

A monoclonal antibody specific for a carbohydrate epitope recognizes an IgE-binding determinant shared by taxonomically unrelated allergenic pollens

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Summary

Background Carbohydrate epitopes are capable of binding human IgE from allergic subjects and these epitopes play a role in the cross-reactivity between allergens from unrelated sources. A monoclonal antibody (5E6), specific for a carbohydrate epitope detectable on components of *Cupressus arizonica* pollen extract, has been produced and characterized.

Objective To study the relationship between the epitopes recognized by the monoclonal antibody and by IgE from allergic subjects. To investigate the presence of such carbohydrate IgE determinant in extracts from 21 pollen species belonging to 16 taxonomically related and unrelated families, by means of the monoclonal antibody.

Methods IgG-depleted fraction from protein G-purified human allergic serum was obtained. The monoclonal antibody and the IgE from the purified fraction were tested on two glycoproteins, polyamine oxidase and ascorbate oxidase, adsorbed on the ELISA plates. The relationship between the monoclonal- and the IgE-recognized epitopes was investigated by ELISA-competition experiments. Analysis of the distribution of this carbohydrate epitope was performed by direct binding of the monoclonal antibody onto the various extracts.

Results The monoclonal antibody and the IgE were able to bind carbohydrate epitopes on the two plant glycoproteins, ascorbate oxidase and polyamine oxidase. Polyamine oxidase shows only one N-glycosylation site whose carbohydrate moiety seems to be composed of a branched chain of seven ordered sugars, i.e. two N-acetyl-D-glucosamine-, three mannose-, one fucose- and one xylose-residues. This structure bears the epitope recognized by mAb 5E6. Human IgE from the IgG-depleted fraction were found capable of inhibiting the monoclonal antibody binding. The allergenic epitope identified was shared by a large number of extracts with different levels of reactivity (OD₄₉₀ ranging from 0.110 to 2.060).

Conclusion Our data support the finding that a monoclonal antibody specific for a carbohydrate epitope of *Cupressus arizonica* pollen extract detects an epitope which is also recognized by IgE from allergic subjects. This characterized reagent could be a useful tool for studying distribution of cross-reactive carbohydrate determinants in allergenic pollen extracts and their components.

Keywords: allergen, carbohydrate, glycoprotein, monoclonal antibody, human serum, IgE, IgG

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Introduction

It is now accepted that carbohydrates are capable of binding human IgE from allergic subjects and that these epitopes play a role in explaining cross-reactivity between allergens from unrelated sources [1–4].

Recently, we demonstrated that carbohydrate moieties present in the *Cupressus arizonica* pollen extract (CaE) are involved in the IgE binding to allergens [5], thus confirming the importance of investigating their role in the widespread cross-reactivities between extracts from various taxonomically related and unrelated sources.

In a recent paper [6] we used monoclonal antibodies (mAbs) to evaluate the presence and nature of cross-reacting epitopes shared by various components of CaE and we also characterized a monoclonal antibody (mAb 5E6) which is specific for a periodate-sensitive epitope represented on different components of CaE.

In the present study, we investigated whether glycidic epitope recognized by mAb 5E6 on CaE is carried by allergenic molecules and whether such epitope is also recognized by human IgE. With this aim, we evaluated mAb 5E6 and IgE reactivity against two glycoproteins, ascorbate oxidase (ASC) and polyamine oxidase (PAO), selected as reference molecules on the basis of their carbohydrate composition and structure. We sought to complete the structure determination of PAO by tryptic cleavage and analysis of the carbohydrate composition of the isolated glycopeptide. Then we investigated the presence of mAb 5E6-specific epitope in other pollen extracts from species belonging to taxonomically related and unrelated families.

Materials and methods

Monoclonal antibody (mAb) 5E6

MAb 5E6, specific for a periodate-sensitive epitope of CaE, was prepared and characterized as reported in Barletta *et al.* [6]. In the capturing experiments, mAb 5E6 was affinity purified on a protein A column as previously described [6].

