

Olive pollen allergy: searching for immunodominant T-cell epitopes on the Ole e 1 molecule

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Summary

Background The amino-acid and nucleotide sequence of Ole e 1 (the major antigen of olive pollen) has been described and the IgE antibody response to this major allergen was associated with DR7/DQ2 antigens. With this previous data we try to define the T-cell epitopes implicated in Ole e 1 reactivity.

Objectives To study the recognition of T cells (derived from allergic and non-allergic Ole e 1 patients) to Ole e 1 synthetic peptides in order to define immunodominant T-cell epitopes.

Methods We have compared the proliferative response of the peripheral blood mononuclear cells from Ole e 1 sensitized patients vs. non-sensitized controls, induced by 14 Ole e 1 synthetic peptides. Thirty subjects were classified in two groups: group 1 (non-responders against Ole e 1, $n = 16$) and group 2 (Ole e 1 responders, $n = 14$), according to their clinical parameters and the presence or not in their sera of the significant Ole e 1 IgE antibody levels.

Results Our results shown that it is possible to find T cells reactive to Ole e 1 peptides in patients with and without significant levels of Ole e 1 IgE antibodies. However, the percentage of response was higher in patients with IgE antibodies (71.4% vs 25%), and the recognition profile was different: the control group showed a broad reactivity pattern, in contrast, the response by the 'Ole e 1 responders' group was mainly directed against three peptides of the carboxi-terminal region, peptides 10 (91–102), 12 (109–120) and 13 (119–130), with a response frequency of 35.7, 28.5 and 28.5%, respectively. By direct and inhibition test no antibody response was found against the synthetic peptides.

Conclusions Our data suggest that the regions between 91 and 102 and 109–130 amino-acids on the Ole e 1 molecule are immunodominant T-cell epitopes. These epitopes are not recognized by IgE antibodies.

Keywords: Ole e 1, HLA class II, DR7/DQ2, T-cell epitopes, olive pollen sensitization, main allergen of olive pollen

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Introduction

One of the critical problems in the response of IgE antibodies against ubiquitous antigens is the fact that only a

small percentage of patients are able to synthesize IgE antibodies while the majority is not, although both are exposed to the same allergenic determinants. On the other hand, it is well known that the allergy is a multifactorial illness involving both genetic and environmental factors [1].

The generation of cellular immune response depends upon recognition by the antigen receptors on T cells

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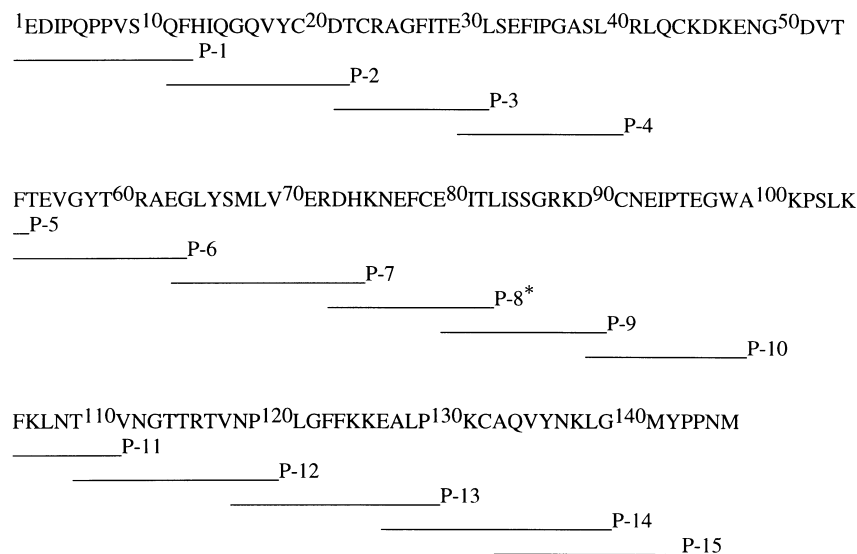


Fig. 1. Panel of overlapping peptides used in the proliferation study, that cover the whole sequence of Ole e 1, based on the amino-acid sequence described by Villalba *et al* [20].* It was not possible to synthesize this peptide.

(TCR) of peptidic fragments derived from processed foreign proteins, associated with products of the major histocompatibility complex (MHC) on the antigen presenting cells (APCs) surface [2]. Due to the implication of HLA class II antigens in the recognition of soluble antigens (such as the allergens), different authors [3–5] thought that this antigenic complex could be the key for the production of specific IgE antibodies.

Several reports have demonstrated the association between IgE antibody response to highly purified allergens, and specific DR and DQ alleles, revised by Blumenthal in [6]. However, although these associations are clearly confirmed, the HLA class II antigens do not seem to be the only element implicated in the IgE status 'responders vs non-responders' to an antigen.

