

Identification of an immunodominant region of the major house dust mite allergen Der p 2 presented by common human leucocyte antigen alleles

L. R. Crack,* H. W. Chan,* T. McPherson*† and G. S. Ogg*†

*MRC Human Immunology Unit, University of Oxford, Weatherall Institute of Molecular Medicine, Oxford, UK; and †NIHR Biomedical Research Centre, Oxford, UK

doi:10.1111/j.1365-2230.2011.04227.x

Summary

Background. Better understanding of the relevance of the immune response to common environmental allergens, such as the major house dust mite (HDM) allergen Der p 2, requires characterization of constituent T-cell epitopes.

Aim. To identify CD4+ T-cell epitopes within Der p 2 recognized by commonly expressed human leucocyte antigen (HLA) alleles.

Methods. HLA-blocking antibodies, peptide pools and truncations were used in ELISpot assays to establish restricted T-cell epitopes.

Results. People with and without atopic dermatitis have detectable Der p 2-specific T cells in the peripheral blood, which can proliferate in response to Der p 2 peptides. Interleukin-4-specific responses, both *ex vivo* and cultured to Der p 2 peptides, had a significant positive correlation with HDM-specific serum IgE. Within one pool of Der p 2 peptides, the 20mer D11 was found to induce multiple responses restricted through several alleles, including HLA-DPB1*0401 and HLA-DRB1*01.

Conclusions. We have identified an immunogenic region of Der p 2 presented by common HLA class II alleles, including the most commonly expressed HLA allele DPB1*0401. Identification of such epitopes may be of future value in peptide immunotherapeutic approaches.

Introduction

Atopic dermatitis (AD) is a common chronic inflammatory skin condition. Adaptive immune responses to ubiquitous environmental allergens, such as those of the house dust mite (HDM) are frequently associated with the disease. The prevalence of AD has been steadily increasing over the past few decades in Europe, so that up to 15–30% of children and 2–10% of adults are affected, with 15% of the population being affected at some stage of their childhood.^{1–3} HDM hypersensitivity

is an important and common association of atopic disease,⁴ including allergic rhinitis, asthma and AD, with > 50% of all patients allergic to HDM.⁵ Within the major European species, *Dermatophagoides pteronyssinus*, HDM-sensitive people are usually responsive to one or both of two major immunogenic allergens, Der p 1 and Der p 2.⁶ Numerous studies have mapped major histocompatibility complex class II-restricted T-cell epitopes to protein antigens, supporting the hypothesis that T cells are primed by a small number of immunodominant epitopes within the protein antigens.

It has been suggested in several studies that > 80% of HDM-allergic people are sensitive to Der p 2.^{7–10} Group 2 allergens, including Der p 2, have been shown to have allergenic activity, through high levels of IgE binding,^{6,9} and are present in high concentration in mite faeces.¹¹ Recognition of *D. pteronyssinus* allergens by T cells of atopic people has been reported in several studies.^{12,13}

Correspondence: Professor Graham Ogg, Cutaneous Immunology Group, Weatherall Institute of Molecular Medicine, Oxford OX3 9DS, UK
E-mail: graham.ogg@ndm.ox.ac.uk

Conflict of interest: none declared.

Accepted for publication 1 August 2011

Human leucocyte antigen (HLA)-DRB1 is associated with total IgE and Der p 2-specific IgE; in particular, a strong association was shown between presence of HLA-DRB1*1501 and titres of Der p 2 IgE,¹⁴ supporting a role for T cells in influencing the Ig repertoire through B-cell interaction. Compared with Der p 1,¹⁵ the T-cell epitopes of Der p 2 have been less well studied, but have been identified over the entire length of the protein. Proliferation assays of polyclonal T-cell responses to overlapping synthetic peptides were used to show that peptides were recognized throughout the length of Der p 2, and proliferative responses were higher in atopic people than their nonatopic counterparts.^{16,17} However, studies examining Der p 2-specific CD4+ T-cell epitopes have not to date defined responses restricted through HLA alleles that are commonly expressed in diverse populations. Such identification would aid the future development of peptide immunotherapy (PIT), as large numbers of the population would potentially be covered.

In this study, we sought to define an immunodominant region of Der p 2 restricted through common HLA class II alleles, including the commonest HLA allele found worldwide, HLA-DPB1*0401.¹⁸

Methods

The study was approved by the Oxford Clinical Research Committee, and all participants gave informed consent.

Subjects

In total, 22 AD adult patients with AD were recruited through the Department of Dermatology, Churchill Hospital (Radcliffe Hospitals NHS Trust, Oxford, UK). AD was confirmed using the validated UK Working Party criteria for diagnosis of AD.¹⁹ Control samples were obtained from 24 normal controls. The control group had no history of eczema or other atopic disease. HLA typing was undertaken using PCR with sequence-specific primers (PCR-SSP) phototyping.²⁰ HDM-specific IgE levels were measured in all subjects (Unicap system; Phadia AB, Uppsala, Sweden) at the Department of Immunology, Churchill Hospital.

Der p 2 peptides and recombinant protein

Peptides used in enzyme-linked immunosorbent spot (ELISpot) and intracellular cytokine staining (ICS) assays were synthesized in the in-house facility at the Weatherall Institute of Molecular Medicine (Oxford, UK) on an automated peptide synthesizer (396 MPS, Advanced Chemtech, Louisville, KY, USA) by

conventional solid-phase Fmoc chemistry, then analysed for purity by reverse-phase high-performance liquid chromatography (HPLC) (> 90% required). This method was used to generate overlapping 20-mer peptides of Der p 2 and any subsequent truncations from its published amino acid sequence (P49278; Swiss-Prot; <http://www.ebi.ac.uk/swissprot/>). Der p 2 recombinant protein (RE-DP2A-1; Indoor Biotechnologies, Warminster, Wiltshire, UK) was used to stimulate peripheral blood mononuclear cells (PBMCs) with and without lipopolysaccharide (LPS) in interferon (IFN)- γ ELISpot assays.

