

Allergy to olive pollen: T-cell response from olive allergic patients is restricted by DR7-DQ2 antigens

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Summary

Background We have recently described the association between the IgE antibody response to *Ole e* I (the major antigen from olive tree pollen) and the DR7-DQ2 haplotype in a Spanish population.

Objective and methods Due to the linkage disequilibrium between DR7 and DQ2, and thus the difficult distinction between the role of these two antigens in the T-cell activation response, we decided to solve this question by two approaches:

1. The study of another ethnic group, individuals of Arabic origin, with a presumably distinct disequilibrium linkage between DR and DQ antigens. Genomic DNA typing was performed in 46 subjects (allergic and non-allergic) by Restriction Fragment Length Polymorphism (RFLP) and results showed that patients with specific IgE antibodies α -*Ole e* I, were DR7 and/or DQ2. These data show a similar restriction pattern to those previously described for Spanish patients. The phenotypic frequency of DR7 antigen is significantly greater than in the non-allergic population, with a corrected P (P_c) value of 0.03.

2. The analysis of the genetic requirements of *Ole e* I response, using T-cell lines specific for this antigen. This was first carried out by blocking the proliferative response of these T-cell lines with specific anti-human HLA class II antibodies and then testing the genetic restriction of this response using a panel of histocompatible and histoincompatible Antigen Presenting Cells (APCs). Both experiments corroborate the hypothesis that DR7 and DQ2 are implicated in the recognition of *Ole e* I.

Keywords: olive pollen allergy, HLA class II, genetic restriction, *Ole e* I, DR7-DQ2, Arab typing

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Introduction

Allergy is a multifactorial illness involving both genetic and environmental factors [1].

Induction of the allergic inflammatory response depends on the activity of CD4⁺ T cells [2], and as has been reported for different allergens, HLA-class II

gene products (principally DR) are able to restrict the recognition of allergen determinants of antigen-specific T-cell lines and clones [3,4]. Several studies have reported the association between HLA class II antigens and the IgE response against purified allergens [5–9]. The association between MHC class II antigens and the response to allergens has been focused on in two ways: first, by epidemiological or population studies, important for establishing the association or prevalence of an antigen in relation to a particular disease and secondly, by

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studying the genetic restriction using specific T-cell antigen [10,11].

The occurrence of a particular DR subregion associated with a particular DQ subregion on the same chromosome is much more frequent than would be expected by chance, this phenomenon is called disequilibrium linkage. When this situation occurs and the corresponding haplotype is associated with a genetically restricted response, it must be determined whether both these antigens are implicated or if the presence of one of them is a statistical artefact. Some investigators suggest that the best solution is to increase the sample size or to investigate other ethnic groups with a presumably different genetic background, until a significant number of patients possessing only one of these factors is found [12].

Olive pollen is one of the main causes of allergic diseases in the Mediterranean area [13]. The major antigen of this pollen, *Ole e* I, has been recently sequenced [14,15] and described as being closely related to the main antigen of other members of the Oleacea family [16,17].

We have recently described a strong association between the immunoglobulin E (IgE) antibody response to *Ole e* I and the DR7-DQ2 haplotype [18] in a sample from a white Spanish population. In addition, a protective role could be postulated for DR4 in this population. Here, we analyse the mentioned class II antigens, in a group of Arabic patients from Israel, as either risk or protective factors, in order to determine which of these antigens are implicated in the IgE response against *Ole e* I. Also, using T-cell lines specific to *Ole e* I, derived from two patients sensitized to *Olea europaea* and with IgE antibodies to *Ole e* I, we performed two kinds of assays: inhibition of the proliferative response to *Ole e* I with HLA class II specific antibodies; and a study of the genetic restriction using a panel of B-cell lines transformed with Epstein Barr Virus (EBV) as APC.

The final objective of this report is to gain further insight into the IgE antibody response to *Ole e* I.

Materials and methods

Patients and controls

The population samples were collected in Um-El-Fahem, a village in northern Israel, where the olive tree is extensively grown.

Clinical assessment was done by: family and personal history, physical examination, and skin tests (prick technique) to the most common allergens in Israel (*Dermatophagoides*, pecan, *Amarantus*, date, *Asteraceae*, *Parietaria*, grass, milk and egg compounds) and *Olea europaea* crude extract. According to these criteria,

the 65 individual studies were classified into three groups: group I (non-allergic), group II, allergic patients without sensitization to olive pollen (non-related allergic) and group III (*Olea europaea* allergic patients). Only the third group ($n = 13$) was selected for the specific antibody study against the major antigen of *Olea europaea*. We used the radio allergosorbent test (RAST) (Pharmacia Cap System, Pharmacia, Uppsala, Sweden) for detecting IgE α -*Olea europaea* crude extract and double antibody radioimmunoassay (DARIA) for determining patients that had IgG and IgE to highly purified *Ole e* I, as previously described [18].

