

Characterization of T-cell responses to the house dust mite allergen *Der p II* in mice. Evidence for major and cryptic epitopes

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SUMMARY

Major histocompatibility complex (MHC) congenic strains can be defined as high and low responders to the major house dust mite allergen *Der p II* on the basis of the ability to sensitize T cells for *in vitro* lymphokine release. Mice of the H-2^b haplotype were high responders, H-2^k were intermediate and H-2^d low responders. Like responses to other proteins, only a limited number of epitopes could be located by the response of T cells from mice immunized with allergen to a series of overlapping peptides. The epitopes for H-2^b mice were 11-35, 78-104 and 105-129, 36-50 and 78-104 for H-2^k mice and 36-60 for H-2^d. Immunization with the peptides however revealed that spleen-adherent cells were required for lymph node cells to recall responses to the whole protein and in addition that mice could be sensitized by cryptic epitopes defined by peptides 22-50 and 1-20 for H-2^b mice. Peptides containing these cryptic epitopes did not normally induce responses in mice primed with the allergen, but when they were used for immunizing they could prime mice for responses to the peptide and the whole allergen. The results both help to define a model for studying the presentation of allergens and have significant implications for peptide-based immunotherapy.

INTRODUCTION

There have been a number of studies mapping major histocompatibility complex (MHC) class II-restricted T-cell epitopes based on the murine response to a range of protein antigens. These have included the use of phylogenetically conserved proteins such as lysozyme,^{1,2} myoglobin³ and cytochrome *c*,^{4,5} and phylogenetically distant molecules such as staphylococcal nuclease^{6,7} and the λ cI repressor protein.^{8,9} In addition, studies have also been performed on antigens involved in disease processes such as the *Amb a I* allergen of ragweed,¹⁰ influenza virus haemagglutinin¹¹ and myelin basic protein.¹² Together, all these support a common finding that T cells become primed to a relatively small number of immunodominant peptides on an antigen. Several factors have been identified that can determine the immunogenicity of a peptide epitope. These include (1) antigen-processing by antigen-presenting cells (APC),¹³⁻¹⁵ (2) the association of peptide with the class II MHC molecule,¹⁶⁻¹⁸ (3) V gene repertoire of the responding T cells¹⁹⁻²¹ and (4) competition for binding of a foreign peptide to the class II

molecule from other peptides.^{22,23} Experiments on the ability of peptides to immunize animals show a range of results with some epitopes immunizing for responses to the protein^{2,8} and others showing poor activity.^{2,3} Some peptides termed cryptic epitopes have been found to be able to induce T-cell responses which cannot be induced or recalled by the protein antigen.² These epitopes are apparently not universal because they could not be demonstrated in a systematic study of responses to the λ cI repressor protein.^{8,9}

In recent years the primary amino acid sequences of many of the major allergens have become available²⁴⁻³² and the concept of using peptides to divert allergen-specific T-cell responses from producing lymphokines such as interleukin-4 (IL-4) has been mooted.³³ In order to enable further investigation of model systems, studies on the T-cell responses and epitope mapping of the murine response to the house dust mite allergen *Der p II* are reported. MHC congenic mouse strains immunized to the allergen could be conveniently divided into high, intermediate and low responders and peptide epitopes could be demonstrated for each haplotype. Features of this investigation are the demonstration that lymph node cultures from mice immunized with peptide could not recall responses to the protein antigen without the addition of splenic adherent cells, and the finding of cryptic epitopes. The cryptic epitopes differ from those described for lysozyme because T cells from mice immunized with the peptide could recall responses to the whole protein and such a finding could have important implications for the development of peptide-based immunotherapy.

Abbreviations: APC, antigen-presenting cell; CFA, complete Freund's adjuvant; MHC, major histocompatibility complex; SMM, spent mite medium.

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MATERIALS AND METHODS

Reagents

L-Glutamine and 2-mercaptoethanol (2-ME) was purchased from Sigma Chemical Co. (St Louis, MO). Dulbecco's Modified Eagles medium (DME) was obtained from Gibco (Grand Island, NY). Complete Freund's adjuvant (CFA) was purchased from Difco (Detroit, MI). Foetal calf serum (FCS) was bought from the Commonwealth Serum Laboratories (CSL) (Parkville, Victoria, Australia). Glass-fibre filter mats were purchased from Enzo Diagnostics Inc. (New York). [^3H]Thymidine was from Amersham Australia (North Ryde, Sydney). Ninety-six-well flat-bottom tissue culture plates were purchased from Becton Dickinson Labware (Lincoln Park, NJ) and gentamycin was bought from David Bull Laboratories (Mulgrave, Victoria, Australia).