Human sera

Human sera were obtained from patients allergic to Cupressaceae pollen, as proven by results of skin prick tests and specific *in vitro* IgE detection (0.2–2.8 optical densities range in ELISA for *C. arizonica*). Sera were stored in aliquots at –20 °C until used. The subjects were free from allergen-specific immunotherapy and their informed consent to participate in the study was obtained. One out of these sera, serum 1619 was chosen on the basis of its high reactivity towards various CaE components

evaluated by preliminary IgE immunoblotting experiments (data not shown), to be used in the allergen capturing assay and for the preparation of serum fractions.

Allergen-capturing assay

CaE components were captured by the Protein-A affinity-purified anti-CaE mAb 5E6 bound to ELISA plates (polystyrene microtitre 96-well, Greiner, Frickenhausen, Germany), essentially as already described in Barletta *et al.* [6]. Plates were coated with two-fold dilutions of the purified monoclonal, starting from 10 µg/mL to 0.15 µg/mL (100 µL/well), blocked by phosphate buffered saline (pH 7.2)-0.05% Tween (PBS-T) and then incubated with 20 µg/mL of CaE. After washing, the human serum 1619 diluted 1:10 in PBS-T was added. IgE binding to CaE was detected by secondary peroxidase-labelled goat anti-human IgE (KPL, Gaithersburg, MD). An irrelevant monoclonal antibody specific for the *Parietaria judaica* major allergen was used in the coating step as negative control.

Purified glycoproteins

ASC and PAO were selected as reference glycoproteins on the basis of their carbohydrate composition and structure [7,8]. ASC from *Cucurbita pepo* was purchased from Boehringer Mannheim (Mannheim, Germany), PAO from maize was isolated and purified to homogeneity according to Binda *et al.* [8]. This enzyme is glycosylated; its electron-density map shows that the glycosylation site is Asn 77. A branched chain of five ordered sugars, two N-acetyl-D-glucosamine residues, a fucose and two mannose residues, has been modelled [8]. To fully characterize the carbohydrate structure, a glycopeptide from PAO was isolated following carboxymethylation and trypsin digestion of the protein, according to Tavladoraki *et al.* [9]. The peptide mixture was directly loaded on a Vydac C8 RP-HPLC column (2.1 × 130 mm, 5 µm particle size) and eluted with a 10–60% linear gradient of acetonitrile in 0.05% trifluoroacetic acid at a constant flow rate of 1 mL/min, using a LabService HPLC apparatus.

Different split aliquots of the column effluent were simultaneously monitored by absorbance at 220 nm and by mass spectrum analysis, collected in peak continuum mode over the mass range of 50–500 amu, with an electrospray ion trap mass spectrometer (LCQ, Finnigan, San Jose, CA) operating in positive ion mode. Automated Edman degradation of isolated peptide was performed on an Applied Biosystems model 476 A gas-phase sequencer according to the Manufacturer's instructions (Applied Biosystems, Foster City, CA).

MAb 5E6 reactivity in ELISA with native and deglycosylated glycoproteins

MAb 5E6 binding on purified glycoproteins was evaluated on ELISA plates-adsorbed antigens. ELISA was performed as described by Barletta *et al.* [6]. On the basis of previous titration experiments performed on PAO and ASC antigens, plates (96-well polystyrene, Dynex, Chantilly, VA) were coated with 1 µg/mL of PAO and with 9.8 µg/mL of ASC. MAb 5E6 was diluted 1:100 in PBS-T. To further characterize the nature of the epitope recognized by mAb 5E6 on purified glycoproteins, these samples were subjected to sodium metaperiodate oxidation after adsorption on ELISA plates, according to the method described by Woodward *et al.* [10]. After washing in PBS-T, the samples were rinsed with 50 mM sodium acetate buffer (pH 4.5), then experimental wells ('deg') were exposed to 30 mM sodium metaperiodate (NaIO₄) in sodium acetate buffer for 1 h at r.t. in the dark, whereas control wells ('mock') were treated with the buffer only. Control and experimental wells were rinsed again with sodium acetate buffer and then exposed to 50 mM sodium borohydride in PBS for 30 min.

