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The Importance of Dominant Negative Effects of Amino Acid Side Chain Substitution in Peptide-MHC Molecule Interactions and T Cell Recognition

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Abstract. Previous studies on the role of specific residues of the peptide or MHC molecule in Ag presentation have revealed the sensitivity of this complex system to even small changes in structure. In our study, we have analyzed the effect of amino acid substitution in a major CD4+ T cell determinant (T1) of HIV-1 gp160 on binding and recognition in the context of various EαEβ MHC class II molecules. Individual alanine substitutions at all but three positions had little or no negative effect on either MHC binding or recognition by a specific T hybridoma, whereas substitutions with larger side chains often diminished reactivity. A poly-alanine peptide containing only four of the original residues was an effective MHC class II binder and in vivo immunogen, although lacking the ability to stimulate the hybridoma. Replacement of a glutamic acid in T1 with alanine or a size-conservative, uncharged glutamine, but not a negatively charged aspartic acid produced a peptide at least 100-fold more potent than the parent peptide, indicating an inhibitory effect of the negative charge. Conversely, substitution of a glutamic acid for valine at position 29 in the floor of the peptide binding site of the EαEβ molecule decreased functional presentation of this peptide by more than 2 logs. However, these two effects of glutamic acid were not complementary and were mediated by distinct mechanisms, as the change in the peptide altered the extent of binding to class II, but the change in the MHC molecule decreased recognition without inhibiting peptide binding. Taken together, the data all suggest the conclusion that changes in side-chains of peptides and MHC molecules affect Ag presentation and T cell stimulation most often by introducing dominant negative or interfering groups that prevent or alter the pattern of binding events primarily mediated by a very limited number of other residues in the Ag or presenting molecule. These results have important implications for understanding the biochemistry of peptide-MHC-TCR interactions and for the possible design of vaccines both more potent and less subject to allele-specific limitations on immunogenicity. *Journal of Immunology,* 1993, 150: 331.

Priming and restimulation of T cells is dependent on both quantitative and qualitative aspects of the binding reactions among antigenic peptides, MHC-encoded molecules, and the T cell αβ receptor (1, 2). The extent to which any given peptide and MHC molecule stably associate is dependent on the primary sequence of the peptide and on allelic variation in the

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residues that line the binding groove of class I and class II MHC molecules (3, 4). Poor peptide binding by specific MHC molecules shows a very good correlation with failure to induce primary T cell responses in vivo (5, 6). This allele and peptide-specific limitation on Ag presentation appears to underlie most classical immune response gene defects and much of the phenomenon of MHC-restricted T cell Ag recognition (5, 7, 8).

More recently, experimental evidence has accumulated indicating that alterations in the structure of the immunizing peptide-MHC pair can interfere with the stimulation of primed T cells by qualitative, not quantitative, changes in the ligand available to the receptor (8–17). This can involve direct receptor contact sites on the original peptide or MHC molecule, or alterations of other sites that by conformational or steric effects prevent effective interaction with the preselected TCR. Changes in the structure of the peptide-ligand pair that occur without alteration in overall ligand density on the APC most likely account for the observation that certain amino acid replacements in either synthetic peptide Ag or MHC molecules permit stimulation of some but not other T cells specific for the starting peptide-MHC combination.

The latter findings, when combined with the emerging evidence in class I models for a very limited number of key peptide residues essential to effective binding to specific allelic class I products (18–21), raise the possibility that much of the fine specificity of T cell responses revealed by peptide or MHC substitution studies corresponds to interference with a small subset of necessary binding interactions rather than the elimination of one among many necessary positive binding events. Corollaries of this hypothesis are that substitution of small side-chain residues at any of many positions in a peptide would have little effect on binding or recognition, that removal of bulky charged or hydrophobic residues might even improve peptide-class II interaction, and that peptides in which most residues are replaced by such noninterfering amino acids would retain their immunogenicity, even if generating T cell responses of somewhat different specificity.

To date, limited experimental support for each of these possibilities has been reported (18, 22–26). In our study, we demonstrate all these predicted effects in a single model system involving a previously defined 16 residue HIV-1 gp160 determinant (27), the EαEβ^k molecule, and a T hybridoma specific for this pair. Individual alanine substitution at all but three positions had little effect on T cell stimulation, whereas bulkier substitutions at other positions also diminished activity. Alanine substitution at as many as 8 of 12 positions in a truncated analog had little effect on peptide binding or in vivo immunogenicity. A single change in the MHC molecule peptide binding site decreased T cell responses by $>10^2$ to 10^3 and a single change in the peptide increased responses by $>10^2$ to 10^3. These changes were not complementary, and peptide competition studies suggested that the peptide change altered binding but the MHC molecule change altered T cell recognition. Taken together, these results support a model in which there are only a few specific residues in peptides required for effective MHC molecule binding. Changes in other positions mediate their effects on priming and restimulation of T cells by interference with the binding or with the recognition dependent on these few key residues.