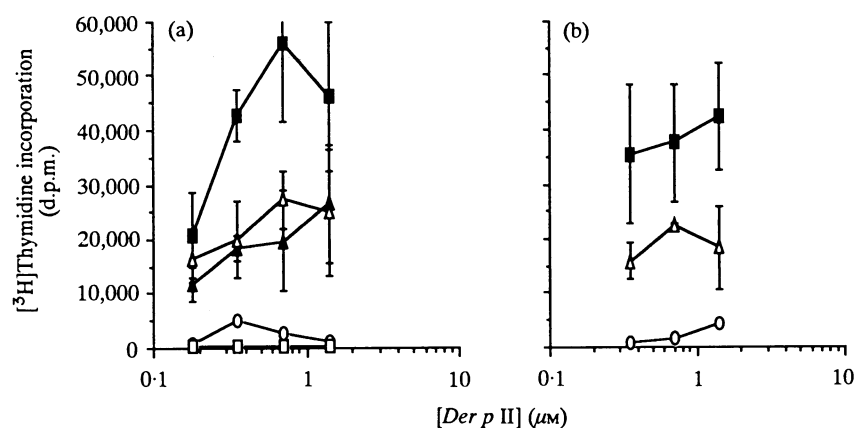
Mice

Female B10 and BALB congenic mice, and inbred strains

BALB/c and C57BL/6J were purchased from the Animal Resource Centre (Murdoch, Western Australia) at 6–8 weeks of age. A description of the different MHC alleles displayed by the different B10 congenic strains is listed in Fig. 1.

Antigens

Ovalbumin (OVA)-crystalline grade V was purchased from Sigma. *Der p* II was affinity purified from 20% spent mite medium (SMM) dissolved in 0.1 M Tris-HCl pH 8.4 (SMM was provided by CSL) [the monoclonal antibody (mAb) used in the purification of the allergen was provided by Dr M. Chapman, University of Virginia, VA]. Overlapping synthetic peptides spanning the entire sequence of *Der p* II and the H-2^d-restricted T-cell epitope of OVA, OVA₃₂₃₋₃₃₉ were synthesized using an Applied Biosystems peptide synthesizer, supplied by Dr M.-C. Kuo. The peptides used for the analysis of *Der p* II T-cell epitopes included: 1-20, 11-35, 22-50, 36-60, 51-77, 61-86, 78-104, 87-112 and 105-129.



(c) Description of MHC alleles in B10 congenic mice

Strain	H-2 haplotype	alleles at H-2 loci						Class II MHC expression	
		K	A α	A β	E β	E α	D	I-A	I-E
B10	b	b	b	b	b	b	b	+	-
B10.A	a	k	k	k	k	k	d	+	+
B10.BR	k	k	k	k	k	k	k	+	+
B10.D2	d	d	d	d	d	d	d	+	+
B10.A(4R)	h4	k	k	k	k	b	b	+	-
B10.A(5R)	i5	b	b	b	b	k	d	+	+

Figure 1. T-cell responses of MHC congenic mice to *Der p* II. B10 (a) or BALB (b) congenic mice were immunized subcutaneously with 100 μg of *Der p* II in CFA. Eight days later the draining lymph nodes were removed and after 24 hr culture the supernatants were assayed for IL-3/GM-CSF activity. The data represent the mean response of three individual mice/group $\pm$ standard deviation. These experiments have been repeated with eight to 10 animals with equivalent results. IL-3/GM-CSF responses of lymph node cells in the absence of lymph node cells in the absence of antigen were < 700 c.p.m. (a) B10 congenic strains tested include B10 (■), B10.BR (▲), B10.A(5R) (△), B10.A(4R) (□) and B10.D2 (○). (b) BALB congenic strains tested include BALB/H-2^b (■), BALB/H-2^k (▲) and BALB/c (○). The *in vitro* concentrations of *Der p* II used in this study are listed as μM values. This was done so a comparison could be made with the concentrations of protein and peptides used. These values are equivalent to 20, 2, 0.2 and 0.02 $\mu\text{g}/\text{ml}$ of *Der p* II. (c) MHC alleles expressed in different B10 congenic strains.

Immunizations

Mice were immunized with 100 µg of protein or 50 µg of peptide emulsified in CFA in a volume of 100 µl at the base of tail.

Culture medium

Cells were cultured in DME supplemented with 2% FCS, 50 µM 2-ME, 2 mM L-glutamine and 20 µg/ml gentamycin.

T-cell assays

For measuring responses to both protein and peptides, the periaortic and inguinal lymph nodes were collected and pooled. Cell suspensions were made by expressing them through a stainless steel wire mesh. The cells were washed and cultured at 4×10^5 cells in 100 µl of culture medium in 96-well flat-bottom tissue culture plates. Native protein or peptide was added and the cells cultured for 24 hr at 37°. Supernatants were collected and assayed for IL-3 granulocyte-macrophage colony-stimulating factor (GM-CSF) activity.

