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An immunogenetic analysis of the T-cell recognition of the major house dust mite allergen Der p 2: identification of high- and low-responder HLA-DQ alleles and localization of T-cell epitopes

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SUMMARY

Cellular reactivity to Der p 2, a major allergen of the house dust mite (HDM) *Dermatophagoides pteronyssinus*, was studied in a group of 41 symptomatic HDM sensitive patients, using fresh peripheral blood mononuclear cells (PBMC) and assays of proliferation. Sixty per cent of the patients responded to Der p 2, with reactivities being greater in patients with asthma as one of their clinical manifestations and also in those who had skin-test reactivity to a number of allergens. HLA-DR and -DQ serotyping was undertaken in 39 of the patients and the magnitude of T-cell proliferative responses to Der p 2 were found to be positively associated with DQ7 and negatively associated with DQ2. T-cell determinants within the Der p 2 molecule were identified by assays using a series of overlapping peptides (15- to 19-mers) spanning the entire protein. Fifty-nine per cent of the 41 HDM-sensitive patients responded to one or more of the peptides. All of the peptides were antigenic for at least one of the individuals, indicating the heterogeneity of the human repertoire reactive with Der p 2. There was a substantial variability in the number and location of epitopes recognized by T cells from the different allergic patients, the mean number per patient being 2·3 ± 1·3 (SD). The most frequently recognized peptide was that spanning residues 111–129, being stimulatory in 66·7%, the other peptides were each recognized by between 8 to 25% of individuals. There was no correlation between the epitope recognized and the presence of particular HLA-DQ antigens.

INTRODUCTION

Allergens from the house dust mite (HDM) are known to be important causes of human allergic diseases including asthma, rhinitis and eczema. *Dermatophagoides pteronyssinus* (Dpt) and *D. farinae* are two major species of HDM present in varying proportions in most countries, Dpt being more important in coastal and humid climates.[1] Der p 1 and Der p 2 are generally considered to be the most immunologically reactive allergens of Dpt with the majority of HDM-sensitive individuals recognizing one or both.[2] Both have been cloned and sequenced[3,4] and studies have been undertaken on the nature and specificity of human cellular and serologic responses to these allergens.[5–7]

As IgE responses to allergens are dependent on T-cell help,[8] and as helper $CD4^+$ T-cell responses to allergens are restricted by specific human leucocyte antigen (HLA) class II molecules,[9] it is possible that associations between responses to purified HDM allergens and specific HLA class II molecules may be identified. These have been demonstrated for DRB1 alleles with a number of pollen allergens, including DR2 with Amb a 5, DR5 with HDAmb a 6, DR3 with Lol p 1, 2 and 3, DR7 with Jun s 1 and DR11 with *Par o* 1 in research undertaken by Marsh and colleagues.[10,11] Association with tree and mold allergens, including Ole e 1 with DR7[12] and Alt a 1 with DR4,[13] have also been identified. Despite a number of studies, there has been no strong association shown between specific DR and DQ alleles and responses to HDM allergens. This may be because major histocompatibility complex (MHC) class II genes other than DR and DQ are the restricting elements and, in fact, studies by Caraballo *et al.*[14] did reveal some evidence of a DP association. In support of this, Higgins *et al.* have previously shown HLA-DP restriction of recognition of HDM allergens by specific T-cell clones.[15] Nevertheless, a more recent and extensive study from Young *et al.*, working in Cookson's laboratory and using genetic-typing techniques, revealed no evidence of any HLA-DP association with serologic HDM reactivity.[16] Alternatively the lack of HLA association may result from the fact that there are many immunoreactive molecules within the HDM, all of which contain multiple

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Table 1. Clinical details, Der p 2 reactivity and HLA specificities of HDM-sensitive patients

