

Cross-reactivity of T-cell responses to *Dermatophagoides pteronyssinus* and *D. farinae*. Studies with group 1 and 7 allergens

B. J. HALES*†, H.-D. SHEN‡ and W. R. THOMAS*

*TVW Telethon Institute for Child Health Research, PO Box 855, West Perth WA 6872, Australia, †Department of Microbiology, The University of Western Australia and ‡Department of Medical Research, Veterans General Hospital, Shih-pai, Tapei, Taiwan, 11217, Republic of China

Summary

Background Comparative information on T-cell responses to allergens from different *Dermatophagoides* species is limited even though differences in the epitypic recognition have been described.

Objective To compare the level of T-cell proliferation and cytokine production to allergens from the mite species, *D. pteronyssinus* and *D. farinae*.

Methods Freshly isolated peripheral blood mononuclear cells (PBMC) from house dust mite (HDM)-allergic and HDM-nonallergic donors were stimulated with the group 1 and group 7 allergens of *D. pteronyssinus* and *D. farinae* and the level of proliferation as well as IL-5 and IFN γ production were measured.

Results The proliferative response and the level of IL-5 produced after *in vitro* challenge with group 1 and group 7 allergens were equivalent for the allergens from both mite species even though *D. farinae* is not detected in the environment where the study population live. As expected the level of IL-5 production to the individual allergens was higher for the allergic donor group than for the nonallergic donors, however, there was no difference in the level of T-cell proliferation between the different donor groups.

Conclusion The proliferative and cytokine response to the group 1 and group 7 allergens for *D. pteronyssinus* and *D. farinae* indicates that there is a large degree of T-cell cross-reactivity between the whole purified allergens from each species. This is despite previous reports demonstrating different responses to synthetic peptides representing Der p 1 and Der f 1 in a similar study population.

Keywords: cytokines, *D. farinae*, Der f 1, Der f 7, Der p 1, Der p 7, *Dermatophagoides pteronyssinus*, house dust mite, T cells.

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Introduction

Mites of the *Dermatophagoides* genus were identified as a significant source of allergen in house dust over 30 years ago [1–2]. The allergens produced from these mites are now known to cause sensitization in between 10 and 30% of most

populations and the sensitization is a major independent risk factor for developing asthma [3,4–10]. Two species of *Dermatophagoides* mites have consistently been found to be important in urban temperate regions. *D. pteronyssinus* is found in coastal regions of Australia, New Zealand, Europe and most of the USA [11–14]. In the continental regions of Europe and the USA, *D. pteronyssinus* frequently co-exists with *D. farinae*, a mite species that prefers drier environmental conditions [13–16]. Many of the allergens from each

Correspondence: W. R. Thomas, Division of Molecular Biology, TVW Telethon Institute for Child Health Research, PO Box 855, West Perth WA 6872, Australia

species have been characterized by cDNA cloning. The amino acid sequence of the group 1 cysteine protease allergens from the two mite species show about 81% identity [17] and the majority of the other allergens show a similar degree of homology [18–21]. The allergens produced by the different mite species are usually serologically cross-reactive owing to this high level of sequence homology so it has been difficult to ascertain the degree of species-specific sensitization by IgE-binding assays [22–23]. This is particularly since the IgE from individual patients can show a 1000-fold difference in the degree of cross-reactivity [24]. Previously we have used the fine specificity of T-cell activation and overlapping synthetic peptides of the group 1 mite allergens to study the T-cell cross-reactivity between Der p 1 and Der f 1 peptides, as well as to peptides representing Eur m 1 from *Euroglyphus maynei* [25]. The studies were conducted in a population living in an area where *D. pteronyssinus* often co-exists with the less abundant *E. maynei* species, and *D. farinae* is rarely detected. The responses to the Der p 1 peptides were found to be larger and more frequent than to the Der f 1 or Eur m 1 peptides, and although detected, the majority of the Der f 1 responses overlapped with responses to a neighbouring or homologous peptide from Der p 1 or Eur m 1. The response to the Eur m 1 peptides were independent of responses to the homologous Der p 1 peptides. This finding indicated allergic sensitization detected by the peptides was caused by the mites in the environment and was not due to some form of molecular mimicry. Aside from this study there are very few studies comparing T-cell responses to allergens from different mite species even though it is well established that T cells are essential in the initiation and regulation of IgE synthesis and eosinophilia. O'Brien *et al.* compared T-cell reactivity between Der p 2 and Der f 2 and showed that the level of T-cell proliferation to the group 2 allergens was usually well correlated for the individual donors, however, there was some discordance in 2/11 donors who only had strong reactivity to Der p 2 [26].