Isolation of APC

Spleen-adherent cells were isolated from naive mice using the method of Bland and Warren.³⁴ Briefly, spleen cells were depleted of red blood cells using hypotonic lysis with sterile distilled water. The cell debris was removed by passing the solution through a sterile cotton wool column and the cells were washed once with DME. The cell pellet was resuspended in DME + 10% FCS and the cells were incubated on plastic culture dishes for a minimum of 2 hr at 37°. The non-adherent cells were removed and the surface of the plate was carefully washed twice with prewarmed media. Ice-cold phosphate-buffered saline (PBS) containing 5 mM EDTA was then added and the plate was incubated on ice for 15 min. Adherent cells were then removed by vigorous pipetting using a wide bore pasteur pipette. The cells were then washed twice with DME and then used in culture.

Purification of antigen-specific or peptide-specific T cells

Mice were killed 8 days after immunization and cell suspensions made from a pool of periaortic and inguinal lymph nodes. T cells were enriched by incubating the lymph node cells on a nylon wool column³⁵ for 2 hr at 37° and then culturing the T cells on a plastic culture dish for a further 1 hr at 37° to remove any accessory cells not retained on the column.

APC assays

4×10^5 T cells were cultured unless otherwise stated with 8×10^4 APC in 96-well flat-bottom tissue culture plates with varying doses of protein or peptide for 24 hr at 37°. Supernatants were collected and assayed for IL-3/GM-CSF activity.

Lymphokine assays

FDC-P1 cells proliferate maximally in response to IL-3 and GM-CSF and submaximally to interferon-γ (IFN-γ) or IL-4.³⁶ 2×10^3 cells were added in 50 µl DME + 5% FCS to 50 µl of culture supernatant in 96-well flat-bottom tissue culture plates. The cells were incubated for 40 hr at 37° and then pulsed with 1 µCi [³H]thymidine ([³H]TdR) for another 4–6 hr at 37°. The cells were then harvested onto glass-fibre filter mats and samples counted for [³H]thymidine incorporation using liquid scintillation spectrometry.

The CTLL-2 cell line will proliferate maximally with IL-2

but only poorly in the presence of IL-4. Supernatants were cultured with 5000 CTLL-2 cells/well for 24 hr at 37° and pulsed with 1 µCi of [³H]TdR. Cells were harvested onto glass-fibre filter mats and the amount of radioactivity incorporated was determined by liquid scintillation spectrometry.

The WEHI-279.1 cell line is a B-cell lymphoma whose growth is inhibited in the presence of IFN-γ.³⁷ Supernatants were incubated with 5000 WEHI-279.1 cells for 3 days at 37° before being pulsed with 1 µCi [³H]TdR. To determine that the inhibition was indeed caused by IFN-γ and not due to another IFN subclass, a 1/10 dilution of an anti-IFN-γ neutralizing mAb (HB170) was added to the test supernatant. If the growth inhibition was reversed by >90% then the supernatants were considered to contain IFN-γ. A 24 hr Con A supernatant was used as a source of IFN-γ in these assays. The FDC-P1 and WEHI 279.1 cell lines were provided by Dr A. Kelso (Walter and Eliza Hall Institute, Melbourne, Australia).

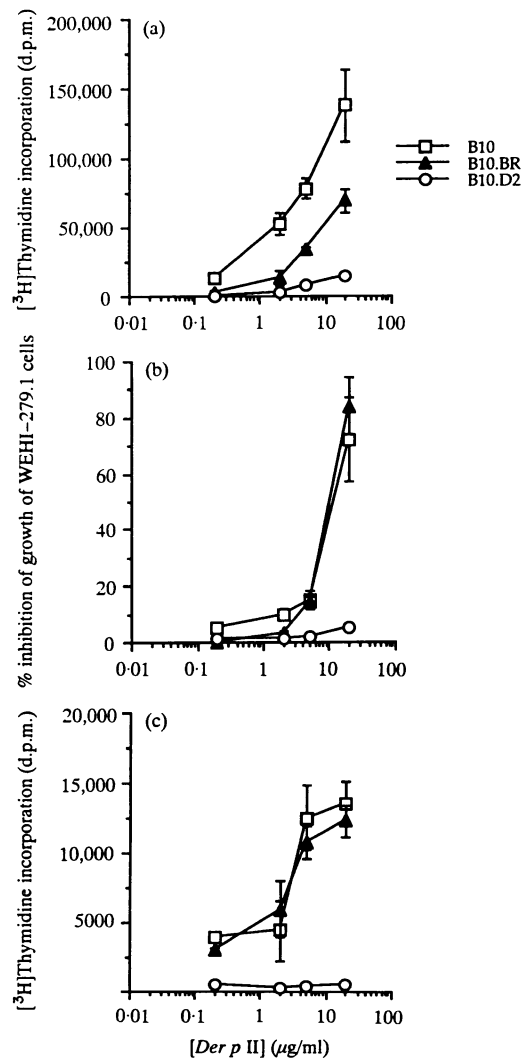


Figure 2. Lymphokine production by B10 congenic mice immunized with *Der p II*. B10 (□), B10.BR (▲) and B10.D2 (○) mice were immunized with 100 µg of *Der p II* and the draining lymph nodes were collected 8 days later. Cell suspensions were cultured with varying concentrations of *Der p II* for 24 hr at 37°. Supernatants were assayed for (a) IL-3/GM-CSF, (b) IFN-γ and (c) IL-2. The results show the mean response of three individual mice $\pm$ standard deviation.