Materials and Methods

Peptides

Peptides were synthesized either by the tea-bag method (28) or using an Applied Biosystems (Foster City, CA) model 430 A peptide synthesizer, using conventional t-BOC chemistry (29, 30), and cleaved from the resin by liquid HF. They were analyzed by reverse phase HPLC on a C18 column using a gradient of 0.1% TFA in water:0.1% TFA in acetonitrile, and where necessary were further purified by gel filtration on Biogel P4 columns (Bio-Rad, Richmond, CA) in 9% formic acid and/or by preparative reverse phase HPLC using a similar gradient. The peptides were shown not to have any nonspecific toxicity for T cells by testing their effect on the response of the T1-specific hybridoma to anti-CD3 mAb (data not shown). The sequences of the original T1 peptide (27) and the variant peptides used in this study are given in Table I.

Transfectants

Mouse DAP.3 L cells expressing various wild-type or mutant class II $\alpha\beta$ pairs were produced, characterized, and maintained as previously described (7, 8, 31).

Mice

Mice of the indicated strains (male or female) were bred either at Bioqual, Rockville, MD, at Biocon, Rockville, MD, or purchased from The Jackson Laboratories, Bar Harbor, ME. Mice were 6 to 12 wk of age at the time of immunization.

T cell hybridomas

The method of Kappler et al. (32) was adapted for the production of T cell hybridomas specific for the T1 peptide. C3H/HeJ (H-2^k) mice were immunized in base of the tail and the rear footpads with 10 μg of T1 emulsified in CFA. Eight to 10 days later, the mice were killed and the draining lymph nodes were removed for preparation of single cell suspensions. These lymph node cells were stimulated in vitro with 3 μM T1. Four days later, 10% rat T cell monoclone (Collaborative Research, Bedford, MA) was added and after 3 days, the viable blasts were collected on a Ficoll gradient. They were fused by standard

Table I
Peptides used in this study[a]

	428	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443
T1	K	Q	I	I	N	M	W	Q	E	V	G	K	A	M	Y	A
28	–	A	–	–	–	–	–	–	–	–	–	–	–	–	–	–
29	–	H	–	–	–	–	–	–	–	–	–	–	–	–	–	–
30	–	K	–	–	–	–	–	–	–	–	–	–	–	–	–	–
31	–	–	A	–	–	–	–	–	–	–	–	–	–	–	–	–
32	–	–	F	–	–	–	–	–	–	–	–	–	–	–	–	–
33	–	–	G	–	–	–	–	–	–	–	–	–	–	–	–	–
34	–	–	–	A	–	–	–	–	–	–	–	–	–	–	–	–
35	–	–	–	F	–	–	–	–	–	–	–	–	–	–	–	–
36	–	–	–	G	–	–	–	–	–	–	–	–	–	–	–	–
37	–	–	–	–	A	–	–	–	–	–	–	–	–	–	–	–
38	–	–	–	–	Q	–	–	–	–	–	–	–	–	–	–	–
39	–	–	–	–	K	–	–	–	–	–	–	–	–	–	–	–
40	–	–	–	–	–	A	–	–	–	–	–	–	–	–	–	–
41	–	–	–	–	–	L	–	–	–	–	–	–	–	–	–	–
42	–	–	–	–	–	F	–	–	–	–	–	–	–	–	–	–
43	–	–	–	–	–	–	A	–	–	–	–	–	–	–	–	–
44	–	–	–	–	–	–	F	–	–	–	–	–	–	–	–	–
45	–	–	–	–	–	–	I	–	–	–	–	–	–	–	–	–
46	–	–	–	–	–	–	–	A	–	–	–	–	–	–	–	–
47	–	–	–	–	–	–	–	N	–	–	–	–	–	–	–	–
48	–	–	–	–	–	–	–	K	–	–	–	–	–	–	–	–
49	–	–	–	–	–	–	–	–	A	–	–	–	–	–	–	–
50	–	–	–	–	–	–	–	–	D	–	–	–	–	–	–	–
51	–	–	–	–	–	–	–	–	Q	–	–	–	–	–	–	–
52	–	–	–	–	–	–	–	–	–	A	–	–	–	–	–	–
53	–	–	–	–	–	–	–	–	–	I	–	–	–	–	–	–
54	–	–	–	–	–	–	–	–	–	F	–	–	–	–	–	–
55	–	–	–	–	–	–	–	–	–	–	A	–	–	–	–	–
56	–	–	–	–	–	–	–	–	–	–	S	–	–	–	–	–
57	–	–	–	–	–	–	–	–	–	–	K	–	–	–	–	–
58	–	–	–	–	–	–	–	–	–	–	–	A	–	–	–	–
59	–	–	–	–	–	–	–	–	–	–	–	R	–	–	–	–
60	–	–	–	–	–	–	–	–	–	–	–	E	–	–	–	–
61	–	–	–	–	–	–	–	–	–	–	–	–	S	–	–	–
62	–	–	–	–	–	–	–	–	–	–	–	–	L	–	–	–
63	–	–	–	–	–	–	–	–	–	–	–	–	Q	–	–	–
64	–	–	–	–	–	–	–	–	–	–	–	–	–	A	–	–
65	–	–	–	–	–	–	–	–	–	–	–	–	–	F	–	–
66	–	–	–	–	–	–	–	–	–	–	–	–	–	L	–	–
67	–	–	–	–	–	–	–	–	–	–	–	–	–	–	A	–
68	–	–	–	–	–	–	–	–	–	–	–	–	–	–	F	–
69	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I	–
KNQK	K	A	A	A	N	A	A	Q	A	A	A	K				

