MECHANISMS INFLUENCING THE IMMUNODOMINANCE OF T CELL DETERMINANTS

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T cells recognize foreign antigens usually as peptides associated with self-molecules encoded by the MHC (reviewed in reference 1). Thus, the binding of immunogenic peptides to MHC molecules is a prerequisite for T cell activation (2). As demonstrated for a class I molecule, each MHC glycoprotein appears to have a single binding site for peptides (3). Because the number of antigenic peptides to be presented to T cells is very large, each MHC molecule must have the ability to bind many peptides of unrelated sequences, for example, several of those generated by the processing of a single protein antigen (4, 5). It would therefore be expected that T cells respond to several different epitopes on a single foreign protein. However, this is not the case: T cell responses are generally specific for a few, and often only one, of the peptides derived by processing of a protein antigen. These T cell epitopes are referred to as immunodominant. Immunodominance of selected T cell epitopes has indeed been demonstrated for several proteins including cytochrome *c* (6), lysozyme (7, 8), myoglobin (9), ovalbumin (10), influenza virus hemagglutinin (11), staphylococcal nuclease (12), ragweed allergen (13), bacteriophage λ repressor (14), and acetylcholine receptor (15). Furthermore, epitope immunodominance has been observed not only in class II MHC-restricted T cell responses, but also in class I–restricted cytotoxic T cell responses (16).

The molecular events involved in antigen processing are still poorly understood, but the emerging view indicates that protein antigens undergo a limited degree of proteolysis in an acidic intracytoplasmic compartment, the resulting peptides associate with MHC molecules and are transported to the cell surface complexed with these molocules (17). Accordingly, several mechanisms can be anticipated to influence T cell responsiveness to antigenic determinants; (*a*) availability of a given peptide after antigen processing, (*b*) its ability to bind to MHC molecules, (*c*) competition between different peptides derived from the same protein antigen (or other proteins) for binding to MHC molecules, (*d*) presence of T cells in the repertoire able to recognize the peptide-MHC complex, and (*e*) preferential activation of helper rather than suppressor T cells by a given peptide. The results presented here provide evidence for four of these five mechanisms operating during the T cell response to hen egg-white lysozyme (HEL).[1]

[1] *Abbreviation used in this paper:* HEL, hen egg-white lysozyme.

Materials and Methods

Mice. C3H and B10.A(4R) mice of either sex were obtained from Olac Ltd. (Bicester, UK) and used when 2–3 mo old.

Antigens. HEL three times recrystallized was obtained from Sigma Chemical Co. (St. Louis, MO). Peptides were synthetized by the Merrifield technique (18) and purified by reverse-phase high-pressure liquid chromatography.

T Cell Proliferation. Mice were immunized subcutaneously at the tail base and into the hind footpads with 3.5–7 nmol antigen (HEL or peptides) emulsified in CFA containing H37Ra mycobacteria (Difco Laboratories, Detroit, MI). Eight days after immunization, lymph nodes draining the injection site were removed and 5×10^5 cells were cultured in wells of microtiter plates (Costar, Cambridge, MA) in RPMI 1640 medium (Gibco Laboratories, Basel, CH) supplemented with 2 mM L-glutamine, 50 μM 2-ME, 50 μg/ml gentamicin (Sigma Chemical Co.), and 2.5% pooled human AB serum with the indicated antigen concentrations. Cultures were set up in triplicates from pooled lymph node cells of two mice per group. Cultures were incubated for 3 d in a humidified atmosphere of 5% CO_2 in air and were pulsed 10 h before harvesting with 1 μCi [^{3}H]TdR (40 Ci/mmol; The Radiochemical Center, Amersham, UK). Incorporation of [^{3}H]TdR was measured by liquid scintillation spectrometry. The MHC class II molecule involved in peptide 1–18 recognition by T cells was determined by inhibition of cell proliferation with anti-Ia mAbs. Ascitic fluid from hybridoma 11-5.2 (anti-I-A^k) or culture supernatant from hybridoma Y17 (anti-I-$E^{b,k,r,s,v}$) were added to cultures in the presence of antigen at a final dilution of 1:200 or 1:2, respectively.

T Cell Hybridomas. T cell hybridomas were established by polyethylene glycol–induced fusion of lymph node cells with the thymoma line BW5147. Lymph node cells were obtained from mice immunized with HEL-CFA and restimulated in vitro with 7 μM HEL for 3 d before cell fusion. After fusion, cells were cultured in selective medium and growing hybrids were screened for IL-2 production in response to HEL and syngeneic APC. Cultures containing 5×10^4 T hybridoma cells and 2.5×10^4 LK 35.2 (H-$2^{k/d}$) cells (obtained from the American Type Culture Collection, Rockville, MD) were set up, without antigen or with 7 μM HEL, in 0.2 ml of culture medium. After 24 h of culture, 50-μl aliquots of supernatant were transferred to microculture wells containing 10^4 CTLL cells and, after an additional 24 h of incubation, the presence of IL-2 was assessed by [^{3}H]TdR incorporation during the last 4 h of culture. Positive hybridomas were then screened on a panel of synthetic HEL peptides. Hybridomas reactive to a HEL peptide were cloned by limiting dilution and individual clones were retested for IL-2 production. The Ia molecule involved in antigen recognition by individual T cell hybridomas was determined by comparing the IL-2 production obtained in the presence of irradiated (2,400 rad) spleen cells from C3H (expressing I-A^k and I-E^k molecules) and B10.A(4R) (expressing only I-A^k molecules) mice.