Other works have focused on the study of T-cell epitopes on the molecule of a particular antigen, in order to find immunodominant regions responsible for these differences [7–13]. Most of these reports have shown that the reactivity of T cells against a specific allergen is not limited to the allergic population, because it is possible to detect allergen reactive T cells in both allergic and normal subjects [11–13]. When the T-cell epitopes recognized among both groups are compared, there are no significant differences. In spite of the fact that T cells are present in both situations, the profile of cytokines secreted by specific T cells derived from allergic and non-allergic is different, with the production of IL-4 (cytokine produced by type 2 T helper) being predominant in the allergic responders and the IFN γ (cytokine produced by type 1 T helper) production in the non-allergic responders [14]. Thus, the pathway of IgE regulation has been described as being based on reciprocal activities of IL-4 and IFN γ cytokines.

The olive tree (*Olea europaea*) is a species widely

distributed throughout the Mediterranean area and its pollen is regarded as a major cause of respiratory allergy in the Mediterranean countries [15]. The major allergen of this pollen, Ole e 1, is a protein of 18–20 kDa [16,17], of a heterogeneous nature [18], that has recently been cloned and the complete amino-acid sequence described [19,20]. On the other hand, Martín-Orozco *et al.* [21] using six MoAbs directed against Ole e 1 found that Ole e 1 has at least four B-cell epitopes on its molecule, identified by competition assays. Finally, we reported the relationship between HLA class II haplotypes and alleles, and the IgE antibody response to highly purified Ole e 1 in two populations (Spanish and Arabic people), and we found a strong association between DR7 (DRB1*0701/2) and DQ2 (DQB1*0201) and this response [22,23].

The present report studies the proliferative response against Ole e 1 synthetic peptides from PBMCs of Ole e 1 allergic and non-allergic subjects in order to search immunodominant T-cell epitopes on this antigen in a context of HLA class II, analysing the possible differences between both groups, in order to gain a better understanding of this allergic response.

Materials and methods

Patients

Individuals referred to the Allergy Department from the Hospital General de Especialidades Ciudad de Jaen (Jaen) or from the Allergy Department at Fundación Jiménez Díaz (Madrid), and volunteer blood donors from our laboratory, were considered for inclusion into this study. Informed consent was obtained from all subjects prior to enrolment in the study. Clinical assessment was done by family and

personal history and physical examination. Immediate hypersensitivity to *Olea europaea* crude extract was determined by skin-prick testing. A positive control with histamine 1 mg/mL and negative control with a saline buffer solution were also performed for each of the subjects. Thirty millilitre of peripheral blood was obtained from all the subjects, for serological and cellular determinations.

Antibody measurement and serum IgE levels

Total serum IgE levels were measured by Pharmacia IgE enzyme immunoassay (Uppsala, Sweden). Specific *Olea europaea* extract IgE antibodies were determined by Pharmacia CAP system RAST (Uppsala, Sweden).

Specific IgG and IgE antibodies to highly purified allergen Ole e 1 were measured by double-antibody-radioimmunoassay (DARIA) as previously described [22]. The comparison of different serological determinations between groups was analysed by Student's *t*-test.

HLA class II typing

Genomic DNA was extracted from peripheral blood following the salting out method [24].

Polymorphism of the DRB1 locus was studied by reverse dot-blot, according to the indications of the manufacture protocols (R&D), and polymorphism of the DQB1 locus was determined by PCR-SSO, according to the methods previously described [25].

Statistical significance of antigen frequencies between groups was measured by Fisher's exact test.

Antigen and peptide purification

Ole e 1 was obtained after gel filtration and HPLC purification by reverse-phase chromatography of crude olive pollen extract (Allergon, Angelholm, Sweden), following a previously described method [26].

Overlapping dodecapeptides ($n = 14$) that spanned the entire Ole e 1 molecule (Fig. 1) were generously synthesized by Dr J.P. Albar, from the Centro Nacional de Biotecnología, Madrid, using the Fmoc strategy. Peptide concentration and purity were determined by reverse phase high performance liquid chromatography methods, obtaining a purity greater than 90%.

The Ole e 1 peptides were synthesized according to the amino-acid sequence previously described for the Ole e 1 molecule [20].

Proliferation assays

Peripheral blood mononuclear cells (PBMCs) were isolated by gradient centrifugation on Lymphoprep (Nycomed

Pharma, Oslo, Norway) from 25 mL of peripheral blood. After three washes in RPMI 1640 supplemented with 5% human AB serum, 4 mm L-glutamine, 50 U/mL penicillin, 50 µg/mL streptomycin, and 1 mM sodium pyruvate (Flow Laboratories, Irvine, UK). PBMCs were cultured in triplicate in 96-well round-bottom microtitre plates, at 2×10^5 cells per well, in a 200 µL volume, in the absence or presence of stimulus (antigens or mitogen) at 37° and 5% CO₂ in humidified air. After 5 days of culture, [³H] thymidine (New England Nuclear, Boston, MA, USA) was added, and its incorporation was measured after 16–18 h in a β scintillation counter (Beckman LS 2800; Palo Alto, CA, USA). The antigens or mitogens were added at the commencement of the cultures at different concentrations: PHA (2.5 µg/mL), Ole e 1 molecule (5 µg/mL), and Ole e 1 peptides (0.1–10 µg/mL range).

Data are presented as stimulation index (SI), defined as the mean value of the triplicate (dpm) of proliferative responses against the antigen or mitogen (Ole e 1 molecule, Ole e 1 peptides or PHA) divided by the dpm value of the medium control (PBMCs without antigen). For determining the SI of peptides, the highest proliferation value of the three concentration tested, was considered. Only SI values greater than or equal to 2, and with a coefficient of variation less than 20%, were considered positive.

