

Genetic variation of Der p 2 allergens: effects on T cell responses and immunoglobulin E binding

B. J. Hales, L. A. Hazell, W. Smith and W. R. Thomas

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

Summary

Background Der p 2 is a highly polymorphic allergen that shows a distinct pattern of sequence divergence. The effect of the variations on T cell and antibody responses has not been compared.

Objectives To compare IgE antibody binding and T cell proliferation and cytokine release induced by variants of Der p 2.

Methods Peripheral blood mononuclear cells (PBMC) from 19 allergic and 15 non-allergic people were stimulated with recombinant variants of Der p 2. IL-5, IL-10, IL-13 and IFN- γ were measured by a time resolved fluorescence (TRF) assay. Serum IgE antibody was measured using a solid-phase TRF assay.

Results Overall the most prevalent variant of Der p 2 (Der p 2. 0101) was the highest or approximately equal highest inducer of T cell proliferation and IL-5, IL-10, IL-13 and IFN- γ release. The most divergent variant 0104 induced the next highest responses. The variants 0107 and 0108 showed interesting changes especially when the allergic status was considered. Responses to 0107 showed poor Th1/Th2 polarization and, except for IL-10 release, cytokine responses to 0108 were low for non-allergic subjects. The variant 0101 showed similar monoclonal antibody binding but moderately less IgE binding than the other variants.

Conclusions The most prevalent variant, Der p 2. 0101, was the most active for T cell stimulation and although its IgE binding was slightly less than other variants that was highly correlated. The variant Der p 2. 0104 which contains the known common polymorphic changes had a response which was similar to Der p 2. 0101 and thus these two variants were the most stimulatory representations of Der p 2. The T cell responses to the less common variants 0107 and 0108 however, showed consistent differences demonstrating that changes in the sequence could change the cytokine response.

Keywords allergen, Der p 2, IgE, mite, polymorphism, T cell

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Introduction

The major Der p 1 [1, 2] and Der p 2 [2–7] allergens of *Dermaphagoides pteronyssinus* are highly polymorphic, a property which may contribute to the high frequency of IgE reactivity to these proteins. Most cDNA sequences obtained for these allergens have been unique while, for comparison, sequences obtained for the allergens such as Der p 4 [8] and 5 [9, 10] have shown little variation. For Der p 2, 8/19 of the reported sequences have been different. The pattern of variation found for Der p 1 and Der p 2 has, however, been different and this may be important for the study of immune responses. The amino acid position 124 of Der p 1 was regularly substituted as an alanine or valine but the substitutions at other positions

were sporadic so particular changes were infrequent [2]. The common sequence of Der p 1 could thus be represented by a single core sequence with two variants, 124 alanine and 124 valine. For Der p 2, however, there was an evolutionary pattern of variation with a divergence which was defined by a pattern of amino changes at positions 40, 47, 111 and 114 [2]. There is therefore a possibility that Der p 2 has diverged into distinct polymorphisms which can vary in their allergenicity. Since it has been found that T cell epitopes are frequently found within the regions 22–50 [10] and 105–129 [11, 12] it is likely that the substitution could affect responses and furthermore, there is evidence that polymorphisms with changes at positions 47 and 114 may have altered IgE antibody binding [13].

The immunological significance of the polymorphisms could be several fold. The different amino acid sequence would increase the scope for major histocompatibility complex (MHC) and T cell receptor binding of the allergens thus increasing overall responsiveness in the population. It is also possible that the different polymorphism could activate T cells with low affinity interactions which can drive Th2 responses [14].

Correspondence: Professor Wayne R. Thomas, Centre for Child Health Research, University of Western Australia, TVW Telethon Institute for Child Health Research, PO Box 855, West Perth 6872, Western Australia. E-mail: wayne@icmr.uwa.edu.au

The geographical distribution of mites with the different alleles may also vary and thus the type of allergen encountered may vary in different regions. Knowledge of the variants and the immune responses to the variants is critical for new developments such as peptide immunotherapy, and for the use of recombinant and modified allergens [15, 16]. Coincidentally, Der p 2 is a convenient molecule for investigating polymorphic proteins. It can be readily produced as a highly allergenic recombinant protein which has been used for X-ray crystallography and other conformational analyses [17–20]. Similar structural studies have been performed for Der f 2 [21]. The results of this study show that T cell and antibody responses to the variants can differ. The most frequent variant, Der p 2. 0101, induced the most consistent Th1 and Th2 cytokine responses even though the IgE binding was slightly lower. The variant Der p 2. 0104 which contains the known common polymorphisms had T cell responses similar to Der p 2. 0101. T cell responses to less common polymorphisms, however, showed that some amino acid substitutions could affect the response to the whole allergen.