[a] The sequences of T1, the various mono-substituted analogs, and the polyalanine analog designated KNQK are given in single letter amino acid code. Numbers at the top of each column refer to positions in the HIV-1 IIIB gp 160 protein. Dashes in each row indicate that the residue at that position in that peptide is the same as T1; letters indicate the substitution contained in the analog. For KNQK, the lysine at 439 is the C-terminal residue in the peptide.

procedures to the $\alpha\beta$ BW5147 variant BW1100 (33) and selected in medium containing HAT. The resulting hybridomas were screened using a growth inhibition assay (34). One or two $\times 10^4$ hybridoma cells in 200 µl medium were cultured in the wells of 96-well plates in the presence of 0.5 to 1 $\times 10^5$ irradiated syngeneic spleen presenting cells or 2 $\times 10^4$ transfected L cells (tk-). After 18 to 24 h, 1 µCi of [^{3}H]TdR was added and the cells incubated another 4 h. The cultures were harvested and the incorporated [^{3}H]TdR measured. This method, or the production of IL-2 as measured in a standard CTLL bioassay (8), was used to evaluate the stimulatory capacity of various peptide-MHC molecule combinations.

In vitro competition assay of peptide binding

Peptide binding was measured indirectly using the functional competition method as described by Adorini and Nagy (35). APC (the B cell hybridomas LK 35.2, LB 27.4, or LS 102.9 (36)) were washed three times in PBS and fixed for 45 s in 0.025% glutaraldehyde. The fixation was stopped by addition of 0.2 M glycine in PBS, and the cells were extensively washed. Five $\times 10^4$ fixed cells were incubated for 4 h at 37°C with competitor peptide, then stimulating peptide at a predetermined suboptimal concentration was added and the mixture incubated overnight. The next day the cells were washed and used as stimulators for the T cell hybridoma.

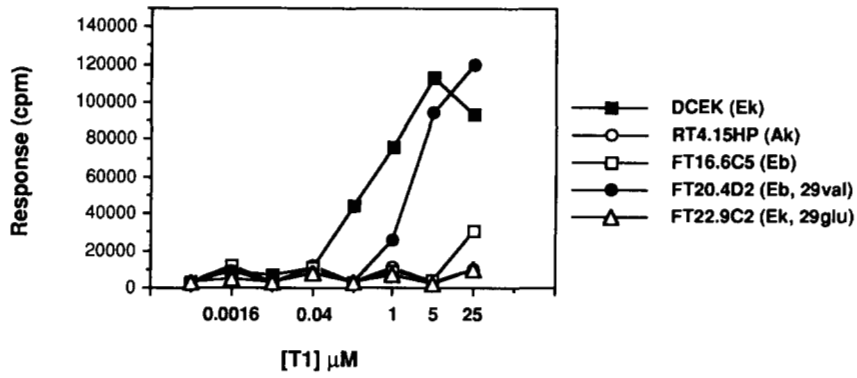

FIGURE 1. Importance of MHC class II polymorphism in presentation of T1. Cytokine production of the C1.1 T hybridoma in response to T1 peptide presented by transfected L cells expressing various MHC class II molecules. Data are expressed as cpm incorporated by the indicator line CTLL exposed to a 1/6 dilution of the supernatant of the stimulated hybridoma.

In vivo immunogenicity assay

Mice were immunized as described for the original T1 determinant above. Primed lymph node cells were purified over nylon wool and 4×10^5 cells were cocultured with 2×10^5 irradiated syngeneic spleen cells in the presence of the indicated concentration of various peptides. On day 4, the cultures were pulsed with 1 μCi of [^{3}H]TdR and incorporation of the label assessed 16 h later.

Results

Effects of MHC class II polymorphism on presentation of T1

T1 is a 16 residue peptide whose sequence corresponds to positions 428–443 of the HIV-1 IIIB gp160 molecule, and which has been shown to be an immunodominant determinant in this protein for presentation by MHC class II molecules in several strains (27, 37). The H-2^k haplotype was a particularly good responder in the initial strain survey, and therefore C3H/HeJ H-2^k mice were immunized to provide a source of primed T cells for preparation of a T1-specific T hybridoma. One such hybrid, C1.1, has been used to study the effects of MHC and peptide residue substitutions on Ag recognition.

