

Stimulatory and inhibitory epitopes in the T cell responses of mice to Der p 1

A. G. Jarnicki and W. R. Thomas

Centre for Child Health Research, University of Western Australia, TVW Telethon Institute for Child health Research, West Perth, Western Australia

Summary

Background The responses of mice to the mite allergen Der p 1 have been used to study the mechanisms of allergic sensitization and the development of new types of immunotherapy. Many of the studies require a knowledge of the T cell epitopes, and because Der p 1 is polymorphic, the effect of natural amino acid substitution in the allergen. The intranasal administration of peptides containing T cell epitopes can induce a mucosal tolerance but it is not known if the major activity is limited to stimulatory peptides and if, as found for autoimmunity, some epitopes are not inhibitory.

Objective To determine and compare the sequences of Der p 1 which contain stimulatory epitopes for the high responding H-2^b and H-2^d mice and the sequences which induce tolerance by intranasal administration of peptides.

Methods T cell responses of mice immunized with Der p 1 were measured by *in vitro* T cell stimulation assays so an extensive study of epitope recognition and intranasal tolerance could be made. Synthetic peptides were used to examine the stimulatory and inhibitory ability of all Der p 1 sequences and to map the major H-2^b epitope in detail. This included the effect of the common polymorphic amino acid 124 substitution found within this epitope.

Results Three and two regions, respectively, were found to contain stimulatory T cell epitopes for H-2^b and H-2^d mice. The peptides in these regions were also the most active at inducing intranasal tolerance for the responding haplotype. The correspondence between inhibitory and stimulatory peptides was maintained for the fine mapping of the major H-2^b epitope. This was found about a core region of 118–126 which was overlapping but separate to a consensus sequence for the binding of endogenous peptides. Peptides with alanine at the naturally polymorphic residue 124 stimulated and inhibited responses to Der p 1 more effectively, while peptides with the valine 124 variant were immunogenic but poorly cross-reactive.

Conclusions The intranasal administration of peptides representing each of five epitopes recognized by two strains of mice were able to induce mucosal tolerance and the major tolerizing activity was limited to these epitopes. The position of the core major epitope for C57 mice, which differs from a previously predicted epitope, and its specificity for the natural alanine 124 variant is described.

Keywords Der p 1, epitopes, intranasal tolerance, mice, peptides, polymorphism, T cell,

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Introduction

The responses of mice to the mite allergen Der p 1 have been used extensively to study allergic sensitization [1–4] and experimental immunotherapy [1,5–8]. The induction of IgE [1,3], delayed hypersensitivity [9] and eosinophilic pulmonary inflammation [2,4] have been described as well as the induction of sensitization via the nasal mucosa [8]. It has been possible to inhibit the responses to the allergen by intranasal administration of the whole protein [1] or of the peptides containing T cell epitopes [5] and by feeding recombinant peptides [10]. Other strategies examined for immunotherapy have been to deviate immune responses by coimmunization with Th1 adjuvants [7]

or with cytokines [11]. The recent production of recombinant Der p 1 in a serologically active form [12] and the description of B cell epitopes [13] and anti-idiotypes will widen the scope for further investigation [14].

Minor changes in amino acid sequence can affect the ability of peptides to sensitize mice and to elicit *in vitro* recall responses [15–19]. It is therefore critical to define the epitopes particularly for studies with peptides and recombinant allergens. While many aspects of T cell epitopes have been studied for the responses to Der p 1, the analyses have not unequivocally covered the entire molecule and have only been studied for one MHC haplotype. Moreover, Der p 1 is polymorphic [20,21] and the T cell epitopes may differ for the different sequence isoforms as found for humans [21]. The effect of polymorphic residues is critical information for the correct design peptides, recombinant molecules and DNA vaccines. A priority is to ensure the epitopes are correctly represented, but it may also be necessary to incorporate the diversity into the study of allergy.

Correspondence: Professor Wayne R. Thomas, Centre for Child Health Research, University of Western Australia, TVW Telethon Institute for Child Health Research, PO Box 100, West Perth 6872, Western Australia. E-mail: wayne@icmr.uwa.edu.au

A key area of interest for immunotherapy is whether the T cell epitopes which stimulate *in vitro* T cell responses are appropriate targets for new desensitization strategies. The use of stimulatory epitopes would limit the strategies to be employed, but for humans, the standard procedure has been to determine T cell epitopes by *in vitro* T cell stimulation assays. The studies here have used a basic T cell stimulation readout to perform a systematic study of the location of epitopes recognized by H-2^b and H-2^d mice and to fine map the major epitope recognized by the high responder H-2^b mice; the strain which has been used in most studies. Other commonly used H-2^d and H-2^k mice are known low responders. The major epitope for H-2^b mice overlapped but was distinct from the hitherto assumed epitope [22], at a location deduced from a MHC binding consensus sequence for natural peptides [23]. The major epitope also encompassed a natural and frequent polymorphic substitution of the Der p 1 sequence and this affected both stimulatory responses and inhibition induced by inhaled peptide. A comparison at several levels, including a mapping with a complete array of peptides, showed that peptides which were the most stimulatory for *in vitro* stimulation were the most active for the induction of mucosal tolerance.

Methods

Animals

Six to eight-week-old mice were obtained from the Animal Resources Centre (Murdoch, Western Australia) and used according to the guidelines specified by the National Health and Medical Research Council of Australia.

Antigens and peptides

Der p 1 was isolated by affinity chromatography from spent mite medium (SMM, CSL Ltd, Parkville, Victoria, Australia) using the 4C1 mAb kindly provided by Dr M. Chapman, Charlottesville, VA, USA. Peptides were synthesized by Chiron Mimotopes Pty. Ltd. (Victoria, Australia). The 12–19 mer peptides used to map T cell epitopes and immunize mice contained a diketopiperazine (DKP) linker at the carboxyl terminus synthesized as part of a peptide set in a 96 well format. The Der p 1 peptide p114–128, was constructed with a free acid at the carboxyl terminus. The peptides were analysed for purity and yield by RP-HPLC and mass spectrometry by the manufacturer. The peptides were resuspended in PBS for use. Except where mentioned the peptides containing position 124 contained the alanine variants (see Results).

