

An immunogenetic analysis of T-cell reactive regions on the major allergen from the house dust mite, *Der p* I, with recombinant truncated fragments

Richard M. O'Brien, FRACP, PhD^a [\[MEDLINE LOOKUP\]](#)

Wayne R. Thomas, PhD^b [\[MEDLINE LOOKUP\]](#)

Brian D. Tait, PhD^c [\[MEDLINE LOOKUP\]](#)

Footscray, Perth, and Melbourne, Australia

Sections

- [Abstract](#)
- [Methods](#)
- [Results](#)
- [Discussion](#)
- [References](#)
- [Publishing and Reprint Information](#)

Abstract

Proliferation assays were used to localize the T-cell reactive sites on the major allergen from the house dust mite *Dermatophagoides pteronyssinus*, *Der p* I. Seven overlapping recombinant fragments of the *Der p* I molecule, synthesized with the pGEX expression vector system, were used to stimulate peripheral blood lymphocytes from 35 HDM-sensitive individuals. The fusion fragments were from 39 to 114 amino acids in length and spanned the entire *Der p* I molecule. Significant proliferative responses to one or more of the fragments were evident in 18 of the allergic individuals, and each of the fragments led to T-cell stimulation in at least one of the subjects. Although T-cell reactive regions were located throughout the molecule, in 12 of the 18 responsive individuals, major immunogenic sites were contained within the 56 amino acids of the N-terminus, and in 11 of these individuals T-cell reactive regions were only present between amino acid positions 1 and 94. In two individuals reactive sites could be mapped in the C-terminal half of the molecule, and in five subjects, epitopes were present in both N- and C-terminal regions. HLA class I and class II DR and DQ specificities were determined serologically in 16 of the 18 individuals, and no strong pattern of association between HLA type and the T-cell immunogenic region could be detected. (J ALLERGY CLIN IMMUNOL 1994;93:628-34.)

GST Glutathione-s-transferase

HDM House dust mite

PBMCs Peripheral blood mononuclear cells

SI Stimulation index

Key words: [House dust mite](#), [Dermatophagoides pteronyssinus](#), [T-cell epitopes](#), [proliferation assay](#), [HLA](#), [immunogenetics](#), [allergy](#), [recombinant](#)

The house dust mite (HDM) is now recognized as one of the most important causes of allergic respiratory disease worldwide and has become the focus of much research, both clinical and laboratory-based. Studies in which patients' sera were used have identified several immunoreactive allergens from the HDM, and a number of these have now been cloned and sequenced. Despite this increased knowledge of the structure of mite allergens, our immunotherapeutic capability against mite-induced allergic disease remains clumsy and not universally effective.

Because IgE responses to aeroallergens with subsequent tissue sensitization are strictly dependent on T-lymphocyte help and because T lymphocytes also directly participate in the inflammatory allergic response, ¹⁻⁴ studies analyzing T-cell recognition of the major HDM allergens, and more particularly those designed to ascertain the critical T-cell reactive sites, are essential. With knowledge of the location and prevalence of immunogenic regions, the development of more effective immunotherapeutic strategies, possibly incorporating synthetic peptides, should be feasible.

Der p I is a major allergen of the HDM, with serologic reactivity being present in a majority of individuals allergic to mites. ⁵ Moreover, up to 60% of the specific IgE in HDM-allergic serum has been shown to bind to the *Der p I* protein. ⁶ We have previously cloned and sequenced *Der p I* and also expressed the gene as a fusion protein with glutathione transferase with the use of the pGEX vector system. ⁷ In the studies reported here, we have used fragments of the *Der p I* protein, generated with convenient restriction enzyme sites, and also expressed as fusion proteins, to explore the T-cell repertoire of individuals allergic to *Dermatophagoides pteronyssinus*. The results shown that peripheral blood mononuclear cells (PBMCs) respond to epitopes in the molecule differently than those previously reported with synthetic peptides and T-cell clones or lines.