Interferon- γ and interleukin-4 enzyme-linked ImmunoSpot assays

The IFN- γ -specific capture antibody (3420-2 A; IFN- γ ELISpot Kit; Mabtech AB, Nacka Strand, Sweden) was diluted with phosphate-buffered saline (PBS) to 15 μ g/mL. Microtitre 96-well plates (MAIPS4510; Multiscreen IP-plates; Millipore, Billerica, MA, USA) were precoated with 35% ethanol (50 μ L/well) for 1 min, then rinsed with distilled water. The wells were then coated with 50 μ L/well of the diluted capture antibody, closed with sealing film (Parafilm; Pechiney Plastic Packaging Company, Chicago, IL, USA) and stored at 4 °C overnight. Plates were washed six times with RPMI, and subsequently blocked with RPMI supplemented with 50 μ g/mL penicillin, 50 μ g/mL streptomycin, 2 mmol/L-glutamine and 10% fetal calf serum for 1 h at 37 °C with 5% CO₂. PBMCs were added to the plates in RPMI supplemented with 50 μ g/mL penicillin, 50 μ g/mL streptomycin, 2 mmol/L L-glutamine and 10% human serum (type AB) (this medium is hereafter referred to as hR10) at 1×10^5 cells/well. Pools of overlapping Der p 2 20mer peptides or truncated Der p 2 peptides were added to the appropriate wells at a final concentration of 4 μ mol/L. Phytohaemagglutinin (PHA) (08E2701, BioStat, UK) was used as a positive control at 2 μ g/mL per well, and RPMI was used as a negative control. All conditions were set up in triplicate wells, and the mean number of spot-forming units was used for analysis. The plate was incubated overnight at 37 °C with 5% CO₂.

After incubation, the plate was washed six times with 200 μ L of PBS plus 0.05% Tween (4370829; BDH Merck Ltd, Poole, Dorset, UK) per well. IFN- γ detection antibody was diluted in PBS to a concentration of 1 μ g/mL, then 100 μ L of the diluted antibody was added to each well and incubated for 2–4 h at room temperature. The washing step was repeated, then 100 μ L/well of diluted streptavidin (1 μ g/mL) was

added, and the plates were incubated at room temperature for a further 1–2 h. The washing step was repeated for a final time, and 100 µL of developing buffer (170-6432; Biorad, Hemel Hempstead, Hertfordshire, UK), made in accordance with the manufacturer's instructions, was added per well, and the plate incubated for 10–20 min at room temperature until spots appeared. Plates were then washed with tap water, and inverted on paper towels to dry.

Once dry, plates were analysed using ELISpot software (version 4.0; AID, Strassberg, Germany). Interleukin (IL)-4 ELISpot assays were completed with an identical method to IFN- γ except that the IL-4 capture antibody (IL-4 ELISpot kit 3410–2 A; Mabtech, Nacka Strand, Sweden) was diluted in bicarbonate buffer: 1.59 g of Na₂CO₃ (S7795; Sigma-Aldrich, Gillingham, UK) and 2.93 g of NaHCO₃ (S6297; Sigma-Aldrich) dissolved in 1 L of distilled water (adjusted to pH 9.6 with 1 N HCl) instead of PBS (pH 7.4).

Cultured enzyme-linked immunospot

PBMCs were suspended in hR10 at 2×10^6 /mL, then the suspension was placed into sterile 24-well plates (3524, Corning Incorporated, Corning, NY, USA) at a volume of 2 mL/well. Cells were stimulated with 4 µg/mL of appropriate peptide, and plates were incubated at 37 °C with 5% CO₂. On days 3, 7 and 10, the cell medium was replenished with fresh RPMI supplemented with hR10 plus 200 IU/mL IL-2. On day 13, cells were removed, washed twice with RPMI, and resuspended in hR10. Cells were returned to clean tissue-culture wells, and incubated overnight at 37 °C with 5% CO₂ to rest before ELISpot assays were carried out as described above and elsewhere.²¹

Human leucocyte antigen-blocking antibodies

Short-term T-cell lines were incubated with 10 µL of 0.2 mg/mL monoclonal antibodies against HLA-DR (L243), HLA-DQ (SPV-L3) and HLA-DP (B7.21) (H127; Leinco Technologies, St Louis, MO, USA) at 37 °C with 5% CO₂ for 1 h before peptide addition for ELISpot.²²

Statistical analysis

The unpaired *t*-test, Mann–Whitney *U*-test, Spearman rank correlation, and the Kruskal–Wallis test with Dunn multiple comparison, were performed as appropriate using GraphPad Prism (version 4.0 for Windows; GraphPad software, San Diego, CA, USA). The D'Agostino–Pearson omnibus test was used to test the normality of

data distribution. In all cases, if data were normally distributed, parametric methods were used, and if the data were not normally distributed, then nonparametric methods were used. All ELISpot assays were included if there was a response in the positive-control well, which was defined as the number of spot-forming units (SFU) being greater than the mean plus twice the SD of the background of the entire population.

Results

Responses to overlapping Der p 2 peptides

To test the breadth, strength and polarization of Der p 2 allergen-specific T-cell responses between patients with AD and healthy controls, PBMCs from donor patients and controls were stimulated with overlapping 20-mer Der p 2 peptides, which were frame-shifted by 10 amino acids, and grouped into pools of up to three peptides, to screen the entire length of Der p 2 for *ex vivo* IL-4 and IFN- γ responses (Fig. 1).

Ex vivo IL-4 positive responses occurred more frequently in the patient group than in the control group; the combined pooled average response was significantly higher than the control stimulation in 77% of the patient group but only 29% of the control group.

There was a significant difference between the AD and non-AD IL-4 *ex vivo* ELISpot responses ($P = 0.04$, two-tailed Mann–Whitney *U*-test). *Ex vivo* IFN- γ ELISpot-positive responses were less frequent in the patient group than in the control group [33% (4/12) vs. 43% (3/7)], although there was no significant difference between the means of the combined peptide responses ($P = 0.17$, two-tailed Mann–Whitney *U*-test).

Kruskal–Wallis tests were performed to determine whether the variation between column medians was significantly higher than that expected by chance. Subsequently, Dunn multiple-comparison tests were carried out to compare the responses to each peptide pool and the negative control with each of the other peptide pools and the negative control. Significant variation between the medians was found for *ex vivo* IL-4 responses in the AD group ($P = 0.04$) (Fig. 1a) but not in the control group ($P = 0.59$) (Fig. 1b). Dunn multiple-comparison *post hoc* test showed a significant difference between the *ex vivo* IL-4 response to pool 4 and the negative control ($P < 0.05$) (Fig. 1a). Similarly, for *ex vivo* IFN- γ responses to Der p 2 peptide pools, significant variation between the medians of the Der p 2 peptides and negative control was seen for the patients with AD ($P = 0.01$) (Fig. 1c) but not for controls ($P = 0.52$) (Fig. 1d).

