

Population analysis of cellular responses to synthetic peptides of *Der p* II, a major allergen molecule of *Dermatophagoides pteronyssinus*, in allergic and nonallergic subjects

Okano M, Nagano T, Kino K, Yasueda H, Baba Y, Saito C, Masuda Y, Ohta N. Population analysis of cellular responses to synthetic peptides of *Der p* II, a major allergen molecule of *Dermatophagoides pteronyssinus*, in allergic and nonallergic subjects.

Allergy 1994; 49: 436–441. © Munksgaard 1994.

Responses of peripheral blood mononuclear cells to synthetic oligopeptides of *Der p* II, one of the major allergen molecules of *Dermatophagoides pteronyssinus*, were compared between allergic and nonallergic subjects. Healthy subjects showed positive responses to crude extracts of *D. pteronyssinus*, but only allergic subjects showed elevated cellular responses to *Der p* II. We synthesized three oligopeptides of *Der p* II in which motifs of a possible T-cell epitope were included. Of 14 subjects with positive response to *Der p* II, three responded to all three peptides, while five did not respond to any peptide tested. In 11 allergic patients who showed positive response to *Der p* II, responsiveness to the peptide K33-T47 was significantly higher than that to other peptides ($P < 0.05$). All the responding patients were also positive for scratch test to *Der p* II, suggesting that those epitopes induced IgE-promoting helper T-cell response in allergic persons. On the other hand, the *in vitro* cellular responses were not necessarily correlated to IgE production against *Der p* II in healthy subjects.

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Key words: *Dermatophagoides* spp.; *Der p* II; house-dust mite allergy, human T cells, synthetic peptides; T epitope.

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Accepted for publication 14 October 1993

House-dust mite (HD) is one of the most important sources of environmental allergens, and high titers of HD-specific IgE antibodies are commonly observed in patients with various allergic diseases (26). *Dermatophagoides* is the most important genus of HD, and serologic studies have revealed the presence of several major allergen molecules in crude mite antigens (3, 9, 15, 24). Since production of specific IgE is controlled by T cells (10, 13), the presence of HD-specific IgE reflects activation of helper T cells specific to HD molecules. T cells recognize antigens in the context of HLA expressed on antigen-presenting cells (6), and the target epitopes are usually different from those for B cells. Information on T-cell epitopes of allergen molecules is thus essential to uncover regulatory mechanisms of allergic hypersensitivities.

Recent research suggests the existence of characteristic primary sequences of peptides as essential

motifs for T-cell recognition (11, 22). It is therefore thought to be possible to predict T-cell epitopes in the primary sequence by their expected conformation (23). In the case of *Der p* II, one of the major allergens of *D. pteronyssinus*, three parts of the sequence were identified as candidates for T-cell epitopes. It is interesting to test whether some of those peptides carry dominant functions for induction of *Der p* II-specific IgE antibodies in many, if not all, allergic patients. Modulation of such a T-cell epitope (or epitopes) could be the main target in future trials of the immunotherapy of allergic diseases.

In this study, we synthesized three oligopeptides, each of which included the predicted T-cell epitope of *Der p* II. By the use of these peptides as well as a whole *Der p* II molecule, the responses of the peripheral blood mononuclear cells (PBMC) of aller-

gic and nonallergic subjects were compared. We discuss the role of T-cell epitopes of *Der p* II in the onset of allergic hypersensitivity to *D. pteronyssinus*.

Material and methods

Subjects

We tested 16 Japanese patients (11–32 years old) with allergic rhinitis and 16 Japanese healthy volunteers (21–46 years old) who had no history of allergic diseases. All the patients were shown to have specific IgE against *D. pteronyssinus* by radioallergosorbent test (RAST). The lowest Pharmacia RAST unit (PRU) was 13.5 U/ml, and the highest scores were >100 U/ml. No patient used immunosuppressive drugs during the study.

Antigens

Antigens used in the study were crude extracts of *D. pteronyssinus* (Dp-c) (21), purified *Der p* II (27), and three synthetic oligopeptides of *Der p* II. As a positive control antigen, we used purified protein derivative (PPD) (Nihon BCG Seizo Co, Tokyo, Japan). Protein contents were measured by the method of Lowry et al. (14). Each antigen was stored at -20°C before testing.