Because T-cell recognition of allergen occurs in association with the Class II major histocompatibility complex proteins, it would be expected that the individual allergens (or specific epitopes within these) responsible for allergic disease would be presented by particular alleles. With this in mind, we have determined the HLA serotype of the allergic individuals studied to determine whether any specific association pattern exists. Additionally, we report here the HLA serotyping of a group of 51 HDM-allergic individuals, including 16 of the 18 who were responsive to the *Der p I* fusion fragments, undertaken as a preliminary study, and compare this to results obtained from 119 healthy control

subjects. [8](#)

METHODS

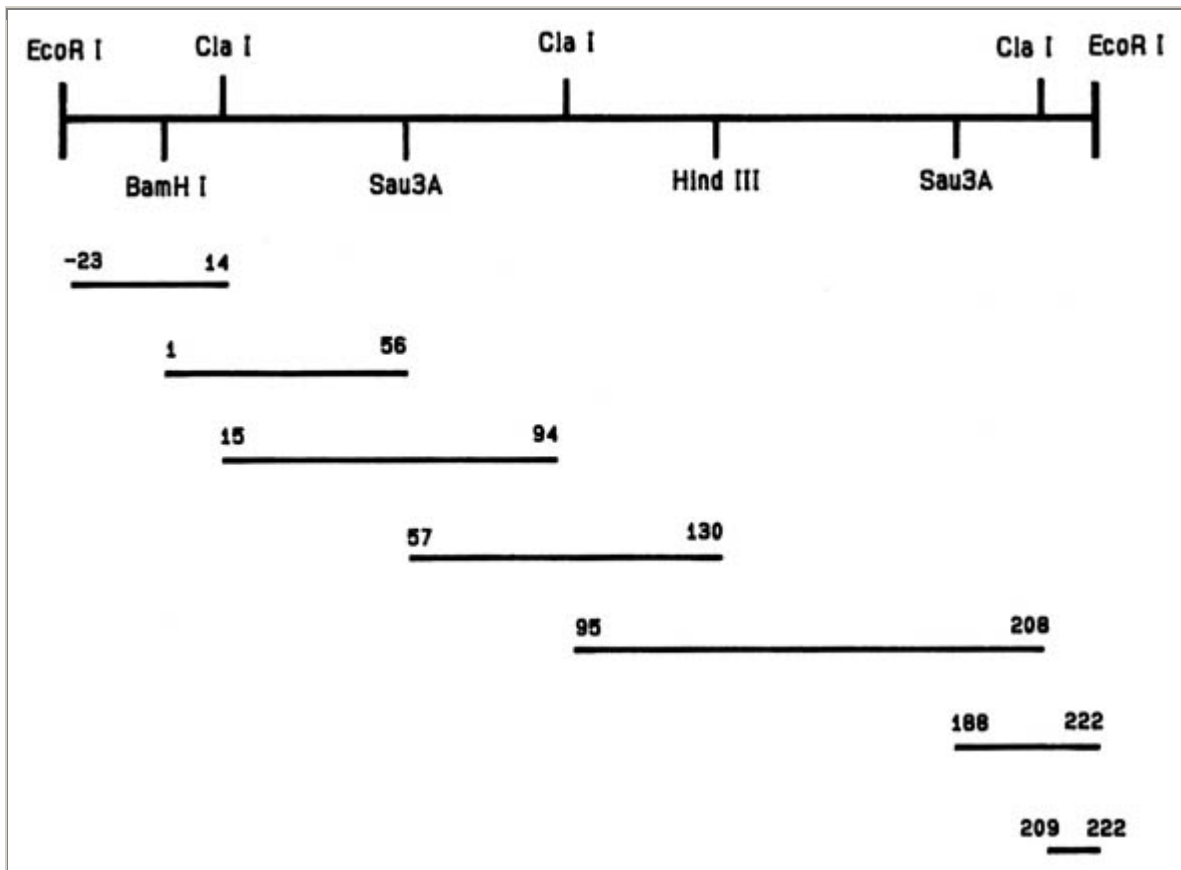
Patients

Adult patients referred to the Allergy Clinics at either the Royal Melbourne Hospital or Western Hospital Footscray were recruited for this project. Immediate-type hypersensitivity to HDM was determined by skin prick testing and then intradermal injection with extracts from Dome/Hollister-Stier (Sydney, Australia). A positive control test with histamine acid phosphate (1 mg/ml) and a negative control test with a buffered solution of the preservative alone were always performed. Total IgE levels and RAST determinations of sensitivity to HDM and other allergens were performed on serum samples from all patients in the Biochemistry Laboratory, Royal Melbourne Hospital. Patients were only included in this study when there was a concordance of skin testing and RAST results. HLA class II typing of DR (DRB1, B3, and B4 gene products) and DQ specificities was performed in the Tissue Typing Laboratory, Royal Melbourne Hospital, by conventional microlymphocytotoxicity. A total of 51 HDM-sensitive individuals were typed, including those studied for patterns of T-cell recognition. These results were compared with those from a control group of 119 healthy individuals, typed as a part of a separate study. [8](#)