DARIA inhibition assay using Ole e 1 synthetic peptides

The DARIA inhibition technique, with minor modifications, for the inhibition of the IgE antibodies α-Ole e 1 with the 14 overlapping Ole e 1 peptides, was used. Firstly, sera of the patients with significant levels of IgE antibodies against Ole e 1, were diluted in IgE myeloma protein to achieve 50% of Ole e 1 binding. Afterwards, they were incubated with different concentrations of competitive peptide (1, 10 and 50 µg/mL), 1 h at room temperature. Then, 1 ng of Ole e 1 radiolabeled with ¹²⁵I, was added 100 µL in (BBS), borate buffer saline), and incubated for 4 h at room temperature. The antigen antibody complexes were precipitated by addition of an appropriate amount of rabbit anti-human IgE for 16 h at 4 °C. The precipitates were washed extensively with BBS, and the radioactivity retained was measured in a gamma counter. Arbitrary units were assigned as described in [22]. As a positive control of inhibition, instead of the peptides, non-labelled Ole e 1 antigen (0.5 µg) was used.

IgG antibodies against Ole e 1 peptides

The IgG antibody level against the Ole e 1 peptides 10, 12 and 13 in the sera of the 40 subjects from this study was measured. We performed the IgG antibody determinations and not determinations for IgE antibodies because, as described in [5], if there are no IgG antibodies against an

Table 1. T cell reactivity against Ole e 1 molecule and Ole e 1 peptides, antibodies determinations and HLA class II haplotype

No (Sex)	Olea europaea IgE (Class)	Ole e 1 IgG (AU/ml)	Ole e 1 IgE (AU/ml)	T cell reactivity (SI)		HLA class II Haplotype
				Ole e 1	Ole e 1 peptides	
a. Ole e 1 non-responders group (Group 1)						
C-1 (F)	1	480 ± 8	243 ± 4	–	–	DRB1*11-DRB1*11/13
C-2 (F)	0	15221 ± 2328	694 ± 236	–	2(4.6), 3(4.5), 4(3.2), 5(3.3), 6(2.6)	DRB1*13-DRB1*13/14
C-3 (M)	0	1360 ± 64	380 ± 29	–	10(3.2), 11 (5.5)	DRB1*01-DRB1*03 DQB1*0201
C-4 (F)	0	1684 ± 38	766 ± 99	–	–	DRB1*04
C-5 (M)	0	1798 ± 161	598 ± 74	–	2(5.6), 3(6), 7(7.8)	DRB1*15-DRB1*0701 DQB1*0201
C-6 (F)		2187 ± 217	219 ± 66	–	–	DRB1*11-DRB1*15
C-7 (M)		940 ± 92	137 ± 10	3	–	DRB1*04-DRB1*11
C-8 (F)	1	548 ± 156	128 ± 29	–	–	DRB1*01-DRB1*04
C-9 (F)	2	1771 ± 593	157 ± 2	11.7	–	DRB1*15/16
C-10 (M)	0	541 ± 263	156 ± 41	–	–	DRB1*13-DRB1*0701 DQB1*0201
C-11 (F)	0	836 ± 35	135 ± 7	–	–	DRB1*01-DRB1*04
C-12 (F)	0	168 ± 15	143 ± 28	2	9(3.8)	DRB1*11-DRB1*0701 DQB1*0201
C-13 (F)	0	538 ± 65	139 ± 46	–	–	DRB1*15/16-DRB1*04
C-14 (F)	2	1379 ± 162	508 ± 61	–	–	DRB1*04-DRB1*03 DQB1*0201
C-15 (M)	2	1158 ± 229	213	–	–	DRB1*01-DRB1*03 DQB1*0201
C-16 (M)	2	745 ± 64	632 ± 2	–	–	DRB1*14-DRB1*13/14
n = 16 (10F, 6M)	0.71 ± 0.91	1959 ± 3583	328 ± 231	(3/16)	(4/16)	
b. Ole e 1 responders group (Group 2)						
A-1 (F)	2	8897 ± 288	4047 ± 105	4.4	7(4.1)	DRB1*11-DRB1*0701 DQB1*0201
A-2 (M)	2	18140 ± 1861	3622 ± 1122	2.1	10(3.7)	DRB1*11-DRB1*0701 DQB1*0201
A-3 (F)	5	25488 ± 78	12434 ± 834	2.4	12(2.7)	DRB1*11-DRB1*13/14
A-4 (M)	4	17366 ± 751	5490 ± 711	2.6	7(2.28), 10(2.6)	DRB1*0.4-DRB1*10
A-5 (F)	3	4297 ± 213	3889 ± 258	3.79	12(3.9), 13(2.8)	DRB1*01-DRB1*0701 DQB1*0201
A-6 (M)	6	3343 ± 218	4983 ± 24	3.4	9(9.5), 10(7.2), 11(7.6), 12(8.6), 13(7.5)	DRB1*04-DRB1*03 DQB1*0201
A-7 (F)	2	1502 ± 72	1596 ± 46	2.07	–	DRB1*0701 DQB1*0201