The aim of this study was to measure the T-cell cross-reactivity of *D. pteronyssinus* and *D. farinae* allergens by measuring the level of T-cell proliferation and the cytokine profile (Th1/Th2) to the affinity purified group 1 and group 7 allergens. The subjects selected for study were from an environment where a comprehensive study found abundant *D. pteronyssinus* but failed to detect *D. farinae* [27]. The results showed there was little difference in the proliferative, IL-5 and IFN γ response to the group 1 and group 7 allergens of each species. This differed from the results found in our previous studies using peptides which showed that 19-mer peptides induced only limited T-cell cross-reactivity between these species. There are several explanations for this phenomenon which will be discussed.

Methods

Study population

Peripheral blood was obtained fresh from 26 donors (18 females, eight males; age range 21–50) recruited from laboratory staff and the general population. Serum was extracted for measurement of specific IgE to house dust mite (HDM) and rye pollen using the Pharmacia CAPS system (a RAST score ≥ 2 on a scale of 1–6 was considered positive), and for the measurement of specific IgE against Der p 7 by dot-blot analysis. The RAST analysis was kindly done by Dr Richard Loh of the Immunology Department at Princess Margaret Hospital for Children (Perth, Western Australia). Fifteen of the 26 subjects had no symptoms of allergic disease (asthma, rhinitis or dermatitis) and were RAST-negative to dust mite and pollen. The remaining 11 subjects were RAST-positive to the HDM allergen and displayed one or more allergic symptoms.

Culture Medium

Cells were cultured using AIM-V lymphocyte medium (Gibco, New York, USA) which contains DMEM and F12 media, supplemented with unspecified amounts of human albumin, insulin, transferrin, lipids, L-glutamine, streptomycin, gentamycin, and HEPES buffer. The addition of 2-mercaptoethanol (4×10^{-5} M; BDH Laboratory Supplier, Poole, Dorset, UK) was performed before use.

Allergens

Natural Der p 1, Der f 1, Der p 7 and Der f 7 were purified by affinity chromatography from spent mite medium using the 4C1 mAb against the group 1 allergen and the WH9 mAb against the group 7 allergen using the method of Chua *et al.* [28]. Tuberculin purified protein derivative (PPD) and the HDM extract were from the Commonwealth Serum Laboratory Limited (Melbourne, Australia) and were included in each tissue-culture assay as positive controls at 30 μ g/mL protein, respectively. Coomassie-stained SDS-PAGE showed the group 7 allergens had the expected sizes of 31, 30 and 26 kDa and the group 1 ran at 25 kDa. The purified allergens were used at 30 μ g/mL. Preliminary work found optimal T-cell proliferation and cytokine release was induced at this concentration.

Cell extraction and proliferation assays

Blood was collected and diluted with an equal volume of RPMI containing preservative free heparin. PBMC were isolated on a density gradient (Lymphoprep, Nycomed, Norway), washed three times in RPMI, and resuspended

in AIM-V culture medium. The cells from 10 allergic and 15 nonallergic donors were seeded at 2×10^5 cells/well in 96-well round-bottom trays in a volume of 200 μ L. The allergens and positive controls were added in triplicate to the cell cultures at 30 μ g/mL, and control wells contained no antigen. Following incubation at 37 °C in 5% CO₂ for 5 days, DNA synthesis was measured by adding ³H thymidine (0.5 μ Ci; DuPont). Thymidine uptake was assayed by harvesting the cultures onto glassfibre filter mats 16–18 h later and counting thymidine incorporation using a Packard Matrix 9600 beta counter (Packard Instrument Co, Meriden, CT, USA). The allergens were considered to be causing proliferation if the stimulation index (SI) ≥ 3.0 , where SI = mean of wells containing antigen/mean of control wells.

