of $NP_{50–57}$ were recovered. Expressing either of the determinants as VV-encoded cytosolic or ER-targeted minigene products resulted in the recovery of an astounding ~55,000 complexes per cell. The minigene-enhanced generation of complexes was associated with greatly enhanced primary anti-peptide responses, as assessed by ex vivo cytotoxic activity (particularly for $NP_{147–155}$), yet only a slight increase

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in the generation of memory T_{CD8+} (59). Even the threefold enhancement of $NP_{147–155}$ generation by $NP_{1–168}$ was associated with an enhanced primary T_{CD8+} response. The use of a T-AG specific for the K^b-$Ova_{257–264}$ complex revealed that ~3500 complexes were expressed on the surface of cells infected with a rVV-expressing chicken ovalbumin (OVA) whereas rVVs expressing cytosolic or ER-targeted minigene products expressed more than 65,000 complexes per cell (26). The abilities of these rVVs to elicit primary and secondary T_{CD8+} responses were similar (59).

These findings lead to several conclusions:

1. The enormous increase in peptide–class I complex formation obtained with cytosolic minigene products relative to full-length proteins demonstrates that the liberation of antigenic peptides from full-length gene products is probably always a limiting factor in the generation of peptide–class I complexes.

2. The extent to which this limits T_{CD8+} responses depends on exactly how inefficient peptide liberation is and on how many complexes are required to obtain maximal responses. For $NP_{147–155}$, 30 complexes are limiting for primary T_{CD8+} responses to VV-NP, and immunogenicity is enhanced by even a threefold increase in complex formation, with further gains coming from an additional 50-fold increase. In the case of $Ova_{257–264}$, 3000 complexes are sufficient to obtain maximal responses, whereas for $NP_{50–57}$, 9300 complexes/cell are insufficient, and primary responses are enhanced by a sixfold increase in complex number. Similarly, it was found that mouse primary and secondary T_{CD8+} responses to rVV- or plasmid DNA-encoded HIV proteins were increased if peptide generation was enhanced by producing rapidly degraded forms of the protein (60).

3. In responding to the same antigen in different contexts, primary T_{CD8+} responses need not parallel memory T_{CD8+} response in magnitude. Thus, there was much less difference in the abilities of rVVs encoding NP and $NP_{147–155}$ minigene product to prime for memory versus primary T_{CD8+} responses. This also demonstrates the conditional nature of immunodominance: Expressed by IV or as a VV-encoded minigene product, $NP_{147–155}$ is a IDD, whereas when expressed as a VV-encoded full-length protein it is a SDD (because primary in vivo responses are low to undetectable by ex vivo ^{51}Cr-release assays).

The great variation in the abundance of IDDs appears to be common. Using a leukemic T cell line transfected with the HIV genome, the A*0201-restricted viral peptides $gag_{77–85}$ (group antigen) and $RT_{476–484}$ (reverse transcriptase)

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were present, respectively, at ~400 and 12 copies per cell (61). Unlike the situation in inbred mice, where there is little variation in which peptides are immunodominant, individuals usually respond to one or the other of these peptides. In any event, in some individuals, the less prevalent determinant is preferred over the more prevalent (42).

Similarly, in the course of a remarkable series of experiments characterizing K^d-restricted responses to the intracellular bacterium *Listeria monocytogenes*, Pamer and colleagues (62–66) have shown that the IDD is the least-abundant determinant. By Elispot analysis, primary T_{CD8+} responded to three peptides—$LLO_{91–99}$ (listeriolysin O), $p60_{217–225}$, and $p60_{449–457}$—at ratios of 20:10:1 (62). This ratio remained unchanged over the subsequent 6 weeks, providing an example in which memory T_{CD8+} responses parallel primary responses. The T_{CD8+} response to each peptide was also measured using soluble strepavidin-tetramerized, peptide–class I complexes (63). This detected specific T_{CD8+} at levels similar to those of the Elispot analysis and revealed that at the peak of primary responses, 1.4% of all T_{CD8+} recognized $LLO_{91–99}$, with 5-fold and 20-fold fewer cells, respectively, recognizing $p60_{217–225}$ and $p60_{449–457}$. During secondary in vivo responses, the number of cells responding to each determinant increased approximately 10-fold. Quantitation of peptides recovered from a macrophage cell line harboring endosomal bacteria revealed that these frequencies were actually inversely related to peptide abundance, with 700 $LLO_{91–99}$, 2700 $p60_{217–225}$, and 9000 $p60_{449–457}$ determinants recovered per cell. By functional competition assay using K^d-restricted T cells specific for a fourth party peptide, the three peptides blocked recognition with a similar molar efficiency, which suggests that they bound to K^d with equal affinity. They were also equally effective at sensitizing target cells for lysis by their respective T_{CD8+}. These last two findings suggest that similar amounts of the three complexes are required for triggering T_{CD8+} responses. When the stabilities of endogenously produced or synthetic peptide–induced complexes were examined (64), complexes containing either of the two dominant peptides were stable ($t_{1/2} > 6$ h) whereas the K^d-$p60_{449–457}$ complex disappeared with a $t_{1/2}$ of 1 h, which is consistent with the correlation of van der Burg et al (52) between immunodominance and complex stability. The great abundance of $p60_{449–457}$ in the face of its rapid turnover is explained by its amazing efficiency of formation. Taking full advantage of unique features of the bacterial system to quantitate the turnover of precursor proteins delivered to the APC cytosol, the efficiency of peptide generation per degraded protein molecule was calculated to be 5%–10% for $LLO_{91–99}$, 2.5%–3% for $p60_{217–225}$, and 25%–30% for $p60_{449–457}$ (65). The specificity of TAP may contribute to these figures because $p60_{449–457}$ is a sixfold more efficient competitor than $p60_{217–225}$ for TAP-mediated transport of a reporter peptide. The relationship between T_{CD8+} responses and abundance