RESULTS

Response of MHC congenic mice to *Der p* II

As a background to further study, the response of mice from B10 and BALB MHC congenic strains to the *Der p* II allergen were examined. Mice were accordingly immunized with 100 μ g of *Der p* II in CFA at the base of tail and depending on the experiment the draining lymph nodes were removed after 8–10 days and examined for the production of IL-3/GM-CSF in response to antigen. From Fig. 1 it can be seen that in both congenic series H-2^b mice were high responders, H-2^d low responders and H-2^k were intermediate. For the B10 series, responses of B10.A(4R) and B10.A(5R) mice were also examined with the former strain being low responders while the latter strain were high responders. B10.A mice (not shown) gave the same level response as B10.BR, so at least for the H-2^k mice the responsiveness to this allergen maps into the I-E region of the MHC as deduced from the haplotypes shown in Fig. 1c. The IL-3/GM-CSF response was initially chosen because these lymphokines are produced by all T cells.³⁶ To measure the correlation with other lymphokines IFN- γ and IL-2 were measured in several experiments. Results from one such experiment indicated that in comparison to IL-3/GM-CSF (Fig. 2a). B10 and B10.BR mice produced similar levels of IFN- γ while B10.D2 mice could not (Fig. 2b). When IL-2 production was measured H-2^b mice were high responders and H-2^d mice were low responders. The IL-2 response of H-2^k mice, however, varied

from high to low in different experiments. IL-4 could not be detected in any of the supernatants using the CT.4S cell line (data not shown).

Localization of T-cell epitopes by responses to overlapping peptides

The T-cell epitopes recognized by the B10 congenic strains following immunization with *Der p* II were located using overlapping synthetic peptides. The lymph node cells from three mice were pooled for each group and responses to the peptides delineated. Epitopes were characterized as responses to a single peptide or were defined as an area of overlap between two stimulatory peptides. They hence are not necessarily the minimal length T-cell epitopes, since this information can only be accurately deduced using the appropriate truncated peptides. These epitope studies have been repeated at least three times with nine to 12 mice per haplotype. The high responder B10 group recognized three epitopes, the major response was to peptides 78–104 and 87–112 (which means the minimal epitope is somewhere between 87 and 104), 11–35 and 105–129 (Fig. 3a). The B10.A(5R) mice recognized similar determinants as the B10 mice, i.e. 87–104 and 105–129 (Fig. 3b) B10.BR mice recognized two epitopes with 36–50 eliciting strongest responses (an overlap between peptides 22–50 and 36–60), while the other was 87–104 (Fig. 3c). Sensitized T cells from the low-responder H-2^d mice (B10.D2) mice recognized weakly, a single peptide 36–60 (Fig. 3d), while B10.A(4R) mice did not produce any significant