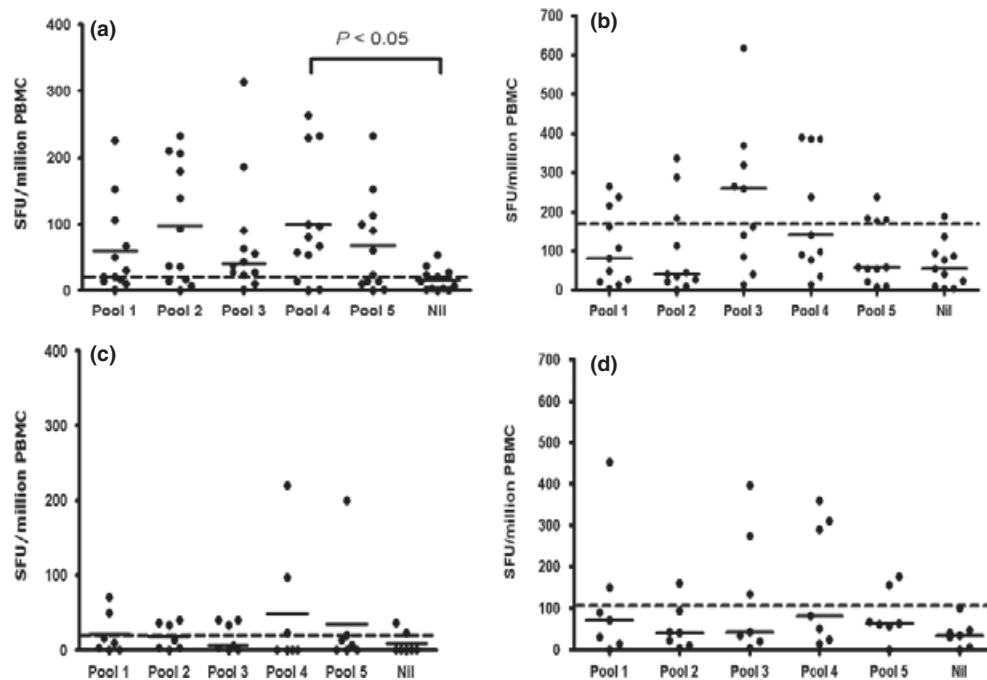


Figure 1 *Ex vivo* T-cell responses to pools of overlapping Der p 2 peptides in individuals with and without atopic dermatitis (AD). (a) Interleukin (IL)-4 and (b) interferon (IFN)- γ responses of patients with AD. (c) IL-4 and (d) IFN- γ responses of controls. The dashed horizontal bar represents the cut-off for a positive response, measured as the mean plus 2SD of the null control. Medians for each peptide and the null control are indicated by horizontal bars. Data are presented as the mean of multiple values. Comparisons of the means of the responses to the combined peptide pools was carried out using the Mann–Whitney *U*-test, and comparisons of the median responses were tested using the Kruskal–Wallis and Dunn multiple-comparison tests. SFU, spot-forming units.

Having detected the expression of cytokines by allergen-specific T cells in PBMCs from both allergic patients and healthy controls, we sought to examine cultured T-cell responses to Der p 2 peptides (Fig. 2). PBMCs from patients with AD and non-AD controls were stimulated with the pools of overlapping Der p 2 peptides, and cultured for 14 days before ELISpot analysis. There was a trend towards increased IL-4 responses in patients (Fig. 2a) compared with controls (Fig. 2b) (64% vs. 42%, respectively, had a total pool response); however, the difference was not significant ($P = 0.27$, two-tailed Mann–Whitney *U*-test).

Comparing the average responses of the IFN- γ combined pools, a positive response was recorded more frequently for patients than for controls [67% (7/11) vs. 46% (6/13)]. No significant difference was found between the patient (Fig. 2c) and control (Fig. 2d) groups in terms of the means of the peptide pool responses ($P = 0.68$, two-tailed Mann–Whitney *U*-test). In general, the IFN- γ cultured ELISpot responses were higher than the IL-4 responses, in terms of SFU/million cells. Kruskal–Wallis tests did not determine any significant differences between the medians of the peptide

pool and the negative responses for both groups and for both cytokines.

The breadth of T-cell responses to Der p 2 peptides was further analysed (Fig. 3). The numbers of Der p 2 peptide pools that elicited an *ex vivo* positive IL-4 (Fig. 3a) and IFN- γ (Fig. 3b) response for both groups were calculated. More than half of the participants had Der p 2-specific IL-4 responses, suggesting that T-cell responses are not focused upon a dominant epitope. In most of the cultured IL-4 ELISpot assays, the control samples failed to produce an IL-4 response to any of the Der p 2 peptide pools, whereas most of the patient samples did respond to at least one peptide pool (Fig. 3c). Cultured IFN- γ responses were less common in both patients with AD and controls, with most of the samples from both groups failing to respond to any of the pools (Fig. 3d).

To dissect T-cell responses by T-cell subtype, T-cell lines grown to the Der p 2 pools and yielding a positive response on ELISpot assays were stained with the T-cell surface markers CD3, CD4 and CD8. In all samples investigated, this response was found to be CD4+ and T cell-mediated (data not shown).