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of $p60_{217–225}$-K^d complexes was examined by mutating $p60_{216}$ to residues that modify peptide generation (66). Reducing peptide generation efficiency 10-fold prevented generation of both primary and secondary T_{CD8+} responses, whereas a twofold reduction or twofold enhancement had no effect on the magnitude of primary or secondary responses. In contrast to some of the viral systems described above, in which graded responses can be observed, this provides a more quantal example of T_{CD8+} responses to a determinant within a complex pathogen.

In humans, A*1101-restricted T_{CD8+} responses to EBV may also be influenced by the stability of peptide–class I complexes. Two EBV determinants are frequently recognized in association with A*1101, $EBNA3B_{416–424}$ (Epstein Barr nuclear antigen) and $EBNA3B_{399–408}$. $EBNA3B_{416–424}$-specific T_{CD8+} dominate both primary and secondary responses, being present at up to 20-fold higher frequencies in both primary (67) and secondary responses as measured by LDA (68). The peptides bind to surface class I molecules with similar affinities (determined by blocking lysis of cells sensitized for lysis with a third party peptide) and sensitize target cells with similar efficiency. This suggests that similar amounts of the two complexes are required for stimulation of their respective T_{CD8+}. Quantitation of peptides from different EBV-transformed B cells revealed that the immunodominant peptide is present at 5- to 40-fold higher levels, depending on the cell line. This difference probably stems, at least in part, from the low stability of complexes formed with the SDD, whose $t_{1/2}$ on the cell surface (assessed by biochemical recovery of detergent-solubilized complexes formed by viable cells incubated with peptide at 26°C) was measured to be at least threefold less than the dominant peptide. This effect may not be intrinsically related to the binding of the subdominant peptide to A*1101 because with soluble class I molecules, the two complexes demonstrate similar stability at 37°C and similar resistance to acid treatment. This may mean that membrane and soluble class I molecules can exhibit selective differences in their binding to certain peptides. Alternatively, as cells are capable of internalizing peptide–class I complexes into endosomal compartments (where they are destroyed), it may mean that APCs can preferentially internalize class I molecules bearing certain peptides.

Given the limited number of quantitative studies that have appeared, and the uncertainty regarding the relevance of in vitro APCs to in vivo APCs, it is not possible to reach firm conclusions regarding the relative abundance of IDDs, SDDs, and "cryptic determinants" (CD). These studies do, however, confirm estimates based on peptide titration curves that IDDs are not always the most abundantly expressed complexes and may, in fact, be expressed in very low numbers. Obviously, much more work is needed in this area, which given the increasing use of T-AGs and multivalent TCRs will surely be forthcoming.

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FACTORS THAT AFFECT PEPTIDE GENERATION *Limiting steps in antigen processing* As discussed above, the liberation of a determinant from its full-length gene product can greatly limit its immunogenicity. This does not necessarily mean that the determinant is liberated less efficiently than other more immunogenic determinants; only that peptide liberation is a limiting step in creating the number of complexes required to optimally activate naïve T_{CD8+}. Peptide liberation in the cytosol is, of course, just one step in the process of creating the trimolecular complex, which includes transport of determinant to the ER, where the peptide may be trimmed to a higher affinity form, and loading onto class I molecules (the last two steps could occur in reverse order). Because it is not yet possible to measure the rate of peptide liberation in the cytosol, to conclude that peptide liberation in the cytosol is the limiting step requires knowledge that the other steps in antigen processing occur at "normal" levels.

Although it is possible to measure the efficiency of TAP-mediated transport using synthetic peptides in semi-intact cells, a potential drawback to this assay is its uncertain relevance to the situation in living cells, where peptide delivery to TAP may be facilitated by molecular chaperones and possibly even coupled to peptide generation. There is now evidence, however, that the efficiency of TAP-mediated transport of VV-encoded minigene products with short flanking residues correlates with the number of peptide–class I complexes generated (although the latter is inferred from indirect methods) (69). A more serious problem is that because peptide trimming can occur in the ER, the composition of the peptides transported by TAP that are generated from physiological antigens (as opposed to minigene products) is uncertain. Measuring the efficiency of the two remaining steps in the pathway, peptide trimming and assisted loading onto class I molecules, is an even more difficult problem. There is as yet only indirect evidence that TAP-transported peptides are trimmed in the ER, and the current evidence for facilitated loading amounts to little more than reasonable, if inspired, speculation.