Cytokine assays

The cells from 11-allergic and 14-nonallergic donors were seeded at 3×10^6 cells/well in 24-well plates in a 2-mL volume for the measurement of IL-5 and IFN γ production. The allergens and positive controls were added to the cells individually at 30 μ g/mL. Supernatant was harvested on day 4 and day 6 for the measurement of IL-5 and IFN γ , respectively. IL-5 was measured using the BAF-BO3 bioassay [25] which was sensitive to 1 pg/mL. IFN γ was measured using the Pelikine ELISA kit (CLB, Amsterdam, the Netherlands) and the sensitivity of the kit was approximately 10 pg/mL.

Statistical analysis

Differences between experimental groups were assessed by either the paired Wilcoxon signed rank test or by the unpaired Mann–Whitney *U*-test. Probabilities less than or equal to 0.05 using the InStat statistical package (GraphPad Software, CA, USA) were considered significant.

Results

Proliferative T-cell responses to allergens of *D. pteronyssinus* and *D. farinae*

PBMCs from 10-allergic and 15-nonallergic donors were stimulated with 30 μ g/mL of the group 1 and group 7 allergens of *D. pteronyssinus* (Dp) and *D. farinae* (Df). The responses were presented as a stimulation index (SI). As will be reported in more detail elsewhere, T-cell proliferation to the group 1 allergens compared to the group 7 allergens of both mite species were similar. Similar reactivity was found for both the allergic and nonallergic donor groups. There was an extremely strong correlation between the SIs to the same allergen groups derived from the different mite species (Fig. 1). The correlation coefficient of SIs of the individual PBMCs between Der p 1 and Der f 1 was 0.951 ($P < 0.001$) and the correlation coefficient between Der p 7 and Der f 7 was 0.921 ($P < 0.001$). As indicated by the slope of the correlations there was a tendency for the Dp responses to be larger than the Df response which was significant ($P < 0.05$) for Der p 7.

Cytokine responses

To examine the level of Th1/Th2 cytokine production to allergens from each mite species, PBMCs from 11-allergic and 14-nonallergic donors were stimulated with the group 1 and group 7 allergens of *D. pteronyssinus* and *D. farinae*. Supernatant was collected on day 4 and day 6 for the measurement of IL-5 and IFN γ , respectively. A summary of the level of T-cell proliferation and cytokine production to the individual allergens is listed in Table 1. The production of IL-5 was significantly higher ($P < 0.005$) for the allergic donors than for the nonallergic donors for all the allergens. Similarly the ratio of IFN γ /IL-5 production was

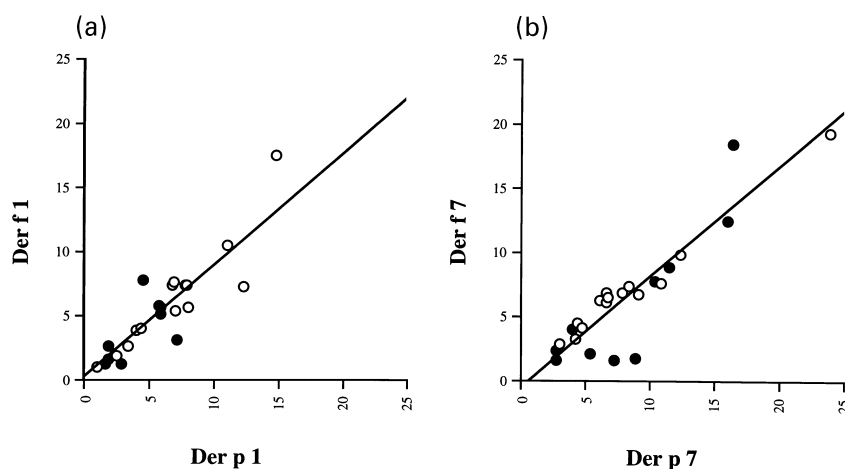


Fig. 1. Correlation of SIs of PBMCs from allergic (●) and nonallergic (○) donors in parallel assays to (a) Der p 1 and Der f 1 ($r = 0.951$; $P < 0.001$) and (b) Der p 7 and Der f 7 ($r = 0.921$; $P < 0.001$).