L cell transfectants were used to determine whether the $A\alpha^kA\beta^k$ or $E\alpha^kE\beta^k$ molecules presented the T1 determinant to C1.1. Figure 1 shows that this hybrid responds to T1 plus $E\alpha E\beta^k$ but not T1 plus $A\alpha^kA\beta^k$. This hybrid also does not respond to T1 presented by the very closely related class II molecule $E\alpha E\beta^b$. Because such clear-cut discrimination of Ag presented in the context of E^k vs E^b was uncommon in our previous studies (7), we further examined the C1.1 response to T1 presented by recombinant Eβ-chain containing molecules. As in the case of cytochrome *c* determinants (7, 8), we found that residue β29, modeled to lie in the floor of the peptide binding groove in a region inaccessible to the TCR (4), determined the response of this hybridoma of T1. The presence of the $E\beta^b$-derived glutamic acid at this position conferred on an otherwise *k* molecule the nonstimulatory phenotype of the molecule containing $E\beta^b$, whereas a class II molecule containing the β29 valine from *k* in the context of the remaining polymorphic residues from *b* was capable of effective C1.1 activation.

In the cytochrome model, two effects of β29 polymorphism can be discerned (8). For the pigeon cytochrome determinant, the β29val to β29glu change interferes with the ability of the molecule to bind peptide. For moth cytochrome, however, this exchange preserves quantitative binding but alters the structure of the peptide-MHC complex formed so that T cells with certain TCR can no longer respond. Attempts to discriminate between these possibilities were hampered by a failure of the T1 peptide to show significant inhibition of cytochrome binding in a classical functional competition assay or for a biotinylated form of T1 to show detectable binding to transfected L cells expressing $E\alpha^kE\beta^k$ (data not shown). The eventual classification of the defect as one of recognition rather than binding required identification of a variant T1 peptide with significantly better binding capacity to E^k (see below).

Effects of single alanine substitutions on C1.1 hybridoma responses to T1

To examine the role of individual residues and groups of residues on the ability of the T1 determinant to bind to class II molecules and to be recognized by T cells, we produced variant 16 residue peptides containing three distinct substitutions at each position except for the amino-terminal lysine and carboxyl-terminal alanine. Residues were individually replaced by alanine (or if already alanine, by serine), to determine the effect of minimizing side chain function by deleting all of the side chain except the β-carbon. Each analyzed position was also replaced by a nonconservative amino acid or a chemically conservative choice, to help distinguish requirements for positive function from possible interfering effects of inappropriate residues. Table 1 summarizes the set of peptides used for these experiments and Figure 2 presents the results of dose-response titrations of the ability of the peptides to stimulate the C1.1 hybridoma in the context of E^k. The most notable finding was that only at three positions