Intranasal administration of proteins and peptides

Mice were lightly sedated and 20 µL volumes of antigen suspension were applied dropwise from a micropipette onto the nares.

T cell assays

Mice were injected subcutaneously with 100 µg of Der p 1 emulsified in 100 µL of complete Freund's adjuvant (CFA) (Difco Laboratories, Detroit, MI, USA) at the base of the tail. Ten days later the draining inguinal and periaortic lymph nodes were removed and single cell suspensions were obtained by passing the lymph nodes through a stainless steel sieve and then washed in DMEM (Gibco, Grand Island, NY, USA)

supplemented with 3.7 g/L of NaHCO₃, 40 mg/L gentamicin (Delta West, Bentley, WA, Australia), 50 mM L-glutamine (Gibco), 50 µM of 2 mercaptoethanol (ME) (BDH Laboratory Supplies, Poole, UK) and 20 mM Hepes (ICN Biochemicals Co., Cleveland, OH, USA), pH 7.2. The cells were seeded at 5×10^5 cells in 200 µL of supplemented DMEM with 2% FCS (CSL) per well in 96 well round bottomed plates (Nunc, Roskilde, Denmark), and stimulated with antigen at the concentrations indicated for 24 h at 37 °C with 5% CO₂ atmosphere. Supernatants were removed and stored at –20 °C until assayed.

Cytokine assays

The IL-2 responsive CTLL-2 cell line was used to detect IL-2. Fifty µL aliquots of supernatants removed from lymph node cell stimulated cultures were added to 5×10^3 CTLL-2 cells per culture-well in a 96 well plate in a total of 100 µL of DMEM supplemented media with 10% FCS. The cultures were incubated at 37 °C for 24 h and then pulsed with 1 µCi of ³H-thymidine (Dupont, Wilmington, DE, USA) for the final six hours. Cells were harvested onto glass fibre mats and the amount of radioactivity incorporated was determined by using a Packard Matrix 9600 beta counter.

GM-CSF/IL-3 was measured by the proliferative response of the FDC-P1 cell line. Fifty µL of supernatants were cultured with 2×10^3 FDC-P1 cells per well for 48 h at 37 °C in 96 well plates and then pulsed with 1 µCi of ³H-thymidine for the final six hours. Cells were harvested onto glass fibre mats and the amount of radioactivity incorporated was determined by using a Packard Matrix 9600 beta counter.

Statistical analysis

All statistics were performed using Prism graph software (version 2.0b, GraphPad Software, Inc., San Diego, CA, USA). Data points were expressed as the mean $\pm$ standard deviation ($\pm$ SD). Comparisons of sample means were performed using Student's *t*-test and *P* < 0.05 was considered significant.

Results

Stimulatory epitopes of C57BL mice

Lymph node cells from C57BL6 mice immunized with Der p 1 in CFA were pooled and stimulated for *in vitro* cytokine release with 19-mer peptides spanning the 221-residue sequence. Each peptide had a sequence which progressed by five residues from the first peptide, creating an overlap of 14 residues between successive peptides. The responses, measured in replicate experiments by IL-2 (Fig. 1a) and GM-CSF/IL-3 (not shown) were the same. Peptides in the regions 30–53, 75–98 and 110–133 stimulated cytokine release. There was no evidence of stimulation with peptides containing the sequence 15–29 which has been reported to stimulate T cells [24] or for a C-terminal epitope. The most stimulatory region, 110–133, was further examined with 19-mer peptides beginning with 104–122 and progressing by one residue to 116–134. The peptides 104–122 through to 107–125, all of which contained an I-A^b binding consensus sequence 112–125 [23], were not stimulatory by both IL-2 and GM-CSF/IL-3 assay (Figs 2a and b). The sequences, beginning from 108 to 126 induced low IL-2 release (Fig. 2a) which increased with successive peptides to the final

