Commentary

The Essence of Epitopes

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Driving the rapid advance in knowledge of peptide-binding to major histocompatibility complex (MHC) molecules is the prospect of predicting which peptides from foreign proteins are likely to be antigenic, and thus effective immunogens in vivo. Despite the numerous peptides that theoretically could be generated from a foreign protein, in practice cytotoxic T lymphocytes (CTL) focus upon just a few immunodominant peptide epitopes presented by self MHC class I molecules (1). Underlying this selection of particular peptides, also called determinants, is the sequence polymorphism that epitomizes class I heavy chains (2). In general, differences between alleles map to the peptide-binding site of the class I molecule and serve to change the types of peptide bound.

Molecular insight comes from two lines of evidence: three-dimensional structures of class I molecules bound to defined peptides, and amino acid sequences of endogenously bound peptides (3). Conserved residues in the peptide-binding site enmesh the peptide termini in hydrogen bond networks, interactions that lay a foundation for high affinity binding. Polymorphic pockets of the class I–binding site then add sequence specificity to the interaction by selecting for particular amino acids at two "anchor" positions within a peptide sequence of eight to nine residues. The identity of the anchor residues and their position define minimum "allele-specific motifs," the relevance of which is demonstrated by their ability to confer class I binding when incorporated into a polyalanine peptide backbone (4, 5). This knowledge raised hope that scanning foreign protein sequences for the presence of particular motifs would facilitate prediction of CTL epitopes. Indeed in notable instances this has been achieved. The H-2K^d peptide-binding motif was used to identify a protective CTL epitope from *Listeria monocytogenes* (6, 7), and Hill et al. (8) found a CTL epitope from *Plasmodium falciparum* that may protect individuals who express HLA-B*5301 from severe malaria.

Although most functionally defined CTL epitopes possess appropriate class I–binding motifs (9), a minority of motif-containing peptides from a given foreign protein are actually immunogenic. For example, although ovalbumin (a favorite model antigen) contains six potential epitopes with the H-2K^b-binding motif (shown in Table 1) (10), the CTL response is dominated by just one, OVA$_{257-264}$ (12, 13). Immunodominance is dictated by more than just the presence of an appropriate class I–binding motif, which seems hardly surprising because antigen presentation involves many steps before the binding of class I and peptide: antigenic protein undergoes proteolysis, transport to the endoplasmic reticulum, and competes there with other peptides for binding to class I molecules. Seeking an explanation for the immunodominance of OVA$_{257-264}$, Jameson and Bevan (10) showed that synthetic peptides corresponding to the six ovalbumin sequences vary widely in their ability to bind H-2K^b, as measured by ability to promote stable class I assembly. Interestingly, OVA$_{257-264}$ was the most efficient, suggesting epitope selection reflects superior binding affinity.

Recent investigations evaluate the contribution of peptide affinity for class I molecules to epitope selection, with the goal of improving identification of potential CTL epitopes. One such study is reported in this issue by Chen et al. (14) who find that immunization of mice with high concentrations of ovalbumin leads to CTL against a second epitope, OVA$_{55-62}$. In pursuit of the basis for dominance of the OVA$_{257-264}$–specific CTL response over that to OVA$_{55-62}$ under less extreme conditions of immunization, they showed the relatively poor response to OVA$_{55-62}$ could not be attributed to bias in the T cell repertoire as equivalent numbers of CTL respond to both epitopes. Thus, the onus was placed on differences in peptide generation and/or presentation. This suspicion was confirmed by comparison of T cell hybridomas specific for OVA$_{257-264}$ and OVA$_{55-62}$, which showed the dominant epitope was processed and presented some 20–50-fold more efficiently than the sub-dominant epitope. Furthermore, a synthetic version of the dominant epitope strongly out competed T cell hybridoma recognition of the sub-dominant epitope and not vice versa. On this basis, the source of dominance was attributed to differences in binding to H-2K^b. Quantitative affinity measurements were therefore undertaken using biosensor-based technology that employes surface plasmon resonance (SPR) to monitor binding of immobilized peptide analogues to a soluble form of H-2K^b (Fig. 1) (15). Although several methods have been developed for studying peptide-MHC binding, they are based on measurement of affinity constants at equilibrium. SPR offers the advantage of direct and continuous monitoring of changes in concentration of soluble class I molecules at the biosensor surface, enabling association and dissociation kinetics to be measured as they occur (in real time). The association rate for the OVA$_{257-264}$ analogue was found to be approximately 10-fold faster than that of OVA$_{55-62}$, and a two to three-fold difference in the dissociation rates of the two peptides was also observed. Thus, Chen et al. (14) conclude that functional dominance of OVA$_{257-264}$ in vivo can be explained almost completely by its high affinity binding to H-2K^b.