IgE reactivity in ELISA with native and deglycosylated glycoproteins

Human sera were tested in ELISA with ASC and PAO glycoproteins before and after chemical deglycosylation with sodium metaperiodate. ELISA plates (Greiner) were coated with 1 µg/mL of PAO and 9.8 µg/mL of ASC in 0.2 mL/well of 0.1 M carbonate buffer, pH 9.6. Amounts of coated antigens were again determined on the basis of preliminary titration experiments. Plates were blocked with PBS/0.1% Tween. Deglycosylation treatment with sodium metaperiodate was performed as reported above for the mAb 5E6, with the exception of the NaIO₄ concentration (10 mM instead of 30 mM). Sera were diluted 1:5 in PBS-T/1% gelatin wt/Vol.

IgE- and IgG-enriched serum fractions

Serum 1619 was separated into an IgE (IgG-depleted) fraction (effluent) and IgG (IgE-depleted) fraction (eluate) by protein G affinity column (1 × 12.5 cm, flow rate 0.8 mL/min) (Protein G Sepharose 4 Fast Flow, Sigma), according to the Manufacturer's instructions. The sample was applied in 20 mM sodium phosphate buffer pH 7.5 and the IgG were eluted from the column by 0.2 M glycine-HCl, pH 2.5. The eluate was neutralized by addition of 1.5 M Tris-HCl, pH 8.8. Serum 1619 and the IgE fraction (effluent) were evaluated for IgE and IgG reactivity by titration on purified glycoproteins adsorbed on the ELISA plates. Antigens titration was carried out with IgG-depleted

fraction (effluent). The amount of the coating antigens and the dilution of the IgE fraction giving optimal results (1:10) were chosen and used in the competition experiments. ELISA was performed as already described for whole sera.

ELISA competition

To evaluate whether mAb 5E6 recognizes IgE-binding epitopes, competition experiments were carried out. ELISA plates (Greiner) were coated with PAO and ASC and blocked as already described. The IgE fraction (IgG-depleted-effluent) was added diluted 1:10, 1:20 or 1:40 in PBS-T and incubated 3 h at r. t. After washing, mAb 5E6, diluted 1:200 in PBS-T on the basis of preliminary experiments, was added and incubated 3 h at r. t. The residual binding was then detected by incubation with peroxidase-labelled anti-mouse IgG (Bio-Rad, Hercules, CA) diluted 1:6000, overnight at r. t. The results were expressed as per cent inhibition of the mAb 5E6 binding to the two glycoproteins.

MAb 5E6 reactivity to pollen extracts

Recognition of the different pollen extracts by mAb 5E6 was evaluated on solid phase-adsorbed antigens. The following allergenic extracts were tested.

Pollen from *C. arizonica* (Cupressaceae) was purchased from Allergon (Angelholm, Sweden) and extracted as previously described [11]. Pollen from *Juniperus oxycedrus* (Cupressaceae) was collected and extracted as previously described [12]. *Olea europaea* (Oleaceae) and *Parietaria judaica* (Urticaceae) pollen extracts were prepared essentially according to Giuliani *et al.* [13]. Other extracts from several plant sources were purchased from Bayer Laboratories (Bayer, Spokane, Washington): eastern red cedar (*Juniperus virginiana*, Cupressaceae) and Utah juniper (*Juniperus osteosperma*, Cupressaceae), bald cypress (*Taxodium disticum*, Taxodiaceae), white oak (*Quercus alba*, Fagaceae), tree of heaven (*Ailanthus altissima*, Simarubaceae), rape (*Brassica rapa*, Cruciferae), salt cedar (*Tamarix gallica*, Tamaricaceae), pinyon pine (*Pinus edulis*, Pinaceae), date palm (*Phoenix dactylifera*, Palmae), bermuda grass (*Cynodon dactylon*, Poaceae), perennial ryegrass (*Lolium perenne*, Poaceae) and orchard grass (*Dactylis glomerata*, Poaceae), common mugwort (*Artemisia vulgaris*, Compositae), eastern sycamore (*Platanus occidentalis*, Platanaceae), bluegum (*Eucalyptus globulus*, Myrtaceae), black locust (*Robinia pseudoacacia*, Leguminosae), and hazel (*Corylus avellana*, Corylaceae). The protein concentration of the extracts was assayed according to the method of Bradford [14]; the sugar content was evaluated according to Dubois *et al.* [15].