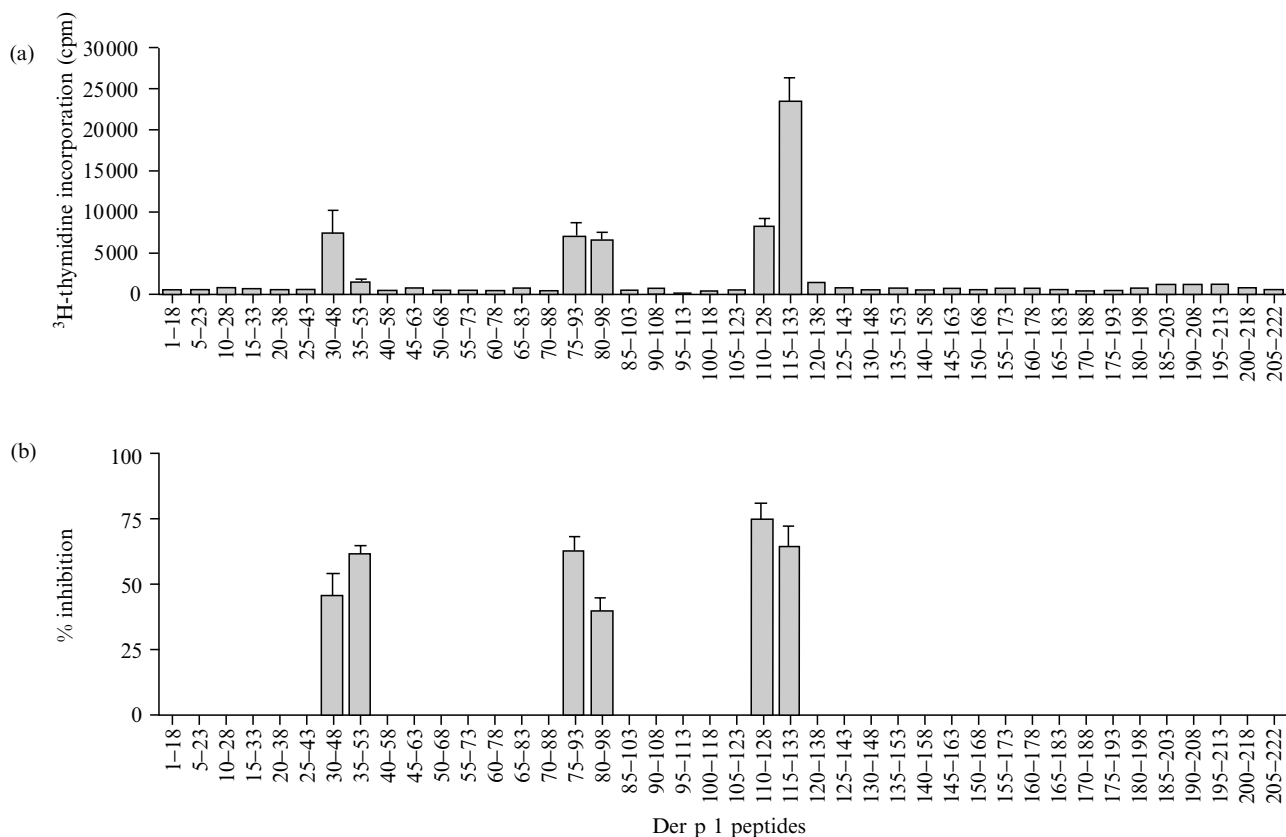


Fig. 1. Mapping of stimulatory and inhibitory Der p 1 epitopes with 19-mer synthetic peptides for C57Bl/6 J mice. To map stimulatory epitopes (a), mice ($n = 3$) were immunized subcutaneously with 100 μg of Der p 1 in CFA and draining lymph nodes harvested on day 10 and pooled. Cells were stimulated with each of the 19-mer peptides (30 $\mu\text{g}/\text{mL}$) and 24 h supernatants were assayed for IL-2. Data are mean ($\pm$ SD). For inhibition studies (b), mice ($n = 3$) were given 10 μg of the appropriate 19-mer peptide intranasally on three consecutive days and 10 days later immunized sc with 100 μg of Der p 1 in CFA. The draining lymph nodes were harvested 10 days after immunization and each mouse was treated individually. Cells were stimulated with Der p 1 (30 $\mu\text{g}/\text{mL}$) and 24 h supernatants were assayed for IL-2. Data are percentage inhibition (mean $\pm$ SD). The percentage inhibition was calculated by subtracting all background (unstimulated) responses and expressing the difference between the PBS and peptide treated mice as a percentage of the PBS control.

116–134. The cytokine release measured by GM-CSF/IL-3 assay was quantitatively different than that for IL-2. All the peptides from 108 to 1126 induced high cytokine release (Fig. 2b).

To map the region more precisely 13-mer peptides beginning with 109–118 and progressing by one residue to 123–135 were used for stimulation (Figs 2c and d). Both the IL-2 and GM-CSF/IL-3 assays showed that the ability to stimulate cytokine release was found for peptides with residues 120–126, with 118–126 inducing maximal release. Mapping with smaller peptides showed the epitope for maximum stimulation was in peptide 117–128 with similar results for IL-2 (Fig. 3) and GM-CSF/IL-3 assays (not shown).

To examine if the responses centred about 118–126 were to a single epitope, mice were immunized with either 114–126 or 120–132, peptides at the N- and C-termini of the 13-mer array or with whole Der p 1. Recall responses were measured by *in vitro* responses to the array of 13-mer peptides (Fig. 4). A core region of responsiveness was seen in that 116–128 induced the highest recall responses of cells from mice immunized with either peptide or with Der p 1, and the responses were higher than the responses to the immunizing peptides. There was however, a bias for the pattern of responses towards the respective N- or C-terminal position of the immunizing

peptide, and the range for each peptide was narrower than that found after immunization with whole Der p 1.

Mucosal inhibition of responses of C57BL mice

Initial experiments showed that the intranasal administration of 3×10^6 μg doses of peptide delivered on consecutive days, produced a 70–80% inhibition of IL-2 responses to Der p 1. To locate inhibitory epitopes, the 19-mer-peptide array, encompassing the entire molecule was examined. Every three adjacent peptides were pooled and administered to groups of three mice which were immunized with Der p 1 in CFA. After 10 days the cytokine responses of the draining lymph nodes cells were measured. Peptides from the pools which induced inhibition in repeated experiments were then administered individually to groups of mice, and the individual mice examined. The peptides which produced significant inhibition (Fig. 1b) were the same peptides which contained the stimulatory epitopes.