Materials and methods

Allergens and antigens

cDNA of different variants of Der p were amplified by polymerase chain reaction (PCR) from pUC18 plasmids with high fidelity *Pfu* polymerase. The DNA was cloned into pET19b (Novagen, Madison, WI, USA) with an N-terminal six histidine tag fused with the natural amino acid terminus except that 0101 was cloned into pET11d modified to add the six histidine tag. Pigeon cytochrome c (PCC) was similarly cloned into the pET 11d vector. The recombinant allergens and PCC were produced from the plasmids transformed into *Escherichia coli* strain BL21-pLysSE (Novagen, Madison, WI, USA) and solubilized from inclusion bodies and purified by Ni-NTA and size exclusion chromatography to remove aggregates according to the method of Mueller [17]. For this the affinity purified allergens were eluted from a Sephadex G75 column and a single peak corresponding to 14 kDa was recovered and concentrated. Endotoxin was measured with E-toxate reagents (Sigma Chemicals Co., St Louis, MO, USA) according to the manufacturer's instructions. The concentrations were below 0.125 Eu/mL.

The Der p 2 variants selected for study were the most abundant Der p 2. 0101, the evolutionary divergent Der p 2. 0104, Der p 2. 0107 which carried a single substitution (from 0101) at

position 47 and Der p 2. 0108 which had a different change to 0104 in the C-terminal region (Table 1).

Study population

Peripheral blood mononuclear cells were obtained from 34 donors (15 males, 19 females, age range 19–56) who were recruited from laboratory staff and the general population. An assay for IgE binding to Der p 2 was used as an allergy test. Serum was not available from three subjects who were classified non-allergic but they were skin test-negative with no history of allergic disease. All of the subjects with positive sera to Der p 2 reported allergic symptoms ranging from moderate asthma to rhinitis and conjunctivitis.

Cell culture and proliferation assays

Blood was diluted with an equal volume of RPMI (Gibco, New York, NY, USA) and peripheral blood mononuclear cells (PBMC) were isolated on a density gradient (Lymphoprep, Nycomed, Norway). The cells were cultured in 96-well round bottom trays in a final volume of 200 µL AIM-V culture medium (Gibco) for 5 days at 37 °C in 5% CO₂. The variant Der p 2 allergens were added at 30 µg/mL, a concentration determined by preliminary titration and one consistent with other studies [22]. For proliferation assays, wells were cultured in triplicate with no-allergen and pigeon cytochrome c (PCC) controls. Thymidine uptake was measured by pulsing the cells with ³H-thymidine (0.5 µCi, DuPont, Wilmington, DE, USA) for 16–18 h then harvesting the cells onto glass fibre mats after 16–18 h. Thymidine incorporation was measured using a Packard Matrix 9600 beta counter (Packard Instrument Co., Meriden, CT, USA). The results here are expressed as a stimulation index of the allergen to the non-stimulated control. The PCC controls gave negligible stimulation (SI 1.6 ± 0.09).

Cytokine assays

PBMCs were seeded at 3 × 10⁶ cells per well in a 24-well plate in a 2-mL volume of AIM-V medium with the antigen added at 30 µg/mL. The cells were cultured as above and the supernatants harvested on day 6. Cytokines were measured in thawed supernatants by immunoassay. IL-4, IL-5, IL-10, IL-13 and IFN-γ were measured using paired antibodies from Pharmingen (Becton Dickinson Pharmingen, North Ryde, NSW, Australia). The assays were developed by DELFIA[®] assay using europium conjugated streptavidin (PerkinElmer, Boston, MA, USA) and counted on a Wallac 1420 Victor multilabel counter (PerkinElmer) for time resolved fluorescence (TRF). The sensitivities of the immunoassays were as follows: IL-4, 0.1 pg/mL; IL-5, 5 pg/mL; IL-10, 1 pg/mL; IL-13, 2.5 pg/mL; IFN-γ, 5 pg/mL. The PCC stimulations induced a low level of cytokine release (IFN-γ 11.5 ± 6.0; IL-5 17.9 ± 8.4; IL-10 0.15 ± 0.26; IL-13 4.8 ± 2.1).