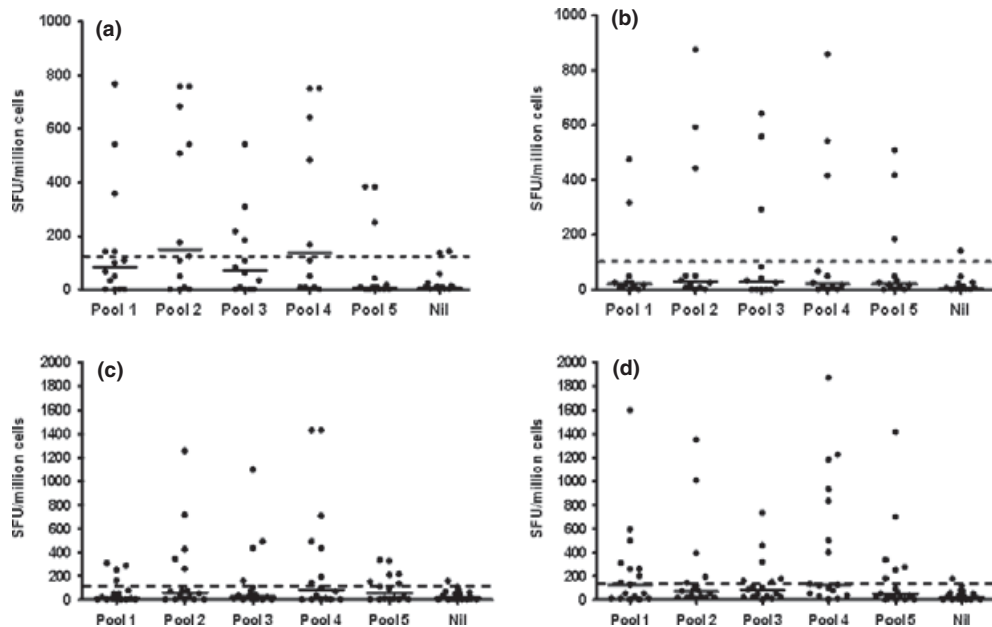


Figure 2 Cultured T-cell responses to pools of overlapping Der p 2 peptides in individuals with and without atopic dermatitis (AD). (a) Interleukin (IL)-4 and (b) interferon (IFN)- γ responses of patients with AD. (c) IL-4 and (d) IFN- γ responses of controls. The dashed horizontal bar represents the cut-off for a positive response, measured as the mean plus 2SD of the null control. Medians for each peptide and the null control are indicated by horizontal bars. Data are presented as the mean of multiple values. Comparisons of the means of the responses to the combined peptide pools was carried out using the Mann-Whitney *U*-test, and comparisons of the median responses were tested using the Kruskal-Wallis and Dunn multiple-comparison tests. SFU, spot-forming units.

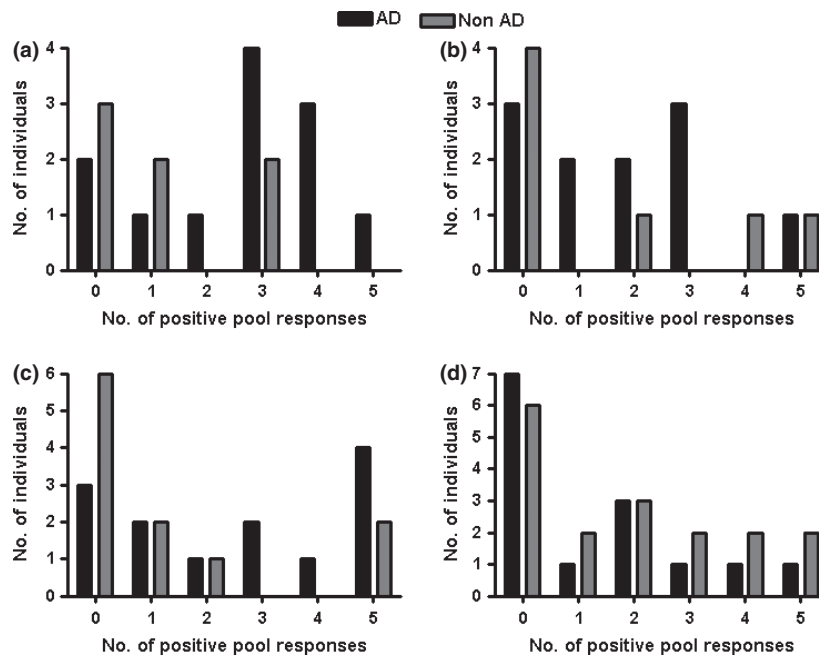


Figure 3 Frequency of positive T-cell responses to Der p 2 peptide pools. (a,b) *Ex vivo* T-cell responses to (a) interleukin (IL)-4 and (b) interferon (IFN)- γ . (c,d) Cultured T-cell responses (c) IL-4 and (d) IFN- γ .

Characterization of potential epitopes

The cultured and *ex vivo* ELISpot assays showed that pool 2 (spanning amino acids 31–70) and pool 4 (spanning amino acids 91–130) of overlapping Der p 2 peptides elicited responses more frequently in both groups and for both assayed cytokines. Pool 4 was selected for further analysis, as it typically produced higher response levels.

The IFN- γ and IL-4 ELISpot responses of short-term T-cell lines grown to Der p 2 pool 4 peptides were shown to respond more frequently to peptide D11 (101–120 QQYDIKYTNVVPKIAPKSEN) than to the other peptides in that pool (D10: 91–110 and D12: 111–130) (Fig. 4a).

HLA class II blocking antibodies were added to establish the HLA restriction of the D11 response. PBMCs from three subjects homozygous for DPB1*0401

were stimulated with the D11 peptide, and cultured for 14 days. The patients had mixed HLA-DRB1 types, but one was homozygous for HLA-DRB1*01 (Table 1). The IL-4 and IFN- γ ELISpot responses to peptide D11 of Der p 2 were substantially reduced by anti-DP and anti-DR antibodies, but not by anti-DQ antibodies (Fig. 4b). As the data were not evenly distributed, a Kruskal–Wallis test was used to compare the differences in the medians. A significant difference was found in the comparison of the medians for both IL-4 ($P = 0.05$) and IFN- γ ($P = 0.03$). These data suggest that the D11 20-mer peptide contains several T-cell epitopes, including both DPB1*0401- and DRB1*01-restricted epitopes.

Progressive truncations of the D11 20-mer peptide (positions 101–120) from both the N terminus and C terminus of the peptide were generated to attempt to determine the optimal epitopes restricted by HLA