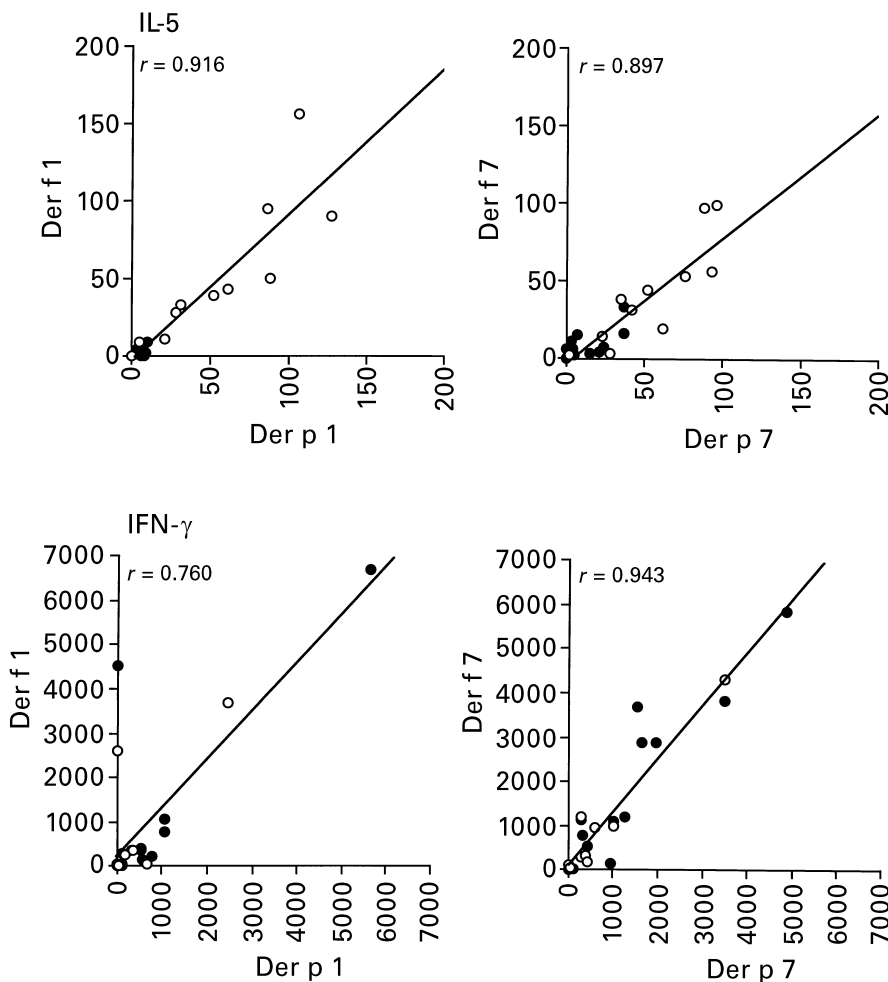


Fig. 2. The level of IL-5 and IFN γ release from PBMCs to the group 1 and group 7 allergens of *D. pteronyssinus* and *D. farinae* was well correlated for the allergic (○) and nonallergic (●) donors.

significantly higher for the nonallergic group. Although the production of IFN γ was higher for the nonallergic than for the allergic group, this did not reach statistical significance.

IL-5 production to the group 1 allergens of both mite species was strongly correlated ($r=0.916$; $P<0.001$), as was the correlation between Der p 7 and Der f 7 ($r=0.897$; $P<0.001$) (Fig. 2). Similarly, a strong correlation was found between the IFN γ responses to the group 1 ($r=0.760$; $P<0.001$) and group 7 ($r=0.943$; $P<0.001$) allergens of each mite species. For the allergic donor group, the level of IL-5 production to Der p 7 was of a slightly higher magnitude than to Der f 7 ($P<0.05$), however, the level of IL-5 production to the group 1 allergens of both mite species were equivalent. There was no difference in the ratio of IFN γ /IL-5 production to the group 1 and group 7 allergens of *D. pteronyssinus* and *D. farinae*.

As found previously for Der p 1 and Der p 7, the cells of the allergic donors produced similar amounts of IL-5 to both Der f 1 and Der f 7 even though the group 7 allergen is reported to bind to IgE at a lower frequency than the group 1 allergen (Fig. 3). Moreover, there was an extremely high

correlation between the level of IL-5 production to the group 1 and group 7 allergens of *D. pteronyssinus* ($r=0.959$; $P<0.001$) and *D. farinae* ($r=0.922$; $P<0.001$) (Fig. 4).