DNA extraction and HLA class II typing

Genomic DNA was extracted from peripheral blood following the salting out method [19], with minor modifications.

DNA typing was performed by RFLP, and the haplotype was assigned according to the methods previously described [20].

Statistical analysis

Statistical significance of antigen frequencies between patients and controls was measured by the Fisher test, and the probability was corrected (P_c) for the number of comparisons (three for DR antigens, and one for DQ antigens). Relative risk (RR) and aetiological fraction (EF) were calculated according to the method [21].

Generation of *Ole e* I-specific T-cell lines

Peripheral blood mononuclear cells (PBMCs) were obtained by gradient centrifugation on Lymphoprep (Nycomed Pharma, Oslo, Norway) from two *Olea europaea* allergic individuals (DB: DR7-DQ2, DR7-DQ2 and GM: DR3-DQ2, DR1-DQ5). The T-cell lines were established by cycles of stimulation with *Ole e* I ($5 \mu\text{g/mL}$) and IL-2 alone, 15 U/mL) as previously described [22] with a minor modification. Briefly, 10^5 PBMC were stimulated with antigen on microtitre plates. Seven days later, human rIL-2 was added at a concentration of 15 U/mL . After 7 days the T cells were restimulated with antigen and APCs. This cycle was repeated three times and the cells were used for assay after 7 days without antigen.

APC Two sources of APC have been used in these studies. T cells were restimulated with autologous irradiated PBLs (3000 rads), and the proliferation assays were done with autologous PBLs or a panel of irradiated EBV transformed cell lines (5000 rads),

Table 1. Classification of the Arabic subjects studied

	Group I (Non-allergic)	Group II (Non-related allergic)	Group III (<i>Olea europaea</i> allergic)
<i>n</i>	12	40	13
Age (years)	30 ± 16	14 ± 14	18 ± 17
Serum IgE (IU/mL)	34.1 ± 8	383.4 ± 465	847 ± 743
Skin test	0	1	2

*0 = Skin test negative. 1 = *Olea europaea* negative, but other common allergen positive. 2 = *Olea europaea* positive.

generously provided by Professor J. Lamb from Imperial College of Science Technology and Medicine, London, UK [23]. The EBV transformed cell lines were DBB (DR7 DR53 DQ9(3)), COX (DR17(3) DR52 Dw24 DQ2), SWEIG (DR11(5) Dw5 DR52 Dw25 DQ7(3)) and JETHROW (DR1 DQ5) (this last line was provided by Dr M. Kreisler, Hospital Pta de Hierro, Madrid, Spain).

Proliferation and inhibition of T-cell response to Ole e I

T-cell lines were incubated, 10^4 cells/well, in triplicate with or without antigen, in the presence of either irradiated (3000 rad) autologous APC (7×10^4 cells/well) or with a panel of previously typed EBV-transformed B cells (5000 rad). After 48 h in culture, cells were pulsed with [H^3] Thd, harvested 16–18 h later and counted. In the inhibition assays, monoclonal antibodies were added at the start of culture. For the inhibition studies, anti-human MHC class II monoclonal antibodies (MoAbs) were used: anti-DR (MAB1762), murine IgG3 antibody purified from ascites, and for DQ, murine MoAb IgG1 (MAB1764), also purified from ascitic fluid. As a non-related control, monomorphic anti-human class I was used (w6/32). These monoclonal antibodies were purchased from Chemicon International (Temecula, CA, USA). Anti-DR7 and anti-DQ1 were commercial monoclonal antibodies (Prilab, Izasa, Barcelona, Spain). Anti-DQ2 was a polyclonal antibody purchased from Prilab.

Results

Studies on the arabic population antibody results

A total of 65 individuals were studied which were allotted to one of three generic groups (Table 1).

Group I (non-allergic). All these had a normal IgE serum level and no reactivity to the skin test. This group was considered as the control population.

Group II (non-related allergic). The level of serum IgE was elevated (383.48 ± 465 IU/mL) and the patients showed skin reactivity to one or more of the commercial extracts, but not for *Olea europaea* crude extract.