With the available technology it is, therefore, not possible to precisely separate the contribution of individual steps in antigen processing to the compromised immunogenicity of SDDs and CDs. There are a number of studies, however, that have laid the foundation for the future understanding of this question. The first (and still the most compelling) demonstration that the regions immediately flanking a determinant (flanking sequence) in a full-length protein can influence its immunogenicity is the work of Del Val et al (70). Inserting a murine CMV (MCMV) determinant into VV-encoded HBV core antigen, it was shown that flanking residues greatly influenced the in vitro presentation of the determinant (up to a 16-fold difference was detected in the amount of peptide recovered from acid extracts of infected cells) and its ability

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to induce a protective T_{CD8+} response to a challenge with a dose of MCMV lethal to nonimmunized animals. Importantly, it was shown that this was not simply a consequence of increasing the overall degradation rate of chimeric protein.

The effects of flanking sequences on immunogenicity have been most thoroughly characterized in K^b-restricted T_{CD8+} responses to OVA. The IDD $Ova_{257–264}$ binds to K^b with high affinity ($K_d \sim 1$ nM). T_{CD8+} responses to a SDD ($Ova_{55–62}$) can be elicited if animals are immunized with cells osmotically loaded with amounts of OVA in excess of the minimal amount required to obtain responses to $Ova_{257–264}$; in fact, the number of memory T_{CD8+} generated for the two determinants is similar under these conditions (71). Clones specific for each determinant were obtained that require similar amounts of the respective peptides for half-maximal activation. Because $Ova_{55–62}$ binds to K^b with a K_a of ~50 nM, this implies that the $Ova_{55–62}$-specific clone is far more sensitive than the $Ova_{257–264}$-specific clone. Despite this, 50-fold greater amounts of electroporated OVA were required to achieve a similar degree of stimulation, which implies that processing of OVA results in a ratio of K^b-$Ova_{257–264}$ complexes to K^b-$Ova_{55–62}$ complexes of ~2500. Obviously, the lower affinity of $Ova_{55–62}$ for K^b could contribute to its poor presentation. On the other hand, this affinity is well within the range observed for IDDs restricted by other class I molecules.

The role of flanking sequences in the difference in efficiency of producing these two determinants was examined by measuring T_{CD8+} recognition of cells cytosolically loaded with 22-mer synthetic peptides composed of one of the determinants with natural flanking residues or flanking residues from the alternative peptide (72). For $Ova_{257–264}$, substitution with the flanking residues of $Ova_{55–62}$ decreased its processing efficiency more than twofold; the converse manipulation only slightly enhanced the presentation of $Ova_{55–62}$. Most importantly, the efficiency of presentation correlated with the efficiency at which purified 20S proteasomes were able to liberate the determinant from the respective synthetic substrates. $Ova_{55–62}$ was cleaved internally by proteasomes, and this could not be rectified by substitution with the $Ova_{257–264}$ flanking sequences. Conversely, the $Ova_{55–62}$ flanking sequences created cleavage sites within the $Ova_{257–264}$ determinant.

The idea that proteasomal destruction of potential determinants is a frequent contributor to immunodominance is supported by two additional studies. Tevethia and colleagues (73) examined a D^b-restricted determinant from simian virus 40 tumor antigen (Tag), $Tag_{489–497}$, that is nonimmunogenic in the context of Tag produced by simian virus 40 transformed cells or by VV-Tag. T_{CD8+} are easily elicited by a rVV encoding the ER-targeted peptide, but not a rVV encoding the cytosolic peptide. This correlates with the efficiency of

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presentation in vitro by rVV-infected cells: Cells expressing the cytosolic peptide are not lysed. This is not due to inefficient transport of $Tag_{489–497}$ by TAP, as inferred from its ability to block TAP-mediated transport of an indicator peptide in a biochemical assay using permeabilized cells. Rather, the problem appears to be proteasomal destruction because presentation of the cytosolic minigene product is enabled by treating cells with a proteasome inhibitor. The addition of flanking sequences to the cytosolic minigene product as simple as $(Ala)_2$ at either end of the peptide, or inclusion in a full-length protein, also enhanced its presentation—presumably by enhancing the generation of a precursor that was not destroyed by proteasomes and could be transported by TAP.

Ossendorp et al studied responses to the $p15E_{574–581}$, the K^b-restricted IDD in the AKV/MCF type murine leukemia virus (MuLV) (74). The homologous protein in FMR type MuLV has six amino acid substitutions, one (Lys to Arg) at the NH_2-terminus of the peptide and the other five located at least 10 residues from the peptide (it was assumed that these were too distant to affect peptide generation). The mutation within the determinant does not affect peptide affinity for K^b and does not affect recognition by T_{CD8+} raised to the AKV/MCF peptide. The two peptides are similarly immunogenic as synthetic peptides. Despite this, mice fail to mount a $p15E_{574–581}$-specific T_{CD8+} response to FMR MuLV, and cells expressing FMR p15 are not recognized by p15-specific T_{CD8+}. In vitro 20S proteasome digestion of synthetic 26mer peptides corresponding to the respective AKV/MCF and FMR sequences liberates the AKV/MCF peptide with a two-residue amino terminal extension and destroys the FMR peptide. The AKV/MCF 10mer peptide was an efficient competitor of TAP-mediated indicator peptide transport, whereas the 8mer was at least 30-fold less efficient. Based on these findings, the authors concluded that the poor antigenicity and immunogenicity of AKV/MCF p15 peptide was due to the Lys to Arg substitution resulting in proteasomal destruction and that cells naturally produced the 10mer, which was then trimmed in the secretory pathway. Although these conclusions are reasonable, they depend on the assumptions that the activities of purified 20S proteasomes accurately reflect proteasome cleavages within the cell and that the other amino acid differences between AKV/MCF and FMR p15E do not affect antigen processing. The latter possibility is more than pure conjecture because in extensive studies regarding the K^d-restricted presentation of rVV-encoded fragments of IV $NP_{147–155}$, Yellen-Shaw et al found that the effects of flanking residues on peptide presentation can extend more than 50 residues from the determinant (75).