Recombinant proteins

The restriction sites of the *Der p I* complementary DNA clone, as indicated in [Fig. 1](#), were used to generate a series of overlapping fragments as previously described. [9](#) Restriction enzymes (Toyobo Co. Ltd., Osaka, Japan) were used according to the manufacturer's instructions. All fragments were blunt-ended with either Klenow or T4 DNA polymerase (Toyobo) and ligated into the *Sma I* site of pGEX vectors, which were used to transform *Escherichia coli* HB101. The *Der p I* peptide fragments, fused to glutathione-s-transferase (GST), were expressed from pGEX and affinity-purified with glutathione agarose.

FIG. 1. Restriction map of the full-length *Der p I* cDNA showing location and length of the overlapping recombinant fragments used in these experiments. All fragments were produced as fusion proteins attached to the GST protein of *Shistosoma japonicum*.



Cellular proliferation assays

Thirty milliliters of peripheral blood was obtained from the allergic patients and of this, 10 ml was allowed to clot, and the serum was used for measuring specific and total IgE. Twenty milliliters was anticoagulated in ACD tubes (Becton Dickinson, Mountain View, Calif.) and of this, 10 ml was sent to the tissue typing laboratory and 10 ml was used for proliferation assays. ¹⁰ After isolation on Ficoll-Hypaque (Pharmacia, Uppsala, Sweden), mononuclear cells were resuspended in RPMI-1640 medium (Gibco, Grand Island, N.Y.) containing glutamine, penicillin, streptomycin, and 10% pooled human AB serum. Cultures were set up in triplicate in a 200 μ l volume in 96-well round-bottom trays, generally at a concentration of 5×10^5 cells/ml.

The antigens or mitogens were added to the wells at the commencement of the culture, which was performed in a humidified incubator containing 5% CO₂. Fusion proteins were used at a concentration range of 1.0 to 100 μ g/ml, a range shown in preliminary experiments to be sufficient to include the maximal response. Negative controls of cells alone and cells with GST alone, at a range of concentrations, were included in each assay. A positive control of purified protein derivative (10 μ g/ml) was also performed with each assay. Cultures were incubated for 7 days, a duration previously established to yield optimal results. ¹⁰ DNA synthesis was measured by adding 0.5 μ Ci of tritiated thymidine during the final 16 hours of culture. Cells were harvested with a semiautomatic multiple harvester, and thymidine incorporation was assayed in a liquid scintillation counter.

Because background proliferation to GST varied significantly among individuals, responses were expressed as stimulation indexes (SIs), calculated as the ratio of the maximal mean counts per minute of the triplicate assays from wells containing allergen to the maximal mean counts per minute of the wells containing the medium or GST alone.

SIs are only presented here if the differences between the mean maximal counts of the triplicate wells containing the fragments and the triplicate wells containing GST alone were significant at the $p < 0.05$ level (Student's t test).

RESULTS

HLA typing of HDM-sensitive patients

As a preliminary step in our analysis of the immunogenetics of HDM hypersensitivity, HLA class I and II tissue typing, by microlymphocytotoxicity, was performed on 51 patients with an allergic disorder caused by HDM sensitivity. These serotypes were compared with those obtained from a group of 119 healthy control subjects, who were tissue typed as a part of a separate study.⁸ There was no difference in the distribution of HLA DRB1 or DQ specificities. The distribution of HLA DRB3 and DRB4 specificities appeared to be different with DR52 being present in 70.6% of the HDM-sensitive individuals and 58.8% of the healthy control subjects. This, however, was not significant at the 5% level as determined by chi square analysis.