Discussion

The primary finding of this study was that the level of T-cell cross-reactivity between the natural allergens of *D. pteronyssinus* and *D. farinae* was high. This is in contrast to differences in responses to overlapping synthetic peptides of Der p 1 (Dp) and Der f 1 (Df) reported previously. Moreover, the correlation between the level of IL-5 and IFN γ production to the natural allergens of both mite species was extremely high. *D. farinae* is rarely detected in the environment of the study population so it is unlikely that the donors are sensitized to *D. farinae*. The previous studies with peptides showed that T cells from a similar group of subjects to that described here responded to many more peptides representing Der p 1 than peptides representing Der f 1 and there was often little cross-reactivity [25]. There are several

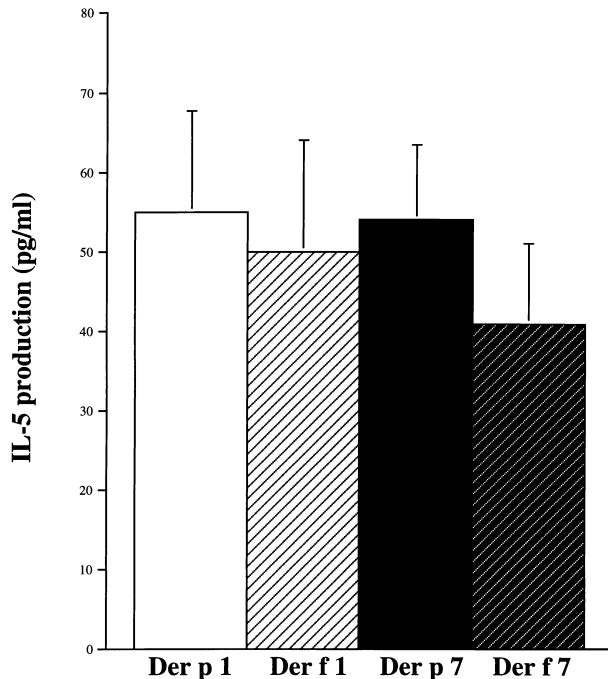


Fig 3. IL-5 production of PBMC from allergic donors to allergens from *D. pteronyssinus* and *D. farinae* were similar, however Der p 7 induced marginally higher levels of IL-5 than Der f 7 ($P < 0.05$).

possible explanations for this difference. Peptide processing and the use of an arbitrary array of peptide may decrease the efficiency of epitope presentation below a threshold level where responses to the sensitizing Dp epitope can be readily detected, but responses to the Df homologue are undetectable. The presentation of the peptide after natural processing may induce responses above the activation threshold for both epitopes. Residues outside those which bind to the groove are important on determining the efficiency of peptide presentation (possibly influenced by the peptide structure or the structure which originally bound to the

MHC). Accordingly, the use of larger peptides has been found to be more efficient at inducing stimulation than smaller peptides [29]. It should be noted here that the responses to Df were marginally less than Dp for both allergens. The natural allergens in the environment may also contain multiple isoforms that are cross-reactive between the mite species and all of the isoforms may not have been represented in the peptide studies. Amino acid polymorphisms within the group 1 allergen occur frequently and the different isoforms are known to significantly influence T-cell recognition of an allergen [30]. The polymorphic substitutions for Der p 1 are frequently to residues found in Der f 1 [31]. As yet the complete number and range of polymorphisms in mite extracts and the environment are unknown. Therefore, it is possible that the individual donors are recognizing T-cell epitopes of the *D. farinae* allergen that are either identical or cross-reactive with the *D. pteronyssinus* allergen. It is unknown whether allelic polymorphisms are found within the group 7 allergen. It is also possible that the combination of low cross-reactivity to several Df epitopes may provide enough stimulation and help for the *in vitro* stimulation to be triggered. The *in vivo* significance is unknown but Hoyne found via cytokine release that mice immunized with Der f 1 did not respond well to Der p 1 in *in vitro* cultures (mice immunized with Der p 1 did respond to Der f 1 but not as well as to Der p 1) showing that species-specific responses can be induced *in vivo* [32]. However, the class II MHC of humans is more diverse than the mouse and sensitized humans have received continuous stimulation rather than one injection. It is also worth noting that in the peptide studies, four of the six donors did have some Der f 1 peptide stimulation either cross-reactive with Der p 1 or Eur m 1.