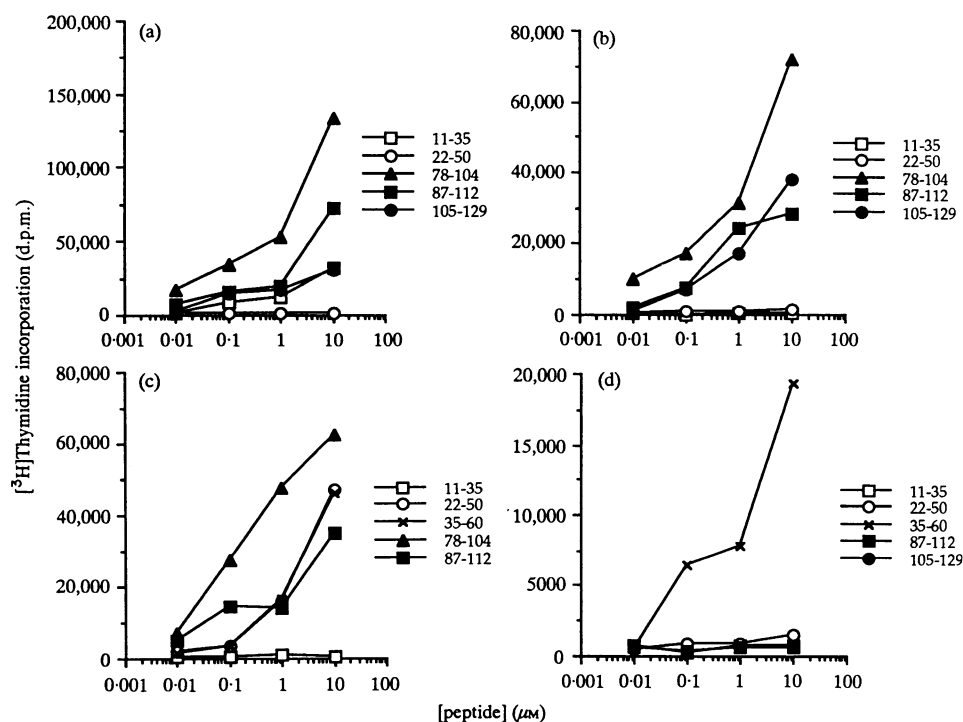


Figure 3. Localization of T-cell epitopes of *Der p* II recognized by different murine haplotypes. Epitope mapping was carried out using three mice per group which had been primed with 100 μ g of *Der p* II in CFA and 8 days later the draining lymph nodes of these mice within each group were pooled and the cells cultured *in vitro* with the relevant peptides (10–0.01 μ M) for 24 hr at 37°. The supernatants after this time were collected and measured for IL-3/GM-CSF activity. Shown are peptides containing the T-cell epitopes recognized by (a) B10 and (b) B10.A(5R), (c) B10.BR and (d) B10.D2 mice. The data represent the mean IL-3/GM-CSF response of triplicate cultures (d.p.m.). The standard deviation of these results was < 10% and the background response of the lymph node cells in the absence of peptide was < 900 c.p.m. These experiments have been repeated at least three times.

reactions. There is a possibility that T cells from H-2^b and H-2^k mice may be responding to two distinct epitopes within the 87-104 (and 36-50 for H-2^k) region and hence finer mapping is required for these areas in both haplotypes. T cells from B10.A(5R) mice fail to recognize the 11-35 epitope on *Der p* II despite possessing the I-A^b restriction element presumably used by B10 mice. The results of these mapping studies are summarized in Fig. 4.

Lymph node cells from peptide-primed mice respond to protein *in vitro* providing it is presented on spleen-adherent cells

Because experiments were to be conducted using peptides to manipulate allergen response, the authors were interested in examining the ability of allergen-derived peptides to prime lymph node T cells and if so, to determine if they could respond to protein. B10 and B10.BR mice were immunized with peptides 78-104 or 105-129 and 36-60 or 78-104 respectively (which represent peptides spanning immunodominant regions), the lymph node cells taken from these mice could respond efficiently to the peptide (Table 1, columns 1, 2 and 3), but showed a very poor ability to be stimulated with the native antigen. Even increasing the *in vitro* concentration of the protein 10 times above that used for the peptide still did not enable the lymph node APC to present it to the T cells. However, they could present the peptides very well with T-cell stimulation at only 0.1 μ M. In the case of B10 mice immunized with p105-129 the lymph node cells failed to respond to both the peptide and the protein (Table 1, column 2). Response to the protein could however be recalled if spleen-adherent cells were added to the culture. This is shown in Table 2 for B10 response to p78-104 and p105-129 and for B10.BR to p22-50 and p78-104. The addition of spleen-adherent cells also permitted cells from mice immunized with p105-129 to respond to both the peptide and protein *in vitro*. To

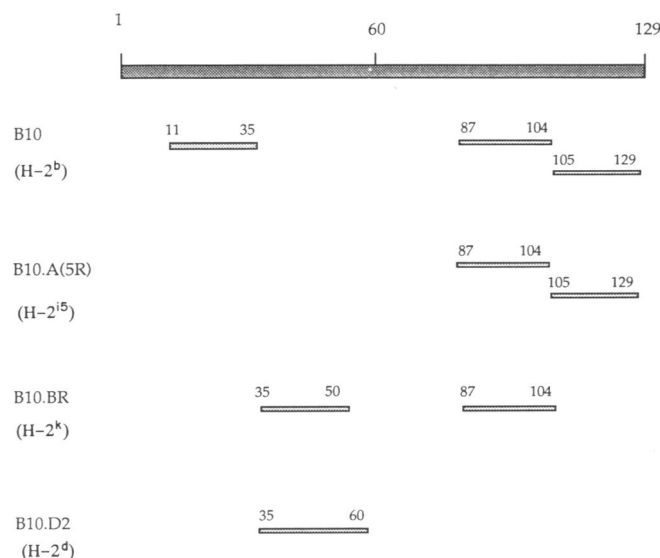


Figure 4. T-cell epitope map for the *Der p* II allergen. This is a summary of the epitope-mapping studies and highlighted are the peptides containing the major immunogenic regions on the allergen which are recognized by murine T cells from different haplotypes. The epitopes 87-104 and 35-50 are based on an assumed overlap.

determine if this was a characteristic shared only by the *Der p* II peptides, the lymph node response was, examined in BALB/c mice primed with the immunodominant H-2^d-restricted peptide OVA₃₂₃₋₃₃₉ (Table 3). Some responses were detected in this case to the OVA protein but they were markedly increased by the addition of spleen-adherent cells to levels stimulated by the immunizing peptide (Table 3).