Antibody assays

For human IgE antibody, EIA plates (Corning Costar, Acton, MA, USA) were coated overnight with 5 µg/mL Der p 2 or control PCC in carbonate buffer 0.1 M pH 9.6, then blocked with 0.5% BSA in Tris-HCl pH 7.4. After washing with Tris-HCl/0.05% Tween 20, serum samples were added in 100 µL volumes and incubated overnight. The wells were then washed and incubated with 100 µL of biotinylated monoclonal

Table 1. Alignment of Der p 2 sequence polymorphisms from cDNA isolated from Perth environmental mites

	40	47	98	111	114	116*
Der p 2. 0101	V	T	A	M	D	V
Der p 2. 0104	L	S	A	L	N	V
Der p 2. 0107	V	S	A	M	D	V
Der p 2. 0108	V	S	A	M	D	A

The amino acid numbering is based on the reference sequence Der p 2. 0101 (GenBank accession number, P49278). The polymorphic residues are highlighted in bold type.

anti-mouse IgE 34612D (BDPharmingen Torrey Pines, CA, USA). The reaction was developed by the Wallac DELFIA® assay using europium conjugated streptavidin TRF (PerkinElmer). The binding results were interpolated to a titration of a standard high titre pool of sera and expressed as TRF binding units relative to the standard. Sera was considered positive if the relative IgE units were more than three standard deviations higher than the mean counts of the non-allergic donors. To ensure reproducibility the assay was repeated for several donors and different preparations of the Der p 2 variants were used.

For human IgG antibody the sera were incubated overnight at a 1/10 dilution in 5% human serum albumin/Tris-HCl/0.05% Tween 20. The assays were developed with biotinylated anti-IgG1 and IgG4 (35052D and 35102D BD Pharmingen) and europium conjugated streptavidin as per the IgE assay. Human myeloma IgG1 (3889) and IgG4 (4639) (Sigma) were used as negative controls.

A monoclonal antibody assay was used to determine the effective plating efficiency of the different Der p 2 variants. Biotinylated 7A1 monoclonal antibody, known to react with all isoforms (Smith 2001, Haakart 1998) supplied by Professor Martin Chapman, Indoor Biotechnology, Charlottesville, VA, USA and an in-house biotinylated mAb 10B2 were added to the wells of plates coated with Der p 2 and a DELFIA® assay performed with europium labelled streptavidin.

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

T cell stimulation

The T cell proliferative response and cytokine release of PBMC to Der p 2. 0101, 0104, 0107 and 0108 was determined for 19 allergic and 15 non-allergic subjects. The combined results for allergic and non-allergic subjects (Table 2) shows that all the

variants significantly stimulated the PBMC but the proliferative, as well as IL-5, IL-13 and IFN- γ responses to Der p 2. 0108, were significantly lower than the other variants. The reverse was found for IL-10 where 0108 induced the highest release that was significantly higher than 0104 and 0107 but similar to 0101. Der p 2. 0101 induced the highest release of IL-5, IL-13 and IFN- γ and was almost the highest for proliferation and IL-10 release. IL-4 was not detected in any of the culture supernatants.

The responses induced by 0104, 0107 and 0108 were compared to 0101 by regression analysis [Figs 1–5]. Overall 0101