To map the inhibitory epitope in the major epitopic region, 110–133, the inhibition induced with the finer 19-mer and 13-mer arrays were measured. As for the stimulation analysis, the 19-mer peptides began with 104–122 and progressed by one residue and the 13-mer peptide began with 109–118 and progressed by one residue. All the peptides that had previously been found to be stimulatory were inhibitory (Figs 5a and b). For the

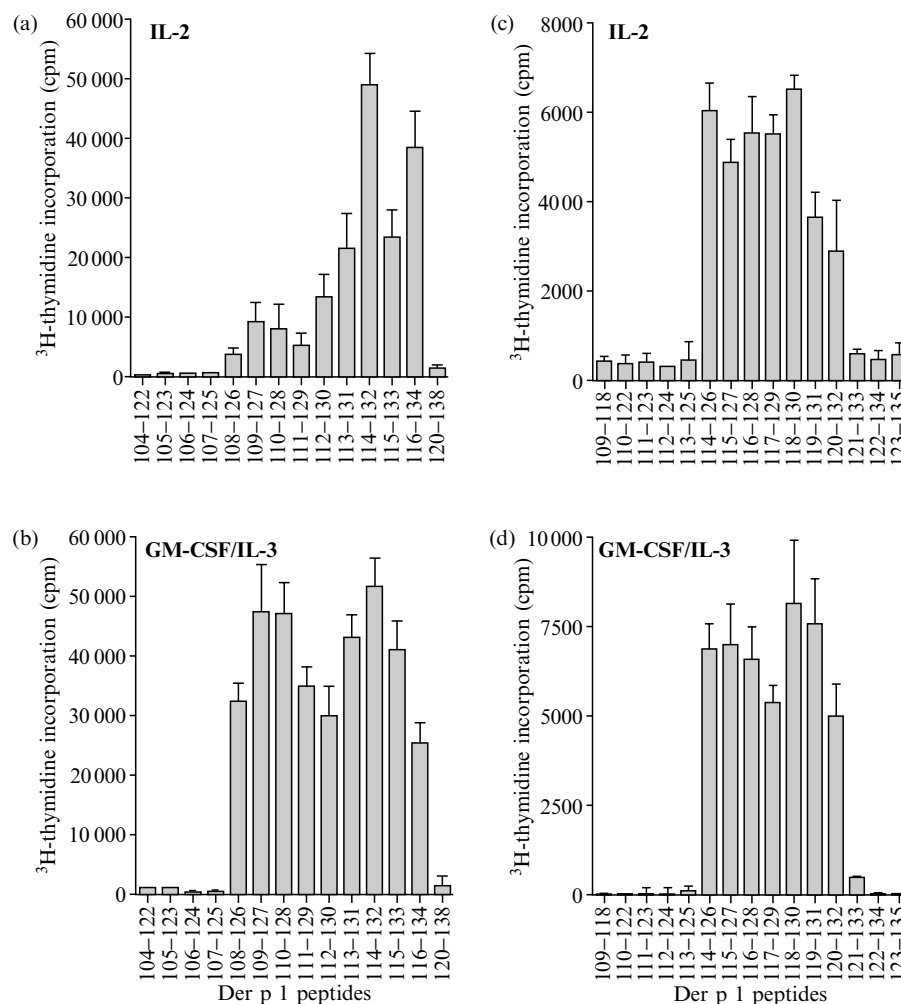


Fig. 2. 19- and 13-mer peptide mapping of the major Der p 1 epitope for C57Bl/6 J mice. C57Bl/6 J mice ($n = 3$) were immunized sc with 100 μ g of Der p 1 in CFA. The draining lymph nodes were harvested on day 10 and pooled. Cell suspensions were stimulated with each of the 19- (a and b) or 13- (c and d) mer peptides (30 μ g/mL) and 24 h supernatants assayed for IL-2 and GM-CSF/IL-3. Data are mean ($\pm$ SD).

19-mer array all peptides from 108 to 126-115-133 were equally inhibitory, which differed quantitatively from the stimulation pattern for IL-2 release where the N-terminal peptides were less stimulatory. For the 13-mers the inhibitory pattern was the same as the stimulatory pattern.

Stimulatory and inhibitory peptides for responses of DBA/1 mice

The location of the stimulatory and inhibitory peptides for responses of a second high responder MHC haplotype, DBA/1 (H-2^q), was examined. The ability of 19-mer peptides to stimulate recall responses to Der p 1 immunization and to inhibit responses to Der p 1 were examined with the procedures used for the C57BL mice. The peptides which stimulated IL-2 responses were in the regions 1–23 and 70–93 which differed from C57 responses (Fig. 6a). The GM-CSF/IL-3 assay gave similar results (not shown). The inhibitory activity of intranasal peptides (Fig. 6b) mapped to the same regions.

Stimulation and inhibition induced by Der p 1 peptides with the natural 124 A/V amino acid polymorphism

cDNA analysis has shown that 50% of Der p 1 molecules will contain an alanine residue at position 124 and about 50% will

have valine. To examine if this could affect the responses to the core 118–126 epitope recognized by H-2^b mice, C57BL mice were immunized with Der p 1 and stimulated with peptides containing either valine or alanine. The cells showed the highest responses to the alanine (A)-variant (Fig. 7a). When the ability of the peptides to induce intranasal tolerance to responses to Der p 1 was examined the same effect was found. The intranasal administration of A-variant inhibited IL-2 responses to Der p 1 to a greater degree than the administration of the valine (V)-variant (Fig. 7b). The ability of A and V-variant peptides to immunize mice, and the ability of intranasal peptides to inhibit responses to peptide immunization was then examined (Fig. 8). Lymph node cells from mice immunized with the high responder A variant produced large recall responses when stimulated with the A-peptide and low responses when stimulated with the V-peptide. Intranasal inhibition of mice immunized with the A-peptide was best achieved by the A-peptide, but some inhibition was produced by the V-peptide. Lymph node cells from mice immunized with the low responder V-variant produced large recall responses but primarily only when stimulated with the V-variant. Intranasal administration of the V-variant also inhibited responses of mice immunized with the V-variant and to a lesser degree responses of mice immunized

with the A-variant. Intranasal administration of the A variant followed by immunization with V peptide left high recall responses to the V-peptide and mice which had been given the A peptide intranasally and immunized with the V peptide had high *in vitro* responses to the A peptide.