Competition for Antigen Presentation. Competition for antigen presentation was performed on fixed LK 35.2 cells. Cells were fixed by resuspending them in 0.025% glutaraldehyde (Fluka, Buchs, Switzerland), and after 30 s the reaction was stopped by addition of 0.2 M glycine. Fixed LK 35.2 cells (5×10^4/well) were incubated with the indicated concentration of competitor (1–200 μM) and with a suboptimal concentration (0.5–1 μM) of antigenic peptide for 18 h at 37°C. The cells were then washed three times and 5×10^4 T hybridoma cells were added. After 24 h, 50-μl aliquots of culture supernatant were transferred to microculture wells and IL-2 production was quantitated as described before. The degree of competition for antigen presentation was determined by the regression line obtained by plotting percent inhibition of antigen presentation against the concentration of the inhibitory peptide used.

Results

Responsiveness of Mice Expressing I-A^k and I-E^k Molecules to HEL Peptides. C3H mice expressing both I-A^k and I-E^k molecules and B10.A(4R) mice expressing only I-A^k molecules (19) were immunized with HEL, and their lymph node cells were restimulated in vitro with a panel of 17 synthetic peptides encompassing the entire HEL sequence (Fig. 1). T cell proliferation was observed in both strains to peptides 46–61,

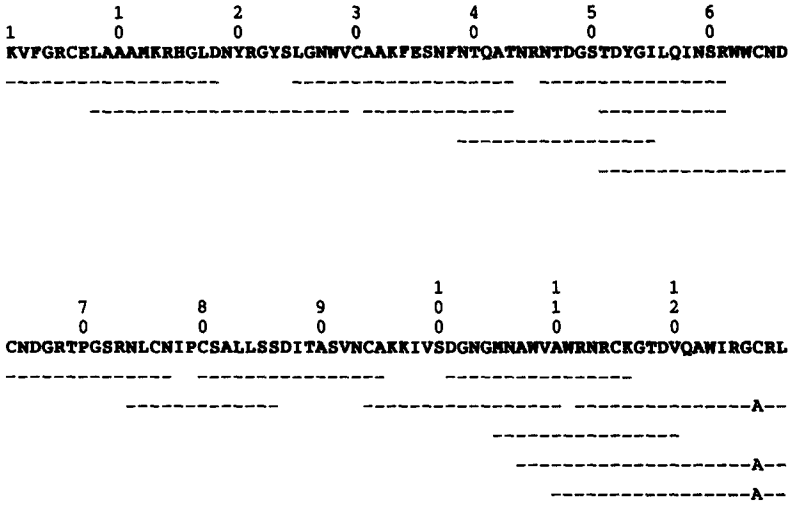

FIGURE 1. Synthetic HEL peptides used in the present study. The HEL amino acid sequence is shown by the single letter code. In peptides 107–129, 110–129, and 112–129 the cysteine at position 127 has been substituted with alanine to prevent formation of a disulfide bridge with the cysteine at position 115.

51–66, 107–129, 110–129, and 112–129 (Fig. 2). In addition, cells from C3H mice responded to peptide 1–18. Thus, at least three immunodominant T cell epitopes could be identified by using these peptides. Since T cell proliferative responses are restricted by class II MHC molecules (20, 21), the finding that both C3H and B10.A(4R) mice respond to sequences 51–61 and 112–129 indicates that these peptides are recognized in association with the class II molecule shared by these strains, that is, I-A^k. In contrast, the T cell determinant included in the sequence 1–18 is recognized in association with I-E^k molecules, since it restimulated HEL-primed T cells from C3H mice, but not those from B10.A(4R) mice. All the other peptides tested failed to restimulate HEL-primed T cells.