Group III (*Olea europaea* allergic). Composed of 13 patients (20% from total population), that showed reactivity to at least *Olea europaea* crude extract by skin test. The sera of these 13 patients were analyzed by RAST (see Table 2). Results showed that 11 patients (84.6%) had IgE antibodies to α -*Olea europaea* crude extract (2 ± 0.63 RASTU/mL).

The specific IgG and IgE α -*Ole e I* were studied by DARIA. The results are shown in Table 2. Five individuals, Group IIIa, had neither specific IgG nor specific IgE. Eight patients had specific IgG α -*Ole e I*: 268 ± 114 Arbitrary Units (AU)/mL. Six of these patients (Group IIIb) had IgE α -*Ole e I* (75% compared to individuals with specific IgG), with a mean value of 3.62 ± 2.09 AU/mL.

Typing of DNA by RFLP

We performed the typing of HLA class II antigens by RFLP in 46 subjects. The distribution of the individuals was nine from group I (this was considered as the normal population), 26 from group II (non-related allergic patients) and 11 from group III, five patients of group IIIa (olive positive, *Ole e I* negative), and six patients from group IIIb (IgE α -*Ole e I* responders).

Only the antigens relevant for IgE antibody response to *Ole e I* were determined as demonstrated in our previous study: DR7, DR3, DR4 and DQ2. Statistical analyses were performed using the Fisher's test and the results indicated that the phenotypic frequency of DR7 ($pf = 83.3\%$) was increased in patients with specific IgE

Table 2. Allergic parameters and haplotype of Group III (*Olea europaea* allergic patients)

No	Rast (Olive crude extract)	t IgE (UI/mL)	α -Ole e I IgG	α -Ole e I IgE	Haplotype
P-1	2+	70	N	N	DR11 DQ7
P-2	2+	383	N	N	DR11 DQ7 DR7 DQ2
P-3	1+	78	N	N	DR7 DQ2 DR15 DQ6
P-4	1+	209	N	N	DR3 DQ2 DR13 DQ7
P-5	3+	1503	N	N	DR6 DQ7 DR15 DQ6
P-6	–	143	163	N	DR3 DQ2 ND
P-7	–	> 2000	160	N	ND
P-8	2+	1774	178	3.24	DR11 DQ7 DR7 DQ2
P-9	2+	298	294	4.78	DR13 DQ6 DR7 DQ2
P-10	3+	> 2000	290	7.28	DR3 DQ2 DR7 DQ2
P-11	2+	221	201	2.66	DR15 DQ6 DR3 DQ2
P-12	2+	963	470	2.17	DR7 DQ2 DR7 DQ2
P-13	2+	1272	391	1.62	DR7 DQ2 DR13 DQ5

P-1 to P-5 are patients from group IIIa. P-8 to P-13 are patients from group IIIb. Each result is expressed in the units that correspond as is indicated in Materials and methods. N is negative and ND is not determined.

(group IIIb) compared with control group (group I (pf = 11.1%), the *P* value was 0.01 ($P_c = 0.03$), RR = 40 and EF = 0.81).

There was no statistically significant difference in the phenotypic frequencies of DR3 and DQ2 antigens in these groups, but more DQ2 was observed in group IIIb (83.3% vs 55.5% in control group). For DR3, pf was 33% in patients vs 44.4% in the control group. DR4 was not present in the groups of *Olea* responder patients (groups IIIa and IIIb), and the phenotypic frequency in the normal group for this antigen was 44.4%, with this difference being only marginally significant ($P = 0.09$).

The distribution of these antigens in the other groups were no different from those found in the normal group: in the non-related allergic group (group II) the phenotypic frequencies were 23% for DR7, 61.5% for DQ2, 30.7% for DR3, 29.4% for DR4 and in Group IIIb (olive pollen allergic but without specific IgE) 20% for DR7, 40% for DQ2, 20% for DR3 and 0% for DR4.

These results are similar to those reported for Spanish patients, but in this case, only DR7 is statistically increased.

Cellular results

Genetic restriction in the α -Ole e I IgE response was examined by two methods: first, by inhibition of the proliferative response to this antigen with a panel of human α -MHC class II antibodies. As shown in Fig. 1, we obtained a significant inhibition (dose dependent, data not shown) of the proliferative response when we used α -DR and α -DQ generic monoclonal antibodies (Fig. 1 H and I, respectively). The greatest inhibition was observed with α -DR7 monoclonal antibody and α -DQ2 polyclonal antibody (Fig. 1 D and E). The non-related monoclonal antibodies, α -class I (G) and α -DQ1 (F) did not produce any significant decrease in the proliferative response. These results are consistent with our view that DR7 and DQ2 are both specifically implicated in the *Ole e I* response.