Patient number	Age (years)	Allergic manifestations*	Other allergic sensitivities†	Response to Der p 2‡	HLA Serotype		
					DR	DQ	DR52/53
1	8	C, R	nil	5·7 (1·0)	nt	nt	nt
2	18	A, R	C§, GP§	6·8 (1·0)	2, 11	1, 7	52
3	50	R	C, GP	9·3 (0·1)	1, 4	1, 7	53
4	17	A, R	C, D§, GP	4·6 (1·0)	7, 13	1, 2	52, 53
5	28	A	C, D, GP, molds¶	11·6 (1·0)	10, 13	1	52
6	38	A, R, E	C, GP, molds	5·5 (1·0)	3, 4	2, 8	52, 53
7	36	A	C, D, alt**	7·5 (10)	1, 7	1, 2	53
8	27	C, R	C, GP	—	4, 13	1, 8	52, 53
9	10	R	nil	—	nt	nt	nt
10	37	A, R	C	—	7, 13	1, 2	52
11	44	C, R	C, alt	9·6 (0·1)	11, 15	1, 7	52
12	35	A, R	C, D, GP, molds	12·4 (1·0)	4, 8	4, 7	53
13	20	R	nil	5·6 (1·0)	4, 14	1, 8	52, 53
14	35	R	nil	nt	11, 16	1, 7	52
15	30	A, C, E, R	C, molds	4·2 (10)	3, 15	1, 2	52
16	30	A, R	GP, alt, cld††	16·5 (1·0)	1, 13	1	52
17	29	R	C, GP	4·6 (10)	15	1	—
18	23	A, R	GP, alt	—	7, 11	2, 7	52, 53
19	22	A, E, R	C, GP, molds	13·9 (1·0)	1, 11	1, 7	52
20	36	A, R	GP, alt	7·8 (0·1)	4, 12	7	52, 53
21	51	C, R	molds	—	13, 15	1	52
22	25	E, R	nil	5·3 (1·0)	1, 10	1	—
23	19	A, R	GP, molds	7·6 (10)	4, 13	1, 8	52, 53
24	30	R	nil	3·3 (1·0)	3, 4	2, 7	52, 53
25	39	R	nil	3·5 (0·1)	3, 7	2	52, 53
26	33	R, C	nil	3·2 (10)	7	2, 9	53
27	26	A, C, R	C§, D§, GP	7·9 (1·0)	4, 11	7, 3	53
28	23	C, R	C, D	4·3 (1·0)	1, 15	1	—
29	23	A, R	GP, molds	4·7 (10)	3, 11	2, 7	52
30	21	A, R, E	C, GP	5·3 (1·0)	2, 4	1, 8	53
31	42	R, E	nil	—	3, 4	2, 8	52, 53
32	49	R	nil	—	4	7	53
33	25	C, R	GP, molds	—	7, 15	1, 2	53
34	36	R	C, GP, molds	—	2, 7	1, 4	52
35	24	R	GP, molds	—	1, 4	1, 7	53
36	62	R	nil	—	2, 7	1, 9	53
37	36	R	C, GP, molds	—	4, 7	2, 7	53
38	37	A, R	C, D, molds	—	2, 7	1, 9	—
39	23	R	GP	—	3, 13	1, 2	52
40	47	A	GP	—	1, 8	4, 5	—
41	48	R	GP	—	7, 11	2, 7	52, 53

* A, asthma; E, eczema; C, conjunctivitis; R, rhinitis.

† Determined by skin prick test, all patients were sensitive to the house dust mite (HDM).

‡ Responses are presented as stimulation indices with the concentration (in μg/ml) of Der p 2 producing that response shown in parentheses.

§ C, cat; D, dog; GP, grass pollen.

¶ Patients sensitive to more than two mould spores were designated as moulds; ** alt, alternaria; †† clad, cladosporium.

nt, not tested.

epitopes restricted by differing alleles, hence no overall major restriction specificity is observed. In order to further explore this later possibility, we present in this report an analysis of T-cell responses to the affinity-purified Der p 2 allergen and a series of overlapping peptides, spanning the entire molecule, and correlate these with the HLA-DR and -DQ serotype.

The localization of T- and B-cell epitopes on allergens has been the focus of much recent research, these investigations being essential for subsequent studies into the generation of immune tolerance or 'reprogramming' the qualitative nature of the response using peptide-based therapy. Studies on *Der p* 1, using recombinant fragments and synthetic peptides, have indicated that T-cell epitopes are found at the N- and C-terminal ends and in the central region.[17–19] Interestingly these are the areas of greatest polymorphism when comparing Der p 1 with Der f 1.[20] In this report, we also describe our studies into

the localization of T-cell reactive regions on the Der p 2 molecule and compare these findings with results from other populations.

MATERIALS AND METHODS

Patients

Individuals referred to the Clinical Immunology/Allergy clinics at the Royal Melbourne Hospital or Western Hospital Footscray were considered for inclusion into this study. Patients were referred for management of one or more of the following conditions: rhinitis, conjunctivitis, asthma or eczema. History and clinical findings were recorded for each patient and these are detailed in Table 1. None of the patients studied was receiving or had previously received allergen-specific desensitization.