Table 1. *Sequences of Peptides from Ovalbumin which Contain the H-2K^b-binding Motif*

Motif-containing peptides	Peptide sequences
OVA_{12-19}	CFDVFKEL
OVA_{25-32}	ENIFYCPI
OVA_{55-62}	KVVRFDKL
$OVA_{107-114}$	AEERYPIL
$OVA_{176-183}$	NAIVFKGL
$OVA_{257-264}$	SIINFEKL

Subscript numbers refer to location of the peptide in the ovalbumin sequence. Peptide sequences are given in the single-letter code. The H-2K^b-binding motif proposed by Falk et al. (11) comprises phenylalanine or tyrosine at position 5 and leucine or isoleucine at the COOH-terminal position of an octamer peptide.

Other investigators also conclude that superior class I binding is an influential factor in the selection of CTL epitopes from peptides sharing minimum motifs. Ruppert et al. (16) employed an assay in which the ability of test peptides to compete with a radiolabeled standard peptide for binding to detergent solubilized class I is used to quantitatively assess affinities. Large variations in affinity were detected when an assortment of tumor and viral antigens containing the minimum HLA-A*0201–binding motif were
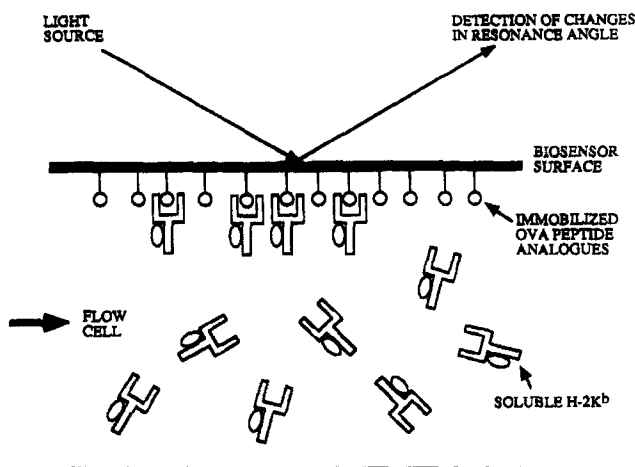
Figure 1. Schematic representation of the biosensor used to quantify the binding kinetics of ovalbumin peptide analogues to H-2K^b molecules. Biosensors perform real time kinetic measurement of molecular interactions. Ovalbumin peptide analogues substituted with cysteine at position 6 were used to facilitate covalent coupling to the dextran-coated gold surface of the biosensor flow cell. Binding of soluble H-2K^b molecules (emptied of endogenous peptides by prior exposure to pH 12.5 to increase peptide binding capacity) to immobilized peptide generates changes in the refractive index of the medium adjacent to the biosensor surface. This in turn induces shifts in the resonance angle of reflected light from the opposite face of the biosensor. Shifts in SPR therefore correlate directly with binding of H-2K^b to immobilized peptide.

assayed, but most known CTL epitopes were among the high affinity binders. Similarly Parker et al. (17) using yet another assay, in which the strength of peptide binding is inversely correlated with the dissociation rate of β_2-microglobulin from HLA-A*0201 molecules reconstituted with the peptide under test, showed that dominant antigenic epitopes are amongst those that bind most tightly.