Synthetic oligopeptides

In the primary sequence of *Der p* II reported by Chua et al. (5), three parts were identified as possible T-cell epitopes: E38–E42, D64–G67, and K109–G112. We synthesized 15–16 *mer*-peptides in which the predicted sequence and its adjacent 10–12 amino acid residues were included (Fig. 1).

Der p II

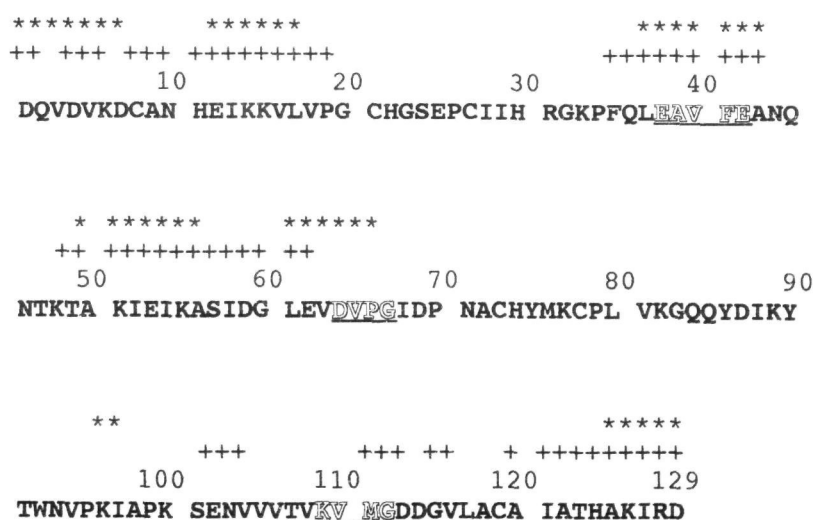


Fig. 1. Primary sequence of *Der p* II reported by Chua et al. (5). Possible α -helix structure estimated by two hypotheses (*ref. 4; ref. 7) are shown. Predicted T-cell epitopes are underlined.

PBMC proliferation assay

Antigen-driven proliferation of PBMC was tested *in vitro* by the method described elsewhere (12). In brief, PBMC were separated by Ficoll gradient centrifugation (specific gravity = 1.077; Pharmacia, Uppsala, Sweden), and were adjusted to be $1 \times 10^6/\text{ml}$ in RPMI-1640 conditioned medium (CM) (Waken Co, Kyoto, Japan), supplemented with 10% heat-inactivated, pooled, human male sera, 100 U/ml penicillin, 100 $\mu\text{g}/\text{ml}$ streptomycin, and 20 mM l-glutamin (Gibco, Grand Island, NY, USA). PBMC were incubated in 96-well, flat-bottom plates (Corning Co, Corning, NY, USA) in the presence or absence of antigens for 7 days in a humidified chamber at 37°C in 5% CO_2 and 95% air. The optimal antigen concentrations were determined in our preliminary experiments (data not shown), and the protein concentrations were 10 $\mu\text{g}/\text{ml}$ for Dp-c, 5 $\mu\text{g}/\text{ml}$ for *Der p* II and peptides, and 2 $\mu\text{g}/\text{ml}$ for PPD. For the final 16 h, 37 kBq of ^3H -labeled thymidine-methyl (TdR) (ICN, Irvine, CA, USA) was added to each well, and incorporated ^3H -labeled TdR was assessed by liquid scintillation spectrometry (Aloka, Tokyo, Japan).

Results were shown as stimulation index (SI), calculated as the ratio of the mean cpm obtained in the triplicate culture with antigen and that obtained in the antigen-free culture. We tentatively set cutoff lines determining positive and negative responses at $\text{SI} = 3.0$ for Dp-c-driven, and 2.5 for *Der p* II or peptide-driven responses.

Skin scratch test

The scratch test for detection of *Der p* II-specific IgE was done with a *Der p* II solution of 10 $\mu\text{g}/\text{ml}$ in saline. Antigen-induced erythema and wheals were measured 15 min after allergen challenge.

Statistical analysis

Statistical analysis was done by the two-sample rank test (8) and the chi-square test with the Yates correction.

Results

Synthetic oligopeptides

In the primary sequence of *Der p* II reported by Chua et al. (5), the α -helix was predicted by two different methods (4, 7), and three parts of the sequence were thought to include possible T-cell epitopes (Fig. 1). With this information, we synthesized three 15–16 *mer*-oligopeptides: K33–T47, I58–C73, and N103–A118.