Immediate-type hypersensitivity to Dpt was determined on all patients by skin prick testing according to the method of Pepys,[21] with extracts purchased from Dome/Hollister–Stier Laboratories (Sydney, Australia). A positive control with histamine acid phosphate (1 mg/ml) and a negative control with a buffer solution of the preservative alone were also performed for each of the subjects.

Patients positive for the HDM allergen who gave informed consent had 40 ml of peripheral blood taken. Ten millilitres was allowed to clot and serum was sent to the Royal Melbourne Hospital Biochemistry Laboratory where total IgE and radioallergosorbent test (RAST) determinations were performed. The other 30 ml of blood was collected into acid citrate dextrose (ACD) tubes (Becton Dickinson, Mountain View, CA) and was used for the HLA typing and cellular proliferation assays.

Serological studies and tissue typing

Specific IgE was measured to HDM by radioimmunoassay (RIA) (Kallestad Laboratories Inc., Austin, TX). Patients were only included in this study when there was both a positive skin prick test and RAST to the HDM.

Individuals were typed for HLA class I and class II DR and DQ antigens in the Tissue Typing Laboratory at The Royal Melbourne Hospital. T cells (class I) and B cells (class II) were isolated from anticoagulated blood samples by the use of magnetic beads and then typed by a conventional microlymphocytotoxicity assay using 200 antisera for class I and 120 antisera for class II.

Antigens and peptides

Native Der p 2 was purified by affinity chromatography from spent mite medium (Commonwealth Serum Laboratories, Parkville, Australia) with a Der p 2-specific monoclonal antibody (mAb) coupled to a Sepharose 4B column (10 ml). Aliquots of the eluted protein were examined for purity by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) with Coomassie blue staining, and only a single band at 15 000 MW representing Der p 2 was detected. Protein concentration was determined by optical density measurement at 280 nm. Peptides that spanned the entire Der p 2 molecule (Table 2) were synthesized with solid-phase techniques on an Applied Biosystems (Foster City, CA) peptide synthesizer as described previously.[22] The purity of the peptides, as determined by high-performance liquid chromatography, was greater than 90%. Concentrations of Der p 2 and the peptides of 0·1, 1·0 and 10 μg/ml, in triplicate, were used in each assay.

Table 2. Analysis of T-cell reactive regions on the Der p 2 molecule recognized by patients 1 to 24, who responded to one or more of the peptides.

		T-cell response Der p 2 Peptides											
Pt	Whole Der p 2	1–15	11–25	21–35	31–47	41–55	51–65	61–75	71–86	81–96	91–105	101–115	111–129
1	5·7												6·0
2	6·8			3·6									3·8
3	9·3			6·5			4·0			8·5			3·0
4	4·6												3·3
5	11·6	4·6								4·0	3·8		
6	5·5				6·5	3·4							
7	7·5		3·0	3·5									4·5
8	—												3·3
9	—												3·0
10	—							3·1					
11	9·6			7·7								3·8	4·6
12	12·4	3·0		8·0	5·0								6·8
13	5·6												4·0
14	nt	3·2							3·6				
15	4·2		3·2	6·0			4·6					3·5	4·5
16	16·5		9·6					3·7	5·8				
17	4·6					7·3	3·4	4·3					3·7
18	—								4·0				
19	13·9					4·0	3·8			3·3		3·6	
20	7·8									3·0	5·4		3·3
21	—		3·3										6·3
22	5·3												3·4
23	7·6						3·4		3·8				5·6
24	3·3								3·0				

We have previously shown (reference 6 and unpublished) that this range of concentrations is sufficient to include the maximal response in the majority of individuals. The actual concentration of Der p 2 producing the highest stimulation index is present in Table 1.

Phytohaemagglutinin (PHA) (Pharmacia Diagnostics, Uppsala, Sweden), at 10 μg/ml, and purified protein derivative (PPD) (Commonwealth Serum Laboratories), at 1 and 10 μg/ml, were included in each assay as positive controls.