Interaction of HEL Peptides with I-A^k and I-E^k Molecules. To investigate the interaction of HEL peptides with class II molecules, we determined the ability of these peptides to compete for presentation with antigenic peptides to T cell hybridomas. Previous work has demonstrated that the competition of peptides for antigen presentation correlates well with their capability to bind to purified Ia molecules (4, 22). In the competition assays we used glutaraldehyde-fixed LK 35.2 cells (expressing both I-A^k and I-E^k molecules) as APC, and two pairs of T cell hybridomas, restricted by either I-A^k or I-E^k, each specific for a different HEL peptide (Table I). Three of these hybridomas recognize immunodominant peptides (we have isolated several independent hybrids with these specificities), whereas the peptide-MHC specificity expressed by hybridoma 2H6.4 is rather rare in our hybridoma collection. Competition was assessed as a decrease in IL-2 production by T cell hybridomas in response to antigen and APC. A typical experiment is shown in Fig. 3 where the response of hybridoma 2C8.4 to peptide 110–129 was used as a read-out system to evaluate the capacity of all nonstimulatory HEL peptides to inhibit antigen pre-

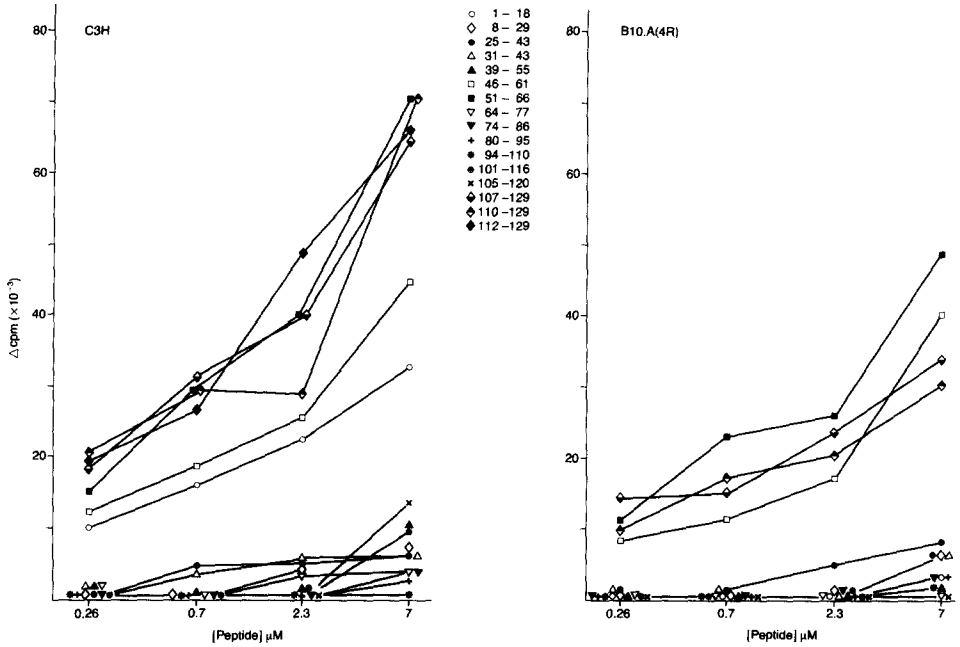

FIGURE 2. I-A^k- and I-E^k-restricted T cell-dependent proliferative response of lymph node cells to HEL peptides. C3H and B10.A(4R) mice were immunized subcutaneously into the hind footpads and at the tail base with 7 nmol/mouse HEL in CFA and 8 d later draining lymph node cells were cultured with different concentrations of the indicated synthetic HEL peptides. Data are presented as mean [^{3}H]TdR incorporation and standard deviation of triplicate cultures with background values (medium alone) subtracted (Δ cpm).

sentation. Strong competition is exerted by peptides 46-61, 51-61, and 51-66, intermediate competition by peptides 25-43, 31-43, and 74-86, and weak competition by peptides 101-116 and 105-120. The other peptides did not exhibit discernible competition in the concentration range used (up to 200 μM). The compilation of data from four to six experiments for each of the four hybridomas is presented in Table II. A very good correlation was found between the competition values obtained for each pair of hybridomas restricted by either I-A^k or I-E^k, irrespective of their peptide specificity. Based on these results, several HEL peptides appear to interact with I-A^k (using the shortest sequences of overlapping peptides in the set examined):

TABLE I

T Cell Hybridomas Used in the Present Study

Strain of origin	Immunization	Denomination	Ia restriction molecule	Peptide specificity*
C3H	HEL	2C8.4	I-A^k	112-129
C3H	HEL	3E11.1	I-A^k	51-61
C3H	HEL	2H6.4	I-E^k	105-120
C3H	HEL	1B8.1	I-E^k	1-18

* Peptide specificity refers to the shortest HEL peptide tested.