Analysis of T-cell reactive sites of *Der p* I

Proliferation assays were performed with PBMCs from a number of HDM-sensitive individuals. The details and validation of the methods used have recently been published.¹⁰ The PBMCs were cultured in triplicate in the presence of one of the recombinant *Der p* I fragments (■Fig. 1) at five to seven different concentrations from 1.0 to 100 µg/ml. The recombinant full-length protein, along with purified protein derivative (10 µg/ml) as a positive control, and the GST protein were also included in each assay. A similar range of concentrations, each in triplicate, was also used for the full-length protein and GST, and the concentrations producing the maximal mean (of triplicate) responses were used for calculating the SI. Fragments were classified as being stimulatory if the SI was greater than or equal to 3 and if the mean of the maximal proliferation induced was significantly different from the mean maximal proliferation generated by GST alone.

In 18 of the 35 individuals studied, good levels of proliferation to one or more of the fragments were evident. All of the fragments were stimulatory in at least one of the individuals, indicating that each had functional activity. ■Table I presents the SIs to *Der p* I and the recombinant fragments for each of the 18 patients who responded to one or more of the fragments. Mononuclear cells from individual patients recognized one to five fusion peptides, with a median of 2; and usually, although not always, it could be concluded from the position of the peptides that they were reactive to different epitopes. The protein concentration producing the maximal response is also shown. It is evident that immunogenic sites are present in a number of locations of the *Der p* I molecule; however, the N-terminal region is recognized the most frequently with 12 or 18 patients reacting to an epitope or epitopes within the first 56 amino acids. In 16 of the patients, reactive sites were present in the 94 amino acids of the N-terminus, and in 11 they were present only in this segment. Five of the patients reacted to epitopes present in both the N- and C-terminal regions of *Der p* I, whereas in only two patients were immunoreactive sites restricted to the C-terminal portion. In two of the patients who responded to one or more of the fragments, there was no reactivity to the entire *Der p* I molecule. Also shown in ■Table I are the other allergic sensitivities for each of the 18 individuals. It is evident there is no

correlation between patterns of allergic reactivity and the location or number of immunogenic regions.

TABLE I. Immunogenetic analysis of SIs of T cells from HDM-sensitive individuals to overlapping fragments of *Der p I*

Patient	Allergen sensitivities*	<i>Der p I</i> response†	<u>Fragment response‡</u>			<u>Fragment response</u>				<u>HLA class II serotype</u>		
			-23-14	1-56	15-94	57-130	95-208	188-222	209-222	DQ	DRB1	DRB3/4
SJ	—	3.0 (10)		21.8 (30)		9.6 (30)		37 (100)				
LC	C,G,M,F	16.2 (3)	8.8 (30)	19 (30)	7.5 (30)					2,8	3,4	52,53
BC	G,M	3.4 (30)			3.0 (30)					1,2	7,15	53
DE	C	—						3.0 (100)		7,13	1,2	52
MJ	—	4.7 (100)	5.0 (30)		6.5 (30)					1,7	11,16	52
AJ	M	3.8 (10)			3.3 (100)		4.1 (10)					
HJ	C,D,G	9.8 (10)		5.4 (30)						1	1,15	
GD	C,D	3.5 (30)		3.2 (30)	3.5 (100)					3,7	4,11	53
TL	G	3.0 (30)		4.8 (30)	3.2 (10)					1,7	4,X	53
GR	C,G	5.2 (3)			3.5 (3)					1	2,13	52
SD	—	—		28.1 (3)						1,7	11,13	52
AH	—	8.4 (30)	3.6 (30)	5.6 (3)	6.5 (10)	6.1 (10)		3.6 (30)		2,7	3,4	52,53
TA	G	3.9 (10)		9.0 (3)						1,7	2,11	52
MR	G,F	4.5 (30)					6.4 (30)	4.0 (100)		7	4	53
DM	G,F	3.3 (30)			7.1 (30)			14.2 (10)	6.4 (3)	2,7	3,4	52,53
JD	G	6.6 (10)		8.7 (10)						1,2	2,3	52
JE	—	9.3 (30)	4.9 (10)							2,4	7,8	52,53
GE	C,D,G	5.2 (30)		6.5 (30)		9.4 (10)		8.8 (10)		1	1,13	52

C. Cat; G. grass pollen; M. molds; F. food sensitivity (one or more); D. dog.

*All patients were sensitive to HDM

†SI of T cells to the recombinant *Der p* I fusion protein versus the GST fusion protein alone. The protein concentration

leading to the maximal SI is given in brackets.