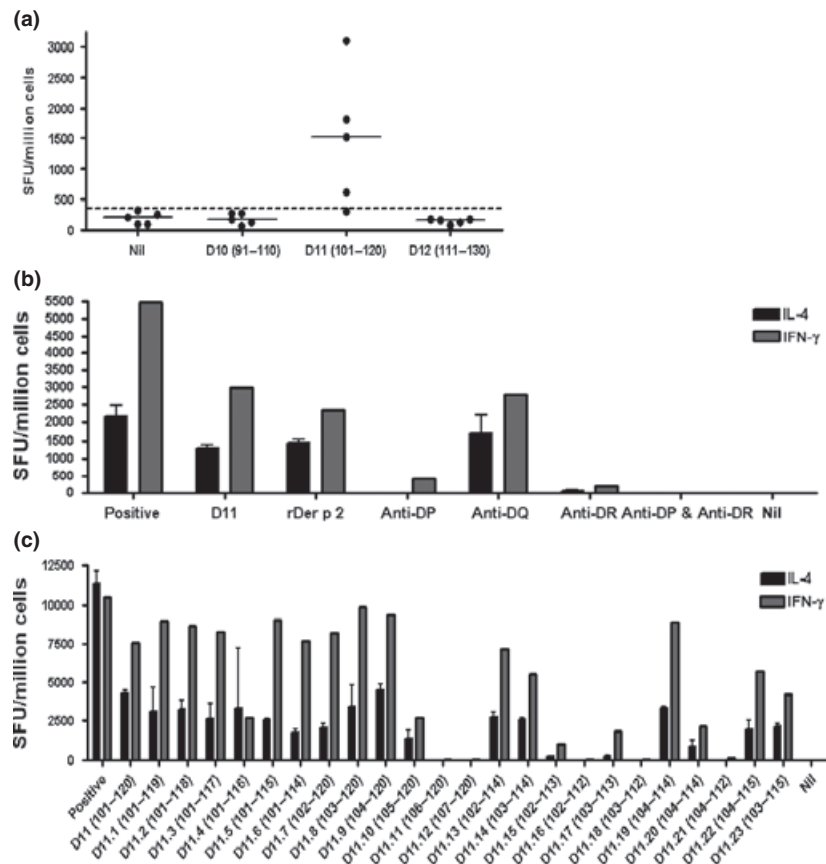


Figure 4 Der p 2 epitope mapping. (a) Combined interleukin (IL) and interferon (IFN)- γ responses to pool 4 constituent peptides. The dashed horizontal bar represents the cut-off for a positive response, measured as the mean plus 2SD of the null control. Medians for each peptide and the null control are indicated by horizontal bars. Data are presented as the mean of multiple values. (b) D11 ELISpot responses with addition of human leucocyte antigen (HLA) class II blocking antibodies. D11 lines stimulated with D11, rDer p 2 and D11 plus anti-HLA antibodies. Data are the means of multiple values, and error bars indicate the SD. (c) ELISpot responses to truncated peptides of D11 20mer. Data are the means of duplicate values. Comparisons of the medians of the truncation responses were tested using the Kruskal–Wallis and Dunn multiple-comparison tests. SFU, spot-forming units.

Table 1 Human leucocyte antigen (HLA)-DR and HLA-DP subtypes of the patients with atopic dermatitis determined using class II blocking antibodies for cultured ELISpot responses.

Patient	HLA-DR		HLA-DP	
1	DRB1*01	DRB1*11	DP*0401	DP*0401
2	DRB1*04	DRB1*04	DP*0401	DP*0401
3	DRB1*01	DRB1*07	DP*0401	DP*0401

class II DPB1 and DRB1 molecules. T-cell lines stimulated with D11 20-mer peptide were tested for IL-4 and IFN- γ responses in cultured ELISpot assays to the D11 truncations generated (Fig. 4c) and the minimal epitope was found to be DIKYTNVPI.

Relationship between house dust mite-specific IgE and Der p 2 responses

Following confirmation of Der p 2-specific responses, the relationship between serum-specific IgE to HDM extract and the observed T-cell responses was investigated. Overall, the patients with AD were found to have higher levels of HDM-specific IgE compared with controls, with

90% of patients having HDM-specific serum IgE (class 0), compared with 40% of controls. High levels of HDM-specific IgE (class 6) were found in 30% of the patient group, but in none of the controls (data not shown).

Ex vivo IL-4 and IFN- γ ELISpot responses detected in both patients with AD and controls were correlated with HDM-specific serum IgE levels by the nonparametric two-tailed Spearman rank correlation coefficient. *Ex vivo* IL-4 responses positively correlated with HDM-specific serum IgE levels ($r = 0.67$, $P < 0.01$) (Fig. 5a), whereas IFN- γ correlated negatively ($r = -0.43$, $P = 0.04$) (Fig. 5b). Cultured IL-4 ELISpot responses also positively correlated with HDM-specific serum IgE ($r = 0.71$, $P < 0.001$) (Fig. 5c), but there was no correlation for cultured IFN- γ responses ($r = -0.02$, $P = 0.94$) (Fig. 5d).

Effect of Der p 2 on lipopolysaccharide-induced responses

To ascertain the effect of LPS on Der p 2 responses, T-cell responses to LPS and Der p 2 were investigated by IFN- γ ELISpot assays. No difference was seen in rDer p

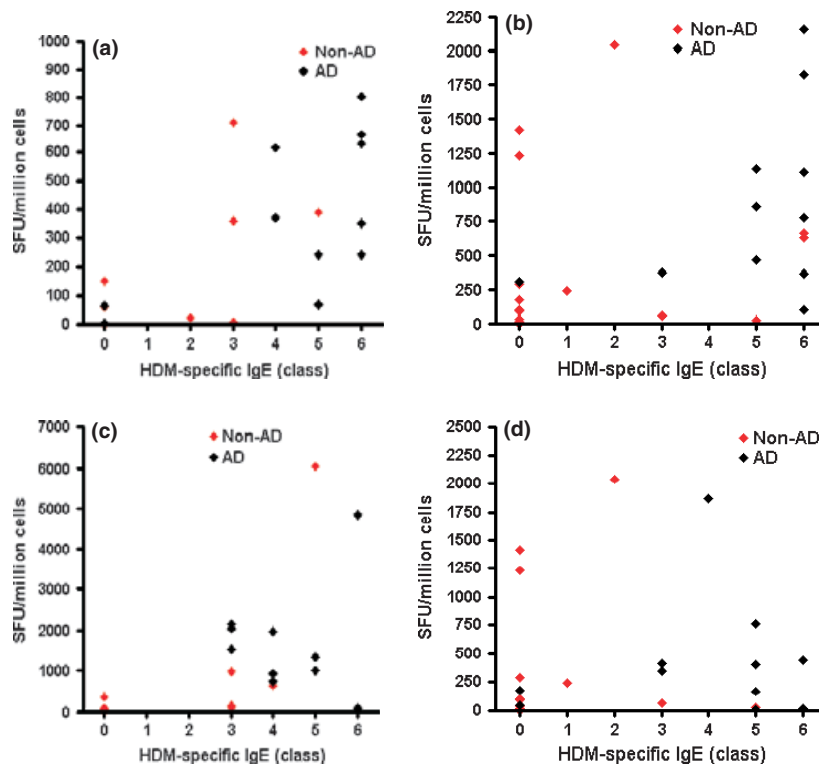


Figure 5 Relationship between combined Der p 2 pooled T-cell responses and Der p-specific serum IgE. Black diamonds, individuals with atopic dermatitis (AD); red diamonds, controls. (a) *Ex vivo* interleukin (IL)-4, (b) *Ex vivo* IFN- γ (c) Cultured IL-4 and (d) Cultured IFN- γ responses. The significance of the correlations was tested using a nonparametric two-tailed Spearman rank correlation test. SFU, spot-forming units.