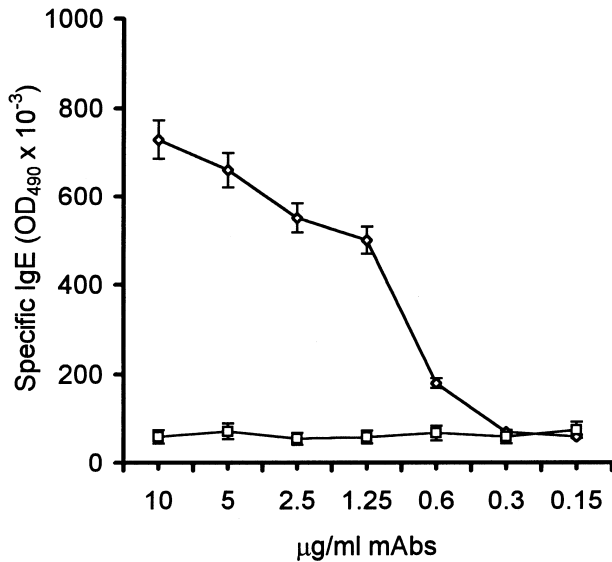


Fig. 1. IgE binding to CaE captured by decreasing amounts of mAbs. Values are the mean of three separate experiments. Bars express the standard error (SE). Symbols: mAb 5E6 = diamonds; mAb anti-*Parietaria judaica* = squares.

ELISA was performed as previously described for glycoproteins. ELISA plates (Dynex) were coated with 2.5 µg/mL of *Cupressus arizonica*, *Juniperus oxycedrus*, *Juniperus osteosperma*, *Juniperus virginiana*, *Quercus alba* extracts and with 20 µg/mL of the other allergenic extracts, on the basis of the sugar/protein ratio determined for each extract. MAb 5E6 was diluted 1:1000 in PBS-T. A negative cut-off value (0.100 OD₄₉₀) was established by replacing in six replicate wells the mAb 5E6 with the diluent (PBS-T) alone.

Results

When the protein A-purified mAb 5E6 was used as capture reagent in ELISA, the results obtained indicated that the mAb adsorbed onto the ELISA plate was able to bind CaE components and allowed them to be recognized by IgE from an allergic patient (serum 1619) (Fig. 1). The titration

of the adsorbed mAb 5E6 yielded a dose-dependent binding of human IgE. No IgE binding to CaE could be detected in the absence of any monoclonal antibody or in the presence of an irrelevant monoclonal antibody specific for a protein epitope of the *Parietaria judaica* major allergen (Fig. 1).

MAb 5E6 was able to recognize the carbohydrate moieties of purified plant glycoproteins, such as ASC and PAO. Due to the significant heterogeneity of the ASC commercial preparations, we used pure maize PAO as additional reference. A PAO tryptic fragment encompassing the Asn residue linked to the oligosaccharide chain was recognized in the HPLC peptide fractions by chemical sequencing. The complete carbohydrate structure was deduced on the basis of crystallographic data and molecular mass of glycan moiety (Fig. 2), according to the following information. Glycosylation of plant proteins is well known to occur in the rough endoplasmic reticulum with the transfer of modular oligosaccharides then modified by several glycosidases and glycosyltransferases. The results of this complex assembling pathway are specific oligosaccharide structures attached to specific glycosylation sites. Data on the molecular mass of the tryptic peptide calculated on the basis of its primary structure (1511.8 Da) and on that measured by mass spectrometric analysis (2683.4 Da) were compared and a mass difference of 1171.6 was obtained. Previous data [8] on glycan composition indicated the presence of five ordered sugars: two N-acetyl-D-glucosamine, two mannose and a fucose, whose total molecular weight is 875 Da; the addition of a fucose and a mannose residue, consistent with the modular glycidic addition, as shown in Fig. 2, and corresponding to a molecular weight of 309 Da, gives a sum of 1171 Da, that is exactly the mass difference obtained. Thus, we can suggest that the PAO glycan epitope has the longest structure established so far as a secretory signal in glycoproteins [16].