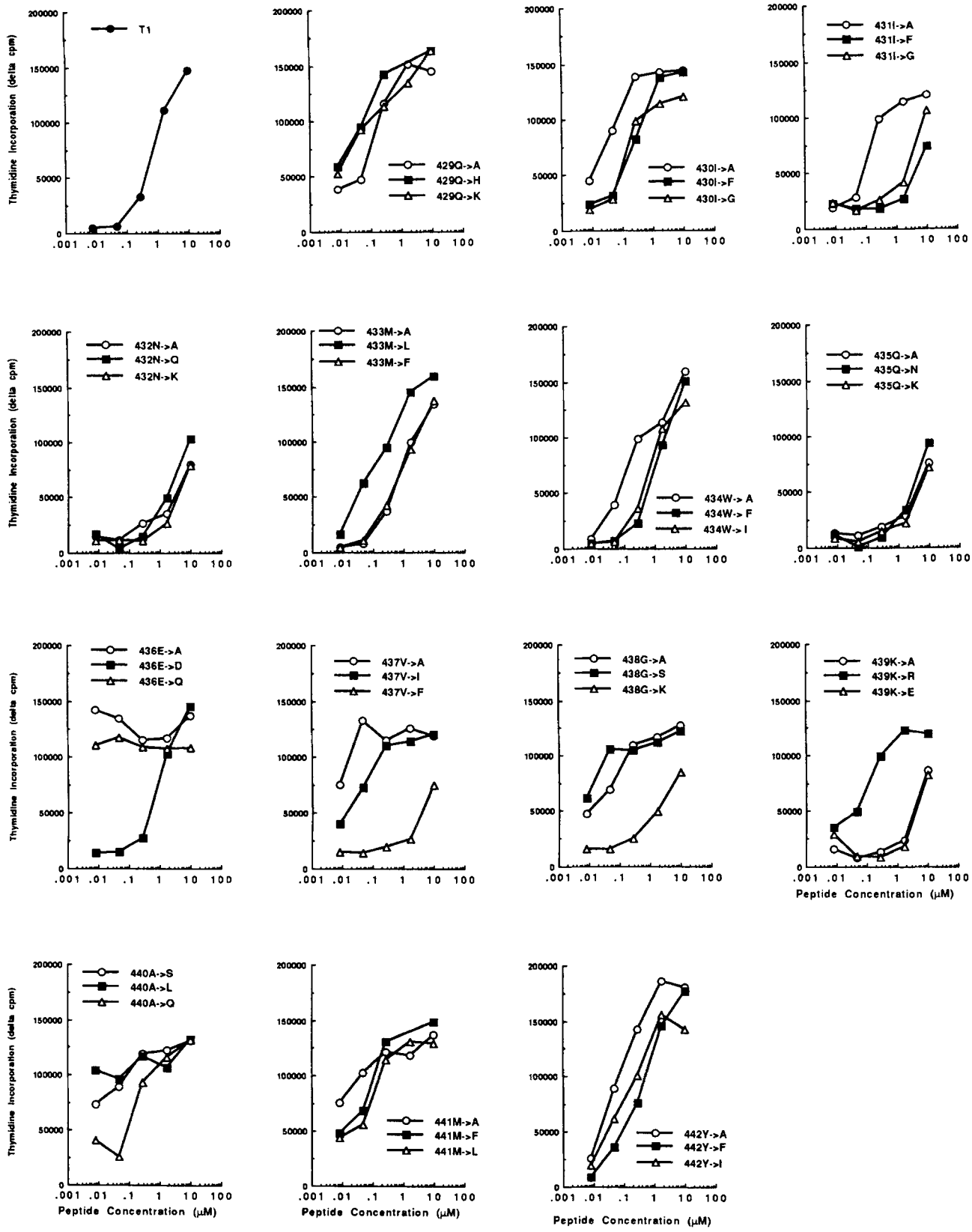

FIGURE 2. Ability of single residue-substituted T1 peptide variants to stimulate the C1.1 hybridoma in the presence of E^k. Cytokine production by the C1.1 T hybridoma was measured after stimulation with various concentrations of the indicated peptides in the presence of E^k-expressing splenic APC. Data are expressed as cpm incorporated by CTLL indicator cells in the presence of a 1/6 dilution of supernatant from the stimulated hybridoma cells.

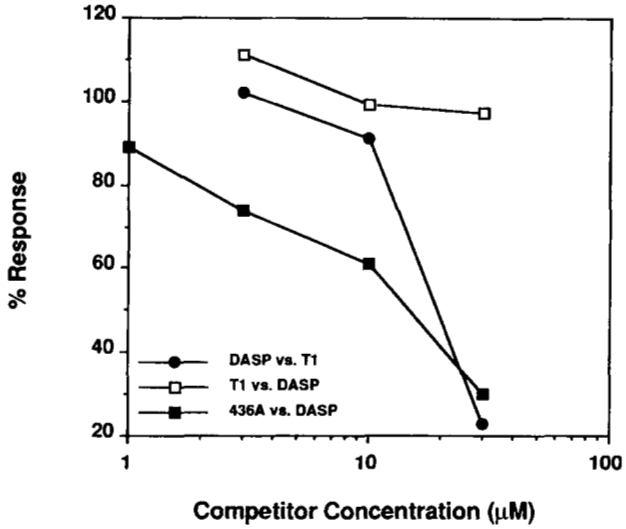

FIGURE 3. The 436A T1 analog but not T1 itself competes with a synthetic cytochrome peptide for MHC class II binding. The relative molar efficiency of peptide binding to the E^k MHC class II molecule was evaluated by measuring the capacity of a peptide to inhibit E^k-dependent Ag presentation of a noncross-reactive peptide to an appropriate T cell hybridoma. Data are expressed as "% response," with 100% being equal to the response of the hybridoma to the stimulatory peptide in the absence of competitor. *DASP,* des-ala-splice form of moth cytochrome (7, 8).

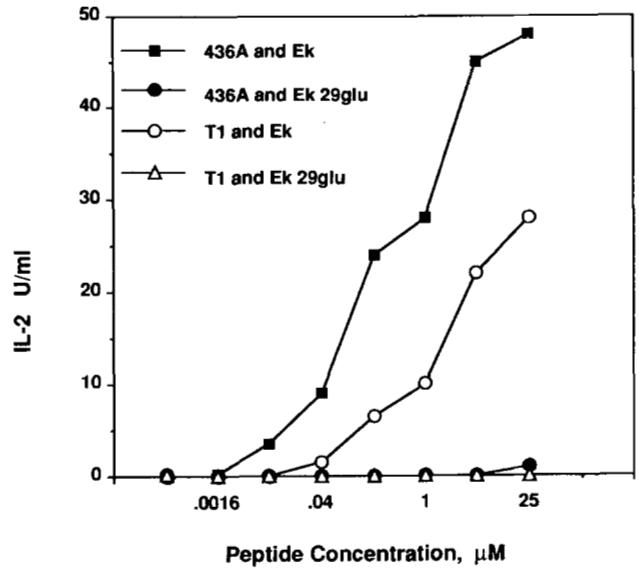

FIGURE 4. Glutamic acid replacements in the T1 peptide and in the E^k MHC class II molecule have noncomplementary effects on Ag presentation. Cytokine production by the C1.1 T hybridoma in response to T1 and the 436A analog presented by either wild-type E^k expressing transfectants or transfectants expressing an MHC class II molecule with an $E\beta^k$ chain in which the valine at residue 29 is replaced by glutamic acid. Data are expressed as IL-2 units.

(432N, 435Q, and 439K) did alanine substitution lead to a markedly decreased response equivalent to the decrease seen with a nonconservative substitution at the same position; two of these were also the positions showing functional loss with a conservative substitution. Thus, these three positions are candidates for residues essential in a positive sense for presentation of the T1 determinant as seen by the C1.1 hybridoma.