Proliferation assays

Fifteen millilitres of peripheral blood was diluted with an equal volume of RPMI-1640. Mononuclear cells were aspirated from the interface after flotation on Ficoll–Hypaque, washed twice in phosphate-buffered saline (PBS), counted, and then resuspended in RPMI-1640 containing penicillin (100 U/ml), streptomycin (100 μg/ml), L-glutamine (2 mmol/l), and 10% pooled human Ab serum. The cultures of mononuclear cells were set up in triplicate in 96-well round-bottom trays in a 200-μl volume, generally at a concentration of 5×10^5 cells/ml. The antigens/mitogen were added to the wells at the commencement of the cultures which were performed for 7 days in a humidified incubator containing 5% CO_2. Preliminary experiments had established that these conditions and this time course was optimal.[6]

DNA synthesis was measured by adding 0·5 μCi of [^{3}H]thymidine during the final 16 hr of the culture. Cells were harvested with a semi-automatic multiple harvester (PHD, model 280, Cambridge Technology Inc., Watertown, MA), and thymidine incorporation was assayed in a liquid scintillation counter. Mean values were calculated for the triplicate assays and standard deviations were generally 20% to 40% with the majority being less than 60% of the mean. As background stimulation varied, results are present as a stimulation index (SI), calculated as the ratio of the maximal mean (of triplicate) counts/min of culture wells containing allergen/mitogen with that of wells containing medium alone. Positive proliferative responses to Der p 2 or the peptides were defined as occurring when the SI was ≥ 3 and the mean of the maximal proliferation induced was significantly different from that present with medium alone. For statistical calculations, a non-significant or non-elevated SI was assigned a value of 1.

RESULTS

T-cell reactivity to the native Der p 2 allergen and to a series of overlapping synthetic peptides was determined in a group of 41 HDM-sensitive patients, all of whom had symptomatic allergic disease and both skin-test reactivity and positive RASTs to the HDM. The clinical details, skin test reactivities, HLA class II DR and DQ serotypes and the responses to Der p 2 are presented in Table 1. Proliferative T-cell responses to the intact Der p 2 protein were present in 24 of the 40 (60%) patients tested. Patients 1 to 24 (59% of the 41) reacted to one or more of the synthetic Der p 2 peptides. Higher SIs were evident in those patients who had asthma as one of their clinical manifestations (mean of 8·3 ± 3·8 for responding patients with asthma, versus 5·4 ± 2·3 for responding patients without; $P = 0{\cdot}05$, Mann–Whitney rank sum test) and also in individuals with skin test reactivities to allergens in addition to the HDM (median of 8·0 ± 3·6 for polysensitive responding patients, versus 4·4 ± 1·2 for monosensitive responding patients; $P = 0{\cdot}028$, Students t-test). No significant association was evident between the level of the SI and age. The median SI was 7·9 ± 3·7 in patients 1–24 who responded to one or more of the peptides and 4·8 ± 1·7 in patients 25–30 who did not recognize any ($P < 0{\cdot}05$, Mann–Whitney rank sum test). Moreover, the greater the number of peptides recognized (Table 2), the larger this stimulatory response ($r = 0{\cdot}59$, $P = 0{\cdot}003$; linear regression analysis of variance). Proliferation present in the medium alone wells varied from 370 to 850 c.p.m. and no significant difference was evident between individuals responsive or non-responsive to Der p 2 or between those reactive or non-reactive to the Der p 2 peptides.

HLA-DR and -DQ serotypes are also presented in Table 1 for 39 of the 41 HDM-sensitive patients. There was no statistically significant difference between the frequency of antigens present in these study patients and those present in a group of 201 healthy controls, tissue typed as a part of a separate study.[23] The most striking finding was an association of particular DQ antigens with either high or low proliferative responses. The strongest association was a negative one for DQ2, with the 15 subjects with this antigen having a median SI of 3·2 ± 2·1 (SD), versus 5·3 ± 4·7 for the 23 DQ2-negative subjects ($P = 0{\cdot}023$, Mann–Whitney U-test statistic). Fourteen DQ7-positive individuals had a median SI of 5·8 ± 4·5, versus 3·9 ± 3·8 in 24 DQ7-negative patients, a difference that was not statistically significant ($P = 0{\cdot}13$). There were five DQ2/7 heterozygotes, three of whom had negative responses to Der p 2 and two had low positive levels (SI 3·7 and 4·3). If these 5 DQ2/7 heterozygotes were excluded, DQ7 positive individuals had a median SI of 7·9 ± 4·4, versus 3·9 ± 3·8 for DQ7 negative patients ($P = 0{\cdot}01$). Furthermore, with the DQ2/7 heterozygotes excluded, the difference between the medians of the DQ7 (7·9 ± 4·4) and DQ2 (3·4 ± 2·3) positive individuals was significant ($P = 0{\cdot}01$). To further investigate the association of the DQ7 antigen with enhanced Der p 2 responsiveness, the frequency of the HLA-DQ7, non-DQ2, class II serotype was calculated in individuals who were strongly Der p 2 positive and compared with Der p 2 weakly positive and negative individuals. Seven out of 11 (63·6%) individuals with an SI > 6·0 were $DQ7^+2^-$, compared with 2/27 (7·4%) individuals with an SI < 6·0 ($P < 0{\cdot}001$, χ^2 with Yates correction).