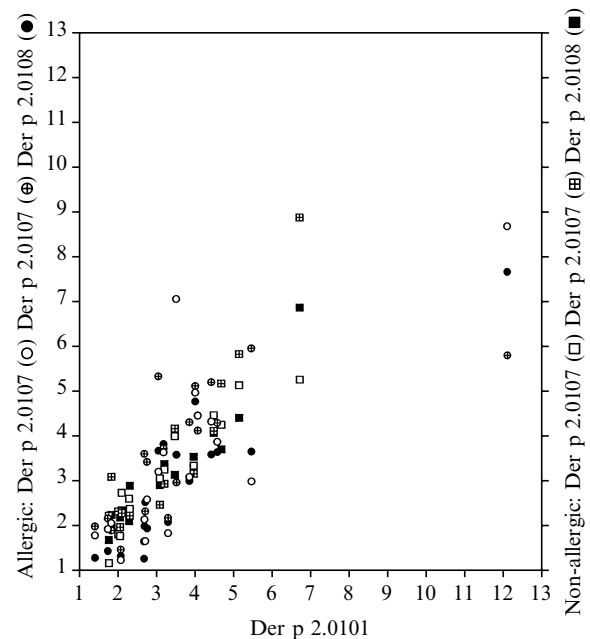


Fig. 1. Proliferative response of peripheral blood mononuclear cells (PBMC) to polymorphic variants of Der p 2. PBMCs from 34 subjects (19 allergic, 15 non-allergic) were stimulated with recombinant variants of Der p 2. 0101, 0104, 0107 and 0108. The mean stimulation indices of the responses of each individual to Der p 2. 0104, 0107 and 0108 are shown plotted against their response to Der p 2. 0101 as a reference. The correlation coefficients vs. Der p 2. 0101 for 0104, 0107 and 0108 were 0.79, 0.68 and 0.88, respectively, for the allergic subjects and 0.94, 0.93 and 0.96 for the non-allergic subjects.

Table 2. Comparison of responses between allergic and non-allergic subjects to the Der p 2 variants

	Donors	Der p 2. 0101	Der p 2. 0104	Der p 2. 0107	Der p 2. 0108
Stimulation Index	Allergic	3.66 ± 0.52	3.33 ± 0.44	3.66 ± 0.34	3.04 ± 0.36†§*
	Non-allergic	3.29 ± 0.37	3.15 ± 0.32	3.55 ± 0.48	3.16 ± 0.34
IL-5 (pg/mL)	Allergic	99.3 ± 18.6††	81.7 ± 12.6††	69.4 ± 16.2	104 ± 33.5††
	Non-allergic	31.5 ± 8.36	38.1 ± 19.2	44.1 ± 14.0	18.9 ± 5.81††‡§**
IL-10 (pg/mL)	Allergic	271 ± 48.7	215 ± 46.0†*¶**	209 ± 52.6†*¶**	271 ± 48.0
	Non-allergic	202 ± 37.7	205 ± 36.4	197 ± 14.0	253 ± 41.9
IL-13 (pg/mL)	Allergic	133 ± 41.9††	127 ± 37.4††	97.5 ± 31.8‡*	96.5 ± 30.9††‡*
	Non-allergic	36.0 ± 6.13	33.6 ± 5.17	39.3 ± 5.65	29.3 ± 4.54
IFN- γ (pg/mL)	Allergic	11 893 ± 3718	9049 ± 2958	6369 ± 2579	6532 ± 2601††‡**
	Non-allergic	3477 ± 1014	2645 ± 657§*	4338 ± 1228	1810 ± 498††‡§*

Results are presented as means (± SEM) of the level of proliferation and cytokine production to the Der p 2 variants. †The level of proliferation or cytokine production was significantly less than that found for Der p 2. 0101. ‡The level of proliferation or cytokine production was significantly less than that found for Der p 2. 0104. §The level of proliferation or cytokine production was significantly less than that found for Der p 2. 0107. ¶The level of proliferation or cytokine production was significantly less than that found for Der p 2. 0108. * $P < 0.05$, ** $P < 0.005$, *** $P < 0.0005$. ††Cytokine production to the Der p 2 variant was significantly higher for the allergic donors compared to the non-allergic donors.

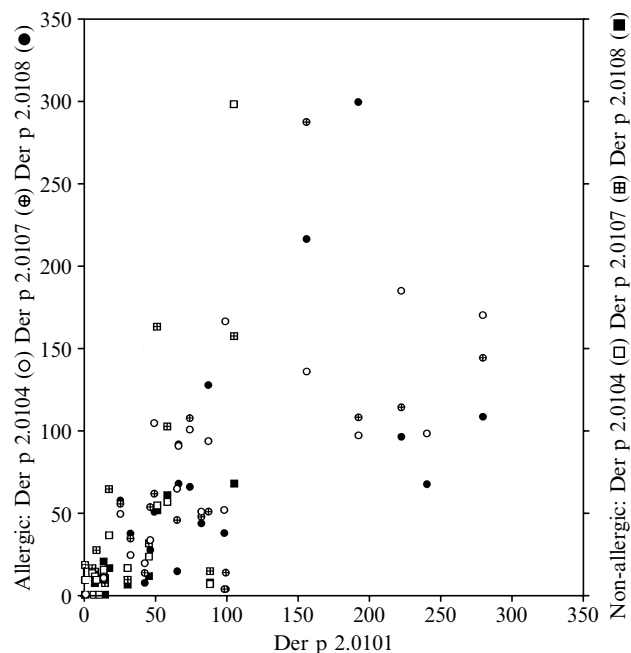


Fig. 2. IL-5 cytokine release of PBMCs to polymorphic variants of Der p 2. PBMCs from 34 subjects (19 allergic, 15 non-allergic) were stimulated with recombinant variants of Der p 2. 0101, 0104, 0107 and 0108. The mean responses (pg/mL) of each individual to Der p 2. 0104, 0107 and 0108 are shown plotted against their response to Der p 2. 0101 as a reference. The correlation coefficients vs. Der p 2. 0101 for 0104, 0107 and 0108 were 0.76, 0.61 and 0.56, respectively, for the allergic subjects and 0.70, 0.64 and 0.68 for the non-allergic subjects.