The findings with $Tag_{489–497}$ and $p15E_{574–581}$ are consistent with the idea that peptide trimming in the ER is an obligate step in the presentation of

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some determinants. In this eventuality, flanking sequences can also potentially play a role in immunodominance by influencing TAP-mediated transport. In thorough studies of TAP-specificity, Neisig et al identified three other optimal class I–binding peptides whose synthetic versions were extremely inefficient competitors of TAP-mediated transport (76). As with $p15E_{574–581}$, extension of one of these poorly transported peptides by inclusion of one or two naturally flanking NH_2-terminal residues greatly improved its interaction with TAP.

It is likely that role of TAP in immunodominance in mice and humans has been obscured by the use of canonical motifs for choosing determinants for study and the concordance between the specificities of TAP and class I molecules for COOH-terminal residues. Because class I molecules are capable of binding some peptide with noncanonical COOH-terminal anchors, it is likely that TAP acts a formidable barrier against the presentation of these peptides. Just how efficient this barrier can be has been elegantly shown by Powis et al (77).

Originally investigating the differential reactivity of a rat class I ($RT1.A^a$)-specific mAb with cells expressing the relevant class I molecule but derived from disparate rat strains, they found that mAb reactivity segregated with what turned out to be the *TAP* locus. The strains in question express TAP alleles that differ greatly in specificity; one of the alleles is unable to transport peptides with a COOH-terminal Arg, a residue that is a (very) dominant anchor for $RT1.A^a$. The supply of peptides provided by the nonpermissive transporter is sufficient to enable the normal assembly and surface expression of other rat class I molecules, but it is so poor for $RT1.A^a$ that export from the ER occurs at 10% the rate of cells expressing permissive TAP. Despite the prolonged period in the ER, which should enable binding of the odd Arg-terminating peptide transported by TAP, peptides recovered from $RT1.A^a$ were nearly completely devoid of COOH-terminal Arg. This latter finding fits neatly with evidence of inefficient ER trimming of COOH-terminal extensions and suggests that the lack of such trimming activity contributes to determinant crypticity by preventing the generation of class I binding peptides from COOH-terminally extended precursors.

Although it has yet to be formally shown that TAP polymorphism in the rat effects the T_{CD8+} response to foreign antigens, there is little doubt that this will be so, particularly because differences in TAP alleles result in alloreactivity. Thus, TAP clearly has the potential to play a role in immunodominance. In humans, TAP may exert a relatively subtle effect on immunodominance because human TAP is relatively promiscuous in its peptide binding: Only a few types of residues are strongly disfavored in the various positions. TAP is polymorphic in humans, however, and there is indirect evidence linking TAP

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alleles to the progression of HIV infection (78). Whether this is due to allelic differences in peptide transport resulting in altered T_{CD8+} responses remains to be demonstrated.

Effects of cytokines on antigen processing Each of the steps in the antigen processing pathway can be affected by exposure of APCs to cytokines. The best-defined effects are induced by interferon γ and tumor necrosis factor α, which increase synthesis of TAP, class I molecules, and several molecular chaperones. Additionally, these cytokines modify proteasomes in two ways (11, 13, 14): first, by enhancing synthesis of three of proteasome subunits that displace homologous subunits during proteasome biogenesis, thereby producing "immunoproteasomes"; and second, by enhancing synthesis of a subset of the regulatory proteins that bind to the ends of proteasomes. These alterations in proteasomes potentially have the most profound effects on immunodominance because the specificity of proteasomes is altered by these modifications. The nature of these changes is controversial, however, and the in vivo effects on the immunogenicity of individual determinants remain largely unexplored. It is important to note that the biological relevance of cytokine-induced qualitative modifications in antigen processing requires that similar alterations in peptide generation occur in both afferent and efferent APCs.

Features of proteins that contribute to immunodominance We have discussed the specific features of potential determinants and flanking sequences that can influence their immunogenicity. A more general issue is whether there are features of individual gene products that favor/disfavor the generation of immunogenic peptides. There are two reasons why this question cannot presently be answered with any degree of precision. First, the least understood portion of the antigen processing pathway is how biosynthesized proteins enter the pathway. Second, as emphasized throughout this review, there is precious little information regarding in vivo presentation of antigens to naïve T_{CD8+}.