Apparent from these studies is that minimum motifs are not the final say in defining a tightly binding peptide. To search for additional clues, Ruppert et al. (16) aligned HLA-A*0201–binding peptides and correlated their sequences with strength of binding. This exercise revealed common "secondary anchors" which when added to the dominant anchors produce an expanded HLA-A*0201 motif that better predicts high affinity binding. Similarly, minimum motifs for HLA-A*0201, HLA-A*0101, HLA-A*0301, HLA-A*1101, and HLA-A*2401 were refined by assessing relative acceptance of different amino acid substitutions at the dominant anchor positions and including those tolerated into an expanded motif (18). Minimum motifs for these alleles predict only 27% of the high affinity binders from 240 possible nonamer peptides of the human papillomavirus type 16 E6 and E7 proteins, but expanded motifs were present in 73% of the high affinity binders (19). Validation that expanded motifs improve epitope identification awaits determination of whether these predicted peptides are dominant epitopes in CTL responses to papillomavirus.

Use of ever-expanding motifs to predict peptide-binding strength presumes each amino acid in the sequence contributes independently to affinity. However, this assumption may sometimes come unstuck. Indeed, in comparing their high and low affinity OVA peptides, Chen et al. (14) could not discern the structural basis for high affinity binding by $OVA_{257-264}$. Reciprocal substitutions between $OVA_{257-264}$ and OVA_{55-62} failed to identify single positions that could explain the affinity differences; rather the effect was attributed to the collective sequence. While the essence of an epitope that gives enhanced affinity for class I resists rationalization, identification of the most immunogenic peptides may still require direct assessment of relative affinity for the presenting class I molecule.

Although peptide affinity for class I molecules clearly has a role in selecting determinants recognized by CTL, consideration of factors besides the chemistry of peptide binding may also be necessary if epitope prediction is to be successful (Table 2). Illustrating this point is a naturally processed decamer peptide from calreticulin bound by HLA-A*0201 which has considerably less affinity than the related but unnatural nonamer peptide lacking the COOH terminal amino acid (20). The failure of HLA-A*0201 to present the nonamer peptide in vivo points to the importance of processes involved in peptide supply.

The influences of proteolytic mechanisms, peptide stability, peptide transport, and the T cell repertoire on CTL responses have been relatively ignored. The ability of 20S proteasomes to process ovalbumin has been examined using an in vitro assay system, and generation of the dominant CTL epitope $OVA_{257-264}$ was observed but the OVA_{55-62} peptide could not be detected (21). Thus although Chen et al. (14) show pep-

Table 2. *Factors during Antigen Processing and Presentation In Vivo which May Influence the Ability of a Peptide to Form a CTL Epitope*

Concentration of antigenic protein at the site of intracellular processing

Ubiquitination of antigenic protein to target it for processing

Specificity of the proteolytic enzymes involved in antigen processing

Rate of peptide generation (influenced by protein unfolding; sequences flanking the peptide)

Stability of peptide (rate of peptide degradation)

Selective transport of peptides into the endoplasmic reticulum

Selective peptide binding by class I molecules

Kinetics of peptide binding and consequent influence on competiton with other class I-binding peptides

Efficiency of peptide presentation to CTL (influenced by the number of class I-peptide complexes and presence of co-stimulatory molecules at antigen-presenting cell surface)

Presence of CTL with appropriate peptide specificity

CTL with appropriate peptide specificity may be rendered anergic due to cross-reactivity with self peptides

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tide affinity for class I can offer a complete explanation for the immunodominance of OVA$_{257-264}$, inefficient epitope generation may also contribute to failure of OVA$_{55-62}$ to induce a strong CTL response. To fully evaluate the latter possibility, further experiments may need to assess peptide generation by 26S proteolytic complexes since processing of OVA is dependent upon ubiquitination of the protein (22). Confirmation that predicted CTL epitopes can be generated in vivo will be crucial if synthetic versions are to be useful as vaccines against intracellular pathogens. There is obviously no point in promoting a CTL response to a predicted antigen if it is never presented during infection in vivo.

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