Table 1. continued

No (Sex)	Olea europaea IgE (Class)	Ole e 1 IgG (AU/mL)	Ole e 1 IgE (AU/mL)	T cell reactivity (SI)		HLA class II Typing
				Ole e 1	Ole e 1 peptides	
A-8 (M)	4	9412 ± 299	13227 ± 11	2	–	DRB1*01-DRB1*03 DQB1*0201
A-9 (F)	3	1635 ± 48	1428 ± 110	4.5	2(3.5), 12(4), 13(4.9), 14(3.6), 15(3.1)	DRB1*11-DRB1*0701 DQB1*0201
A-10 (F)	3	5888 ± 23	7012 ± 306	9.9	3(2.8), 14(2.9), 7(4.1)	DRB1*01-DRB1*03/13/14*
A-11 (M)	4	56865 ± 2026	2922 ± 253	3.1	10(2.04)	DRB1*11-DRB1*0701 DQB1*0201
A-12 (F)	2	2192 ± 97	2114 ± 137	4	–	DRB1*01-DRB1*10
A-13 (F)	3	2821 ± 717	3159 ± 229	2.3	–	DRB1*01-DRB1*03 DQB1*0201
A-14 (F)	2	2199 ± 345	2310 ± 35	5.16	2(3.5), 4(2.7), 10(2.8), 13(5.7), 14(5.7), 15(3)	DRB1*04-DRB1*03 DQB1*0201
	3.2 ± 1.25	11377 ± 15075	4804 ± 3781	(14/14)	(10/14)	

antigen, there will be no IgE antibodies against this antigen either.

To detect IgG antibodies against the peptides 10 (91–102), 12 (109–120) and 13 (119–130) of the Ole e 1 molecule, an enzyme-linked immunoabsorbent assay (ELISA) was performed according to [27], with minor modifications. Microwell plates (EIA plate, Costar, USA) were coated with 100 ng of each peptide per well, overnight at 4°C. All sera were tested at a dilution of 1:10, using duplicate samples. As a second antibody, a peroxidase-conjugated rabbit-antihuman immunoglobulin G (Nordic, Tilburg, The Netherlands) was used, at a dilution of 1:3000. As a positive control, the Ole e 1 antigen (1 µg/well) tested with sera from sensitized patients was used. Results were considered positive when the optical density was twice the value obtained with the sera of normal controls.

Results

Profile of the studied subjects

This study was performed with 30 subjects, classified into two groups: group 1 (non-Ole e 1 responders) and group 2 (Ole e 1 responders), according to the presence or not in their sera of significant IgE antibody levels against highly purified Ole e 1. Serological determination data are indicated in Table 1. The control group, Group 1, was composed of 16 individuals (62.5% females vs 37.5% males). Seven (C-1 to C-7) were non-allergic patients (negative skin test

and RAST against *Olea europaea*) and nine (C-8 to C-16) were patients sensitized against *Olea europaea*, but not against Ole e 1. Group 2 was composed of 14 Ole e 1 sensitized patients (A-1 to A-14), with 64% females vs 36% males. The mean age was statistically higher ($P = 0.03$) in group 1 (32 ± 15) compared with the mean age from group 2 (22 ± 9).

The mean level of IgE antibodies to *Olea europaea* extract was significantly increased in the sera of the second group 3.2 ± 1.25 UI/mL compared with the first 0.71 ± 0.91 UI/mL, with a P -value of $P < 0.0001$.

No statistically significant difference was found between the mean level of total serum IgE of group 1 (110 ± 230 UI/mL) and the corresponding value for Group 2 (172 ± 196 UI/mL).

Analysis of the HLA class II antigens of the subjects (individually represented in Table 1) was based on our previous results of DR7/DQ2 as antigens associated to IgE antibody response to Ole e 1. On this premise, we compared the presence of these antigens in both groups, finding that six subjects (37.5%) in the group of non-Ole e 1 responders (group 1) carried the DQ2 (DQB1*0201) antigen (this phenotype frequency is similar to that previously reported for the Spanish population, 48%). Of these, three were associated with the DR3 (DRB1*03) antigen (18.7%) and three (18.7%) were associated with DR7 (DRB1*0701) antigen. A total of 62.5% (10 subjects) carried an HLA class II antigen (locus DR) other than DQ2.

For the Ole e 1 sensitized group (group 2), the presence of DQ2 was higher, 71.4% (10 individuals), and this antigen

Table 2. Comparison of the mean reactivity to Ole e 1 molecule and Ole e 1 peptides in responders and non-responders to this antigen