‡SI to the various recombinant truncated fragments, also versus the GST fusion protein alone. The protein concentration leading to the maximal SI is given in brackets.

The 17 individuals who did not respond to any of the fragments did not differ in any clinical or serologic respect from the 18 who showed a response to one or more of the fragments. In a previous article we had shown that nonatopic individuals either do not respond or respond poorly to the intact *Der p* I molecule. To ensure that there was no reactivity to the recombinant truncated fragments in individuals without specific IgE, six nonatopic individuals were also studied for reactivity to the seven fragments; there were no significant reactivities in any of these six control subjects (data not shown).

Comparison of HLA type and immunoreactive region recognized

To determine whether there was an association between the immunoreactive regions on *Der p* I and the patient's HLA type, the HLA-DRB1, B3, and B4 types and the DQ serotype of 16 of the 18 patients were also determined and are presented in Table I. No strong pattern of association is evident.

DISCUSSION

The major findings from this study are that recombinant truncated fusion fragments can be used to localize T-cell reactive regions on the major allergen from *D. pteronyssinus*, *Der p* I, and that T cells from individuals sensitive to HDM recognize epitopes throughout the molecule. Although our strategy of using longer fragments was not designed to identify minimal epitopes, it revealed a diversity and positioning of epitopes that differed from data previously reported by two other groups [11](#), [12](#) that used synthetic peptides and T-cell clones. O'Hehir et al. [11](#) demonstrated epitopes between positions 45-73, 89-117, 85-109, and 111-139; and Yssel et al. [12](#) localized epitopes to regions 45-67, 94-104, and 117-143. In our study the most frequent reactivity, and often the strongest in terms of SI, was with the N-terminal peptide 1-56 (10 of 18 responders showed reactivity), which, assuming 10 residues are sufficient for a minimal epitope, would only contain two of seven of the epitopes so far described. However, of the 10 reactors to this fusion, six did not react to region 15-94, indicating that there would be no overlap with the currently described epitopes.

The fusion peptide 15-94, which contained three of seven of the epitopes, was also a frequent reactor producing a positive SI in nine of 18 patients. The most surprising result was that the fusions 57-130 and 95-208, which contained five of seven of the published epitopes, reacted with only three of 18 and two of 18 of the patients, respectively. The C-terminal fusion peptide 188-222, which does not contain any of the described epitopes, reacted with six of 18 patients, being the third most frequent reactor. Although the fusions were generally large and the study was not designed to precisely locate the epitopes, the N-terminal fusion, encompassing only residues 1-14, reacted with mononuclear cells from four of 18 patients, and the C-terminal fusion, encompassing 209-222, reacted with cells

from one.

The sequences of *Der p* I and *Der f* I, the major allergen of *D. farinae*, show that these allergens have about 20% diversity, most of which is found in the N- and C-terminal 1-20 and 200-222 and in the center of the molecule 90-110. ¹³ These regions, based on the structure of the related papain and actinidin, correspond to flexible regions, the junction of which contains the catalytic site. The remaining conserved regions have been shown by crystallography to be two hydrophobic globular domains. ¹⁴ The epitopes previously described for T-cell clones ^{11, 12} have generally clustered in the variable central connecting portion of the molecule and are likely to be species-specific. Our studies have indicated epitopes predominantly in the N-terminal, and (less commonly) in the C-terminal, regions of *Der p* I, which would also probably be species-specific. More precise information would be required to localize cross-reacting epitopes.

This is the first reported study in which recombinant molecular techniques have been used to identify epitopes with fresh ex vivo lymphocytes from a number of individuals allergic to HDM. Previous unreported studies in human beings, in which synthetic peptides were used, had not generated convincing polyclonal responses, and it is possible that this was due to the much shorter sequences generally used. Indeed, in our own studies in mice (unpublished), we likewise found reactivity to the recombinant *Der p* I fragment spanning residues 1 to 56, but not to any overlapping shorter peptides within this region. This is possibly because epitope recognition and presentation may be modified by the absence of flanking sequences in these shorter peptides. Certainly, the degree to which substitutions can be tolerated in stimulation assays with peptide epitopes can depend on the presence of the flanking sequence, ¹⁵ because these regions are of great importance in the processing, binding, and stability of epitopes. ^{16, 17} Two of the 18 individuals who responded to a fusion peptide did not recognize the whole allergen; it is possible that the entire recombinant molecule is processed differently than the natural protein because of its different conformation, and hence, the relevant peptides are not always displayed adequately. The same processing events do not necessarily occur for the fusion peptides. Another possibility is that the actual immunogen in the patient is a polymorphorm or the equivalent allergen from another species. The study by Liu et al. ¹⁸ shows that even a single amino acid change outside an epitope can prevent effective presentation.