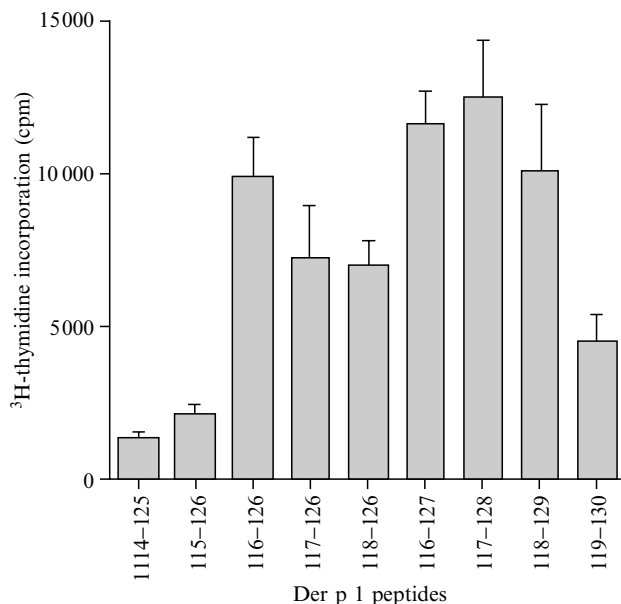


Fig. 3. Determination of a minimal stimulatory peptide. C57BL/6 J mice ($n = 3$) were immunized sc with 100 μ g of Der p 1 in CFA. The draining lymph nodes were harvested on day 10 and pooled. Cell suspensions were stimulated with the 9–12 mer peptides (30 μ g/mL) shown and 24 h supernatants were assayed for IL-2. Data are mean ($\pm$ SD).

Discussion

The results show a systematic study to localize the stimulatory and inhibitory epitopes for mucosal tolerance to Der p 1. It found for two strains of mice that the epitopes that were able to induce mucosal tolerance were those most able to stimulate T cell cytokine recall responses. The location of the three epitopes for H-2^b mice concurs with the responses to peptides shown previously [10]. Previous studies have shown that the intranasal administration peptides from these regions were able to inhibit responses Der p 1 [5]. It is shown here that these peptides were the most active when compared to all the non-stimulatory peptides. It is possible that other peptides, at higher doses and/or with a more frequent administration, can be inhibitory and this has been shown for the subdominant epitopes in 138–166 [5]. The concordance of the inhibitory and stimulatory epitopes was also found for the H-2^q strain DBA/1. It was further demonstrated with fine mapping of the major H-2^b epitope and with the altered activity of this epitope produced by amino acid substitution. The stimulatory responses measured in this system are MHC class II restricted [25] and the results agree with the demonstration that the intranasal inhibition can be induced in CD8 knockout mice [25].

While the ability to induce mucosal tolerance in other experimental systems has correlated with MHC binding activity [26] this is the first systematic study examining peptides representing a whole antigen. All 5 epitopes located here were highly inhibitory and correlated with MHC responsiveness. In contrast the studies with autoimmunity to myelin basic protein found that only one of two strongly stimulatory peptides could induce intranasal tolerance [27]. It is possible that the responses to autoantigens are more complex and it is more difficult to expand autoreactive regulatory clones [28]. A feature of the

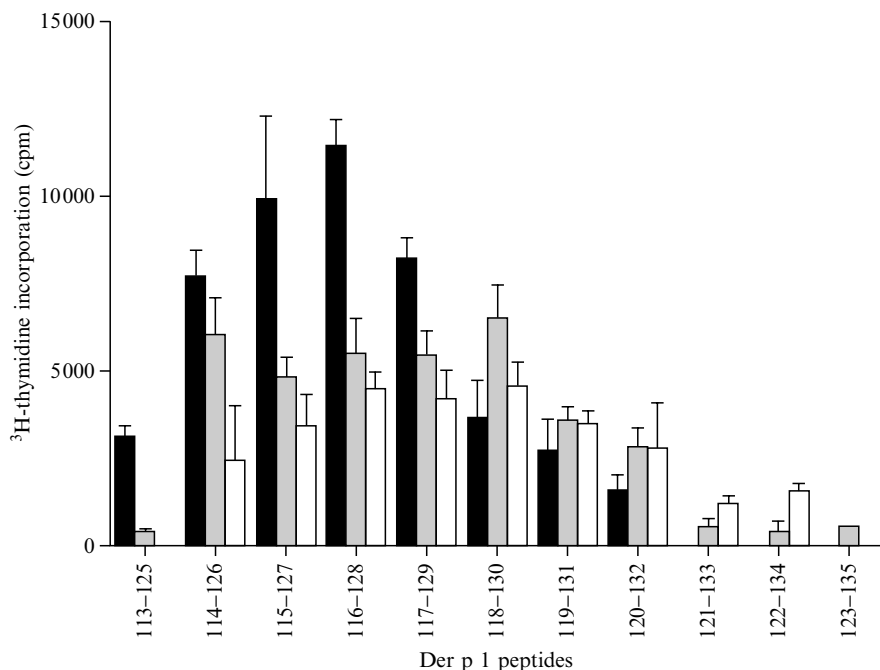


Fig. 4. Epitope responses to peptide immunization. C57BL/6 J mice ($n = 3$) were immunized sc with 100 μ g of either Der p 1 (□), p114–128 (■) or p120–132 (□) in CFA. After 10 days cells from the draining lymph nodes were pooled and stimulated with the array of 13-mer peptides (30 μ g/mL) and 24 h supernatants assayed for IL-2. Data are mean ($\pm$ SD).