Antigens	None-Ole e 1 responders (n= 16)		Ole e 1 responders (n= 14)	
	Frequency of response (%)	Mean SI	Frequency of response (%)	Mean SI
Ole e 1	18.7	5.5 ± 5.3	100*	3.8 ± 2.08
P-1 (1–13)	0	–	0	–
P-2 (11–22)	12.5	5.1 ± 0.7	14.2	3.5
P-3 (22–33)	12.5	5.2 ± 1	7.1	2.8
P-4 (31–42)	6.2	3.2	7.1	2.7
P-5 (42–53)	6.2	3.3	0	–
P-6 (53–64)	6.2	2.6	0	–
P-7 (64–75)	6.2	7.8	21.4	3.4 ± 1
P-9 (81–92)	6.2	3.8	7.1	9.5
P-10 (91–102)	6.2	32	35.7	3.6 ± 2.06
P-11 (99–110)	6.2	5.5	7.1	7.6
P-12 (109–120)	0	–	28.5*	4.8 ± 2.6
P-13 (119–130)	0	–	28.5*	5.2 ± 1.9
P-14 (129–140)	0	–	21.4	4.06 ± 1.4
P-15 (134–145)	0	–	14.2	3.05 ± 0.07

*p < 0.05 (Comparison of the frequency of response between groups).

was associated with DR3 in four patients (28.5%) and with DR7 in six individuals (42.8%). The haplotype was not related with DQ2 in four patients (28.5%).

A comparison of the presence of these antigens between groups did not show any statistically significant difference. However, it is important to note that, again, the frequency of DQ2 in our patients (group 2) was higher than the frequency previously reported in the control Spanish population (71.4% vs 48%).

Cell proliferation against Ole e 1 peptides

The next step was to search for immunodominant epitopes on the Ole e 1 molecule, studying the proliferative response of PBMCs to Ole e 1 molecule and 14 overlapping Ole e 1 synthetic dodecapeptides (Fig. 1), synthesized according to the sequence described in [20]. The proliferative response was determined according to the method described in Materials and methods. All the cells were cultured with PHA mitogen (2.5 µg/mL) as positive control, and alone (without antigen) as negative control. The cell proliferation without stimulus ranged from 356 to 2840 (dpm).

T-cell reactivity from subjects in control group (group 1) was scarce (Table 1), and in general, highest antigen concentrations were necessary for a positive stimulation in this group. Thus, 5 µg/mL of peptide was the most frequent concentration required to produce a significant proliferative

response in group 1, in contrast to 0.1 µg/mL in 'Ole e 1 responder' group (data not shown).

Our results (Table 1) showed that three subjects out of 16 from control group positively responded to Ole e 1 molecule, and from these, one subject showed a statistically significant proliferation against peptide 9. Another three subjects, although with negative response to Ole e 1 molecule, were able to respond against some Ole e 1 peptide.

In the patient group (group 2, responders to Ole e 1) we found that 10 out of 14 responded positively against some Ole e 1 peptide, and four patients responded positively to Ole e 1 molecule, but were not able to proliferate against any Ole e 1 peptide.

The T-cell reactivity comparison between groups (Table 2) showed that there were remarkable differences. Firstly, the percentage of reactivity (frequency of response) against Ole e 1 molecule and Ole e 1 peptides, was always higher in the group of 'Ole e 1 responders', being the frequency of response against Ole e 1 peptides of 71.4% in the patient group compared with the 25% observed in the 'non-Ole e 1 responder group'. Secondly, although the mean number of peptides recognized for both groups was similar (2.7 ± 1.7 group 1 vs 2.7 ± 1.9 group 2), the regions recognized for these subjects were different (Table 2). The peptides recognized most often in group 1 (non-Ole e 1 responders) were peptides 2 and 3, that were recognized only by two of the 16 subjects studied (12.5%)

with a mean stimulation index of 5.1 ± 0.7 and 5.2 ± 1 , respectively. In contrast, the response of group 2 was focused fundamentally against three peptides located in the carboxi-terminal half of this protein, peptide 10 (91–102), recognized by five of the 14 patients studied (35.7%), with 3.6 ± 2.06 mean value of stimulation index, peptide 12 (109–120) with a frequency of recognition of 28.5% (4/14) and a higher mean value of stimulation index 4.8 ± 2.6 , and peptide 13 recognized by 4/14 patients (28.5%) with 5.2 ± 1.9 of stimulation index. Comparison of the reactivities against individual peptides of both groups, analysed by Fisher's exact test, revealed statistical differences with peptide 12 and 13 ($P = 0.03$) that were not recognized by any subject from Group 1, and marginally significant differences in the reactivity of peptide 10 ($P = 0.05$).

In the group of 'Ole e 1 responders', seven of the 10 patients (70%) with a significant proliferative response against Ole e 1 peptides carried DQ2 antigens 5 associated with DR7 and 2 with DR3, two carried other non-related HLA class II antigens: DR5-DR6 (DRB1*11-DRB1*13/14), DR4-DR10 (DRB1*04-DRB1*10) and in one case it was not possible to determine if the patient carried the antigen DR3/DR6 (A-10, Table 1).

Due to the low number of subjects with a proliferative response against the peptides with a DQ2 non-related HLA class II antigen it was not possible to statistically compare results.

Peptide recognition by IgG and IgE antibodies against Ole e 1

In order to assess if the same epitopes that react with T cell could be recognized by specific IgE antibodies, the different peptides were used in inhibition assays.

In DARIA inhibition assays, high concentrations of the 14 Ole e 1 overlapping peptides (1, 10 and $50 \mu\text{g/mL}$) were unable to diminish IgE binding to Ole e 1 (data not shown), suggesting a difference between T-cell epitopes and epitopes that are recognized by IgE antibodies.