Table 1. Proliferative responses of PBMC to Dp-c and PPD *in vitro*

Donor	Allergy	CPM*		
		Antigen (-)	PPD	Dp-c
WA	-	420±54	59 837±7437 (142.5)**	9668±463 (23.0)
AR	-	2251±669	80 204±3681 (35.6)	4613±1145 (2.1)
SA	+	1534±483	65 411±6601 (42.6)	27 521±3025 (17.9)
BB	+	972±56	98 114±7080 (100.9)	59 380±5593 (61.1)

Mean cpm of triplicate culture is shown.

** Stimulation indices are shown in parentheses.

PPD: purified protein derivative.

Antigen-driven PBMC proliferation

Antigen-free culture cpm was less than 3000; representative results are shown in Table 1. All but one subject tested showed strong proliferative responses of PBMC (SI > 10.0) to the positive control antigen, purified protein derivative (PPD). One subject who showed a faint SI to PPD did respond to Dp-c (SI = 19.1). Thirteen of 16 allergic patients (81.3%) and 12/16 healthy controls (75.0%) showed positive responses to Dp-c. The strength of proliferation was high in the patient group; mean SI for Dp-c was 18.1 in the patients and 8.88 in the controls. However, the difference was not statistically significant. Proliferated PBMC were confirmed to be CD3⁺4⁺8⁻ T cells by flow cytometry (data not shown). Responders to Dp-c were tested for *Der p* II-driven proliferation.

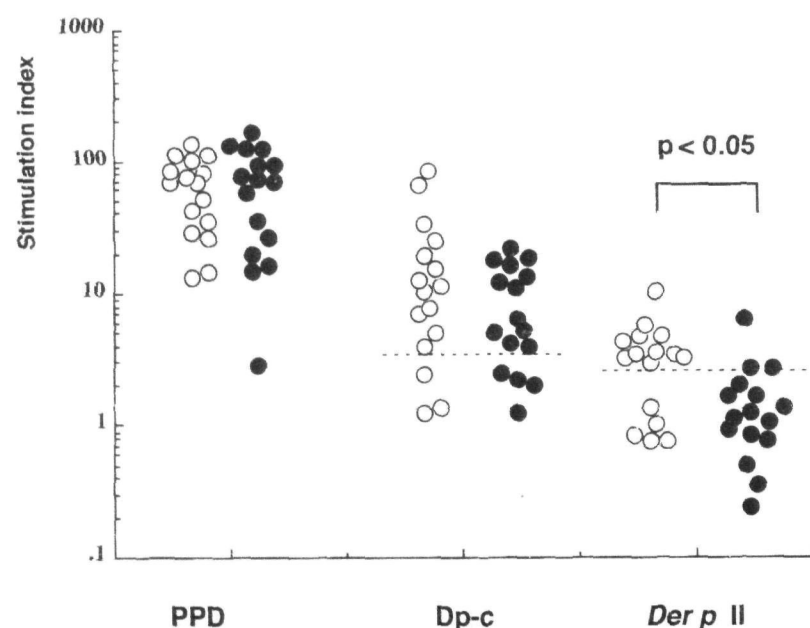


Fig. 2. Proliferative responses of PBMC obtained from allergic (open circle) and healthy (closed circle) subjects. Tentative cutoff lines for Dp-c (SI = 3.0) and *Der p* II (SI = 2.5) are shown as dotted lines. PBMC proliferation against *Der p* II was significantly higher in allergic group than healthy subjects ($P < 0.05$).

eration. Eleven of 13 patients (84.6%) showed positive responses to *Der p* II, whereas positive responses were observed in only three of 12 healthy subjects (Fig. 2). The difference in frequency of positive responders between the two groups was statistically significant ($\chi^2c = 8.06$; $P < 0.01$). The mean SI of *Der p* II-driven proliferation in the patient group was also significantly higher than that in healthy subjects (3.67 for the patients and 1.78 for the controls; $P < 0.05$).

Peptide-driven PBMC proliferation in allergic and nonallergic groups

Peptide-driven proliferation was tested only in subjects who responded to the whole *Der p* II molecule. Six of 11 patients (54.5%) showed positive responses to the peptide K33-T47, and three (27.3%) responded to I58-C73 as well as N103-A118 (Fig. 3). The mean SI for the first peptide was significantly higher than mean SIs for the other peptides ($P < 0.05$). Together with the observation that two of three healthy controls responded to the first peptide, this shows that the peptide K33-T47 might be one of the dominant stimulators regardless of the clinical situations of tested subjects. Subjects with positive responses to *Der p* II were divided into several groups according to their responding patterns. Five of 14 responders did not respond to any peptides tested, while three persons responded to all three peptides. Four persons responded only to the peptide K33-T47, and other patterns are shown in Table 2. In some subjects, the responsiveness was not changed when we tested them at different antigen doses (data not shown).