It is possible, however, to identify some properties of gene products that will influence the generation of peptides by infected APCs. First, and most obviously, for any gene product, the rate of peptide generation will be proportional to the rate of translation (this governs the abundance of the gene product and its byproducts; further, increased synthesis will also result in enhanced cross-priming). This is not to say that IDDs always come from the most abundant viral proteins: Indeed, they don't. Rather, that given a certain inherent efficiency of peptide generation from a protein, expressing more of the protein will result in a concomitant increase in peptide generation. Second, in these circumstances, increased protein turnover favors peptide generation. Third, targeting of the protein to the ER can have positive and negative effects. On the plus side, if

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the protein has a determinant in its signal sequence, there is a good chance that the peptide will be generated very efficiently; indeed, many of the most abundant peptides recovered from class I molecules are derived from signal sequences. On the minus side, if the determinant is present in the lumenal domain of the protein, its presentation will most likely be compromised. Fourth, for proteins expressed by bacteria and simple eukaryotes whose life cycle includes an intracellular phase, a special rule applies. Proteins that are targeted to the cytosol of cells will be preferentially presented. Finally, it has been observed that HIV determinants restricted by different allomorphs cluster in certain regions of several viral proteins (42), which suggests that regions of proteins can have properties that favor efficient peptide liberation.

Features of pathogens that contribute to immunodominance The temporal sequence of viral gene expression can greatly influence immunodominance. In some cases, only a small subset of viral genes may be expressed by APCs. For example, responses to EBV in chronically infected individuals are largely limited to the few gene products that are constitutively expressed in latently infected B cells (79). For rVVs, expression of recombinant proteins expressed under the control of late viral promoters (i.e. after the initiation of viral DNA synthesis) can decrease immunogenicity (and antigenicity), possibly related to viral interference with host protein synthesis (80).

Viruses may also influence immunodominance in more specific ways. *Herpesviridae* are the undisputed champions in this realm. One of the EBV proteins is abundantly expressed in latently infected cells but is infrequently immunogenic, probably because of a region that interferes with proteasome-dependent peptide generation. CMV expresses numerous viral gene products that interfere with antigen processing, including one that specifically blocks the generation of peptides from an abundant viral structural protein, and others that target class I molecules in the ER for destruction, retain class I molecules intracellularly, or interfere with TAP function. HSV expresses a protein that prevents TAP-mediated peptide transport. Adenoviruses interfere with antigen processing by decreasing transcription of class I molecules and accessory antigen processing components, or by expressing a protein that retains class I molecules in the ER.

The effects on immunodominance of such global interference with antigen processing have yet to be investigated in detail, but a simple prediction is that T_{CD8+} responses will focus on those determinants that for whatever reason are less affected by the strategy employed by the virus. In the case of human T_{CD8+} responses to CMV, this mechanism has been proposed to account for the immunodominance of a virion protein of such abundance that a sufficient number of copies are delivered to APCs from input virus to produce immunogenic

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quantities of peptide–class I complexes before viral gene expression can interfere with antigen presentation (45, 81).

The Other Side of the Coin: Contribution of T_{CD8+} Responses to Immunodominance

T_{CD8+} REGULATION: IMMUNODOMINATION A major contributor to immunodominance is immunodomination: the suppression of SDD-specific responses by IDDs. This was one of the initial observations of immunodominance (82, 83), and it is detected as enhanced responses to nondominant determinants under conditions when responses to the IDD are prevented by altering or removing the determinant, its class I restriction element, or IDD-specific T_{CD8+}. Immunodomination occurs in T_{CD8+} responses to virus-transformed cells (84), tumor antigens (50), minor H antigens (85), DNA vaccines (46), and viruses (49, 86). In some circumstances, immunodomination is limited largely to primary responses, the IDD having little effect on the priming of SDD-specific memory T_{CD8+} (87). It is probably more frequent that immunodomination occurs in both primary and memory T_{CD8+} responses. In gauging the effect of immunodomination in secondary responses, it is crucial to stimulate T_{CD8+} with nonlimiting amounts of SDDs in the absence of the potential IDDs because domination can occur in vitro.

There are two general explanations for immunodomination. The first is that the IDD interferes with the generation of the SDD in APCs. Although this possibility has yet to be rigorously eliminated in any system by peptide quantitation, it has been repeatedly observed that T_{CD8+} recognition of SDDs is not affected by the coexpression of the IDD (84, 88). Most IDDs are of such low abundance as to make competition for binding to class I molecules extremely unlikely (in fact, it is difficult to observe such competition except under extreme overexpression of determinants from minigenes). In the unusual circumstance where one peptide (or two overlapping peptides) may be presented by more than one class I allomorph, this mechanism can contribute to the dominance of a response restricted to the allomorph that selectively acquires the peptide (89). Finally, immunodomination is frequently observed between peptides that bind to different class I allomorphs and would, therefore, be unlikely to compete for binding.

The second, far more likely explanation is that T_{CD8+} specific for dominant peptides suppress responses to other peptides. This could occur by multiple mechanisms operating alone or in conjunction, including: reduction of antigen load through the actions of rapidly responding IDD-specific T_{CD8+} such that SDDs are expressed at suboptimal levels for T_{CD8+} activation; competition at the level of APCs for T_{CD8+} activation; and systemic suppression of responses to SDDs by IDD-specific T_{CD8+}.