There are advantages and disadvantages in the use of either synthetic peptides or recombinant (fusion) proteins. Certainly the longer fragments (40 to 100 amino acids) are easily and accurately generated in expression vectors and are much more likely to require processing, which may result in the presentation of different peptides than those used in sets of smaller synthetic peptides. Furthermore, some peptides are processed before presentation (e.g., *Der p* II 105-129 in mice; ¹⁹) therefore, if an inappropriate synthetic peptide is used, the epitopes may be destroyed. On the other hand, synthetic peptides of 13 to 15 residues are easily synthesized and are essential for more precise localization of epitopes. Thus it is evident that a variety of methods are needed to successfully define the relevant epitopes and that these studies with recombinant truncated fragments complement the studies with shorter synthetic peptides.

Because IgE responses are dependent on T-cell help ¹ and because T-cell recognition of allergen only occurs in association with major histocompatibility complex class II proteins, ²⁰ the allergens responsible for allergic disease should be presented by particular major histocompatibility complex class II alleles. The lack of a strong association between class II alleles and HDM sensitivity, as demonstrated by other authors ²¹⁻²³ and by our own data,

could be due to restriction to previously undetermined class II alleles, or it may be that the different allergens within the HDM and/or the subregions within the individual major allergens are all restricted by different class II alleles, and hence, no clear overall pattern of restriction is able to emerge. Our data from examination of T-cell immunogenic regions and HLA-DR and DQ specificities suggest that associations do not exist, but more critical analysis with minimal T-cell epitopes is required. Concerning the possibility that class II genes, apart from DR and DQ, may be relevant in mite allergen hypersensitivity, a recent study by Caraballo et al., [24](#) in which genetic typing techniques were used, indicated that HLA-DP alleles may be critical in HDM allergy. These results, which involved population analysis, have been supported by more recent molecular typing data from a study by Young et al. [25](#)

We thank Miss Vanessa Christopher for performing all the assays and Mrs. Linda Scott for her skillful preparation of this manuscript.

REFERENCES

1. Ishizaka K. Regulation of IgE synthesis. *Annu Rev Immunol* 1984;2:159-83.
2. Orange PR, Austen WG, Austen KF. Immunological release of histamine and slow reacting substance of anaphylaxis from human lung. I. Modulation by agents influencing cellular levels of cyclic 3',5' adenosine monophosphate. *J Exp Med* 1971;134:136S-48S.
3. Plaut M. Antigen-specific lymphokine secretory patterns in atopic disease. *J Immunol* 1990;144:4497-500.
4. Lett-Brown MA, Alam R, Grant JA. Regulation of human basophils and mast cells. Activation by cytokines. In: Cruse JM, Lewis RE Jr, eds. *The Year in immunology 1988. Immunoregulatory cytokines and cell growth*. Basel: S. Karger, 1989:195-204.
5. Tovey ER, Baldo BA. Comparison by electroblotting of IgE-binding components in extracts of house dust mite bodies and spent mite culture. *J ALLERGY CLIN IMMUNOL* 1987;79:93-102.
6. Van Der Zee JS, Van Sweiten P, Jansen HM, Aalberse RC. Skin tests and histamine release with P1-depleted *Dermatophagoides pteronyssinus* body extracts and purified P1. *J ALLERGY CLIN IMMUNOL* 1988;81:884-96.
7. Chua KY, Stewart GA, Thomas WR, et al. Sequence analysis of cDNA coding for a major house dust mite allergen, *Der p I*. *Homology with cysteine proteases*. *J Exp Med* 1988;167:175-82.
8. Barnett AJ, Tait BD, Barnett MA, Toh BH. T lymphocyte subset abnormalities and HLA antigens in scleroderma (systemic sclerosis). *Clin Exp Immunol* 1989;76:24-9.
9. Green WK, Cyster JG, Chua K-Y, O'Brien RM, Thomas WR. IgE and IgG binding of peptides expressed from fragments of cDNA encoding the major house dust mite allergen *Der p I*. *J Immunol* 1991;147:3768-73.