The cytokine responses to the allergens from *D. pteronyssinus* and *D. farinae* were also similar. It has been speculated that low affinity interactions of the peptide for the MHC/TCR molecule leads to a short signal which

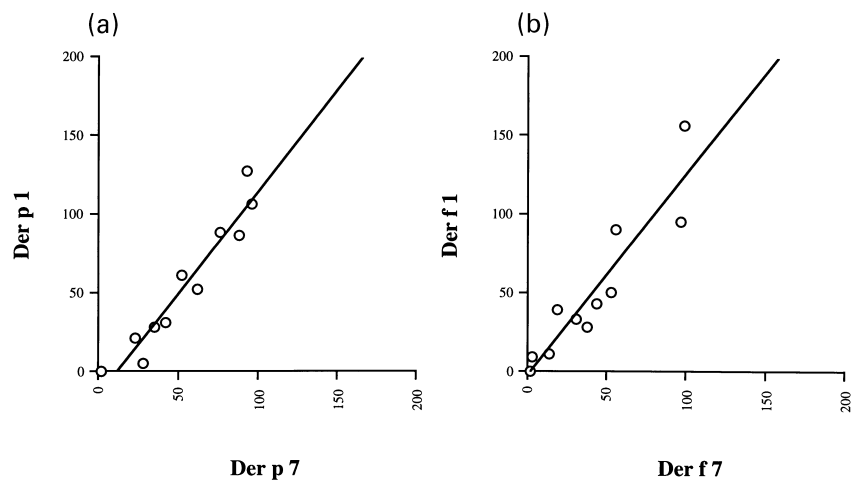


Fig. 4. Correlation of IL-5 production of PBMCs from HDM-allergic individuals to (a) Der p 1 and Der p 7 ($r = 0.959$; $P < 0.001$) and (b) Der f 1 and Der f 7 ($r = 0.922$; $P < 0.001$).

induces a Th2 response, and a high affinity interaction leads to a long signal to induce a Th1 response [33–36]. The IL-5 and IFN γ responses to the allergens of the different species were well correlated and had the expected Th2 preference for the allergic donors. The only significant difference showing that Der p 7 induced more IL-5 than Der f 7 would argue against low affinity interactions inducing Th2 cytokines. It may however, be that low affinity interactions are most effective during the development of sensitization and not in the *in vitro* stimulation of polarized cells as examined here. Given that the allergens from each species have a 20% sequence divergence, it is unlikely that polymorphic substitutions which produce 0.5–2% divergence would affect cytokine production in this type of assay [31]. As in the previous study, responses of nonallergic subjects were readily detected and the responses to the allergen from the different species did not generally differ.

O'Hehir and colleagues have previously identified cross-reactivity of T-cell clones to whole Dp and Df [37]. Six clones were generated by inducing the cells with *D. farinae* and two showed cross-reactivity to *D. pteronyssinus*. A T-cell line from an allergic donor induced with Dp extract was later found to respond well to both Dp and Df extracts by the same investigators, although the Df response was considerably less [38]. It was interesting that T-cell clones generated with Dp peptides from the same donor did not respond to Df.

The fact that strong T-cell responses could be induced by the nonsensitizing *D. farinae* suggests that the allergens of different species can be used, at least on the population level, to measure T-cell responses and possibly modify immunoregulatory responses *in vivo*. The significance of cross-reactivity is highlighted in this study because *D. farinae* has been shown to be almost absent from the environment, and the responses mapped by overlapping peptides do show a bias towards the abundant *D. pteronyssinus*.