ASC and PAO were also chosen on the basis of previous data on the sugar CaE composition [5,11]. Thus, the reactivity of mAb 5E6 was firstly evaluated in ELISA against untreated glycoproteins. A significant binding to PAO and ASC was recorded, as shown in Table 1. A 76%

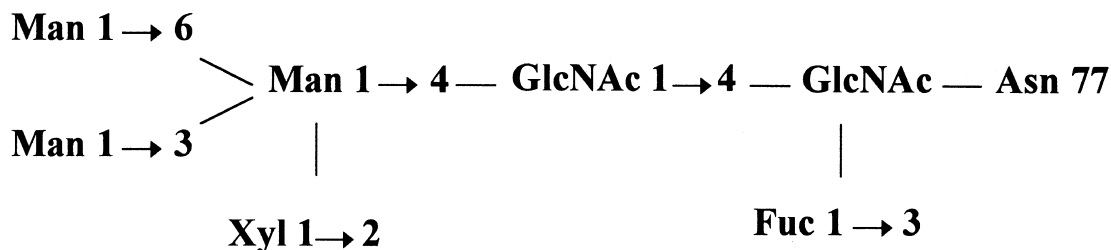


Fig. 2. Putative structure of the N-glycan from maize PAO.

Table 1. MoAb 5E6 and human IgE reactivity (serum 1619) on the glycoproteins PAO and ASC bound on the ELISA plates. The results are expressed in optical densities (OD₄₉₀) (mean from four experiments $\pm$ SE)

	PAO			ASC		
	Untreated	Mock*	Deg**	Untreated	Mock*	Deg**
MoAb 5E6	0.72 $\pm$ 0.005	0.69 $\pm$ 0.02	0.17 $\pm$ 0.005	0.505 $\pm$ 0.005	0.505 $\pm$ 0.015	0.125 $\pm$ 0.005
Human IgE	1.065 $\pm$ 0.035	1.045 $\pm$ 0.065	0.02 $\pm$ 0.01	1.55 $\pm$ 0.02	1.72 $\pm$ 0.055	0.03 $\pm$ 0.01

*Mock: control wells treated as 'deg' but without sodium metaperiodate.

**Deg: experimental wells exposed to sodium metaperiodate.

reduction of this reactivity was obtained when the two glycoproteins were deglycosylated (Table 1). To set ELISA competition experiments, we preliminarily evaluated the IgE reactivity to ASC and PAO glycoproteins. The results reported in Table 1 indicate that IgE from the allergic subject 1619 recognized carbohydrate epitopes on ASC and PAO. In fact an almost total (99%) reduction was recorded when periodate treatment was applied. This finding is not a feature restricted to serum 1619, since comparable results were obtained when a panel of 10 sera from allergic subjects was tested (Table 2).

Preliminary experiments indicated that IgG specific for carbohydrate moieties of ASC and PAO are present in the whole serum together with specific IgE (Table 3). In order to avoid any interference due to IgG competing with the monoclonal antibody, IgG-depleted IgE fraction from serum 1619 was obtained and evaluated for its capability to bind to the glycoproteins coated on the ELISA plates and

therefore to compete with the binding of the mAb 5E6. An increase of IgE reactivity and a complete depletion of IgG were obtained in the effluent. In order to define the experimental condition to be used for competition experiment, the optimal concentration of antigens ASC and PAO was identified by titration experiments with IgE fraction (data not shown). As already demonstrated for human serum 1619 and mAb 5E6, the results with IgE fraction indicated that the ASC coating concentration required to get similar optical densities was 10-fold greater than PAO (Table 3). In such conditions, the IgE fraction was able to inhibit the mAb 5E6 binding on PAO up to 51%, whereas the best inhibition value obtained on ASC was 20% (Fig. 3).

Finally, to identify whether the glycidic allergenic epitope recognized by mAb 5E6 is present on extracts belonging to species from taxonomically unrelated families, we evaluated its reactivity on a panel of 21 allergenic extracts (Table 4). The results suggested that

Table 2. IgE reactivity from a panel of 10 allergic subjects on the PAO and ASC glycoproteins bound on the ELISA plates. The results are expressed in optical densities (OD₄₉₀)

Human IgE	PAO			ASC		
	Untreated	Mock*	Deg**	Untreated	Mock*	Deg**
1	1.04	1.01	0.02	1.10	1.12	0.03
2	0.70	0.71	0.02	0.81	0.82	0.01
3	1.14	1.16	0.08	1.26	1.25	0.05
4	0.47	0.49	0.00	0.60	0.63	0.01
5	0.27	0.29	0.03	0.30	0.31	0.00
6	1.02	0.99	0.01	1.10	1.15	0.02
7	0.70	0.70	0.00	0.72	0.75	0.01
8	0.84	0.86	0.03	0.89	0.88	0.00
9	1.20	1.21	0.05	1.43	1.40	0.03
10	1.40	1.38	0.01	1.48	1.44	0.05

*Mock: control wells treated as 'deg' but without sodium metaperiodate.