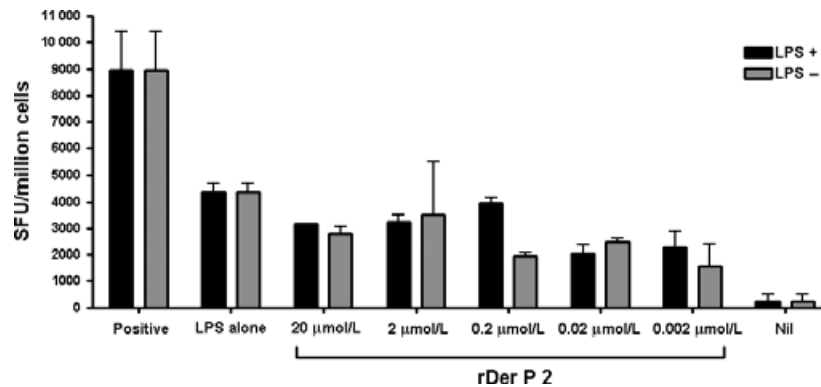


Figure 6 Effect of lipopolysaccharide (LPS) on recombinant Der p 2 interferon (IFN)- γ responses in a patient with atopic dermatitis who was allergic to house dust mite. Peripheral blood mononuclear cells were stimulated with LPS alone, or with varying concentrations of recombinant Der p 2 with and without 10 μ g/mL LPS stimulation. Data represent the mean of triplicate values. SFU, spot-forming units.

2-induced IFN- γ *ex vivo* ELISpot responses of PBMCs from a HDM-allergic patient with AD in the presence or absence of LPS (Fig. 6).

Discussion

In summary, we found that both patients and controls had detectable Der p 2-specific T cells in the peripheral blood, which were able to proliferate in response to Der p 2 peptide pools. Patients with AD produced more IL-4-secreting cells than did healthy controls, whereas IFN- γ responses were more frequent in controls.

Cultured ELISpot assays were shown to improve detection of Der p 2-specific T cells. IL-4-specific responses, both *ex vivo* and cultured to Der p 2 peptides, had a significant positive correlation with HDM-specific serum IgE, although the Der p 2 responses did not correlate with the filaggrin mutations identified within the AD cohort. Within one pool of Der p 2 peptides, the 20-mer peptide D11 (QQYDIKYTNVPKIAKSEN) elicited the highest and most frequent responses; this peptide contained T-cell epitopes restricted through several alleles including HLA-DP*0401 and HLA-DRB1*01.

Several studies have identified the presence of both CD4+ and CD8+ allergen-specific T cells in lesional skin and peripheral blood of people with atopy.^{23–25} Frequencies of allergen-specific T cells in the peripheral blood have previously been found to be as low as 0.0007% of CD4+ T cells.²⁶ Even though ELISpot assays have high sensitivity, allergen-specific T cells are difficult to detect. The use of cultured ELISpot assays has improved the detection rate of allergen-specific T cells. In this study, we investigated both IFN- γ and IL-4 responses to Der p 2 peptides to achieve a clearer picture of the Th1/Th2 balance in allergen-specific

T-cell responses. We found that the IL-4 ELISpot responses in patients with AD had a major Th2 bias.

The potential for immunotherapy using T-cell epitope-containing peptides has been highlighted recently, leading to successful clinical trials with immunodominant T-cell epitopes from several allergens, including bee venom²⁷ and cat.²⁸ Der p 1 and Der p 2 represent important allergens in *D. pteronyssinus* whole extract, because they are recognized by most HDM-allergic patients, and 50–100% of IgE reactivity has been shown to be directed towards these allergens.^{9,29–31}

HLA-DPB1*0401-specific epitopes may prove particularly useful in terms of developing PIT as it shares a similar binding motif with other alleles. HLA-DPB1*0401 and 0402 are known to have a similar binding motifs.³² Another allele, HLA-DPB1*0201, is also similar to both HLA-DP4 alleles, differing by one amino acid in the P4 pocket, with no difference in the P1 and P6 major binding pockets. Therefore, it is predicted that both HLA-DP4 and HLA-DPB1*0201 respond to a similar repertoire of peptides.³² The identification of epitopes that bind several common alleles offers important potential for PIT.

Worldwide, HLA-DPB1*0401 is the commonest HLA class II allele, widespread within all ethnic groups.³³ In the white population, HLA DPB1*0401 occurs at a frequency of approximately 40%.³⁴ Although DP alleles have received less attention than DR and DQ, a single HLA-DP allele mismatch can result in graft-versus-host disease T-cell responses,^{35,36} and thus it is likely that DP alleles play an important and relevant immunological role. The identification of HLA-DP-restricted T-cell epitopes including human immunodeficiency virus,³³ respiratory syncytial virus,³⁷ melanoma³⁸ and Fel d 1¹⁸ further confirms the importance of the HLA-DP loci in the immune response to antigens. The occurrence of

this allele within all racial groups allows for comparison between different populations. For example, it has been estimated that comparison of at least six HLA-DR alleles would be necessary to cover a similar proportion of people.³² Linkage disequilibrium with HLA-DR or DQ alleles does not occur with HLA-DPB1*0401, therefore this allele will occur in people with a variety of DR and DQ alleles.³⁹ HLA-DPB1*0401-specific class II tetramers have been made and used successfully,¹⁸ which allows a further approach to the tracking and phenotyping of DP-restricted responses in allergic people before and after treatment.