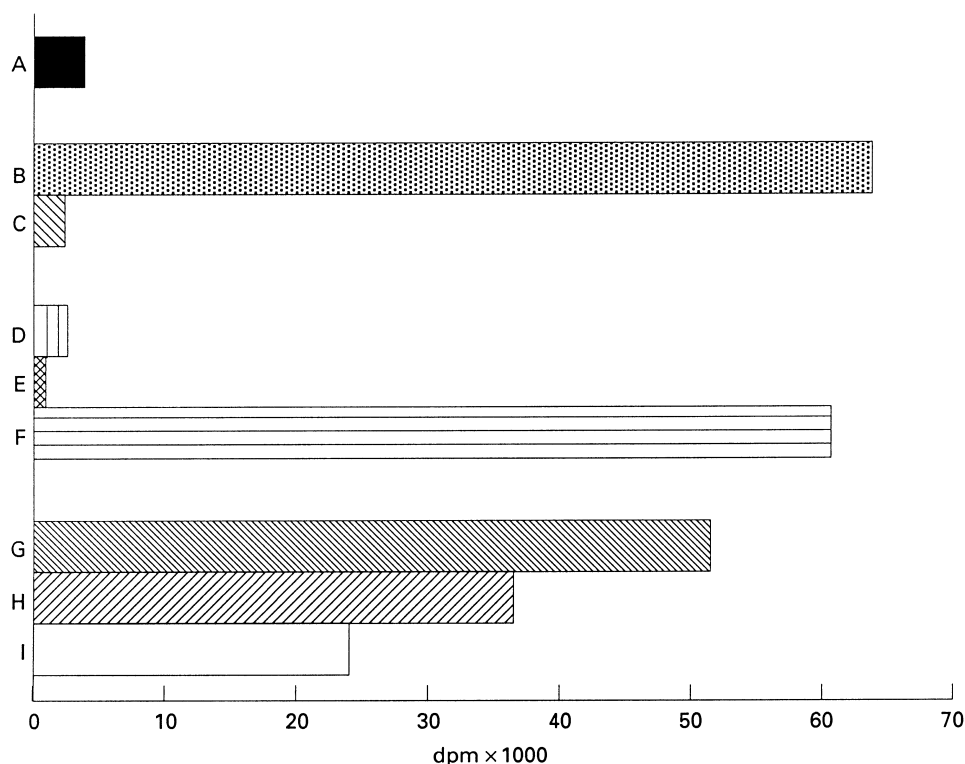


Fig. 1. Inhibition with HLA class II antibodies of the proliferative response of T-cell lines specific to *Ole e I*. A. T-cells line alone (10^4 well). B. + *Ole e I* ($5 \mu\text{g/mL}$). C. + *Sin a I* (non-related antigen, $5 \mu\text{g/mL}$). D. + *Ole e I* ($5 \mu\text{g/mL}$) + α -DR7 monoclonal antibody (1:10). E. + *Ole e I* ($5 \mu\text{g/mL}$) + α -DQ2 polyclonal antibody (1:10). F. + *Ole e I* ($5 \mu\text{g/mL}$) + α -DQ1 monoclonal antibody (1:10). G. + *Ole e I* ($5 \mu\text{g/mL}$) + α -Class I monoclonal antibody (1:200). H. + *Ole e I* ($5 \mu\text{g/mL}$) + α -DR monoclonal antibody ($5 \mu\text{g/mL}$). I. T-cells line + α -DQ monoclonal antibody ($5 \mu\text{g/mL}$). Results are expressed as mean disintegration per minute (dpm) $\pm$ SD of triplicate cultures (see Materials and methods).

The second approach was to test the ability of a panel of histocompatible or histoincompatible APC to present *Ole e I* to T-cell lines derived from *Ole e I* allergic patients. These assays are summarized in Table 3. The results show that the two T-cell lines used, DB and GM, are able to proliferate with *Ole e I*, when the APC is DR7 and/or DQ2, but when we used APCs with a histoincompatible haplotype, the response to *Ole e I* was negative. These results, support the view that DR7 and DQ2 are involved in the recognition of *Ole e I*.

Discussion

The association between HLA class II antigens and the specific IgE response to purified allergens has been reported in several studies [5–9]. Recently, we described a significant association between the IgE antibody response to a highly purified allergen from olive tree pollen (*Ole e I* and HLA class II antigens, DR7 and DQ2, in Spanish patients with seasonal allergic pollenosis [18].