We also investigated the response of fresh polyclonal T cells from HDM-sensitive individuals to overlapping peptides corresponding to all regions of the Der p 2 molecule, results are presented in Table 2. Of the 41 patients, reactivity to one or more of the peptides was present in 24. T-cell responses to all regions of the molecule were observed. Individual members of these 24 patients responded to from one to five of the peptides, with a mean of 2·3 ± 1·3. No peptide was stimulatory in all of the patients, indicating that the stimulation could not be due to mitogen-like activity. As with Der p 2, the concentration of peptide inducing maximal T-cell response varied, being 1·0 μg/ml in 28, 10 μg/ml in 19 and 0·1 μg/ml in nine. The majority of patients who reacted to one or more of the peptides also responded to the whole Der p 2 molecule, with only 5/23 being unresponsive. Similarly, of those 24 patients who responded to Der p 2, 18 or 75% responded to one or more of peptides. Generally the proliferative response to the whole molecule was stronger than to any of the peptides, with the mean for Der p 2 being 5·9 ± 4·7 and that for the overlapping peptides varying

from 3·6 ± 0·9 to 5·9 ± 1·9. The most frequently recognized peptide was that encompassing amino acid positions 111–129, with T cells from 16 of 24 patients (67%) being reactive.

DISCUSSION

In this report we have shown for the first time that specific HLA-DQ antigens may be associated with both high (DQ7) and low (DQ2) T-cell responsiveness to the Der p 2 allergen. This association was only apparent when the magnitude of the response was considered and there was no significant association with absolute frequency of HLA-DR or -DQ antigens. The latter is consistent with findings in previous HLA frequency studies to Der p 1[19] and confirms the work of other authors.[10,16] In addition, we have also demonstrated that the negative effect of the DQ2 antigen is dominant over the positive effect of DQ7.

There are a number of possible mechanisms to account for the inhibitory/stimulatory effects of the DQ alleles in our patient group. Certainly, DQ alleles have been reported to act as 'immune suppression' genes and to code for non-responsiveness to a number of antigens. As early as 1984, an association between HLA-MB1 and diminished T-cell responses to PPD was observed in *in vitro* T-cell responses in normal individuals.[24] Later studies from Matsushita *et al.*,[25] Hirayama *et al.*[26] and from Salgame *et al.*[27] confirmed HLA-DQ related non-responsiveness and provided evidence for the existence of antigen-specific $CD8^+$ cytotoxic T cells, restricted by HLA-DQ. These $CD8^+$ cells may interact directly on $CD4^+$ T cells inducing lysis or possibly on antigen-specific B cells, although immunoregulation by production of cytokines, such as interferon-γ (IFN-γ), and immune deviation to a Th1 response is a more contemporary paradigm.[28] Although 'immune suppressive' effects have generally been associated with HLA-DQ alleles, a recent report from Reid *et al.* has demonstrated a quite strong association between the DR4 allele and decreased IgE responses to mountain-cedar allergen,[13] thus emphasizing the concept that the immune system can generally utilize a variety of mechanisms to achieve a particular result.

Although most reports have implicated HLA-DQ alleles as being associated with negative immune responsiveness, Cardaba *et al.* have demonstrated a heightened IgE response in DR7- and DQ2-positive individuals.[12] Despite the fact that these two alleles are in strong linkage disequilibrium, it did seem that the DQ gene was the major susceptibility locus. Interestingly, the major DQ gene identified in our patients coding for immune non-responsiveness was also DQ2. The lack of any overall HLA-DR or -DQ association with HDM recognition would suggest that other genetic factors determine whether individuals are responsive to the mite. However, our data does indicate that in HDM recognition, the presence of DQ7 enhances the strength of response to the Der p 2 allergen. Likewise the presence of HLA-DQ2 appears to diminish the response to Der p 2 and the low response in DQ2/7 heterozygotes suggests a DQ2-associated dominant suppressive effect.