Two other important results were obtained in this screen. First, several positions (431, 437, 438, 440) in addition to the three noted above were identified at which nonconservative, but not alanine or conservative substitutions, resulted in markedly decreased stimulation. This pattern was consistent with the introduction of a negative effect at a position whose original residue did not contribute in a required manner to the activity of the peptide. Second, at position 436, replacement of glutamic acid with alanine increased the stimulatory properties of the peptide by $>10^2$, an effect also seen with the size-conservative, uncharged glutamine substitution, but not the charge-conservative aspartic acid replacement, indicating a deleterious effect of a negative charge at this position in the peptide.

Characterization of 436A substitution for effects on class II binding vs T cell recognition

This potent stimulatory peptide with an alanine substitution at position 436 was tested in a competition binding assay using a synthetic cytochrome *c* peptide and a cytochrome-E^k-specific hybridoma. In contrast to the parent T1 peptide, which was unable to inhibit cytochrome peptide responses under these conditions, the 436A peptide was an excellent competitor (Fig. 3). Similar results were obtained in an experiment carried out using the T1 and 436A peptides as competitors for binding to purified $E\alpha^kE\beta^k$ molecules in detergent (data not shown), arguing strongly that the substitution did not act merely to protect the peptide against proteolysis during the culture period, but rather that the removal of the glutamic acid had significantly affected binding of the peptide to the class II molecule. Because the effect of position 29 on the pigeon cytochrome response is control of peptide binding, because the alanine substitution at 436 seemed to act by increasing binding, and because it could be imagined that an incompatible charge interaction between the glutamic acid at 436 in T1 and the glutamic acid at β29 in the MHC molecule might account for the failure of presentation with the original T1-E^b MHC class II combination, we examined whether the 436A peptide would be efficiently presented by E^b or E^k-β29glu class II molecules. Figure 4 shows that contrary to expectation, the same 2 to 3 log difference in presentation capacity of E^k vs E^b seen with T1 was retained when examining 436A. This is most compatible with the effect of β29glu being on recognition and not binding. Evidence supporting this conclusion is presented in Figure 5*A*, which shows that the 436A peptide