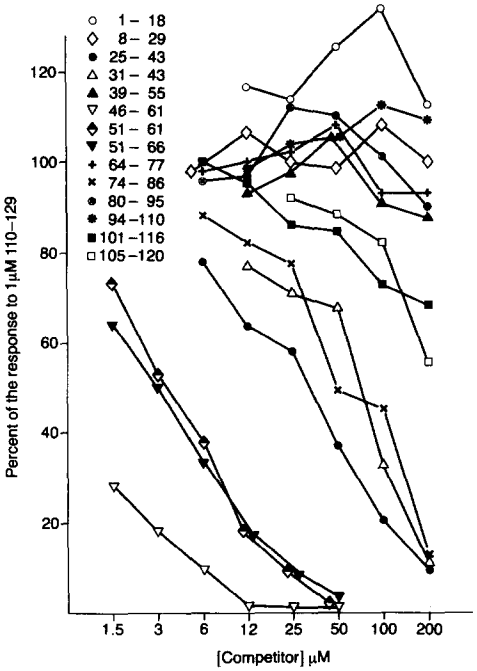

FIGURE 3. Competition of the response of hybridoma 2C8.4 to peptide 110-129 by HEL peptides. Inhibition of antigen presentation was determined by incubating glutaraldehyde-fixed LK 35.2 cells (5×10^4 cells/well) with 1 μM peptide 110-129 and 1.5-200 μM of nonstimulatory HEL peptides for 18 h. The cells were then washed and 2C8.4 hybridoma cells (5×10^4 cells/well) were added. After 24 h, antigen-specific IL-2 production was determined by adding 50-μl aliquots of culture supernatant to 10^4 CTLL cells for an additional 24 h. Data are presented as percent of the [^{3}H]TdR incorporation obtained in response to 1 μM of peptide 110-129. Responses in the presence or absence of peptide 110-129 were 57,963 and 722 cpm, respectively.

51-61, 112-129, 31-43, 74-86, 101-116, and 105-120. Thus, at least five nonoverlapping sequences among the HEL peptides tested are able to interact with the I-A^k molecule.

In terms of competition for I-E^k molecules, 1-18 was found to be the most efficient peptide. This was expected, because the I-E^k-restricted component of anti-HEL response was also directed against this sequence (see in Fig. 2). The T cell determinant appears to map in the NH_2-terminal region of sequence 1-18, since the interaction of peptide 8-29 with I-E^k molecules is weaker than that of 1-18; furthermore, no response can be observed to peptide 8-29 after HEL priming, and hybridoma 1B8.1 also fails to recognize peptide 8-29. In addition to 1-18, the following peptides were found to interact, rather weakly, with the I-E^k molecule: 25-43 (but not 31-43), 51-66 and, as for the I-A^k molecule, all the COOH-terminal HEL peptides tested in the region 101-129 (Table II).

Immunogenicity of Nonimmunodominant HEL Peptides. So far the results have demonstrated that several of the HEL peptides, although capable of binding to class II molecules, cannot restimulate a response of HEL-primed T cells. We therefore determined the immunogenicity of these peptides by injecting them into C3H and B10.A(4R) mice. The control experiment in Fig. 4 confirms that the dominant peptide 1-18 is, as predicted (see Fig. 2), co-recognized only with I-E^k molecules, since it induces a proliferative T cell response in C3H but not in B10.A(4R) mice. Formal evidence for the restriction by I-E^k molecules of the T cell response to peptide 1-18 was obtained by the inhibition of cell proliferation by anti-Ia mAbs (data not shown). An interesting situation is represented by the nondominant peptide (25-43) that binds to both I-A^k and I-E^k molecules, but is much stronger immunogenic in C3H

TABLE II

Inhibition of I-A^k- and I-E^k-restricted Antigen Presentation by HEL Peptides

HEL competitor peptide	Amino acid sequence	Concentration of competitor peptide required for 50% inhibition of antigen presentation by I-A^k 2C8.4 + 110-129	I-A^k 3E11.1 + 46-61	I-E^k 2H6.4 + 105-120	I-E^k 1B8.1 + 1-18
		μM		*μM*	
1-18	KVFGRCELAAAMKRHGLD	>2,000	>2,000	60	ND
8-29	LAAAMKRHGLDNYRGYSLGNWV	>2,000	>2,000	155	625
25-43	LGNWVCAAKFESNFNTQAT	49	60	216	133
31-43	AAKFESNFNTQAT	80	67	>2,000	>2,000
39-55	NTQATNRNTDGSTGYDI	>2,000	>2,000	>2,000	>2,000
46-61	NTDGSTDYGILQINSR	1	ND	>2,000	>2,000
51-61	TDYGILQINSR	5	ND	>2,000	>2,000
51-66	TDYGILQINSRWWCND	3	ND	248	58
64-77	CNDGRTPGSRNLCN	>2,000	>2,000	>2,000	>2,000
74-86	NLCNIPCSALLSS	60	50	>2,000	>2,000
80-95	CSALLSSDITASVNCA	>2,000	>2,000	>2,000	>2,000
94-110	CAKKIVSDGNGMNAWVA	>2,000	>2,000	117	111
101-116	DGNGMNAWVAWRNRCK	150	90	ND	106
105-120	MNAWVAWRNRCKGTDV	490	175	ND	220
107-129	AWVAWRNRCKGTDVQAWIRGARL	ND	20	ND	90
110-129	AWRNRCKGTDVQAWIRGALR	ND	27	ND	65
112-129	RNRCKGTDVQAWIRGALR	ND	25	ND	100