**Deg: experimental wells exposed to sodium metaperiodate.

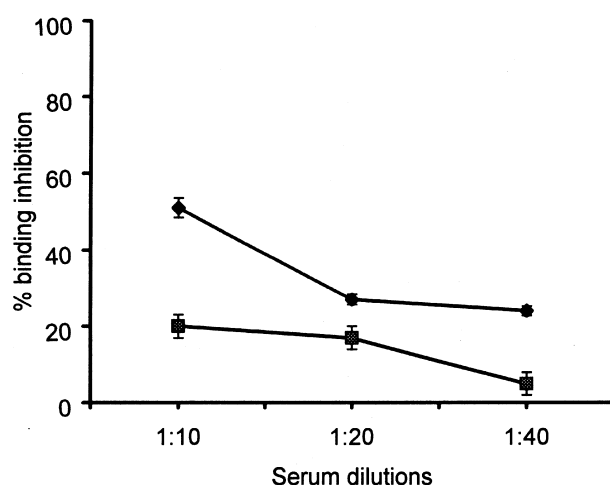
**Fig. 3.** MAb 5E6 binding inhibition by specific IgE to carbohydrate epitopes after specific IgG-depletion of the serum. PAO = diamonds; ASC = squares. Values are the mean of four separate experiments. Bars express the standard error (SE).

Table 3. IgE and IgG reactivity of the human serum 1619 and protein G purified fraction (effluent) to the glycoproteins bound on the ELISA plates. The results are expressed in optical densities (OD₄₉₀) (mean from three experiments $\pm$ SE)

Glycoprotein	Serum IgE	IgG	IgE-fraction IgE	IgG
PAO	0.7 $\pm$ 0.035	1.47 $\pm$ 0.03	1.07 $\pm$ 0.02	0.03 $\pm$ 0.005
ASC	0.89 $\pm$ 0.01	1.4 $\pm$ 0.025	1.02 $\pm$ 0.02	0.02 $\pm$ 0.005

such a carbohydrate epitope is present in a wide number of extracts (19 pollen extracts belonging to 15 related and unrelated families). High and intermediate mAb reactivities were recorded on the Cupressaceae, Taxodiaceae, Fagaceae, Simaroubaceae and Cruciferae extracts. Lower values were obtained with other species, belonging to Pinaceae, Palmae and Tamaricaceae. A decreasing reactivity was displayed by Oleaceae, Urticaceae, Platanaceae, Compositae, Poaceae, Leguminosae and Myrtaceae, thus suggesting that at least minute amounts of allergenic glycidic epitope are present on these extracts. *Dactylis glomerata* and *Corylus avellana* extracts displayed a negligible binding and were considered not reactive on the basis of the established negative cut-off.

Table 4. MoAb 5E6 reactivity on a panel of 21 pollen extracts bound on the ELISA plates. The results are expressed in optical densities (OD₄₉₀) (mean from three experiments $\pm$ SE)