On the other hand, it was not possible to find significant levels of IgG antibodies against the three peptides, determined as immunodominant by proliferative assays (p10, p12, p13), in any of the 40 sera tested by ELISA.

Discussion

This report describes the prevalence of T-cell response from Ole e 1 sensitized patients, against three regions of the Ole e 1 molecule (amino-acids 91–102, 109–120 and 119–130), defined by PBMCs proliferation assays.

Since olive pollen sensitization is one of the major causes of pollinosis in the Mediterranean area, it is interesting to understand the factors involved in this allergic disorder.

The major antigen of this pollen, Ole e 1, is a protein

composed of two polypeptides with molecular weights of 18 and 20 kDa. The fact that the amino-acid and nucleotide sequences of this protein have been recently described [19,20], opens up the possibility of searching for immunodominant T-cell epitopes on the Ole e 1 molecule, using synthetic peptides with a size in accordance to the HLA class II motif (12 aa). Similar analysis has recently been published with several other allergens such as mite-, grass pollen-, tree pollen-, cat-, bee venom- and chicken-derived allergen, as revised in [28].

In this report, we mapped T-cell epitopes by studying the polyclonal T cell response (PBMCs) against Ole e 1 overlapping synthetic peptides in 30 subjects. Based on the serological determinations were divided into two groups, group 2, composed by 14 Ole e 1 IgE antibody responders 4804 ± 3781 AU/mL and group 1 (control group) composed by 16 non-responders against the Ole e 1 molecule (mean IgE antibody level: 328 ± 231 AU/mL). From these, seven were non-allergic subjects (C-1 to C-7) and nine *Olea europaea* sensitized patients (C-8 to C-16). All of them (group 1) were non Ole e 1 responders.

Our cellular results confirm the idea previously reported by several authors [28] that it is possible to find T cells that react against allergens, in both sensitized and non-sensitized subjects. However, the lymphoproliferative response to Ole e 1 molecule and Ole e 1 peptides was different between groups, being statistically higher in the patients group. We found that the PBMCs from both populations proliferated in response to Ole e 1 peptides across the entire sequence of the molecule, but with remarkable differences. Firstly, the percentage of response was different, 75% of patients with IgE antibodies against Ole e 1 (group 2) showed a significant proliferative response against some Ole e 1 peptide. In contrast, only 25% (4/16, and from these, three subjects respond positively to the peptides and none to Ole e 1 molecule) of the patients that do not show Ole e 1 IgE antibodies respond significantly to some Ole e 1 peptide.

On the other hand, although the major part of the peptides were able to produce a significant response in some cases it was not possible to find any proliferative response against peptide 1 1–13 in none of the subjects studies. The peptides 12 (109–120), 13 (119–130), 14 (129–140) and 15 (134–145) were not recognized by the control group, and none of the Ole e 1 sensitized patients were able to respond against 5 (42–53) and 6 (53–64) peptides (Table 2).

On the other hand, we found that the response was generally directed against many epitopes on the Ole e 1 molecule, because most patients responded against more than 1 peptide (mean number of peptides recognized by subject: 2.7 ± 1.7 in control group vs 2.7 ± 1.9 Ole e 1 responders group) and each subject showed a different pattern of response.

The most interesting result, however, was found in comparing the profile of recognition between the two groups (Table 2). This showed how the response is fundamentally directed against peptides located in the carboxy-terminal half of the protein (peptide 10, (91–102), peptide 12, (109–120), peptide 13, (119–130) in the 'Ole e 1 responders', in contrast with the broad reactivity pattern observed in the 'non-Ole e 1 responder' group, and in general, the reactivity against these peptides is very close to the reactivity against the Ole e 1 molecule (Table 1). We found that there is a recognition of different T-cell epitopes, although the subjects from different groups shared the same HLA class II antigens. These three regions are immunodominant epitopes in the Ole e 1 response of sensitized patients. When these regions were analysed in the GenBank we found that they only showed high sequence homology (highest than 90%) with the allergens from *Oleaceae* family (*Syringa*).

Our results are different, but not discordant with previous studies performed with other antigens as Bet v 1 [11], Der p 2 [7], Fel d 1 [12] or phospholipase A₂ (bee venom allergen) [10] which describe that allergic and non-allergic patients recognize the same T-cell epitopes on the allergen molecule. In our study, although one of the immunodominant regions 91–102 is also recognized by one of the control subjects, in contrast with other reports, we found that there are important differences in the frequency of recognition between Ole e 1 IgE responders and Ole e 1 nonresponders. These differences are statistically significant in the case of peptide 12 (region 109–120) and 13 (119–130) (that were not recognized by none of the control subjects).