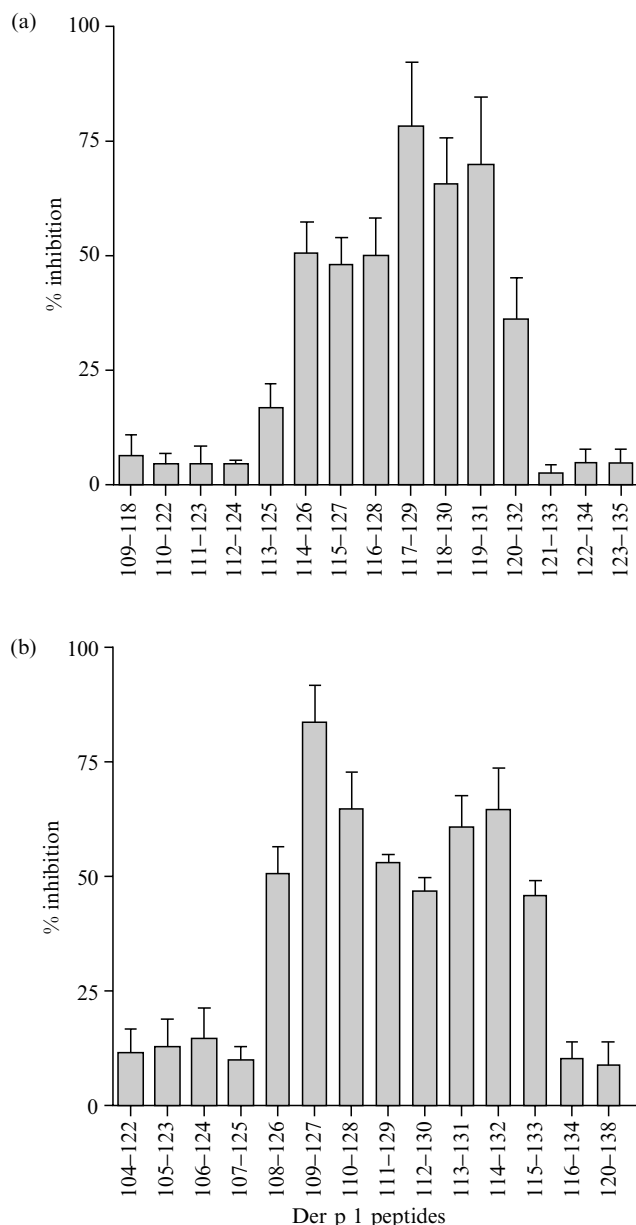


Fig. 5. Inhibitory 19- and 13-mer peptides for the major Der p 1 epitope of C57BL mice. C57BL/6 J mice ($n = 3$) were given with 10 μg of 19- (a) or 13- (b) mer peptide intranasally on three consecutive days and 10 days later immunized sc with 100 μg of Der p 1 in CFA. The draining lymph nodes were harvested 10 days later and each mouse was treated individually. Cell suspensions were stimulated with Der p 1 (30 $\mu\text{g}/\text{mL}$) and 24 h supernatants assayed for IL-2. Data are percentage inhibition (means $\pm$ SD).

experimental autoimmune encephalomyelitis system is the inhibitory activity of the peptide Ac1-9 is increased by mutation which increases its MHC binding activity whereas high affinity clones to autoantigens do not expand [26,28]. The study here with an exogenous antigen shows that all the stimulatory peptides to an exogenous antigen could mediate the mucosal tolerance and thus the mechanisms for non-self tolerogenic peptides may be different.

The responses of cells from H-2^b mice immunized with Der p 1 were found in the regions 30-53, 75-98 and 110-133 in increasing order of magnitude. This confirms the previous results for these regions [10]. No responses to peptides containing the CLIP-like epitope at 15-29 [24] were found so it is not a prominent epitope and likewise no responses were detected to a C-terminal peptide. Assuming the immunodominant regions 30-53 and 75-98 contained a single epitope, the fact that overlapping peptides were stimulatory, would place the epitopes within the residues 35-48 and 80-93. The location of the major epitope in the region 110-133 was studied in detail. Peptides from this region have been used to induce tolerance in a variety of studies on unresponsiveness and Higgins et al. [28] have shown that individual humans recognize several different epitopes in this region, suggesting multiple epitopes could be involved. A further consideration is that the region contains consensus sequences for binding to H-2^b class I (residues 111-119) and class II (residues 113-127) molecules [22]. The class I binding sequence have been shown to contain a functional CD8 epitope which can induce cytotoxic lymphocytes [22]. The use of the I-A^b binding motif with the core binding position of N115 and P121 [22] was suggested by the location of an epitope within positions 113-127. Most studies have used the large peptide 110-133 so experiments can be performed less equivocally with a defined epitope. The data here shows the epitope is in fact located more towards the C-terminal than the consensus sequence and can be separated from the CD8 epitope. The core region was between residues 118-126 and even peptide 120-132 could induce significant stimulation and mucosal tolerance. Furthermore the absence of responses to peptides 113-125 and 107-125 which contain the consensus sequence would suggest it is not used at all. The fact that the region contained a consensus sequence for the binding of endogenous peptides therefore does not make it a preferred epitope. The epitopes which are restricted by I-A^b are, as with other systems, varied [15,16]. No inhibition was found by the intranasal administration of peptides containing the CD8 epitope which is in keeping with the observation that this type of tolerance could be readily produced in CD8 knockout mice [25].

The immunizations with p120-132 and 114-126 were performed to corroborate the results which showed that the mice recognized epitopes centred about a single core sequence between 116 and 128. These peptides represented the extreme N- and C-terminal of the proposed epitope. The T cell responses of the mice immunized with either peptide were heteroclytic in that the recall responses were preferentially induced by peptides based around the core p116-128 rather than the immunizing peptide. The response to p113-125 which contained the consensus sequence for endogenous peptides was low even when the mice were immunized with p114-126. The pattern of the recall responses of mice immunized with each peptide was however, biased to peptides towards the position of the immunizing peptide. The pattern induced by Der p 1 immunization extended across a broad range suggesting a range of epitopes are presented with a single core MHC binding region. It may be of interest to determine if the new populations of T cells induced by the peptides can respond to the whole allergen. This could be useful for reprogramming regulatory responses. This may be relevant because the peptides can inhibit responses of primed mice [5].