Inhibition of antigen presentation was determined by incubating for 18 h glutaraldehyde-fixed LK 35.2 cells with the indicated HEL peptides recognized by the T cell hybridomas and a dose range (1-200 μM) of nonstimulatory peptides. LK 35.2 cells were then washed and the indicated T hybridoma cells were added. After 24 h antigen-specific IL-2 production was measured as in Fig. 2. The degree of competition for antigen presentation was determined by the regression line obtained by plotting the percent inhibition of [^{3}H]TdR incorporation against the concentration of inhibitory peptides used. Data are expressed as the concentration (μM) of competitor peptide required for 50% inhibition of antigen presentation and represent the arithmetic mean of four to six independent determinations. 50% inhibition values between 200 and 2,000 μM were extrapolated. ND, not determinable.

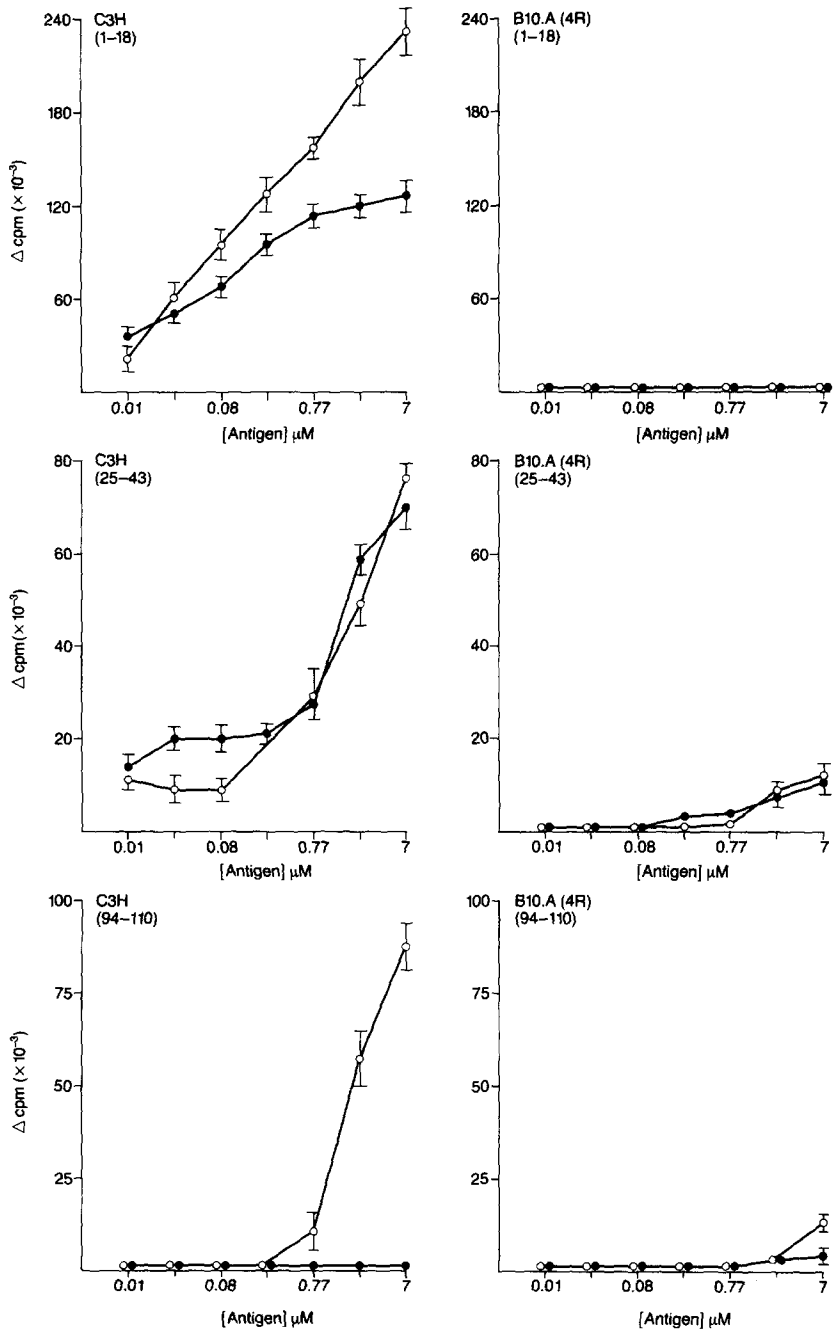

FIGURE 4. T cell-dependent proliferative response of lymph node cells from peptide-primed mice to the immunizing peptide and to HEL. C3H and B10.A(4R) mice were immunized as in Fig. 2 with 7 nmol/mouse of the indicated HEL peptide in CFA and 8 d later the draining lymph node cells were cultured with different concentrations of the immunizing peptide (O) or HEL (●). Data are presented as in Fig. 2.