Previously, Villalba *et al.* [19] described that there are two regions in the Ole e 1 molecule, positions 38–51 and 83–99, where the microheterogeneity is mainly located and these regions were previously described by the same group [20] as the most hydrophilic and immunogenic areas. On the other hand, Lombardero *et al.* [29], confirmed the microheterogeneity of the aa sequence of the Ole e 1, indicating that could be also present in other parts of the allergen. As well, they describe a unique consensus site for N-glycosylation in the position 111–113. Our results suggest that the regions around 91–102, and 109–130 could be the most important regions implicated in the T-cell response to Ole e 1. These results are in accordance with previous works reporting that the immunogenic sites on the Der p 1 allergen are located predominantly in regions of high polymorphism [30,31], and, as explained by Tainer *et al.* [32] this could be due to the fact that polymorphic regions tend to be more flexible and are able to establish contact with MHC molecules more readily.

On the other hand, we have found that most patients who respond to some Ole e 1 peptide carry the DQ2 antigen (75%) from group 1 and 70% from group 2), and regarding

the immunodominant peptides it is worth noting that 4/5 patients (from group Ole e 1 responders) that recognized peptides 10, 4/4 patients that recognized the peptide 13 and 3/4 that recognized the peptide 12 carried the DQ2 HLA class II antigen. In the control group, the only patient that recognized one of these peptides (peptide 10) carried the DQ2 HLA class II antigen. The peptides 12 and 13 were not recognized by any control subject.

Finally, our study of the role of these 'immunodominant' peptides in the IgE antibody response against Ole e 1 revealed that these peptides recognize different epitopes on Ole e 1 molecule than IgE antibodies, because the DARIA inhibition assays were negative. These results are in accordance with previous evidence that the epitopes stimulating allergen-specific T cells are different from the epitopes detected by IgE antibodies [33,34]. On the other hand, we found that it was not possible to detect significant levels of IgG antibodies against these peptides in the sera of any of the subjects studied in this report. Taken together, these results suggest that these peptides could be possible future immunomodulators of Ole e 1 response, since the nonrecognition by antibodies decreases the risk of anaphylactic reactions.

As recently revised by Neerven [28] studies on the ability of allergen-derived synthetic peptides to inhibit cellular responses and humoral responses to allergens have provided evidence that the allergen-specific immune response can be inhibited. Thus, it has been demonstrated that anergy can be induced with supraoptimal concentrations of antigen or allergen-derived peptides. In mice, the immunoglobulin synthesis can also be inhibited *in vivo* by intranasal, oral or subcutaneous administration of peptides. However, there is no evidence in humans to support the contention that vaccination with a single peptide will downregulate the response to an intact protein. In any case, considerable care must be taken in the selection and combination of peptides in designing peptide therapies.

Besides the heterogeneity [19,20] of the Ole e 1 molecule, this report describes three immunodominant T-cell epitopes on this allergen. These results confirm the possibility that immunodominant regions in the allergens can be found using PBMCs studies, in spite of the complexity of these kind of responses. Finally, the results of this study should be considered as a 'starting point' that support the idea that in the future new strategies could be used in the treatment of Ole e 1 sensitization.