10. O'Brien RM, Thomas WR, Wootton AM. T cell responses to the purified major allergens from the house dust mite *Dermatophagoides pteronyssinus*. J ALLERGY CLIN IMMUNOL 1992;89:1021-31.
11. O'Hehir RE, Garman RD, Greenstein JL, Lamb JR. The specificity and regulations of T-cell responsiveness to allergens. Annu Rev Immunol 1991;9:67-95.
12. Yssel H, Johnson KE, Schneider pV, et al. T cell activation-inducing epitopes of the house dust mite allergen *Der p* I. Proliferation and lymphokine production patterns by *Der p* I-specific CD4⁺ T cell clones. J Immunol 1992;148:738-45.
13. Dilworth RJ, Chua KY, Thomas WR. Sequence analysis of cDNA coding for a major house dust mite allergen *Der f* I. Clin Exp Allergy 1991;21:25-32.
14. Stewart GA, Thomas WR, Chua HY, Turner KJ, Geysen HM. An allergen and antigenic mapping analysis of a major mite allergen, *Der p* I. Adv Biosciences 1989;74:297-311.
15. Brown LE, Jackson DC, Tribbick G, White DO, Geysen HM. Extension of a minimal T cell determinant allows relaxation of the requirement for particular residues within the determinant. Int Immunol 1991;3:1307-13.
16. Eisenlohr LC, Yewdell JW, Bennink JR. Flanking sequences influence the presentation of an endogeneously synthesized peptide to cytotoxic T lymphocytes. J Exp Med 1992;175:481-7.
17. Gammon G, Geysen HM, Apple RJ, et al. T cell determinant structure: cores and determinant envelopes in three mouse major histocompatibility complex haplotypes. J Exp Med 1991;173:609-17.
18. Liu Z, Williams KP, Chang Y-H, Smith JA. Single amino acid substitutions alter T cell determinant selections during antigen processing of *Staphylococcus aureus* nuclease. J Immunol 1991;146:438-43.
19. Hoyne GF, Callow MG, Kuo M-C, Thomas WR. Characterization of T-cell responses to the house dust mite allergen *Der p* II in mice. Evidence for major and cryptic epitopes. Immunology 1993;78:65-73.
20. Schwartz RH. T lymphocyte recognition of antigen in association with gene products of the major histocompatibility complex. Annu Rev Immunol 1985;3:237-61.
21. Ishikawa T, Kitao Y. Genetic control of in vivo IgE antibody production specific to *Dermatophagoides farinae*. Int Arch Allergy Appl Immunol 1985;77:210-12.
22. Caraballo LR, Hernandez M. HLA haplotype segregation in families with allergic asthma. Tissue Antigens 1990;35:182-6.
23. Friedhoff LR. Epidemiology of atopic allergy. In: Marsh DG, Blumenthal MN, eds. Genetic and environmental factors in clinical allergy. Minneapolis: University of

Minnesota Press, 1990:53-72.

24. Caraballo LR, Marrugo J, Jimenez S, Angelini G, Ferrara GB. Frequency of DPB1*0104 is significantly decreased in patients with allergic asthma in a mulatto population. *Hum Immunol* 1991;32:157-61.

25. Young RP, Wordsworth BP, Pile K, et al. HLA-DPB1*0401 increased in atopic disease [Abstract]. *Allergy* 1992;47:8.

Publishing and Reprint Information

- From ^aThe University of Melbourne, Department of Medicine, Western Hospital, Footscray; ^bThe Western Australian Research Institute of Child Health, Princess Margaret Hospital, Perth; and ^cTissue Typing Laboratory, Royal Melbourne Hospital, Melbourne, Australia.
- Supported principally by grants from the National Health and Medical Research Council, Australia.
- Received for publication Sept. 9, 1992.
- Revised July 30, 1993;
- Accepted for publication Aug. 3, 1993.
- Reprint requests: R. M. O'Brien, FRACP, PhD, Department of Medicine, Western Hospital, Gordon St., Footscray VIC 3011 Australia.
- Copyright © 1994 by Mosby—Year Book, Inc.
- **1/1/50449**