than in B10.A(4R) mice. This finding indicates that peptide 25–43 is recognized by T cells preferentially in association with I-E^k molecules, probably due to a low precursor frequency of I-A^k-restricted clones specific for this peptide. The T cells induced by peptides 1–18 and 25–43 can be restimulated with HEL, which indicates that the antigenic determinants in these peptides become available upon processing of HEL. Conversely, the priming with peptide 94–110 induced T cells responding to the peptide but not to HEL, which indicates that this peptide is not generated (or destroyed) during processing. The same applies to T cells induced by peptide 74–86, that is, they can be restimulated with the peptide but not with HEL (Fig. 5). As expected, the immunodominant peptide 1–18 induces a higher peptide-dependent proliferative response than the nondominant peptides 25–43 and 94–110. In conclusion, two of the three nonimmunodominant peptides tested are not generated during processing, and therefore, cannot induce T cells upon priming with HEL. However, the third nondominant peptide (peptide 25–43) is available after processing, and thus, the nonresponsiveness to this peptide after priming with HEL must arise by a different mechanism.

In Vivo Competition between HEL Peptides for Interaction with Class II Molecules. Since all HEL peptides tested were found to be immunogenic irrespective of whether they were immunodominant or not, we have examined the possibility that in vivo competition between different peptides for presentation by class II molecules may account for immunodominance. Previous studies have demonstrated that competition between self and non-self peptides for binding to class II molecules can occur in vivo (23). To test the in vivo competition for I-A^k molecules, we immunized B10.A(4R) mice with peptides 51–66, 74–86, 110–129, or with an equimolar amount of peptides 51–66 and 74–86 or 51–66 and 110–129. The results in Fig. 5 demonstrate that a vigorous antipeptide response is induced when each peptide is injected separately into mice. However, when the immunodominant peptide 51–66 is injected together with an equimolar dose of peptide 74–86, the proliferative T cell response to the latter is drastically reduced, whereas the response to 51–66 is affected only marginally. In contrast, injection of the two immunodominant peptides 51–66 and 110–129 together does not result in an appreciable competition in vivo. Surprisingly the response to 51–66 is even increased. Thus, immunodominant peptides can effectively compete with a nondominant one for induction of a response in vivo. It should be pointed out, however, that the nondominant peptide 74–86 tested in this experiment is not available after processing (Fig. 5, and reference 24), and therefore, the latter fact rather than competition is the cause for nonresponsiveness to this peptide after priming with HEL.

To investigate whether competition between HEL peptides in vivo is a physiologically relevant process, we chose a situation in which two peptides, one immunodominant and the other not, were both available after processing of HEL. Peptides 1–18 and 25–43 exemplify this situation since both interact with I-E^k molecules, both induce a T cell response to themselves and to HEL, but only 1–18 is immunodominant. We therefore injected these peptides either separately or mixed in equimolar ratio into C3H mice. The results in Fig. 6 confirm that both peptides are able to induce antipeptide T cell responses when injected separately. However, when peptides 1–18 and 25–43 are injected together a marked decrease in the proliferative T cell response to peptide 25–43 is observed, whereas the response to peptide