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References

- 1 Blumenthal MN, Yunis E, Mendell N, Elston CR. Preventive allergy: Genetics of IgE-mediated diseases. *J Allergy Clin Immunol* 1986; 78:962–8.
- 2 Schwartz RH. T lymphocyte recognition of antigen in association with products of the major histocompatibility complex. *Annu Rev Immunol* 1985; 3:237–61.
- 3 Levine B, Stember R, Fotino M. Ragweed hay fever: Genetic control and linkage to HLA haplotypes. *Sciences* 1972; 178:1201.
- 4 Blumenthal MN, Amos DB, Noreen H, Mendell NR, Yunis EJ. Genetic mapping of Ir locus in man: Linkage to second locus of HLA. *Science* 1974; 184:1303–4.
- 5 Marsh DG, Hsu SH, Roebber M et al. HLA-Dw2: a genetic marker for human immune response to short ragweed pollen allergen Ra5. Response resulting primarily from natural antigenic exposure. *J Exp Med* 1982; 155:1439–51.
- 6 Blumenthal MN. Searching for the asthma and allergy gene—The current status of our knowledge. *ACI News* 1995; 7:15–8.
- 7 O'Hehir RE, Verhoef A, Panagiotopoulou E et al. Analysis of human T cell responses to the group II allergens of *Dermatophagoides* species: localization of major antigenic sites. *J Allergy Clin Immunol* 1993; 92:105–13.
- 8 Bungy Poor Fard GA, Latchman Y, Rodda S, Geysen M, Roitt Brostoff J. T cell epitopes of the major fraction of rye grass *Lolium perenne* (Lol p I) defined using overlapping peptides in vitro and in vivo. I. Isoallergen clone 1A. *Clin Exp Immunol* 1993; 94:11–6.
- 9 Bungy GA, Rodda S, Roitt I, Brostoff J. Mapping of T cell epitopes of the major fraction of rye grass using peripheral blood mononuclear cells from atopics and non-atopics. II. Isoallergen clone 5A *Lolium perenne* group I (Lol p I). *Eur J Immunol* 1994; 24:2098–103.
- 10 Carballido JM, Carballido-Perrig N, Kagi MK. T cell epitope specificity in human allergic and non-allergic subjects to bee venom phospholipase A2. *J Immunol* 1993; 150:3582–91.
- 11 Ebner C, Schenk S, Najafian N et al. Nonallergic individuals recognize the same T cell epitopes of Bet v 1, the major birch pollen allergen, as atopic patients. *J Immunol* 1995; 154:1932–40.
- 12 Mark PG, Segal DB, Dallaire ML, Garman RD. Human T and B cell immune responses to Fel d 1 in cat-allergic and non-cat-allergic subjects. *Clin Exp Allergy* 1996; 26:1316–28.
- 13 Hashiguchi S, Hino K, Taniguchi Y et al. Immunodominance of seven regions of a major allergen. Cry j 2, of Japanese cedar pollen for T-cell immunity. *Allergy* 1996; 51:621–32.
- 14 Romagnani S. Atopic allergy and other hypersensitivities. Editorial overview: Technological advances and new insights into pathogenesis prelude novel therapeutic strategies. *Current Opinion Immunol* 1995; 7:745–50.
- 15 Bousquet J, Guerin B, Hewitt B, Lim S, Michel FB. Allergy in the Mediterranean area III. cross reactivity among Oleaceae pollens. *Clin Exp Allergy* 1985; 15:439–48.
- 16 Lauzurica P, Gurbindo C, Maruri N et al. Olive (*Olea europaea*) pollen allergens-I. Immunochemical characterization by immunoblotting. CRIE and immunodetection by a monoclonal antibody. *Mol Immunol* 1988; 25:329–35.
- 17 Lauzurica P, Maruri N, Galocha B et al. Olive (*Olea europaea*) pollen allergens-II. Isolation and characterization of two major antigens. *Mol Immunol* 1988; 25:337–44.
- 18 Wheeler AW, Hickman BE, Fox B. Heterogeneity of a major allergen from olive (*Olea europaea*) pollen. *Mol Immunol* 1990; 27:631–6.
- 19 Villalba M, Batanero E, López-Otín C et al. Cloning and expression of Ole e I, the major allergen from olive tree pollen. *J Biol Chem* 1994; 269:15217–22.
- 20 Villalba M, Batanero E, López-Otín C et al. The amino acid sequence of Ole e 1, the major allergen from olive tree (*Olea europaea*) pollen. *Eur J Biochem* 1993; 216:863–9.
- 21 Martín-Orozco E, Cárdaña B, del Pozo V et al. Epitope mapping, cross-reactivity with other Oleaceae pollens and ultrastructural localization. *Int Arch Allergy Appl* 1994; 104:160–70.
- 22 Cárdaña B, Vilches C, Martín E et al. DR7 and DQ2 are positively associated with the immunoglobulin-E response to the main antigen of olive pollen (Ole e I) in allergic patients. *Human Immunol* 1993; 38:293–9.
- 23 Cárdaña B, de Pablo R, Vilches C et al. Allergy to olive pollen: T cell response from olive allergic patients is restricted by DR7-DQ2 antigens. *Clin Exp Allergy* 1996; 26:316–22.
- 24 Miller SA, Dykes D, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988; 16:1215–8.
- 25 Kimura A, Sasazuki T. Workshop reference protocol of the HLA DNA typing technique. In: Tsuji K et al., eds HLA 1991. London: Oxford University Press, 1992.
- 26 Villalba M, López-Otín C, Martín-Orozco E et al. Isolation of three allergenic fractions of the major allergen from *Olea europaea* pollen and N-terminal amino-acid sequence. *Biochem Biophys Res Commun* 1990; 172:523–8.
- 27 Paradis K, Dib A, Homberg JC, Alvarez F. Characterization of anti-liver kidney microsome antibody (Anti-LKM1) from hepatitis C virus-positive and negative sera. *Gastroenterology* 1993; 104:1762–7.
- 28 Joost van Neerven RJ, Ebner C, Yssel H, Kapsenberg ML, Lamb JR. T-cell responses to allergens: epitope-specificity and clinical relevance. *Immunol Today* 1996; 17:526–31.
- 29 Lombardero M, Barbas JA, Moscoso del Prado J, Carreira J. cDNA sequence analysis of the main olive allergen, Ole e 1. *Clin Exp Allergy* 1994; 24:765–70.
- 30 O'Hehir RE, Garman RD, Greenstein JL, Lamb JR. The specificity and regulation of T-cell responsiveness to allergens. *Annu Rev Immunol* 1991; 9:67–95.
- 31 O'Brien RM, Thomas WR, Nicholson I, Lamb JR, Tait BD. An immunogenetic analysis of the T-cell recognition of the major house dust mite allergen Der p 2: identification of high- and low-responder HLA-DQ alleles and localization of T-cell epitopes. *Immunology* 1995; 86:176–82.

- 32 Tainer JA, Getzoff DE, Patterson Y, Olsen AJ, Lerner RA. The atomic mobility component of protein antigenicity. *Ann Rev Immunol* 1985; 3:501–25.
- 33 Yssel HK, Johnson PV, Schneider PV et al. T cell activation-inducing epitopes of the house dust mite allergen Der p I. *J Immunol* 1992; 148:738–41.
- 34 Ebner C, Szépfalusi Z, Ferreira F et al. Identification of multiple T cell epitopes on Bet v I, the major birch pollen allergen, using specific T cell clones and overlapping peptides. *J Immunol* 1993; 150:1047–54.