References

- Voorhorst R, Spieksma FTM, Varekamp H, Leupen MJ, Lyklema AW. The house-dust mite (*Dermatophagoides pteronyssinus*) and the allergens it produces. *Journal of Allergy* 1967; 39:325–39.
- Miyamoto T, Oshima S, Ishizaki T, Sato SH. Allergenic identity between the common floor mite (*Dermatophagoides farinae*) and house dust as a causative antigen in bronchial asthma. *J Allergy* 1968; 42:14–28.
- Sears MR, Herbison GP, Holdaway MD, Hewitt CJ, Flannery EM, Silva PA. The relative risks of sensitivity to grass pollen, house dust mite and cat dander in the development of childhood asthma. *Clin Exp Allergy* 1989; 19:419–24.
- Lau S, Falkenhurst G, Weber A, Werthmann I, Lind P, Buettner-Goetz P, Wahn U. High mite-allergen exposure increases the risk of sensitization in atopic children and young adults. *J Allergy Clin Immunol* 1989; 84:718–25.
- Wickman M, Nordvall SL, Pershagen G, Sundell J, Schwartz B. House dust mite sensitization in children and residential characteristics in a temperate region. *J Allergy Clin Immunol* 1991; 88:89–95.
- Sporik R, Chapman MD, Platts-Mills TA. House dust mite exposure as a cause of asthma [see comments]. [Review] *Clin Exp Allergy* 1992; 22:897–906.
- Kuehr J, Frischer T, Meinert R, Barth R, Forster J, Schraub S, Urbanek R, Karmaus W. Mite allergen exposure is a risk for the incidence of specific sensitization. *J Allergy Clin Immunol* 1994; 94:44–52.
- Sporik R, Ingram JM, Price W, Sussman JH, Honsinger RW, Platts-Mills TA. Association of asthma with serum IgE and skin test reactivity to allergens among children living at high altitude. Tickling the dragon's breath [see comments]. *Am J Respiratory Crit Care Med* 1995; 151:1388–92.
- Peat JK, Tovey E, Toelle BG, Haby MM, Gray EJ, Mahmic A, Woolcock AJ. House dust mite allergens. A major risk factor for childhood asthma in Australia. *Am J Respiratory Crit Care Med* 1996; 153:141–6.
- Wahn U, Lau S, Bergmann R, Kulig M, Forster J, Bergmann K, Bauer CP, Guggenmoos-Holzmann I. Indoor allergen exposure is a risk factor for sensitization during the first three years of life. *J Allergy Clin Immunol* 1997; 99:763–9.
- Green WF, Woolcock AJ, Stuckey M, Sedgwick C, Leeder SR. House dust mites and skin tests in different Australian localities [see comments]. *Aust NZ J Medicine* 1986; 16:639–43.
- Hart BJ, Whitehead L. Ecology of house dust mites in Oxfordshire. *Clin Exp Allergy* 1990; 20:203–9.
- Arlian LG, Bernstein D, Bernstein IL, Friedman S, Grant A, Lieberman P, Lopez M, Metzger J, Platts-Mills T, Schatz M. Prevalence of dust mites in the homes of people with asthma living in eight different geographic areas of the United States. *J Allergy Clin Immunol* 1992; 90:292–300.
- Mosbech H. House dust mite allergy. [Review] *Allergy* 1985; 40:81–91.
- Lind P. House dust mites – indoor ecological factors. *Allergy* 1985a; 40:34–6.
- Lind P. Enzyme-linked immunosorbent assay for determination of major excrement allergens of house dust mite species *D. Pteronyssinus*, *D. Farinae* and *Microceras* *Allergy* 1986; 41:442–51.
- 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.
- Chua KY, Doyle CR, Simpson RJ, Turner KJ, Stewart GA, Thomas WR. Isolation of cDNA coding for the major mite allergen Der p II by IgE plaque immunoassay. *Int Arch Allergy Appl Immunology* 1990b; 91:118–23.
- Yuuki T, Okumura Y, Ando T, Yamakawa H, Suko M, Haida M, Okudaira H. Cloning and sequencing of cDNAs corresponding to mite major allergen Der f II. Arerugi – Jap J Allergology 1990; 39:557–61.
- Shen HD, Chua KY, Lin WL, Hsieh KH, Thomas WR. Molecular cloning and immunological characterization of the