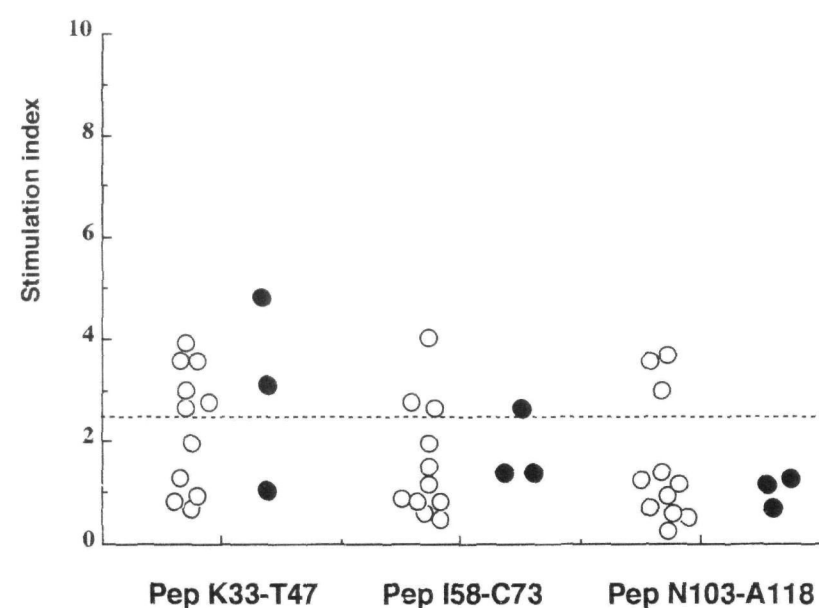


Fig. 3. PBMC proliferative responses to three *Der p* II peptides. Results of allergic (open circle, $n = 11$) and healthy (closed circle, $n = 3$) responders to the whole *Der p* II are shown. Tentative cutoff for positive and negative responses was set at SI = 2.5.

Table 2. Recognition patterns of PBMC obtained from allergic and nonallergic donors against synthetic *Der p II* peptides

	Proliferation against			No. of responders
	K33-T47	I58-C73	N103-A118	
Group 1	+	+	+	3 (21.4%)
Group 2	+	+	-	1 (7.1%)
Group 3	+	-	-	4 (28.6%)
Group 4	-	+	-	1 (7.1%)
Group 5	-	-	-	5 (35.7%)
Total	8/14 (57.1%)	4/14 (28.6%)	3/14 (21.4%)	14

Relationship between cellular responses and scratch test for *Der p II*

Although not all healthy subjects were tested, 9/10 control subjects tested were negative for the scratch test for *Der p II*. Three healthy subjects showed PBMC responses to *Der p II*; however, results of the scratch test were not necessarily concordant with *in vitro* variables in nonallergic subjects. Eight allergic patients were tested in a *Der p II* scratch test. All the patients tested were positive for both scratch test and *in vitro* cellular responses to *Der p II* (Table 3).

Discussion

In this study, we observed the functional *Der p II* epitopes recognized by human T cells of allergic and nonallergic donors. Although we tested only three possible T-cell epitopes of *Der p II*, there were at least five distinct response patterns in the tested sub-

jects. We predicted T-cell epitopes by a hypothesis proposed by Rothbard & Taylor (23); by another method (16), similar parts were also predicted as T epitopes (data not shown). It has been shown that T-cell epitopes may be located in the α -helix structure of antigen peptides, and four parts of *Der p II* seem to be located in the α -helix (Fig. 1). Although three peptides tested in the present study might be included in the main T-cell epitopes of *Der p II*, the observation that one-third of responders to *Der p II* failed to recognize any three peptides suggested the presence of other, undetected T-cell epitopes located in the other α -helical parts of the *Der p II* molecule.