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Immunodomination has been most extensively studied in LCMV infection of H-2^d mice, where L^d-restricted, $NP_{118–126}$-specific T_{CD8+} dominate responses to other determinants, including K^d-restricted $GP_{283–291}$-specific T_{CD8+}. The latter determinant is dominant if mice are infected with a LCMV mutant that was T_{CD8+}-selected for loss of the $NP_{118–126}$ determinant (88), or following infection with wild-type LCMV if mice express NP in the thymus from a transgene, resulting in the deletion of high-affinity $NP_{118–126}$-specific T_{CD8+} (90). Under normal circumstances, T_{CD8+} responses to $GP_{283–291}$ are delayed relative to the response to $NP_{118–126}$, and it appears that clearance of virus by $NP_{118–126}$-specific T_{CD8+} reduces the antigen load to a point that prevents stimulation of $GP_{283–291}$-specific T_{CD8+}. Consistent with this explanation, the response to $GP_{283–291}$ was also suppressed in H-2^d $\times$ H-2^b F1 mice infected with the variant LCMV, presumably because of the presence of T_{CD8+} specific for the major H-2^b-restricted determinant.

This last finding points to a consistent feature of immunodomination: The ability of a determinant to dominate (or be dominated) is relative, and determinants restricted by the same or different allomorphs can be ordered in a hierarchy of domination. This has long been known to occur in T_{CD8+} responses to minor H antigens (91). The hierarchy can be altered by the prior experience of the immune system. This has been neatly shown with T_{CD8+} responses to Sendai virus in H-2^b $\times$ H-2^k F1 mice (87). The IDD in H-2^b mice ($NP_{324–332}$) is rendered subdominant in the F1 mice by the H-2^k–restricted response to undefined (but non-NP) viral determinants. If, however, mice are infected with a rVV expressing NP prior to infection with Sendai virus, $NP_{324–332}$-specific T_{CD8+} now dominate the response. Such a reversal of immunodominance has also been shown in responses to tumor cells (50). The ability of responding memory T_{CD8+} to suppress responses by naïve T_{CD8+} was first demonstrated in experiments in which lymphocytes from virus-infected mice were adoptively transferred into naïve mice (92, 93).

The hierarchical nature of immunodomination has been examined by immunizing mice with mixtures of synthetic peptides that represent IDDs in their respective systems. Secondary peptide-restimulated T_{CD8+} from mice immunized with a mixture of five K^b-restricted peptides focused on two of the peptides (94). This could not be attributed to either peptide affinity for K^b or the numbers of peptide–class I complexes needed to trigger T_{CD8+} lysis. Rather, the hierarchy correlated with a 3.5-fold difference in the numbers of T_{CD8+} that responded to individual peptides, as determined by LDA. If, however, animals were immunized with dendritic cells pulsed with the synthetic peptide mixture, T_{CD8+} responses were distributed equally among the four most immunogenic peptides. These findings led the authors to propose that the number of APCs was limiting following peptide immunization and that immunodomination reflected T_{CD8+}

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competition for APCs. In a prior study (95), it was shown that immunizing mice with five K^d-restricted peptides also led to a hierarchy of responses that could not be attributed to peptide affinity for K^d. Indeed, when two peptides differing 30-fold in their affinity for K^d were coimmunized, the response was dominated by the weaker binding peptide. This could not be ascribed to differences in destruction by serum proteases or to recognition of either of the peptides by T_{CD4+}. Most interestingly, the immunodomination could be eliminated if mice were treated with interleukin-12, pointing to cytokine-mediated regulation of T_{CD8+} responses.

Together, these studies support the concept that activated T_{CD8+} cells can exert a suppressive effect on nonactivated (or possibly less activated) T_{CD8+} and that this can play a role in immunodomination. How localized this effect is remains to be determined (individual APC versus regions within a node, versus entire node etc) and may well differ, depending on the nature of the antigen. It is likely that in some circumstances this suppression would act synergistically with a reduction in antigen load to enhance the domination phenomenon. It is also plausible that in some instances suppression of T_{CD8+} responses is due to other components of the immune response to complex pathogens. Of particular relevance are the remarkable findings that prior immunization with tumor cells, allogeneic splenocytes, or even xenogeneic erythrocytes suppresses T_{CD8+} responses to unrelated alloantigens (96). Suppression is transferable by serum, and the suppressive factor appears to be antibody-bound TGF-β acting in a process that requires its Fc-receptor–mediated binding to macrophages (97, 98). As viruses induce robust antibody responses, it is plausible that this mechanism contributes to the immunodominance of determinants able to induce the most rapid T_{CD8+} responses. The possible role of antibody in immunodominance can be easily examined using knockout mice unable to produce antibody.

It should be noted that although immunodomination is commonly observed in mouse T_{CD8+} responses to diverse antigens, its contribution to immunodominance in human T_{CD8+} responses has not been extensively examined. There is, however, at least one clear example of immunodomination involving B8-restricted, EBV-specific responses that is described below.