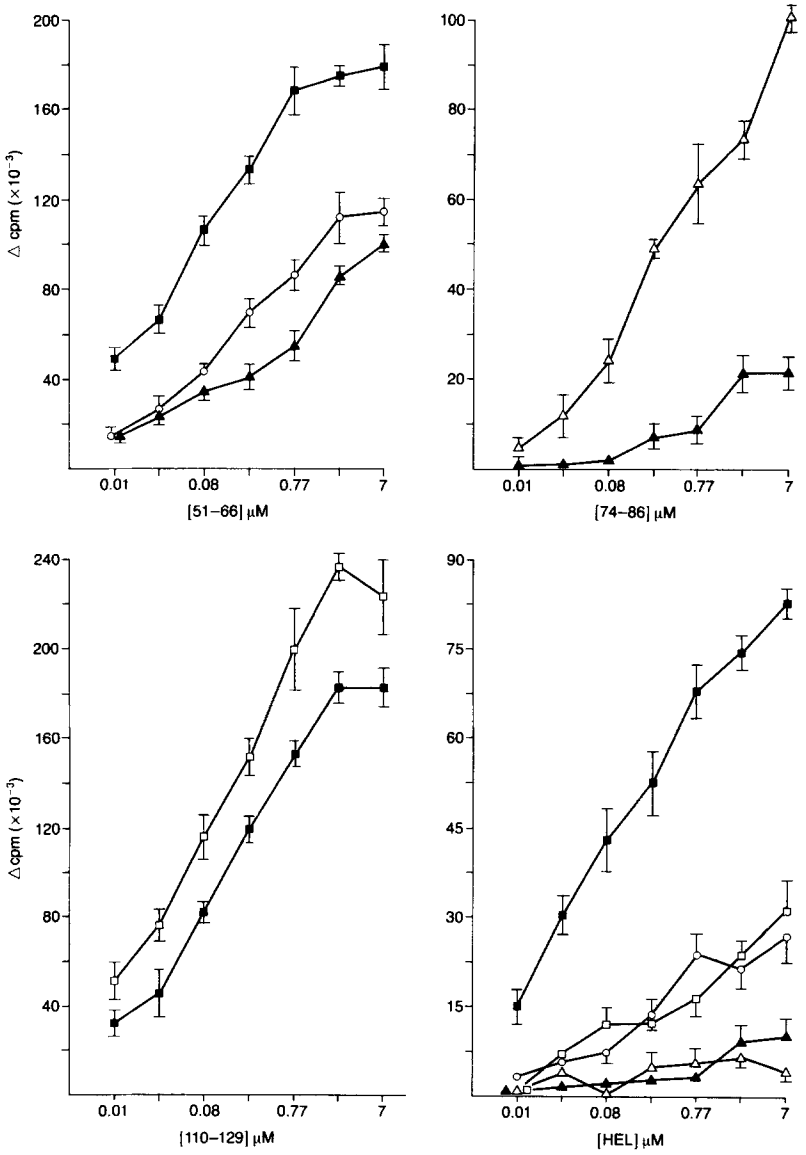

FIGURE 5. In vivo competition between HEL peptides for interaction with I-A^k molecules. B10.A(4R) mice were immunized as in Fig. 2 with 3.5 nmol/mouse peptide 51-66 (○), 74-86 (△), or 110-129 (□). In addition, mice were immunized in the same inoculum with 3.5 nmol peptide 51-66 and 3.5 nmol peptide 74-86 (▲) or with 3.5 nmol peptide 51-66 and 3.5 nmol peptide 110-129 (■). 8 d later draining lymph node cells were cultured with different concentrations of the indicated antigen. Data are expressed as in Fig. 2.

1-18 remains unaffected. These results indicate that competition, in vivo, between different peptides derived from a given protein antigen represents a physiologically relevant mechanism underlying T cell epitope immunodominance.

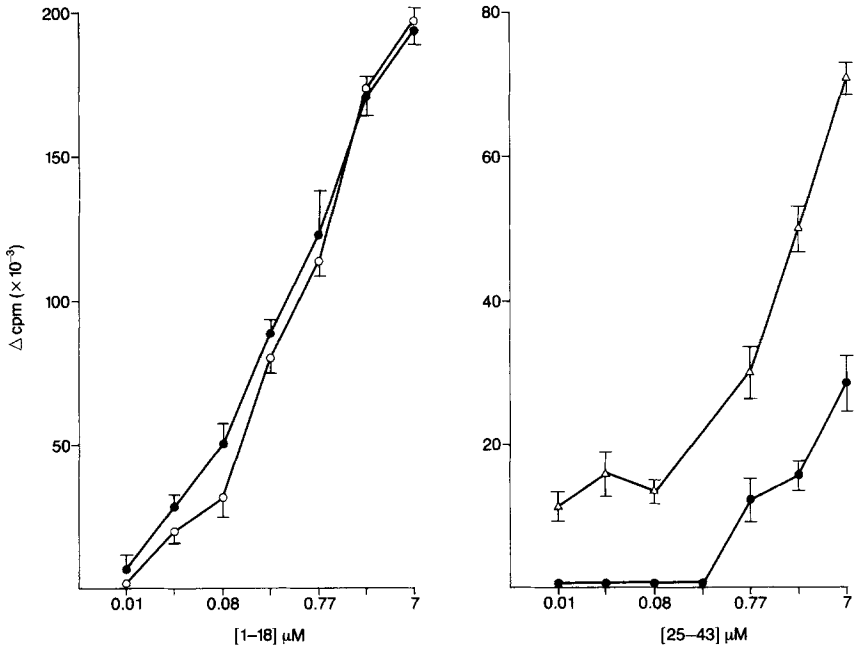

FIGURE 6. In vivo competition between HEL peptides for interaction with I-E^k molecules. C3H mice were immunized as in Fig. 2 with 7 nmol/mouse peptide 1-18 (○), peptide 25-43 (△) or with a mixture of 7 nmol of peptide 1-18 and 7 nmol of peptide 25-43 (●). 8 d later draining lymph node cells were cultured with different concentrations of the indicated peptides. Data are expressed as in Fig. 2.