Table 1. Antigen-presenting cells in the lymph nodes of peptide-primed mice can only present peptides

<i>In vitro</i> antigen	IL-3/GM-CSF response ([³ H]TdR uptake, d.p.m.)			
	B10 mice		B10.BR mice	
	Peptides used for immunizing and for <i>in vitro</i> antigen†			
	p78-104	p105-129	p36-60	p78-104
[<i>Der p</i> II] (μM)				
1.4	3408 ± 756*	1040 ± 233	1605 ± 345	717 ± 450
0.7	7015 ± 2323	1865 ± 185	1404 ± 434	419 ± 225
0.35	4571 ± 259	1050 ± 543	2001 ± 1222	1139 ± 550
0	759 ± 158	971 ± 336	520 ± 218	1070 ± 329
Peptide (μM)				
0.1	83,483 ± 7542	2159 ± 259	90,500 ± 7242	93,305 ± 2267
0.05	58,369 ± 1286	1659 ± 532	67,694 ± 3330	85,199 ± 193
0.01	37,701 ± 5432	1932 ± 115	11,083 ± 1035	49,328 ± 5146

† B10.BR or B10 mice (3/group) were primed with 50 μ g peptide and 8 days later the periaortic and inguinal lymph nodes were removed and cell suspensions made. 4×10^5 cells were cultured in the presence of antigen (peptide and protein) for 24 hr at 37°. Supernatants were assayed for IL-3/GM-CSF activity. The *Der p* II concentration is given in μ M values for ease of comparison against peptide responses. The *Der p* II concentrations listed are equivalent to 20, 2 and 0.2 μ g/ml.

* The data represent the mean response (d.p.m.) of three individual mice $\pm$ standard deviation.

Table 2. Spleen-adherent cells can present protein antigens to peptide-primed T cells

	B10 mice		B10.BR mice	
	p78-104	p105-129	p22-50	p78-104
[Der p II] ($\mu\text{g/ml}$)				
20	142,668 $\pm$ 21,171*	95,581 $\pm$ 12,401	98,582 $\pm$ 7745	46,497 $\pm$ 7365
2	29,273 $\pm$ 1881	16,172 $\pm$ 2235	25,132 $\pm$ 4378	17,128 $\pm$ 4278
0.2	4550 $\pm$ 1019	8169 $\pm$ 1102	18,795 $\pm$ 2110	17,073 $\pm$ 5123
[Peptide] (μM)				
10	252,768 $\pm$ 35,112	44,382 $\pm$ 8712	94,317 $\pm$ 12,730	92,890 $\pm$ 8759
1	115,324 $\pm$ 18,756	17,403 $\pm$ 1889	94,062 $\pm$ 14,027	91,394 $\pm$ 12,316
0.1	42,581 $\pm$ 3108	6980 $\pm$ 750	83,941 $\pm$ 378	43,594 $\pm$ 2567
0	1638 $\pm$ 259	270 $\pm$ 102	3332 $\pm$ 1227	1241 $\pm$ 235

B10 and B10.BR mice were primed subcutaneously with 50 μg of peptide and the draining lymph node cells collected 7 days later. LN T cells were enriched by incubating the cells on a nylon wool column. Adherent cell populations were prepared from spleen cell suspensions collected from naive mice. T cells were cultured with adherent cells in the presence of either *Der p II* or peptide for 24 hr. The supernatants were assayed for the presence of IL-3/GM-CSF. The data shown represent the mean response of triplicate values $\pm$ standard deviation. These results have been repeated twice with equivalent results.

* Data obtained from [^3H]thymidine incorporation (d.p.m.).

Table 3. Presentation of ovalbumin to peptide-primed T cells in the presence of spleen-adherent cells

	Lymph node (alone)	Lymph node (+ spleen adherents)
[Ovalbumin] ($\mu\text{g/ml}$)		
800	18,124 $\pm$ 1402	87,546 $\pm$ 2659
40	7113 $\pm$ 1258	23,554 $\pm$ 1876
4	1008 $\pm$ 412	2113 $\pm$ 579
[p323-339] (μM)		
10	39,455 $\pm$ 3422	55,412 $\pm$ 3356
1	9823 $\pm$ 1743	23,115 $\pm$ 3412
0.1	4513 $\pm$ 775	3499 $\pm$ 1754
0.01	712 $\pm$ 116	1099 $\pm$ 187
0	553 $\pm$ 46	784 $\pm$ 210

BALB/c mice were immunized with 50 μg of pOVA 323-339 and 8 days later the draining lymph nodes were removed. Half the cells were cultured directly without any further treatment, while the other half of the cells were enriched for T cells on a nylon wool column. Purified T cells were then cultured with splenic adherent cells. All cells were cultured with either ovalbumin or peptide and the 24-hr supernatants were assayed for IL-3/GM-CSF. The data represents the mean response of triplicate wells $\pm$ standard deviation. This experiment has been performed twice.