The major allergens of *D. pteronyssinus* have different functional activities. Der p 1, 3 and 9 exhibit significant protease activity, whereas Der p 2 and 5 lack enzymatic activity. Despite Der p 2 being a nonproteolytic allergen, a recent study showed that it can induce several proinflammatory cytokines in bronchial epithelial cells.⁴⁰ By contrast, a previous study reported that Der p 2 had no effect on alveolar A549 cells.⁴¹ Considering that the putative receptor interacting with Der p 2 has not been identified, a proinflammatory role for Der p 2 may be dependent on the cell type. Based on sequence homology, Der p 2 has been shown to be structurally similar to lipid-binding protein myeloid differentiation factor (MD)-2, which binds LPS with high affinity and subsequently associates with TLR4.^{42,43} Recently, it has been shown that Der p 2 exhibits functional homology and structural homology to MD-2, as Der p 2 can reconstitute LPS-induced TLR4 signalling in MD-2 deficient cells and mice.⁴⁴

In this study, Der p 2 was able to maintain IFN- γ responses in an HDM-sensitive individual in the absence of LPS stimulation (Fig. 6). Der p 2 exhibits structural and functional homology with the lipid-binding protein MD-2. MD-2 binds LPS with high affinity and associates with TLR4.^{42–44} Our data concur with these findings. Previous work showed that recombinant Der p 2 can directly activate airway smooth-muscle cells, initiating immune responses through TLR activation.⁴⁵ Exogenous antigen presentation by APCs in the absence of direct TLR stimulation generally leads to tolerance.⁴⁶ The fact that Der p 2 can directly stimulate TLR activation would need to be taken into consideration in the development of immunotherapeutic strategies.

Conclusion

We defined Der p 2 immunogenicity in both patients with AD and controls, and identified an immunodominant region containing an epitope presented by HLA-DP*0401. This allele is the commonest HLA molecule

worldwide, and shares peptide-binding motifs with other common class II alleles. We therefore hope that the data will be of value to further understanding of the role of T cells in disease pathogenesis, and for the further development of PIT approaches.

What's already known about this topic?

- HDM-derived Der p 2 is a target for IgE in individuals with AD, but little is known about the T-cell specificities within Der p 2.
- Identification of immunodominant regions of Der p 2 that are recognized by T cells is of value for studies of disease pathogenesis and for future immunotherapeutic strategies.

What does this study add?

- We have defined immunodominant regions of Der p 2 that are presented by common HLA class II alleles in individuals with AD.
- Th2 responses to these regions show a correlation with allergen-specific IgE levels, which supports their role in contributing to disease pathogenesis.

Acknowledgements

We are most grateful to the Medical Research Council, Misses Barrie Charitable Trust and Oxford NIHR Biomedical Research Centre Programme and Comprehensive Local Research Network for financial support and all patient and control donors who participated in this research.

References

- 1 Schultz Larsen F. Atopic dermatitis: a genetic-epidemiologic study in a population-based twin sample. *J Am Acad Dermatol* 1993; **28**: 719–23.
- 2 Wadonda-Kabondo N, Sterne JA, Golding J *et al.* A prospective study of the prevalence and incidence of atopic dermatitis in children aged 0–42 months. *Br J Dermatol* 2003; **149**: 1023–8.
- 3 Hachem JP, Wagberg F, Schmuth M *et al.* Serine protease activity and residual LEKTI expression determine

- phenotype in Netherton syndrome. *J Invest Dermatol* 2006; **126**: 1609–21.
- 4 Platts-Mills TAE, Chapman MD. Dust mites: Immunology, allergic disease, and environmental control. *J Allergy Clin Immunol* 1987; **80**: 755–75.
- 5 Boulet L, Turcotte H, Laprise C *et al*. Comparative degree and type of sensitization to common indoor and outdoor allergens in subjects with allergic rhinitis and/or asthma. *Clin Exp Allergy* 1997; **27**: 52–9.
- 6 Bruynzeel-Koomen CA, Van Wichen DF, Spry CJ *et al*. Active participation of eosinophils in patch test reactions to inhalant allergens in patients with atopic dermatitis. *Br J Dermatol* 1988; **118**: 229–38.
- 7 Ford SA, Tovey ER, Baldo BA. The spectrum of low molecular weight house dust mite (*Dermatophagoides pteronyssinus*) allergens with emphasis on Der p II. *Clin Exp Allergy* 1990; **20**: 27–31.
- 8 Heymann PW, Chapman MD, Aalberse RC *et al*. Antigenic and structural analysis of group II allergens (Der f II and Der p II) from house dust mites (*Dermatophagoides* spp). *J Allergy Clin Immunol* 1989; **83**: 1055–67.
- 9 Pittner G, Vrtala S, Thomas WR *et al*. Component-resolved diagnosis of house-dust mite allergy with purified natural and recombinant mite allergens. *Clin Exp Allergy* 2004; **34**: 597–603.
- 10 Sporik R, Chapman MD, Platts-Mills TA. House dust mite exposure as a cause of asthma. *Clin Exp Allergy* 1992; **22**: 897–906.
- 11 Park GM, Lee SM, Lee IY *et al*. Localization of a major allergen, Der p 2, in the gut and faecal pellets of *Dermatophagoides pteronyssinus*. *Clin Exp Allergy* 2000; **30**: 1293–7.
- 12 Rawle FC, Mitchell EB, Platts-Mills TA. T cell responses to the major allergen from the house dust mite *Dermatophagoides pteronyssinus*, antigen P1: comparison of patients with asthma, atopic dermatitis, and perennial rhinitis. *J Immunol* 1984; **133**: 195–201.
- 13 Lanzavecchia A, Santini P, Maggi E *et al*. In vitro selective expansion of allergen specific T cells from atopic patients. *Clin Exp Immunol* 1983; **52**: 21–8.
- 14 Cookson WO, Ubhi B, Lawrence R *et al*. Genetic linkage of childhood atopic dermatitis to psoriasis susceptibility loci. *Nat Genet* 2001; **27**: 372–3.
- 15 Ardern-Jones MR, Black AP, Bateman EAL, Ogg GS. Bacterial superantigen facilitates epithelial presentation of allergen to T helper 2 cells. *Proc Nat Acad Sci* 2007; **104**: 5557–62.
- 16 O'Hehir RE, Verhoef A, Panagiotopoulou E *et al*. Analysis of human T cell responses to the group II allergen of *Dermatophagoides* species: localization of major antigenic sites. *J Allergy Clin Immunol* 1993; **92**: 105–13.
- 17 Joost van Neerven R, van t'Hof W, Ringrose JH *et al*. T cell epitopes of house dust mite major allergen Der p II. *J Immunol* 1993; **151**: 2326–35.
- 18 Bateman EAL, Ardern-Jones MR, Ogg GS. Identification of an immunodominant region of Fel d 1 and characterization of constituent epitopes. *Clin Exp Allergy* 2008; **38**: 1760–8.
- 19 Williams HC. Atopic dermatitis: new information from epidemiological studies. *Br J Hospital Med* 1994; **52**: 409–12.
- 20 Bunce M, O'Neill CM, Barnardo MC *et al*. Phototyping: comprehensive DNA typing for HLA-A, B, C, DRB1, DRB3, DRB5 & DQB1 by PCR with 144 primer mixes utilizing sequence specific primers (PCR-SSP). *Tissue Antigens* 1995; **46**: 355–67.
- 21 Jones L, Black AP, Malavige GN, Ogg GS. Persistent high frequencies of varicella-zoster virus ORF4 protein-specific CD4+ T cells after primary infection. *J Virol* 2006; **80**: 9772–8.
- 22 Anderson RP, Degano P, Godkin AJ *et al*. In vivo antigen challenge in celiac disease identifies a single transglutaminase-modified peptide as the dominant A-gliadin T-cell epitope. *Nat Med* 2000; **6**: 337–42.
- 23 Neumann C, Gutgesell C, Fliegert F *et al*. Comparative analysis of the frequency of house dust mite specific and nonspecific Th1 and Th2 cells in skin lesions and peripheral blood of patients with atopic dermatitis. *J Mol Med* 1996; **74**: 401–6.
- 24 Sager N, Feldmann A, Schilling G *et al*. House dust mite-specific T cells in the skin of subjects with atopic dermatitis: frequency and lymphokine profile in the allergen patch test. *J Allergy Clin Immunol* 1992; **89**: 801–10.
- 25 van der Heijden FL, Wierenga EA, Bos JD *et al*. High frequency of IL-4-producing CD4+ allergen-specific T lymphocytes in atopic dermatitis lesional skin. *J Invest Dermatol* 1991; **97**: 389–94.
- 26 Pala P, Message SD, Johnston SL *et al*. Increased aero-allergen-specific interleukin-4-producing T cells in asthmatic adults. *Clin Exp Allergy* 2002; **32**: 1739–44.
- 27 Muller U *et al*. Successful immunotherapy with T-cell epitope peptides of bee venom phospholipase A2 induces specific T-cell anergy in patients allergic to bee venom. *J Allergy Clin Immunol* 1998; **101**: 747–54.
- 28 Norman PS *et al*. Treatment of cat allergy with T-cell reactive peptides. *Am J Respiratory Crit Care Med* 1996; **154**: 1623–8.
- 29 van der Zee JS, van Swieten P, Jansen HM *et al*. Skin tests and histamine release with P1-depleted *Dermatophagoides pteronyssinus* body extracts and purified P1. *J Allergy Clin Immunol* 1988; **81**: 884–96.
- 30 Hales BJ, Martin AC, Pearce LJ *et al*. IgE and IgG anti-house dust mite specificities in allergic disease. *J Allergy Clin Immunol* 2006; **118**: 361–7.
- 31 Meyer CH, Bond JF, Chen MS *et al*. Comparison of the levels of the major allergens Der p I and Der p II in standardized extracts of the house dust mite, *Dermatophagoides pteronyssinus*. *Clin Exp Allergy* 1994; **24**: 1041–8.
- 32 Castelli FA, Buhot C, Sanson A *et al*. HLA-DP4, the most frequent HLA II molecule, defines a new supertype of peptide-binding specificity. *J Immunol* 2002; **169**: 6928–34.