Discussion

A peptide derived from a protein molecule is considered immunodominant when it restimulates a proliferative response of T cells primed with the whole protein (25). To determine the mechanisms underlying immunodominance, we have analyzed the proliferative T cell response elicited by a panel of synthetic HEL peptides in lymph node cells from HEL-primed mice. Three immunodominant T cell epitopes have been identified, in HEL sequences 1-18, 51-61, and 112-129. Epitopes 51-61 and 112-129 are recognized by T cells in association with I-A^k molecules, whereas the epitope in HEL sequence 1-18 is recognized in association with I-E^k molecules. The epitope in sequence 51-61 had also been identified by Allen et al. (26). Several other HEL peptides are not immunodominant, although they are capable of interacting with I-A^k or I-E^k molecules or with both. At least two of them, 74-86 and 94-110, are not available after processing of HEL. Hence, immunization with HEL does not induce responses against these peptides, and conversely, T cells induced by these peptides do not react to HEL.

Most interesting is the case of peptide 25-43. This peptide is not dominant, although it induces a response that can be recalled by HEL, which indicates that the epitope is available after antigen processing. The reason for the lack of dominance is twofold. First, the peptide, although it binds to I-A^k slightly better than to I-E^k molecules, induces only a marginal I-A^k-restricted response. A similar case has re-

cently been noted by Guillet et al. (27). This failure could result from a low frequency of the relevant clones in the T cell repertoire or the T cells induced may have a poor capacity to proliferate. Second, the precursor of I-E^k-restricted T cells specific for peptide 25–43 are not activated because of in vivo competition by the response to the dominant peptide 1–18. Since peptide 1–18 binds to I-E^k molecules better than peptide 25–43, the former is likely to inhibit binding of the latter to the presenting molecule. The two mechanisms together render the response to peptide 25–43 cryptic in H-2^k mice.

The results also demonstrate that the simultaneous presence of two immunodominant T cell epitopes, such as those in peptides 51–66 and 110–129 both interacting with I-A^k molecules, does not result in competition when peptides are injected together at equimolar concentrations. Similarly, no competition was observed when the mouse lysozyme peptide 46–62 was injected together with peptide 112–129 in B10.A(4R) mice, whereas a clear dose-dependent competition was achieved by increasing the amount of mouse lysozyme peptide 46–62 injected (23). Such a coexistence is anticipated between peptides that bind with relatively high affinity to the presenting molecule, and thus, have both the chance to occupy a number of binding sites sufficient for T cell activation. However, we have observed an additional unexpected synergism between these peptides, in that the presence of peptide 110–129 enhanced significantly and reproducibly the response to peptide 51–66. An explanation for this synergy is that these peptides may be further processed (28) and, as a result, both may fit into a single MHC binding site or they may form crossreactive epitopes upon binding to MHC molecules. The possibility of further processing is supported by our observation that the two peptides compete, instead of synergizing, when tested on fixed APC incapable of processing. However, the alternative possibility that these two peptides influence each other in solution before binding to the presenting molecules, also remains open.

The degree of "foreigness" to the mouse immune system does not exert a major influence in determining immunodominance since, for example, the immunodominant HEL sequence 51–61 has only one amino acid substitution as compared with the corresponding mouse sequence (29), whereas the nonimmunodominant peptide 25–43 has seven.

It has been suggested that amphipathicity is an important feature of immunodominant T cell epitopes (30), but other studies (31) argue that the helix formed need not be amphipathic. Among the immunodominant T cell epitopes we have studied, the sequences 51–61 and 1–18 exhibit amphipathicity but 112–129 does not, indicating that this property is not essential for immunodominance.

The immunodominance of a given T cell epitope may also depend on its capacity to induce preferentially helper or suppressor T cells. Although this mechanism has not been addressed in the present work, it appears to operate, under certain conditions, in the anti-HEL immune response (32).

In conclusion, the phenomenon of epitope dominance appears to be the outcome of a complex interplay between physical-chemical rules governing peptide-MHC interactions and a series of biological mechanisms. The present work has demonstrated four distinct mechanisms operating in the determination of dominant epitopes, but leaves the possibility that further ones exist open.

Summary

The preferential recognition of certain amino acid sequences from foreign protein antigens by T cells is referred to as T cell epitope immunodominance. To determine the mechanisms underlying this phenomenon, we have studied the correlation between the interaction of a series of synthetic peptides encompassing the entire hen egg-white lysozyme (HEL) sequence with class II molecules of the H-2^k haplotype, and T cell responsiveness to these peptides. After HEL priming, three immunodominant T cell epitopes were found: two, included in the HEL sequences 51–61 and 112–129, were recognized in association with I-A^k molecules, and one, included in sequence 1–18, in association with I-E^k molecules. Accordingly, these peptides bound to the appropriate class II molecule, as demonstrated by competition for antigen presentation. Several other HEL peptides, although capable of associating with class II molecules, were not immunodominant. The absence of immunodominance has been shown to arise by three different mechanisms: (*a*) competition by an immunodominant peptide for presentation in vivo, (*b*) failure to generate the peptide during antigen processing, and (*c*) an inherently poor capacity of the T cell repertoire to respond to a particular peptide-MHC complex.

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