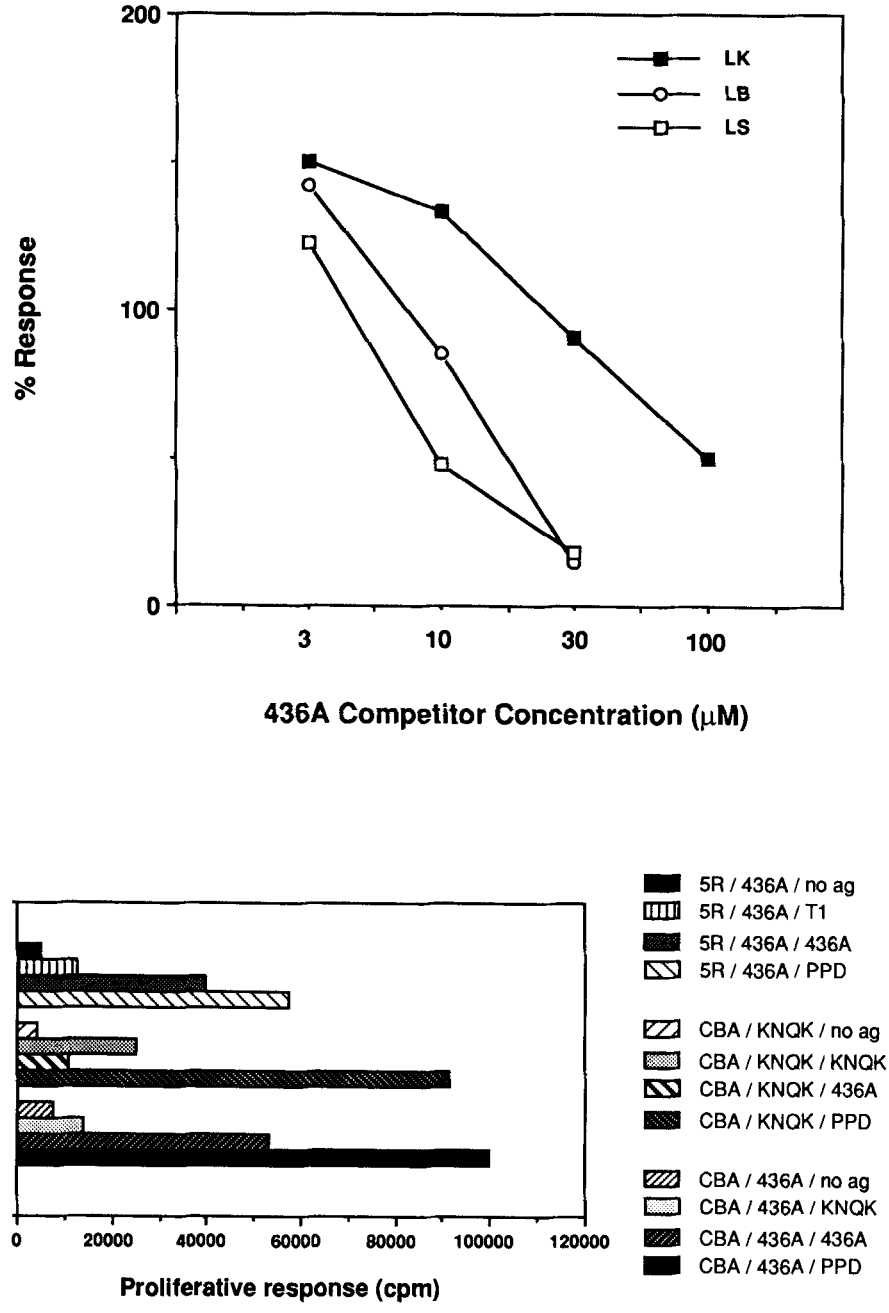

FIGURE 5. *A*, The lack of effective C1.1 stimulation by the 436A T1 analog in the context of $E\beta^b$ is not due to poor peptide binding. A competition assay performed as in Figure 3 was used to evaluate the ability of the 436A peptide to bind to MHC class II molecules containing glutamic acid at position 29 in the Eβ chain. Data are expressed as in Figure 3. *B*, In vivo immunogenicity of substituted T1 peptides. Lymph node T cell proliferative responses to T1, the 436A analog, and the KNQK peptide were evaluated after priming with either the 436A or KNQK peptides in mice expressing either E^k or E^b. Results are expressed as absolute cpm for each culture condition. In the legend, the immunogen is listed after the strain, followed by the Ag used for stimulation in vitro.

competes the binding of a synthetic cytochrome peptide to both E^k and E^b to a similar extent, and Figure 5*B*, which shows that immunization of B10.A(5R) (E^b) mice with the 436A peptide elicits a T cell proliferative response, as expected if binding to E^b was effective.

MHC class II binding and T cell stimulation by peptide retaining only the residues affected by alanine substitution

Given the above results, it appeared that only a few residues of the original T1 peptide were critically required for binding to and effective presentation by E^k molecules, consistent with the results of Maryanski et al. (18) and Jardetzky et al. (26). This was directly examined by synthesizing a truncated 12 mer peptide (residues 428–439, termed KNQK) containing only alanine except for the three residues affected adversely by alanine substitution (432, 435, and 439) plus the NH_2-terminal lysine at 428. The data in Figure 6 show that KNQK is an effective E^k binder, able to compete cytochrome peptide presentation in a manner similar to that of the HIV-1 peptide with only alanine substituted at 436. KNQK was also immunogenic in H-2^k mice, eliciting T cells responding well to restimulation with itself, though only weakly cross-reactive with the 436A peptide (Fig. 5*B*). The C1.1 hybridoma did not

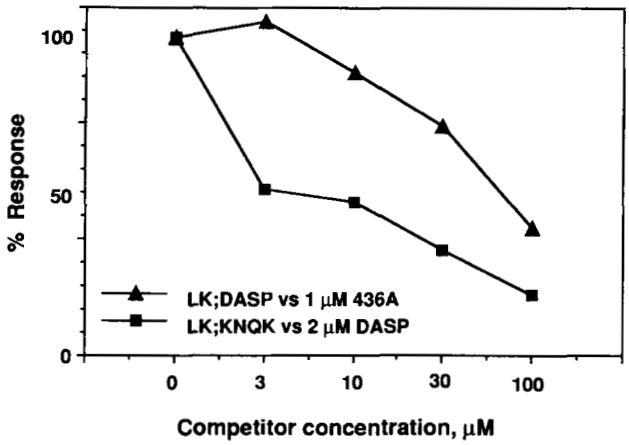

FIGURE 6. Ability of the KNQK peptide to bind to E^k. The competition assay method used in Figure 3 was used to assess the ability of the KNQK peptide to bind to E^k. Data are expressed as in Figure 3.

respond to the multiply substituted peptide at all (not shown). These data indicate that this minimum peptide retains MHC binding capacity, but only partially shares the epitopic structure of the 436A or T1 peptides.

Discussion

The notion that only a few residues are of critical importance to peptide-MHC molecule interaction has recently been given strong support by analysis of peptides eluted from class I molecules. Both sequencing of the bulk eluted material from a single allelic class I product (19) and analysis of individual peptides recovered from class I molecules show that one to two residues (20, 38), one of which is typically C-terminal, are highly conserved among such naturally bound ligands. Our results extend this theme to class II binding, demonstrating that only four of 16 original residues in the T1 determinant need be retained to maintain effective interaction with and presentation by the $E\alpha E\beta^k$ molecule. These findings are similar to previous reports of polyproline/polyalanine peptides with only one or two residues from an immunogenic peptide being required for binding to the class I (18) or class II molecule (26) presenting the original Ag.