- 33 Busson M, Castell FA, Wang XF *et al.* Prediction of CD4+ T cell epitopes restricted to HLA-DP4 molecules. *J Immunol Meth* 2006; **317**: 144–51.
- 34 Al-Daccak R, Wang FQ, Theophille D *et al.* Gene polymorphism of HLA-DPB1 and DPA1 loci in caucasoid population: frequencies and DPB1-DPA1 associations. *Human Immunol* 1991; **31**: 277–85.
- 35 Gaschet J, Lim A, Liem L *et al.* Acute graft versus host disease due to T lymphocytes recognising a single HLA-DPB1*0501 mismatch. *J Clin Invest* 1996; **98**: 100–7.
- 36 Petersdorf EW, Gooley T, Malkki M *et al.* The biological significance of HLA-DP gene variation in haematopoietic cell transplantation. *Br J Haematol* 2001; **112**: 988–94.
- 37 de Waal L, Yuksel S, Brandenburg AH *et al.* Identification of a common HLA-DP4-restricted T-cell epitope in the conserved region of the respiratory syncytial virus G protein. *J Virol* 2004; **78**: 1775–81.
- 38 Mandic M, Castelli F, Janjic B *et al.* One NY-ESO-1-derived epitope that promiscuously binds to multiple HLA-DR and HLA-DP4 molecules and stimulates autologous CD4+ T cells from patients with NY-ESO-1-expressing melanoma. *J Immunol* 2005; **174**: 1751–9.
- 39 Aldener-Cannavá A, Olerup O. HLA-DPB1 typing by polymerase chain reaction amplification with sequence-specific primers. *Tissue Antigens* 2001; **57**: 287–99.
- 40 Österlund C, Gronlund H, Polovic N *et al.* The non-proteolytic house dust mite allergen Der p 2 induce NF-kappaB and MAPK dependent activation of bronchial epithelial cells. *Clin Exp Allergy* 2009; **39**: 1199–208.
- 41 Kauffman HF, Tamm M, Timmerman JM *et al.* House dust mite major allergens Der p 1 and Der p 5 activate human airway-derived epithelial cells by protease-dependent and protease-independent mechanisms. *Clin Mol Allergy* 2006; **4**: 5.
- 42 Inohara N, Nunez G. ML – a conserved domain involved in innate immunity and lipid metabolism. *Trends Biochem Sci* 2002; **27**: 219–21.
- 43 Shimazu R, Akashi S, Ogata H *et al.* MD-2, a molecule that confers lipopolysaccharide responsiveness on Toll-like receptor 4. *J Exp Med* 1999; **189**: 1777–82.
- 44 Trompette A, Divanovic S, Visintin A *et al.* Allergenicity resulting from functional mimicry of a Toll-like receptor complex protein. *Nature* 2009; **459**: 585–9.
- 45 Chiou Y-L, Lin C-Y. Der p2 activates airway smooth muscle cells in a TLR2/MyD88-dependent manner to induce an inflammatory response. *J Cell Physiol* 2009; **220**: 311–18.
- 46 Sporri R, Reis e Sousa C. Inflammatory mediators are insufficient for full dendritic cell activation and promote expansion of CD4+ T cell populations lacking helper function. *Nat Immunol* 2005; **6**: 163–70.