Cryptic epitopes which induce responses to the *Der p II* protein

Because the addition of spleen-adherent cells to lymph node cultures led to a difference in antigen presentation, the presence of epitopes on the *Der p II* peptides was reinvestigated. Groups of B10 mice were immunized with one of the overlapping peptides and responses of lymph node cells to the peptides and the allergen was measured in cultures containing spleen-adherent cells. Large responses were obtained with the peptides 11-35, 78-104, 87-112 and 105-129 which were already known to

contain epitopes. As well, peptides 1-20, and 22-50, not previously thought to contain epitopes, could stimulate lymph node responses that could be recalled by the immunizing peptide and the whole protein (Fig. 5). Peptides 51-77 and 61-86 do not appear to contain T-cell epitypic sequences (Fig. 5). The response to p22-50 was as large as that to the other major epitopes when compared by the peak of the response and the concentration of antigen required. Direct counts of the IL-3 GM-CSF assays can be approximately compared because internal standards had little variation between the experiments reported. Subsequent experiments showed that as for the other epitopes the spleen-adherent cells were only necessary to reveal responses of p22-50 primed lymph nodes to the whole protein.

DISCUSSION

Several studies have been looking at strategies to try to modify or prevent autoimmune disease in mice using synthetic peptides which correspond to dominant T-cell epitopes.^{38,39} Now that allergen sequences are available, similar strategies might also be used to alter the process of allergic sensitization. The primary aim of this investigation was to generate information which might facilitate the development of mouse or transgenic mouse models. This required defining the MHC-linked immune response genes and in determining the T-cell epitopes recognized by individual haplotypes. To do this the standard protocol of immunization in CFA, as has been used in other epitope studies, was adopted and T-cell activity was measured by the release of lymphokines from *in vitro*-stimulated lymph node cultures. The knowledge generated, for example of the T-cell epitypic regions, would enable an informed choice to be made as to the selection of a peptide with the potential to modulate the lymphokine (e.g. IL-4) response of allergen-reactive T cells *in vivo*. At present human T-cell responses to the *Der p II* allergen have been poorly characterized, and therefore these studies could be of advantage in relating to human disease. Since it is possible that APC in

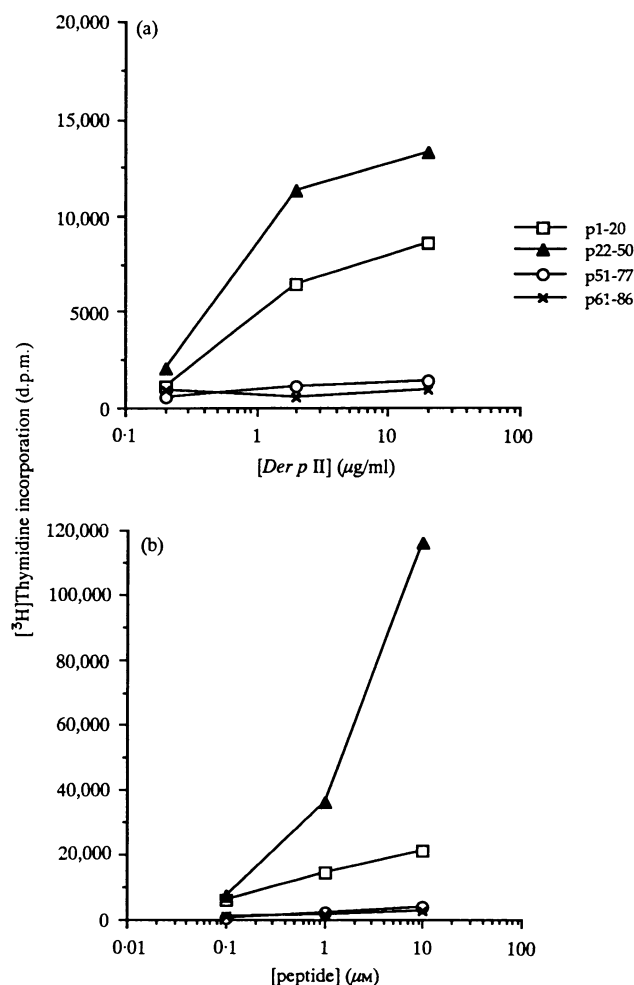


Figure 5. Identification of two cryptic epitopes on the *Der p II* allergen. B10 mice were immunized with 50 μg of either p1-20, p22-50, p51-77 and p61-86 and 8 days later the periaortic and inguinal lymph nodes were removed. T cells purified over a nylon wool column were cultured with splenic adherent cells with the addition of either *Der p II* or peptide. The data shown represent the mean IL-3/GM-CSF response of triplicate wells (d.p.m.) $\pm$ standard deviation. This experiment has been repeated twice with equivalent results. Background responses were < 800 d.p.m.

humans and mice utilize similar hydrolytic enzymes, similarities in antigen processing and presentation could be expected.