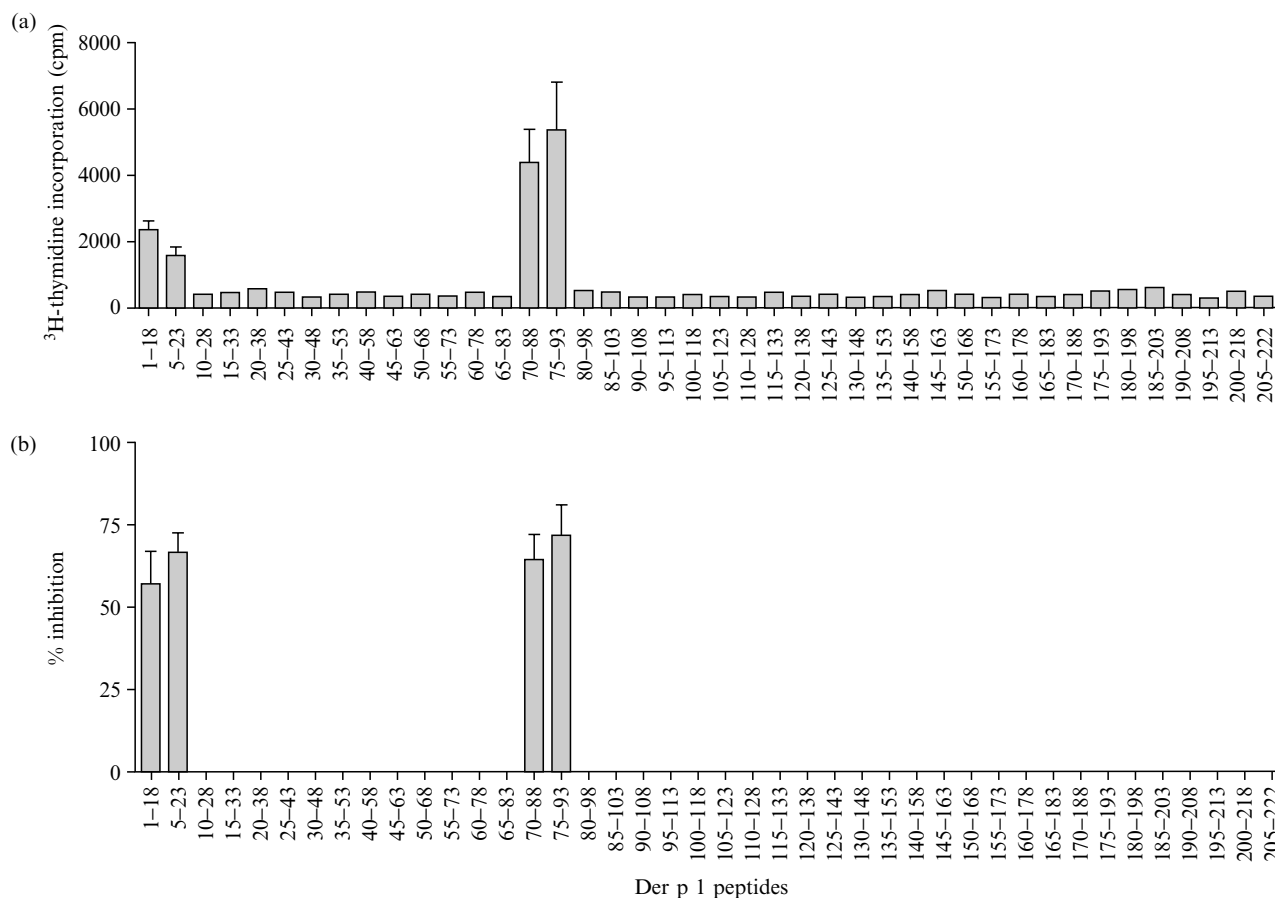


Fig. 6. Stimulatory and inhibitory Der p 1 epitopes with 19-mer synthetic peptides in DBA/1 mice. To map stimulatory epitopes (a), mice ($n = 3$) were immunized sc with 100 μg of Der p 1 in CFA and draining lymph nodes harvested on day 10 and pooled. Cells were stimulated with each of the 19-mer peptides (30 $\mu\text{g}/\text{mL}$) and 24 h supernatants were assayed for IL-2. Data are mean ($\pm$ SD). For inhibition studies (b), mice ($n = 3$) were given 10 μg of the appropriate 19-mer peptide intranasally on three consecutive days and 10 days later immunized sc with 100 μg of Der p 1 in CFA. The draining lymph nodes were harvested 10 days after immunization and each mouse was treated individually. Cells were stimulated with Der p 1 (30 $\mu\text{g}/\text{mL}$) and 24 h supernatants were assayed for IL-2. Data are percentage inhibition (mean $\pm$ SD). The percentage inhibition was calculated by subtracting all background (unstimulated) responses and expressing the difference between the PBS and peptide treated mice as a percentage of the PBS control.