- house dust mite allergen Der f 7. *Clin Exp Allergy* 1995b; 25:1000–6.
- 21 Smith WA, Thomas WR. Comparative analysis of the genes encoding group 3 allergens from *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*. *Int Arch Allergy Immunol* 1996a; 109:133–40.
 - 22 Lind P, Hansen OC, Horn N. The binding of mouse hybridoma and human IgE antibodies to the major fecal allergen, Der p I, of *Dermatophagoides pteronyssinus*. Relative binding site location and species specificity studied by solid-phase inhibition assays with radiolabeled antigen. *J Immunol* 1988; 140:4256–62.
 - 23 Hill MR, Newton MR, Hart BJ. Comparative IgE responses to extracts of five species of house dust mite, using western blotting. *Clin Exp Allergy* 1993; 23:110–6.
 - 24 Horn N, Lind P. Selection and characterization of monoclonal antibodies against a major allergen in *Dermatophagoides pteronyssinus*. Species-specific and common epitopes in three *dermatophagoides* species. *Int Arch Allergy Appl Immunology* 1987; 83:404–9.
 - 25 Hales BJ, Thomas WR. T-cell sensitization to epitopes from the house dust mites *Dermatophagoides pteronyssinus* and *Euroglyphus maynei* [see comments]. *Clin Exp Allergy* 1997; 27:868–75.
 - 26 O'Brien RM, Thomas WR, Wootton AMT. cell responses to the purified major allergens from the house dust mite *Dermatophagoides pteronyssinus*. *J Allergy Clin Immunol* 1992; 89:1021–31.
 - 27 Colloff MJ, Stewart GA, Thompson PJ. House dust acarofauna and Der p I equivalent in Australia: the relative importance of *Dermatophagoides pteronyssinus* and *Euroglyphus maynei*. *Clin Exp Allergy* 1991b; 21:225–30.
 - 28 Chua KY, Dilworth RJ, Thomas WR. Expression of *Dermatophagoides pteronyssinus* allergen, Der p II, in *Escherichia coli* and the binding studies with human IgE. *Int Arch Allergy Appl Immunology* 1990a; 91:124–9.
 - 29 Kammerer R, Kettner A, Chvatchko Y, Dufour N, Tiercy JM, Corradin G, Spertini F. Delineation of PLA2 epitopes using short or long overlapping synthetic peptides: interest for specific immunotherapy [see comments]. *Clin Exp Allergy* 1997b; 27:1016–26.
 - 30 Thomas WR, Smith W-A, Hales BJ. Allergens-sequence diversity and T-cell recognition. *ACI Int* 1998; 10:16–22.
 - 31 Chua KY, Kehal PK, Thomas WR. Sequence polymorphisms of cDNA clones encoding the mite allergen Der, p. I *Int Arch Allergy Immunol* 1993; 101:364–8.
 - 32 Hoynes G. T-cell recognition during mucosal and systemic responses, University of Western Australia: Department of Microbiology, 1992.
 - 33 Pfeiffer C, Stein J, Southwood S, Ketelaar H, Sette A, Bottomly K. Altered peptide ligands can control CD4 T lymphocyte differentiation *in vivo*. *J Exp Medicine* 1995; 181:1569–74.
 - 34 Kumar V, Bhardwaj V, Soares L, Alexander J, Sette A, Sercarz E. Major histocompatibility complex binding affinity of an antigenic determinant is crucial for the differential secretion of interleukin 4/5 or interferon gamma by T cells. *Proc Natl Acad Sci United States America* 1995; 92:9510–4.
 - 35 Tao X, Constant S, Jorritsma P, Bottomly K. Strength of TCR signal determines the costimulatory requirements for Th1 and Th2 CD4+ T cell differentiation. *J Immunol* 1997; 159:5956–63.
 - 36 Leitenberg D, Boutin Y, Constant S, Bottomly K. CD4 regulation of TCR signaling and T cell differentiation following stimulation with peptides of different affinities for the TCR. *J Immunol* 1998; 161:1194–203.
 - 37 O'Hehir RE, Eckels DD, Frew AJ, Kay AB, Lamb JRMHC. class II restriction specificity of cloned human T lymphocytes reactive with *Dermatophagoides farinae* (house dust mite). *Immunology* 1988; 64:627–31.
 - 38 Higgins JA, Thorpe CJ, Hayball JD, Ohehir RE, Lamb JR. Overlapping T-cell epitopes in the group 1 allergen of *Dermatophagoides* species restricted by Hla-Dp and Hla-Dr class II molecules. *J Allergy Clin Immunol* 1994; 93:891–9.