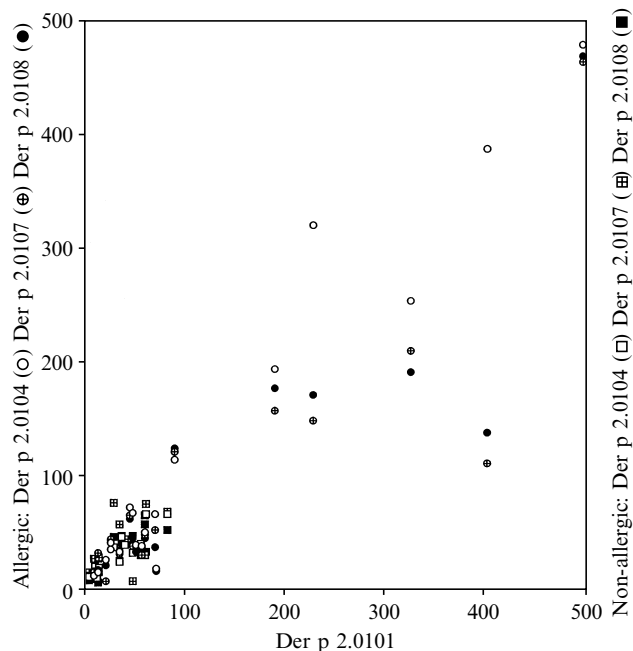


Fig. 4. IL-13 cytokine release of PBMCs to polymorphic variants of Der p 2. PBMCs from 34 subjects (19 allergic, 15 non-allergic) were stimulated with recombinant variants of Der p 2. 0101, 0104, 0107 and 0108. The mean responses (pg/mL) of each individual to Der p 2. 0104, 0107 and 0108 are shown plotted against their response to Der p 2. 0101 as a reference. The correlation coefficients vs. Der p 2. 0101 for 0104, 0107 and 0108 were 0.98, 0.93 and 0.94, respectively, for the allergic subjects and 0.92, 0.50 and 0.83 for the nonallergic subjects.

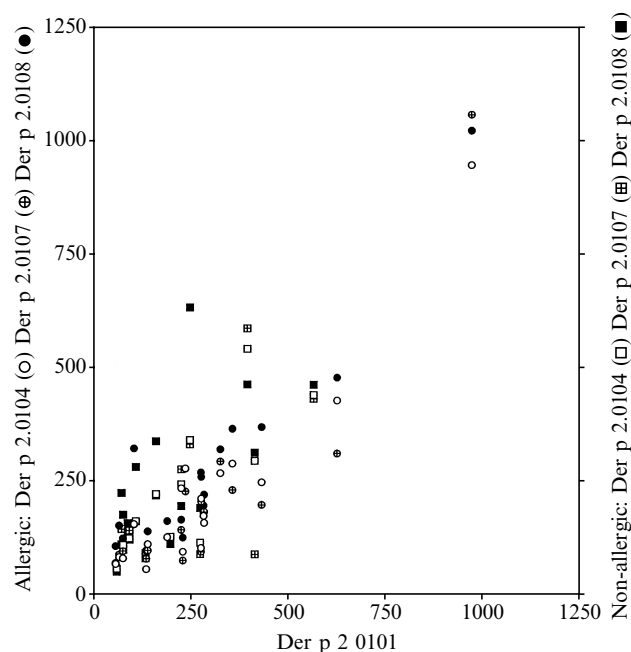


Fig. 3. IL-10 cytokine release of PBMCs to polymorphic variants of Der p 2. PBMCs from 34 subjects (19 allergic, 15 non-allergic) were stimulated with recombinant variants of Der p 2. 0101, 0104, 0107 and 0108. The mean responses (pg/mL) of each individual to Der p 2. 0104, 0107 and 0108 are shown plotted against their response to Der p 2. 0101 as a reference. The correlation coefficients vs. Der p 2. 0101 for 0104, 0107 and 0108 were 0.94, 0.89 and 0.93, respectively, for the allergic subjects and 0.82, 0.64 and 0.62 for the non-allergic subjects.