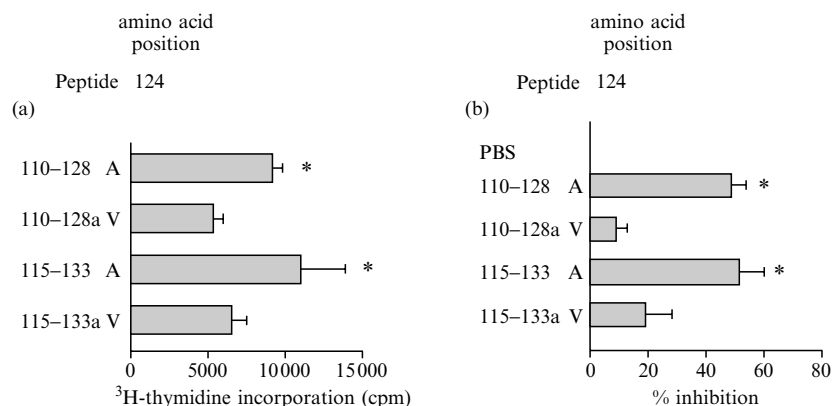


Fig. 7. Stimulation and inhibition of T cell responses with polymorphic peptides containing the major epitope. (a) For stimulation studies, C57Bl/6 J mice ($n = 3$) were immunized sc with 100 μg Der p 1 in CFA. The draining lymph node cells were removed 10 days later and then stimulated with the 19-mer peptides. (b) For inhibition studies, C57Bl/6 J mice ($n = 3$) were given 10 μg of the appropriate peptide intranasally on three consecutive days and 10 days later challenged sc with 100 μg of Der p 1 in CFA. The draining lymph node cells were removed 10 days later and then stimulated with Der p 1 (30 $\mu\text{g}/\text{mL}$). 24 h supernatants were assayed for IL-2 responses. p110-128 and p115-133 induced greater responses and significantly more inhibition (*) when they contained A124 (Students t -test, $P < 0.05$). Data are mean ($\pm$ SD) or percentage inhibition (mean $\pm$ SD).

The 19-mer peptides 108-126, 109-127, 110-127 and 111-127 were relatively poor at stimulating the release of IL-2 even though they contained the core epitope 118-126 and could inhibit responses when administered intranasally. They did however, strongly stimulate the release of cytokine judged

by the GM-CSF/IL-3 assay. This assay has been shown to be less susceptible to inhibitory effects than IL-2 release [5,10] so it may be useful to include in mapping studies because interfering flanking sequences frequently modify responses [17,18].

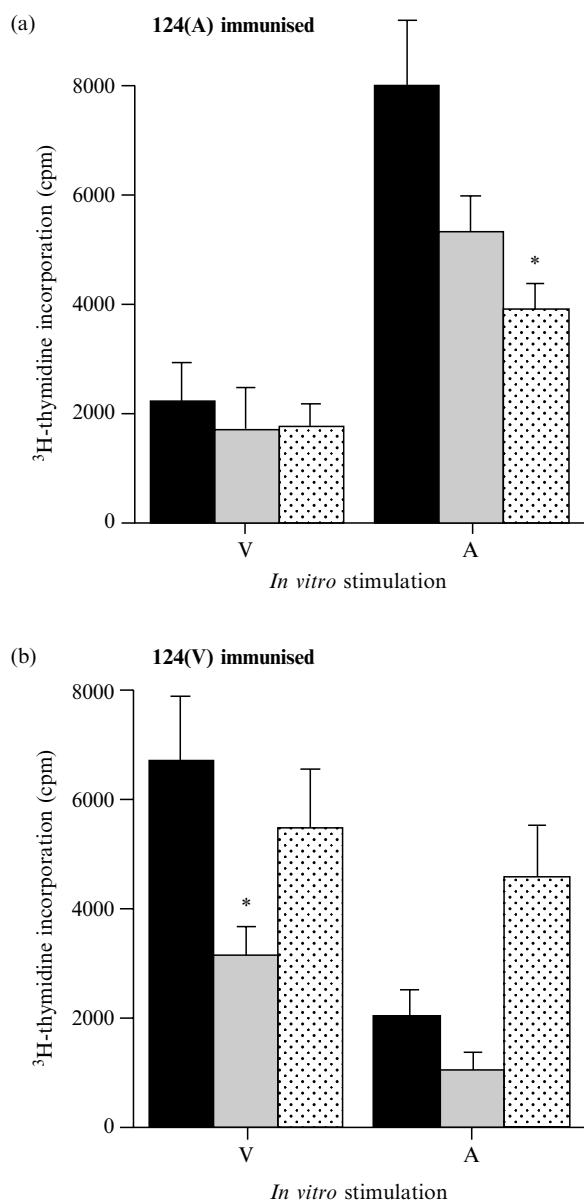


Fig. 8. Cross-reactivity and cross-inhibition with peptides with A or V polymorphisms. C57BL/6 J mice were pretreated intranasally with PBS (■), or p110–128 with either alanine (▨) or valine (□) at position 124 and then immunized sc with 100 µg of the peptide in CFA. After 10 days the draining lymph nodes were harvested and cell suspensions stimulated with either peptide. 24 h supernatants were assayed for IL-2. Data are mean (± SD).

The studies here with the alanine/valine substitution at position 124 have considerable practical importance. Mice immunized with Der p 1 responded better to the peptides with 124 A and mucosal tolerance was easier to induce with 124 A peptides. Peptides with the substitution of 124 V were however, able to immunize mice to respond to V peptide, but suboptimally to A peptides, and could induce intranasal tolerance to immunization with the V peptide. The peptide can therefore be an epitope but with only a small amount of cross-reactivity to the preferred A-variant. The analysis of cDNA sequences show that the A and V 124 polymorphisms are found equally in the environment and

in the commercial source of mites use to prepare the Der p used here [20,21]. It thus suggested that the Der p 1.0101 be used for the C57BL mice. It is fortunate that the first cDNA clone which was an A124 variant provided the information for the initial description of the major epitope. A recent analysis of Der p 1 polymorphisms found that all other amino acid substitutions were sporadic and are unlikely to affect overall responses to natural preparations. While the natural 124 polymorphisms could modulate the production of cytokines, in the way described for altered peptide ligands [19] this was not studied because the effects would be specific for the MHC and the epitope. It is however, critical for studies with peptides and recombinant molecules to recognize the effect of the substitution.

Acknowledgement

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