Our data also indicate that only a few specific amino acids are essential for retention of epitopic structure sufficient for effective TCR engagement and T cell response. This contrasts with previous studies that suggested that many substitutions interfered with such T cell recognition without eliminating effective MHC class II binding. Reexamination of these earlier studies reveals that in most cases, the deleterious effects of single substitutions on response resulted from the introduction of nonconservative substitutions into the peptide. Most, but not all (39), investigations in which alanine or conservative replacement was used as a method show markedly fewer sites that grossly disrupt effective presentation to specific T cells, consistent with our results. Nevertheless, although many single alanine substitutions in the T1 peptide preserved antigenicity, the multiply substituted KNQK peptide, although able to bind to $E\alpha E\beta^k$ and prime T cells, was not able to stimulate the C1.1 hybridoma. This could be due either to removal of several interactions, each of which is dispensable alone, but which when summed prevent T cell stimulation (40), or to the removal of side groups constraining the orientation of the essential epitopic residues, so that a new ligand conformation is presented by KNQK even though all epitopic residues from the original peptide are preserved. It is not possible to discriminate between these models with present methods.

In addition to the lack of negative effects at most positions when alanine was introduced, the marked gain in function on removal of the glutamic acid at 436 also is consistent with this idea of dominant interference. Thus, either alanine or glutamine, which is of similar molecular dimensions to glutamic acid but lacking the negative charge, produced a heteroclitic peptide, whereas aspartic acid, smaller than the original glutamic acid but retaining the negative charge, was a poor stimulator. These data are consistent with the gain in function being due to the removal of a negative effect of the charge of the original residue, rather than to the introduction of a more effective binding residue.

A similar theme emerges from the analysis of the effects of polymorphism in the MHC molecule on presentation of T1-related peptides. E^k but not E^b was able to present T1 to the C1.1 hybridoma. However, competition studies using the 436A analog showed that this was not due to defective binding, but to an inappropriate ligand structure for receptor engagement. The same peptide was bound equally well by the two class II molecules and the difference between the MHC molecules was only at a site believed to be unavailable for direct TCR interaction (4, 8). Thus, the effect of the MHC change was to interfere with appropriate three-dimensional display of those residues necessary for binding. Offering this peptide-MHC complex to other receptors by immunizing naive animals showed that the peptide-MHC complexes were suitable for stimulation of T cells of distinct fine specificity.

Overall, these data indicate that the amino acids of the peptide and MHC molecule that jointly constitute the ligand for the TCR can be divided into two major subsets: a small number of residues whose chemical structure is essential to important binding events between peptide and MHC and between TCR and peptide-MHC complexes, and a larger number of positions that must be permissive for these events but that are not necessary for them (2, 24). Changes in the former sites, even conservative ones, most often result in a drastic loss of function (MHC binding or T cell stimulation, respectively). Alterations in the latter

positions that do not introduce side chains perturbing the proper functioning of the first set of residues have little or no discernable effect on the binding reactions involved in Ag presentation or recognition. However, many nonconservative changes at these latter positions have such perturbing effects, either reducing peptide-MHC association or altering the ligand conformation so that effective TCR binding is eliminated.

This model of peptide-MHC-TCR interaction as requiring few specific binding events, but potentially interfered with by incompatible residues at many other positions is consistent with data from other studies on Ag presentation, as well as investigations of assembly and transport of MHC class II molecules, CD8 coreceptor-MHC molecule binding, and Ag-antibody interactions (41). Wei et al. (42) have found that effective Ag presentation occurs with the presence of any of several residues at a single polymorphic position in a class II molecule, and that no particular residue is required, although certain amino acids at this position are very deleterious to presentation. In the case of class II dimer assembly and transport, alanine substitutions at polymorphic positions in the α1 domain did not affect these parameters (16), whereas replacement with naturally occurring polymorphic amino acids at the same positions interfered with these processes (43–47). For CD8 binding to MHC molecules, alanine substitutions at only a few sites have marked effects on function (48), although substitution of bulky or charged groups at many of the positions where alanine is tolerated is very deleterious.

The importance of amino acids causing negative effects rather than lacking a required binding potential, whether in initial peptide-MHC association or later peptide-MHC molecule restimulation of primed T cells, has significant implications for the design of subunit vaccines. The gain in potency exhibited by replacing 436E with alanine suggests that it may be possible to identify in some or many Ag sites interfering with effective MHC interaction, and by replacing these residues, to both markedly increase the potency of synthetic vaccines and broaden the range of allelic MHC molecules to which binding is effective. If such altered antigenic structures epitopically cross-react with the natural material produced during infection, as was the case here, then such engineered molecules may be highly effective vaccines in the outbred, allelically diverse human population. It has been shown that the number of peptide-MHC complexes needed to restimulate primed T cells is substantially less that required to initiate responses (49), so the decreased ligand density produced on natural infection may still yield a level suitable for restimulation of cells primed using the more potent synthetic Ag.

The ability of MHC molecules to bind a broad range of peptides most likely derives from requiring only a few key positions to nucleate the binding event (18–21, 25, 26). Pathogens may escape immune destruction, not by eliminating such necessary residues, but by introducing interfering residues within the adjacent sequence. The strategy of removing such negative residues from vaccine immunogens may thus overcome this evolved character of natural Ag, permitting more effective immunization than would occur by direct infection. Thus, the T1 (436A) peptide described here might be a much more potent immunogen for priming helper cells for subsequent responses to the HIV-1 gp160 than the natural sequence T1 peptide itself.

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