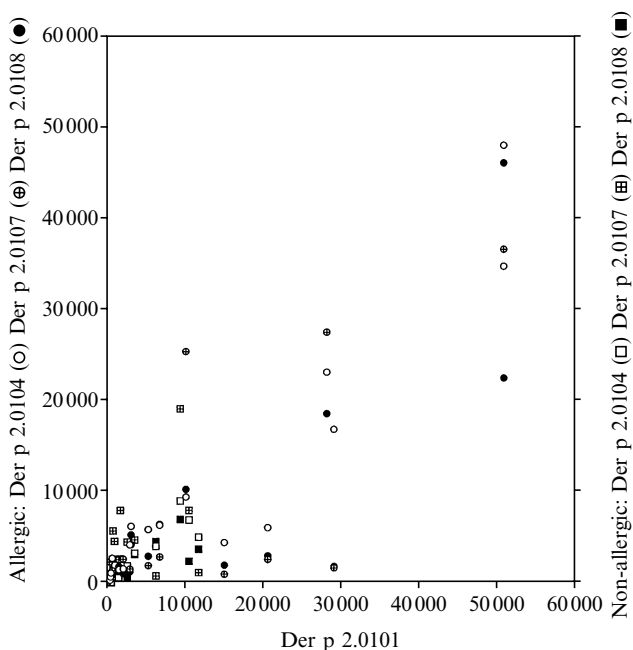


Fig. 5. IFN- γ cytokine release of PBMCs to polymorphic variants of Der p 2. PBMCs from 34 subjects (19 allergic, 15 non-allergic) were stimulated with recombinant variants of Der p 2. 0101, 0104, 0107 and 0108. The mean responses (pg/mL) of each individual to Der p 2. 0104, 0107 and 0108 are shown plotted against their response to Der p 2. 0101 as a reference. The correlation coefficients vs. Der p 2. 0101 for 0104, 0107 and 0108 were 0.95, 0.73 and 0.83, respectively, for the allergic subjects and 0.89, 0.42 and 0.78 for the non-allergic subjects.

was the most stimulatory for all responses with no individual showing low proliferation or cytokine release to 0101 with corresponding high responses to another variant. While there was an overall correlation of responses the most divergent responses can be seen to IFN- γ where the PBMC of several people with high responses to 0101 had low responses to 0104, 0107 and 0108, especially for 0107 and 0108. This can be seen by the observations which would fall well below a line representing a slope of 1. Some individuals also had low IL-5 responses to some variants compared to 0101. The responses to 0104, the variant which contained the most amino acid substitutions compared to 0101, usually had the strongest correlation of responses to 0101.

To analyse the effect on Th1/Th2 bias the responses of the allergic and non-allergic subjects were compared (Table 2). The data shows that the mean responses tended to be lower for the non-0101 variants and overall the pattern did not change in allergic and non-allergic subjects. As expected the allergic donors had higher IL-5 and IL-13 responses, which were significant for 0101, 0104 and 0108 for IL-5. The cytokine release to 0107 did not differ significantly for PBMC from allergic to non-allergic donors. The IL-5 responses of non-allergic subjects to 0108, but not the responses of allergic subjects, were significantly lower than the responses to the other variants, whereas both allergic and non-allergic donors had significantly lower IFN- γ responses to 0108. The IL-10 responses also showed differences between the donor groups, with increased IL-10 response to 0101 and 0108 only being evident for the allergic donors.

Antibody reactivity

The relative IgE binding comparison between the Der p 2 variants showed binding to 0101 was significantly lower on average