Both the B10 and BALB congenic mice showed that H-2^b were high responders and H-2^d low. This was found for IL-3/GM-CSF, IFN- γ and IL-2. In comparison H-2^k mice produce equivalent amounts of IFN- γ as H-2^b mice, but an intermediate IL-3/GM-CSF response and a very variable IL-2 response. No IL-4 could be detected with the CT.4S cell assay but this was expected of the protocol. Taking into consideration the recombinant MHC alleles of the congenic mice used in this study it was possible to map H-2^k responsiveness to I-E and a class II restriction of H-2^b mice with I-A was assumed. In keeping with the T-cell responses in H-2^b mice can be completely inhibited if protein-primed lymph node cells are treated with the anti-I-A^b mAb (M5/114) and complement. The mapping of epitopes produced results consistent with studies on other proteins with one to three epitopes being recognized by each haplotype and

the results shown in Fig. 3 are also representative in that each strain recognized particular epitopes with a consistent hierarchy of responsiveness in terms of the IL-3/GM-CSF assay. Thus, for example, the response of B10 mice was strongest to p78-104 and weakest to p11-35 and p105-129. Despite repeated testing it was not possible to detect a response to 11-35 in the I-A^b B10.A(5R) mice. It is possible therefore that T cells responsive to the peptide are eliminated by thymic selection to the I-E product expressed in these mice and may further explain why these mice consistently give an intermediate response to the allergen.

A priority of these studies was to document the ability of peptides to sensitize T cells which are able to recall responses when challenged with the protein antigen *in vitro*. This activity would seem axiomatic but although peptides have been reported to sensitize for responses to hen egg lysozyme,² influenza haemagglutinin,⁴⁰ cytochrome *c*¹³ and myoglobin³ proteins, the responses have in many cases been suboptimal. Thus the major epitope 102-118 of B10.D2 mice immunized to myoglobin is a mediocre immunogen¹⁴ and the peptides reported for cytochrome *c* responses elicit far better reactions to the peptide.⁴¹ However, in some cases, peptide immunization has been shown to elicit strong responses to a native antigen.^{42,43}

Even against this background the finding that the ability of lymph node cells from mice primed with allergen-derived peptides showed minimal responses to *Der p II* was surprising. The same phenomenon was found for the OVA peptide OVA₃₂₃₋₃₃₉ although not so absolute. The inclusion of spleen-adherent cells from mice however reverses this and permits peptide-primed lymph node cells to respond to protein. Since the lymph nodes from mice primed with peptide obviously contain T cells which can mount large responses to the peptide, one line of investigation was to identify the cell involved in presenting protein in the spleen preparations and further, to determine why lymph node cultures from mice primed with protein and not peptide can respond to native and peptidic antigens. The results to be documented in a further report indicate that spleen dendritic cells are responsible for the presentation of protein and that the lymph node-adherent cells can only present peptide and not protein. Presentation in the lymph node cultures from protein-primed mice was via B cells, presumably possessing antigen receptors specific for tertiary structures on *Der p II*. Special note needs to be taken of the response of B10 mice to p105-129 because they could not respond to either the protein or the peptide following immunization with this peptide without the addition of spleen-adherent cells. Evidence from experiments using metabolic inhibitors shows that this peptide requires processing before presentation and thus it is treated like a protein by spleen cells. It also illustrates that it may be possible to design peptides which can only be presented by restricted cell populations. Finally for this section it should be noted that these results are different to experiments with some peptides of HEL where large anti-protein responses could be obtained without spleen filler cells.²

The difference in the antigen presentation by cultures with spleen-adherent cells prompted an investigation to immunize mice with the full series of *Der p II* peptides and to test the reactivity to the peptide and protein in lymph node cultures with the spleen-adherent cells. In addition to the known epitopes, responses were also detected to p1-20 and p22-50 which do not elicit responses in protein-primed mice. These will be called cryptic epitopes in keeping with the terminology previously

used.² The responses to p22-50 were as large in terms of IL-3/GM-CSF production and the dose of antigen required for activation as those to major epitopes. Like the results with other epitopes, lymph node cells alone primed to a cryptic epitope could only respond to the peptide, but if spleen-adherent cells were added they could respond to the protein. These cryptic epitopes however apparently differ fundamentally from those described for lysozyme in that we were able to demonstrate that they could induce responses which may be recalled by the protein and not just the peptide,^{2,14} indicating the native allergen could be processed in such a way that a sufficient amount of the epitope could be presented on spleen-adherent cells which in turn could elicit a T-cell response. These cryptic epitopes, which in a report also in preparation will be described for *Der p* I, could have important consequences for peptide-based strategies whose aim will be to immunize or modify responses to the whole allergen. They may for example widen the number of peptides capable of being used for immunotherapy and perhaps provide a key to stimulate selectively a naive population of cells uncommitted to a pattern of lymphokine production.

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