T_{CD8+} REPERTOIRE The findings presented in the previous section prompt a question central to understanding immunodominance: Why do IDD-specific T_{CD8+} respond better than SDD-specific T_{CD8+}? The simplest explanation would be that IDDs are the most abundant peptides expressed on the surface of the relevant APC and, consequently, the corresponding T_{CD8+} are most rapidly and vigorously activated. Although this may account for some IDDs, evidence presented above strongly suggests that there is no simple correlation between immunodominance and abundance (again with the caveat regarding the

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unknown properties of the relevant APC in vivo). Rather, it is probable that that in the eyes of the T_{CD8+} repertoire, some peptide–class I complexes are created more equal than others. There are two major questions to be addressed. First, to what extent is this Orwellian bias intrinsic to the TCR repertoire as opposed to being imposed by thymic or peripheral selection (nature versus nurture)? Second, how is the bias executed: increased numbers of T_{CD8+} (either in the number of clones or the average population of each clone) versus more rapid proliferation of an equivalent number of T_{CD8+} (size versus speed)?

Issue 1: nature vs nurture The most direct approach to examine the extent to which the TCR repertoire is intrinsically biased toward IDDs is to produce TCRs independently of positive and negative selection (in a phage display library for example) and determine the frequencies and avidities of TCRs for IDDs and SDDs. This poses numerous and formidable technical difficulties and remains a distant goal.

A less direct (but more accessible) approach to examining this question is to compare immunodominance in responses to a common antigen in organisms expressing a common restriction element but with distinct self antigens and TCR genes. Two such organisms are humans and transgenic mice expressing human class I molecules. Team Sette found a considerable overlap in peptide immunogenicity in humans and transgenic HLA A*0201 or A11 mice (33, 99). Most importantly, immunization of such transgenic mice with viruses has been found to elicit T_{CD8+} that recognize the same IDD determinants as human T_{CD8+} (100–102). In the single study that compared TCR usage in man and mouse T_{CD8+} specific for the same peptide–class I complex, mouse and human TCRs were found to utilize nonhomologous Vα and Vβ segments (103).

These findings indicate that the dominance of many determinants occurs independently of TCR genes and the precise nature of the self peptides operative in thymic and postthymic selection. As described below, within an individual, IDDs are often recognized by T_{CD8+} bearing TCRs composed of different Vβ and Vα chains. Together, these findings strongly suggest that there are special features of some IDDs (possibly also some non-IDDs recognized with similar high affinity by T cells) that enable them to interact favorably with TCRs. This could result from one or a combination of the following factors: (*a*) the orientation or nature of side chains available for interaction; (*b*) the induction of unique conformational alterations in the α helices of the class I binding groove by peptide binding; (*c*) the conformational flexibility (increased or decreased) of the peptide after binding to the groove.

This is not to say that positive and negative selection have no effects on immunodominance. The potential of negative selection for influencing immunodominance has been elegantly shown in human T_{CD8+} responses to EBV

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(104, 105). In most B8-positive individuals, responses are dominated by B8-restricted, EBNA3A$_{325–333}$-specific T_{CD8+} expressing a highly conserved TCR. These T_{CD8+} are strongly alloreactive to HLA B*4402. EBV-infected individuals expressing B8 and B*4402 make a less vigorous B8-restricted T_{CD8+} response to EBNA3A$_{325–333}$ (threefold decrease in CTL number by LDA). Remarkably, these T_{CD8+} do not recognize B*4402 and do not express the conserved oligoclonal TCRs typical of T_{CD8+} in B8-negative individuals. Presumably, immunodomination by B*4402-reactive T_{CD8+} prevents the activation of these alternative T_{CD8+} in B*4402-negative individuals. These findings demonstrate that tolerance to self class I molecules can influence the repertoire of virus-specific T_{CD8} and also that immunodomination even occurs within responses to individual IDDs.

The contribution of tolerance to individual self peptides in shaping the TCR repertoire to foreign antigens is nearly completely undefined. It has been shown that T_{CD8+} specific for IV HA$_{210–219}$ (hemagglutinin) cross-react with a peptide from an immunoglobulin V_H gene (106, 107). HA$_{210–219}$ is a subdominant peptide, but this self reactivity is probably not the critical factor limiting HA$_{210–219}$ immunogenicity because other HA$_{210–219}$-specific T_{CD8+} do not recognize the V_H peptide.

Issue 2: size vs speed Until recently, it was not possible to accurately enumerate T_{CD8+} and measure the diversity of their TCRs. There have been three recent developments that will accelerate progress in this area. First is the use of peptide–class I tetramers to enumerate and isolate T_{CD8+} specific for individual determinants. Second is the commercial availability of fluorochrome-conjugated, Vβ segment specific–mAbs for nearly each of the mouse Vβ segments. Used in conjunction with peptide–class I tetramers, these mAbs provide a broad but extremely useful measure of TCR diversity. As panels of mAbs specific for mouse Vα and human Vα and Vβ segments become available, the discrimination of this method will increase and enable its widespread application to studies of human T_{CD8+} responses. Third are improvements in PCR-based methods and DNA sequencing efficiency that enable sequencing of TCR genes from individual T_{CD8+} isolated using peptide–class I tetramers.