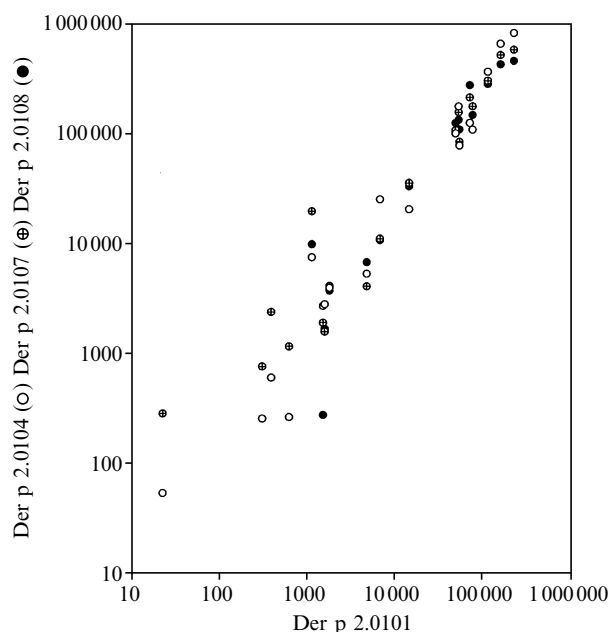


Fig. 6. Relative IgE binding to the Der p 2 variants. IgE binding (relative TRF units) of each allergic individual to Der p 2. 0104, 0107 and 0108 are shown plotted against their binding to Der p 2. 0101 as a reference. The correlation coefficients vs. Der p 2. 0101 for 0104, 0107 and 0108 were 0.97, 0.98 and 0.97, respectively.

than that found for the other variants [Fig. 6]. Nevertheless all positive sera had IgE binding to 0101 and there was a strong correlation of reactivity. There was no significant difference in mean IgE binding between 0104, 0107 and 0108. The plating efficiency of the Der p 2 variants was the same, as shown by binding to the 7A1 and 10B2 monoclonal antibodies using a similar DELFIA assay developed by europium coupled streptavidin (data not shown). Control sera from a cat-allergic subject with 800 IU of IgE bound less than 200 TRF units compared to the binding of 800 000 for positive sera.

Assays for IgG antibodies were conducted with some sera. They did not detect IgG1 but low titres of IgG4 were found to Der p 2. 0101 and Der p 2. 0104 in the sera of 7/12 allergic subjects and in 1/8 sera from non-allergic subjects (data not shown).

Discussion

The major group 1 and group 2 house dust mite allergens are produced from single genes [7, 23] but are characterized by a high degree of allelic variation for both group 1 [1, 2] and group 2 [2–7]. This raises the possibility that the polymorphisms may contribute to the allergenicity of the proteins, and for the application of recombinant allergens, the challenge of determining representative sequences. For Der p 1 the polymorphisms primarily consist of sporadic substitutions [2], so while the variation is frequent there is little accretion of substitutions that diverge from the consensus sequence. For Der p 2, however, the pattern of polymorphism shows a distinct evolutionary pattern centred on residues 40, 47, 111 and 114 [2]. About half the sequences from two geographically separate localities, Perth in Australia and Taipei in Taiwan, have the Der p 2. 0101 sequence consisting of residues VTMD [2, 7]. The other half show progression to substitutions beginning with 47S (Der p 2.0107) and then with changes of 40L and 114N and finally 111L. Der p 2. 0104 contains all these substitutions. The amino acid changes are accompanied by a series of non-coding nucleotide changes supporting the pattern of divergence [2]. The substitutions 47 and 114 are usually found together and occur in close proximity on adjacent loops at one end of Der p 2 molecule [13, 17, 21].

To investigate the effect of these changes on the immune responses the ability of four different variants to stimulate T cell proliferation and T cell cytokine release was examined. When considered at a population level or at the level of individual responses the Der p 2.0101 allergen was superior to or approximately the same as the response elicited by other variants. This was for proliferation, the Th1 cytokine IFN- γ , the Th2 cytokines IL-5 and IL-13 and the regulatory cytokine IL-10. Der p 2. 0101 therefore appears to be the most suitable variant for studying T cell responses and for exploring new types of therapy with recombinant or mutated recombinant allergens. Similar studies in other environments would be of interest although 0101 is also well represented in the CSL culture used for commercial extracts and is found in the Taiwan environment [7].

The variant Der p 2. 0104 with four amino acid differences from the 0101 variant induced only slightly lower cytokine release. Peptides representing the 0101 sequence in the regions of 2–50 and 105–129 were found by O'Hehir et al. [11] to have epitopes recognized by 7/18 subjects, although responses to two

other nonpolymorphic regions were more frequent. O'Brien et al. [12] found the peptide 111–129, which contains three of the polymorphisms investigated here, was the most frequent stimulator and induced responses in PBMC from 16/24 donors. The similar responses to 0101 and 0104 suggest the polymorphisms do not affect responses to the epitopes or the variants induce responses to their own epitopes. Variants with the N114 substitution [2, 7] appear frequent and thus could induce their own responses.