In the present report, we studied this response in an Arabic population, in order to determine whether, in this group with a presumably different genetic background, these antigens are also important for *Ole e I* presentation to T cell as has been demonstrated in a Spanish population group. Also, we wanted to clarify the role of DR7 and DQ2 in this response. The results obtained in this report show that DR7 and DQ2 remain in linkage disequilibrium in this ethnic group. Nevertheless, DR7 has a lower phenotypic frequency in the control group compared with the Spanish population (pf: 11.1% Arabic vs 31.3% Spanish).

As with our preliminary results, the group of responder patients to *Ole e I* had a high phenotypic frequency for this antigen (pf = 83.3%). This increase was statistically significant when the phenotypic frequency of DR7 in this group was compared with this value in the control group ($p_c = 0.03$, RR = 40, EF = 0.81). This result is in agreement with our previous study, and confirms that the DR7 antigen is implicated in the IgE antibody response to the main antigen of olive tree pollen.

Table 3. *Ole e* I recognition by specific T cell lines (DB and GMZ) using a panel of different HLA Class II APCs

T-cell lines	Antigen presenting cells (APCs)			
	Autologous	COX (DR3–DQ2)	DB (DR7–DQ2)	DBB (DR7–DQ9)
DB (DR7–DQ2, DR7–DQ2)	5.9	3.1	5.9	3.02
GM (DR3–DQ2, DR1–DQ5)	2.82	2.4	1.9	NS*

Summary of proliferative response of T-cell lines (10^4 cells/well) to *Ole e* I in the presence of lymphoblastoid B cells transformed with Epstein Barr virus as antigen presenting cells (7×10^4 cells/well). Values shown are stimulation index of the statistically significant proliferations ($P < 0.05$) as determined by [^3H]TdR incorporation. The assays were performed in triplicate, and the standard deviation was $< 10\%$. Negative controls: APC with non-related haplotype, SWEIG (DR11(5) DQ7(3) for both lines and JETHROW (DR1 DQ5) for GM T cell line. In all cases there were no significant proliferation ($\text{SI} = 1$). *Non-significant ($\text{SI} = 1$).

On the other hand, as found in the Spanish population group, DQ2 seems to be a very common antigen (DQ2 phenotypic frequency for the control group was 55% vs 48% in the Spanish control group). This high value, and the fact that the number of people studied was low, could explain why DQ2 was not significantly increased in the patient group in this study. Nevertheless, the DQ2 phenotypic frequency (83.3%), is greater in patients with IgE antibodies α -*Ole e* I compared with the control group (55.5%). This result could signify that this antigen is not implicated in the response, and is only increased as a result of the disequilibrium linkage effect. For this reason we decided to perform the cellular experiments.

The serological inhibition assays show that, in our case, T-cell proliferation is restricted by HLA-DR and DQ. The fact that monomorphic α -DR and α -DQ monoclonal antibodies inhibit cellular activation strongly support these findings. In contrast, inhibition assays with other allergens such as *Amb a* V and *Chi t* I [24,25], show that DQ is not implicated in this response. Also, these data confirm that DR7 and DQ2 specifically are both implicated in this response.

On the other hand, studying the proliferative response of these T-cell lines using a panel of APCs, again demonstrated that only when the APCs are DR7 and/or DQ2 these T-cell lines are able to respond to *Ole e* I. As far as we know, the combined involvement of DR and DQ in the allergen response has only been described in the study of Der p 2 reactive T-cell repertoire in a mite sensitized patient [23], where the authors identified HLA-DR and DQ restricted T-cell epitopes, one of which is able to bind to both DR and DQ molecules.

Finally, another surprising observation was that the DR4 antigen was not present in the group of responders to *Olea europea*, in contrast with the other two groups, 44.4% in the control and 29.4% in the non-related allergic group. This result again suggests that DR4 is a possible protective factor, as previously found in a Spanish allergic population sample.

This is the first study performed with an Arabic population with the inherent difficulty of the lack of data concerning phenotypic frequencies of this population, for this reason we wanted to highlight the need for more studies related with this ethnic group.

This work emphasizes the importance of genetic factors in the allergic response. As described in several reports over the last few years [26–29], antigen specific and non-specific factors are involved in genetic restriction. Until now, none of these factors can be considered as the exclusive determinant of the restriction. Studies related with all these different aspects will thus shed light on these multifactorial atopic diseases.

This report confirms that DR7 and DQ2 are involved in the IgE antibody response to *Ole e* I, but it is necessary to perform more studies with T-cell lines and T-cell clones and peptides of this protein to determine which is the main region implicated in this response, and clarify this complex response.

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