Assessing size verus speed entails determining the numbers of naïve T_{CD8+} capable of responding to a given determinant. This is now theoretically feasible using peptide–class I tetramers, but it remains challenging because of the low frequency of naïve cells in T_{CD8+} populations. The present discussion is limited to studies of activated primary T_{CD8+} or secondary T_{CD8+}, which have provided useful information but leave the major issue largely unresolved.

The diversity of TCR usage in T cell responses was first examined in T_{CD4+} responses to IDDs present in “Sigma” antigens (cytochrome, lysozyme, etc),

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revealing a highly restricted Vβ and Vα chain usage (108). The close evolutionary relationship of these antigens to self proteins, however, no doubt limits the diversity of these responses relative to responses to antigens in pathogens. Such self-tolerance may also contribute to the pauciclonal K^d-restricted T_{CD8+} primary response to a HLA CW3 determinant, which is composed of T_{CD8+} exclusively expressing Vβ10, most often in association with Jβ1.2 segments (109). The repertoire size in different individuals was found to consist of between 15 and 20 clonotypes. This landmark study was the first in which T_{CD8+} fresh from a responding animal were cytofluorographically sorted (based on Vβ10 expression) and the sequence of the TCR expressed by individual cells determined by PCR-based methodology. No doubt, many similar studies will follow in which peptide–class I tetramers are used to isolate T_{CD8+} whose TCRs are sequenced using primers that encompass all possible V segments.

The diversity of TCRs in mouse and human responses to viral IDD determinants have been examined in a number of systems using somewhat less sophisticated and precise methods. The findings fall fairly evenly into two camps: those in which responses are dominated by cells of the same Vβ (or less often Vα) chain (45, 110–113), and those in which responses are composed of cells expressing multiple Vβ and Vα chains (87, 114–116).

It is obviously premature to draw any firm conclusions regarding the contribution of TCR diversity to immunodominance. It may be reasonable to conclude, however, that the relationship will not be simple. A poignant example of the complexity possible is provided by the K^b-restricted response to HSV $gB_{498–505}$ (glycoprotein B) (110). In C57BL/6 mice and other strains with similar TCR genes, T_{CD8+} expressing TCRs with Vβ10 or Vβ8S1 genes dominate the response. In C57/L mice, however, which lack the genes encoding these regions, the response is more diverse. Based on the amount of peptide required to sensitize target cells for T_{CD8+} lysis, the new clones are of the same sensitivity as the original clones and are present among memory cells at only slightly lower frequencies. The TCRs utilized are present in C57BL/6 mice but are presumably dominated by the oligoclonal responders.

The domination of these clones [and in the case of EBV (105), as discussed above] supports the possibility of differential proliferation of naïve T_{CD8+} expressing TCRs of similar affinity for same peptide–class I complex. There are several possible mechanisms that may apply. (*a*) TCRs containing certain V regions may signal better than other TCRs upon binding to a given peptide–class I complex, and these differences may be most (or only) apparent in stimulating naïve T_{CD8+}. (*b*) A subset of naïve IDD-specific T_{CD8+} expressing a given TCR may require increased numbers of peptide–class I complexes because of the presence of tolerizing self peptides specific for the TCR. Once activated,

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however, the cells may be as sensitive as the IDD-specific cells expressing an alternative TCR nonreactive with self peptides.

Questions, Questions, Questions

We have strived to emphasize that immunodominance results from the complex interplay of three major factors: the quantities of peptide–class I complexes expressed on APCs, the repertoire of T_{CD8+} awaiting the complexes, and the ability of IDD-specific T_{CD8+} to suppress SDD-specific responses. It is to be expected that the contribution of these factors to immunodominance varies considerably in an antigen- and allomorph-dependent manner. Ultimately, understanding immunodominance will require answers to the following questions. What is the relevant APC for activating naïve T_{CD8+}, and under what circumstances does it present exogenous versus endogenous peptides? What is the relationship between the abundance of a peptide–class I complex on the relevant APCs and its selection as an IDD versus a SDD? Are some/many IDDs that are intrinsically more immunogenic because of an innate propensity to interact with TCRs, and if so, what is the structural basis for the interaction? How does the T_{CD8+} repertoire contribute to immunodominance and to what extent is this based on clonal diversity, or the size or proliferative capacity of individual clones? What controls the proliferative capacity of individual clones and how much is TCR related (tolerance/peptide antagonism) versus other factors (differences in internal signal transduction pathways or cytokine responsiveness)? What mechanisms underlie immunodomination and what are the roles of cytokines in this process? Why does immunodomination exist and is this an inevitable byproduct of the workings of the immune system or is there an evolutionary edge to using a minimal number of clonotypes to respond to a given antigen? [We previously suggested one possible advantage: minimization of the chance of self reactivity (38). Were this true, vaccines designed to elicit responses to the maximal number of target determinants would result in an increased incidence of autoimmunity.]

Most of these questions address fundamental aspects of T cell biology, fitting final testimony to the central place that immunodominance occupies in T cell responses. Although reasonably complete answers will come neither easily nor rapidly, understanding of immunodominance is poised to increase logarithmically in the next few years because of recent technical advances and newfound interest attendant with the increased urgency to develop vaccines that induce effective T_{CD8+} responses to HIV and other organisms resistant to humoral immunity.

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