The epitope localization studies reported here indicated that, although immunogenic regions existed throughout the Der p 2 molecule, the most frequently recognized region was located within the C-terminal 111 to 129 residues. Interestingly, although specific DQ antigens correlated with high and low T-cell responses to the entire Der p 2 molecule, there was no clear association between HLA class II serotype and a particular epitope recognized or pattern of recognition. This suggests that the association of specific HLA-DQ alleles with high and low responses maybe because DQ acts in a regulatory fashion, rather than as the direct restricting element of T-cell recognition. An alternative hypothesis could be that initial responses may be induced by cross-reactivity with another antigen, e.g. food allergens as proposed by Calkhoven *et al.*[29] The 'immune response' gene effect would then be for the cross-reactive antigen. Finally it remains possible that the processing of the whole Der p 2 molecule by the presenting cell *in vitro* may result in the generation of particular peptides not tested in this study. In this context the use of *in vitro* experiments of synthetic peptides derived from the Der p 2 molecule with sequences containing amino acids at critical residues known to bind preferentially to DQ7 molecules, but not DQ2 molecules, may provide a basis for explaining the DQ associations seen.[30]

A previous study investigating the T-cell epitopes on Der p 2 also used polyclonal T cells and synthetic peptides.[22] Nine atopic and nine non-atopic individuals were investigated and, as with our studies, epitopes were recognized throughout the Der p 2 molecule. Overall, proliferative responses from atopic individuals were greater than from non-atopic subjects. Peptides spanning amino acid residues 61–104 were recognized by 16/18 individuals and nine subjects reacted to peptides spanning 11–50. Reactivity to peptide 105–129 was certainly present but the region was not considered to contain a dominant epitope, although it was recognized by five atopic and four non-atopic subjects. All of the individuals analyzed in this study reacted to the Der p 2 allergen and 95% were responsive to one or more of the peptides. In contrast, only 60% of our HDM-sensitive subjects reacted to the Der p 2 allergen and 75% (18/24) of this group, who were tested, reacted against one or more of the peptides. Moreover of the 24 patients who responded to the peptide, five did not react with the whole Der p 2. Four of these patients, however, only responded to one peptide, suggesting that response may be low and that the peptide is more efficient at stimulating marginal responses. Another possibility is that the sensitization of these five patients is actually to a group 2 allergen from another species, such as *D. farinae*, or a polymorphism of Der p 2. The presentation of the processed peptide in the cultures stimulated by whole allergen may then be competed to undetectable levels (in this assay) by non-stimulatory peptides which bind the MHC but not antigen-reactive cells, which are mostly sensitized to the peptides of another species or polymorphism. This competition would not exist with a single peptide.

Van Neervan and colleagues have also studied T-cell epitopes on Der p 2.[31] They investigated five atopic HDM-sensitive individuals and showed that short-term T-cell lines reacted with at least 10 different epitopes, including one within the C-terminal residues 116–129. T-cell clones were generated from an individual reacting only with this C-terminal region and T-lymphocyte recognition was demonstrated to be restricted by HLA-DR alleles, not HLA-DQ or HLA-DP. The identification of an immunodominant region of the Der p 2 molecule in the C-terminal region is consistent with previous studies on epitope recognition of HDM proteins in murine models.[32] Following Der p 2 immunization, using the high-responder H-2b strain, T cells from draining lymph nodes

recognized only three epitopes and two of these were located in the C-terminus, peptides 87–104 and 105–129.

In previous research into epitope localization on the Der p 1 allergen, immunogenic sites were found to occur predominately in regions of high polymorphism.[17,19] Interestingly, sequencing of a number of different Der p 2 cDNA clones has revealed an additional four closely related variants with a total of five polymorphic residues (manuscript in preparation). Two of these residues are located in the C-terminal region, at positions 114 and 127. Moreover, when comparing Der p 2 with Der f 2, there are 18 polymorphic residues and five of these are in the C-terminal 20 residues. Thus, as with Der p 1, it appears that the most immunodominant regions of the Der p 2 molecule are those with the greatest polymorphism. In terms of mechanisms, there is good evidence now that polymorphic regions tend to be more flexible and hence may establish contact with MHC molecules more readily.[33]

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