Human CD4⁺ T cells recognize antigens in the context of self HLA class II molecules, and specificities of T-cell responses are restricted by HLA class II allotypes, which are highly polymorphic (1). The peptide K33-T47 functions as an active stimulator. Because no strict association between HD allergy and HLA has been reported (17), it is unlikely that the peptide K33-T47 was recognized under the restriction of a particular HLA type's being common in the allergic patients. It is interesting to note that this peptide was recognized by T cells under the restriction of HLA-DR15 (paper in preparation) as well as of HLA-DR1 in Caucasians (25). In view of those observations, this peptide might be a universal peptide which was recognized by various T cells with the restriction of different HLA types (2). Analysis of preferential V gene usage of T-cell receptors of the responding T cells is underway to test this possibility.

When we tested PBMC proliferation against Dp-c, we observed that nearly 80% of healthy subjects tested responded to the mite antigen. The frequency of responders to Dp-c in healthy subjects was comparable to that in allergic patients, although the strength of responsiveness was lower in healthy subjects. It is not surprising that the PBMC of healthy subjects responded *in vitro*, albeit at a lower level, since they are also exposed to HD and produce some IgG antibodies. On the other hand, *Der p II*-driven proliferation was observed only in 18.8% of healthy subjects, whereas 68.8% of all allergic subjects tested reacted to *Der p II*.

Clearly, *Der p II* ought to be more discriminating than Dp-c, because of the complex composition of the latter. Our study serologically demonstrated *Der p II* to be a major allergen of HD. Although almost all subjects, allergic or nonallergic, have had contact with HD, the *Der p II* molecule seemed to induce positive cellular responses only for allergic persons. The reason that the major allergen was selectively recognized by allergic donors is not clear. There might be an immunomodulation mechanism which modulates helper T-cell responses to *Der p II*. Healthy controls also responded to *Der p II* in some cases,

Table 3. Relationship between PBMC responses and skin scratch test for *Der p II*

Subject	Allergy	PBMC response*	Scratch test**
KA	-	+(6.6)	-/-
WA	-	+(2.7)	-/-
MI	-	+(2.8)	7×12/32×37
TA	-	-(1.1)	-/-
OH	-	-(1.9)	-/-
OK	-	-(0.7)	-/-
CH	-	-(1.1)	-/-
HA	-	-(0.8)	-/-
ON	-	-(0.4)	-/-
NI	-	+(0.5)	-/-
BB	+	+(5.7)	4×8/22×24
NA	+	+(9.7)	7×6/30×29
BO	+	+(3.5)	13×15/31×34
MA	+	+(2.8)	8×13/42×49
MT	+	+(2.9)	11×12/47×43
HA	+	+(4.7)	0×0/17×15
OZ	+	+(4.8)	5×7/26×30
TA	+	+(3.7)	8×10/30×36

* SIs for *Der p II* of each subject are shown in parentheses.

** Results of scratch test are shown as size of wheal/erythema (mm×mm).

possibly because HLA-DR15, a responder HLA allele, is one of the most common HLA-DR types in the Japanese population. Even in such cases, those T-cell responses failed to produce HD-specific IgE. Those findings suggest that predisposition to HD allergy is determined in some part by two distinct factors: 1) HLA-controlled T-cell recognition of allergen epitopes, and 2) production of mainly Th2 cells, but not Th1 cells, which is independent of regulation by HLA.

All patients who showed a positive scratch test for *Der p* II also responded to *Der p* II in a PBMC proliferation assay *in vitro*. The concordance of cellular and IgE-producing responses in the patient group strongly suggests that the allergen-driven helper T-cell response is indeed an essential step for the onset of HD allergy, as has been shown by O'Brien et al. (18). On the other hand, one healthy subject showed positive responses to *Der p* II in both cell proliferation and scratch test. The reason that the subject had no episode of allergy is not clear, and again we think that there might be an additional factor or factors, major or minor, in the onset of clinical symptoms. Recent studies on cellular immunology are solving the enigma of antigen-recognition mechanisms during the T-cell response. It seems to be possible to manipulate T-cell responses by artificial modification of the crucial motif of the antigen structure (19, 20). This strategy might be applicable to prevention or treatment of HD allergy through modulation of helper T-cell responses to major HD allergen molecules *in vivo*.

Acknowledgments

The authors thank Dr A. Ishii, of the National Institute of Health, Japan, and Dr E. Nakayama, of Okayama University, for their critical discussion and suggestions. This work was supported in part by a grant-in-aid (no. 02454172) for Scientific Research from the Ministry of Education, Science, and Culture of Japan.

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