The lowest overall, and in some respects quite informative, responses were to 0107 and 0108 which differed from the 0101 by one and two amino acids, respectively. Both contained the common 47S substitution found in almost all the sequences, which diverge from 0101 [2], and the 0108 contained an additional and unusual change at position 116 [2]. Compared to 0101 the release of all cytokines by cells from allergic subjects tended to be lower when induced with 0107 while the responses of PBMC from non-allergic subjects tended to be higher. The differences in responses between allergic and non-allergic subjects were therefore less than that induced by the other variants and was not statistically significant. The feature of the responses to 0108 was the poor reactivity of the PBMC from non-allergic subjects, especially for IL-5 and IFN- γ release, while the responses of PBMC from allergic subjects were similar to the responses induced by the other variants. The results suggest responses of non-allergic subjects to a C-terminal epitope found in this variant favour a non-allergic response. The sequences of Der p 2 found in environmental mites shows that the 0108 variant itself would not be frequently encountered by allergic subjects [2]. It should also be noted that the increased IFN- γ production by PBMC from allergic subjects described here agrees with most studies which have examined responses to house dust mite allergens. When examined directly *ex vivo* PBMC from allergic subjects release more or a similar level of IFN- γ than cells from non-allergic subjects [22, 24–26]. The IFN- γ release may decrease with the severity of disease [27] although this can still be higher than the release from non-allergic subjects [24]. The higher IFN- γ release has also been demonstrated for children and could therefore be a factor enhancing sensitization. As found for other studies [24, 25] IL-4 was difficult to detect in primary cultures stimulated with allergen so the two Th2 cytokines IL-5 and IL-13 were measured. The IL-10 release tended to be higher in the allergic groups. Paradoxically other groups have reported this anti-inflammatory cytokine is highest in allergic and asthmatic subjects [28, 29].

The ability of isoallergens to stimulate T cells differently has been described for tree and grass pollen allergens [28, 29], although the isoallergens are from different gene families and show greater sequence variation than the allelic polymorphisms. For Bet v 1 the isoforms which induced the highest T cell proliferation did not correlate with the isoforms which bound IgE suggesting that the cytokine release would be different and that the low IgE binding isoforms may be useful therapeutic reagents [28]. The sequence differences between the isoforms were found at 6–20 positions of the 160 amino acid protein [30]. Wurtzen showed that T cell clones to Timothy grass pollen group 5 allergens showed a large heterogeneity in their reactivity to different isoallergens [29]. The response of PBMC to the different isoforms was not reported so it is not known how the overall responses of the different individuals would vary.

Different responses to these isoallergens would be expected because the sequences vary by 30–40%, which is similar to interspecies variations [31].

The modest difference in IgE binding activity between Der p 2, 0101 and the other variants agrees with the study of Smith [13] who found a better association between the reactivity of Der p 2, 0102 and 0103 with natural Der p 2 than between Der p 2, 0101 and natural Der p 2. The reduced binding of the sera was evident in the slope of that data and in the IgE binding reported here. It must be emphasized that the variants showed a good correlation and no person was negative for 0101. The difference in binding cannot be accounted for by coating differences because all variants had the same reactivity with two monoclonal antibodies. The 0102 and 0103 variants examined previously differ from 0101 with changes in positions 47 and 114. The asparagine/glutamate change at position 114 would be considered important because of the charge difference and because it has been shown to affect monoclonal antibody binding [13, 32]. The variants 0107 and 0108, however, did not have the asparagine 114 substitution suggesting that the serine/threonine change affects IgE binding. Indeed this was the only amino acid difference between 0101 and 0107. The fact that residues in positions 47, 111 and 114 are closely located in the loop structures suggest a structural unit. The side chain of residue 40 although buried is closely located and may have a subtle effect. The high IgE binding of all the variants of Der p 2 differs from studies with Bet v 1 which show considerable variation in that capacity [30, 32].

The fact that the variants which induced low T cell cytokine release were amongst the highest IgE binders, and bound the monoclonal antibodies as well as 0101, shows that they were folded at least as well as 0101. The T cell responses are therefore unlikely to be influenced by artefactual processing or differences in antigen-presenting cells. Indeed 0104, which had the same elevated IgE binding as 0107 and 0108, induced similar T cell responses to 0101. The results show the 0101 and 0104 variants are the best molecules for eliciting T cell responses. The altered cytokine responses to variants differing by only one and two amino acids suggests the nature of the responses to Der p 2 can be quite dependent on changes in epitope recognition. This will need to be considered in the design of genetically modified allergens.

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