Genus/species	Family	OD ₄₉₀
<i>Juniperus virginiana</i>	Cupressaceae	2.06 $\pm$ 0.03
<i>Juniperus osteosperma</i>	Cupressaceae	2.03 $\pm$ 0.025
<i>Juniperus oxycedrus</i>	Cupressaceae	1.96 $\pm$ 0.035
<i>Taxodium distichum</i>	Taxodiaceae	1.75 $\pm$ 0.02
<i>Cupressus arizonica</i>	Cupressaceae	1.52 $\pm$ 0.09
<i>Quercus alba</i>	Fagaceae	1.37 $\pm$ 0.13
<i>Ailanthus altissima</i>	Simaroubaceae	1.26 $\pm$ 0.12
<i>Brassica rapa</i>	Cruciferae	0.75 $\pm$ 0.045
<i>Pinus edulis</i>	Pinaceae	0.46 $\pm$ 0.04
<i>Phoenix dactylifera</i>	Palmae	0.41 $\pm$ 0.04
<i>Tamarix gallica</i>	Tamaricaceae	0.37 $\pm$ 0.015
<i>Cynodon dactylon</i>	Poaceae	0.33 $\pm$ 0.065
<i>Parietaria judaica</i>	Urticaceae	0.25 $\pm$ 0.025
<i>Olea europaea</i>	Oleaceae	0.24 $\pm$ 0.035
<i>Platanus occidentalis</i>	Platanaceae	0.21 $\pm$ 0.035
<i>Artemisia vulgaris</i>	Compositae	0.15 $\pm$ 0.002
<i>Robinia pseudoacacia</i>	Leguminosae	0.17 $\pm$ 0.015
<i>Eucalyptus globulus</i>	Myrtaceae	0.15 $\pm$ 0.03
<i>Lolium perenne</i>	Poaceae	0.11 $\pm$ 0.005
<i>Dactylis glomerata</i>	Poaceae	0.09 $\pm$ 0.005
<i>Corylus avellana</i>	Corylaceae	0.075 $\pm$ 0.005

Discussion

Several papers report that glycidic moieties are recognized by human IgE and support the idea that common carbohydrate epitopes are responsible for wide IgE cross-reactivities between not related allergenic extracts. IgE to cross-reactive carbohydrate determinants (CCDs) have been reported in 30–40% of the pollen allergic population [4,17]. The absence of biological activity seems to be characteristic of these IgE [4,18], thus influencing the *in vitro* diagnosis of the allergic disease [4].

In a recent paper we reported that carbohydrate epitopes on CaE reactive components were involved in the binding of IgE from Cupressaceae allergic subjects [5]. We have also demonstrated that the carbohydrate epitopes recognized by human IgE are present on several purified glycoproteins, such as bromelain and honeybee venom phospholipase A₂ as well as on several plant-derived extracts [5].

In the present paper we further studied the issue of carbohydrate epitopes using the mAb 5E6, induced by immunization with CaE and specific for a carbohydrate epitope [6].

Thus, we firstly verified if such epitope was present on CaE allergenic components. The results of ELISA-capturing experiments demonstrated that IgE from an allergic patient, chosen on the basis of his high reactivity to CaE, was able to bind the CaE components captured by the mAb with a dose/response fashion according to the mAb concentration.

Then, as already shown for human IgE [4,5], it was demonstrated that mAb 5E6 was able to recognize the carbohydrate moiety of purified plant glycoproteins such as ASC and PAO (Table 1). Since ASC commercial preparations exhibit significant heterogeneity, with the dominant glycoform lacking the fucose residue [19], we introduced, as an additional reference glycoprotein, maize PAO, which is available in a highly purified homogeneous form. PAO shows only one N-glycosylation site whose carbohydrate moiety has now been characterized as composed of a branched chain of seven ordered sugars, i.e. two N-acetyl-D-glucosamine-, three mannose-, one fucose- and one xylose-residues. This structure bears the epitope recognized by the mAb 5E6. Titration experiments revealed that, as

compared to PAO, 10-fold more ASC is required to obtain a similar binding with both IgE fraction and mAb 5E6.

Since the structure of the carbohydrate moiety is identical, the main differences between ASC and PAO are related to the presence in the former of isoforms displaying different glycidic residues added to the common biantennary backbone. These isoforms, characterized by various percentages of Fuc α 1–3-linked and Xyl β 1–2-linked, could differently influence the affinity/avidity of the binding of IgE fraction and mAb 5E6. This hypothesis could be supported by the results obtained in the competition experiments, where IgE are able to inhibit mAb binding onto PAO carbohydrate epitopes up to 51% while IgE cannot efficiently compete when ASC is used as antigen (20%), possibly indicating a weaker binding of IgE onto the latter molecule. Taken together, the competition data between human IgE and mAb 5E6, obtained in the experimental condition used, support the idea that a fraction of the IgE population present in the serum used, recognizes the same epitope detected by the monoclonal antibody.

The characterized mAb 5E6 was used to evaluate the presence of the carbohydrate cross-reactive allergenic epitope in various extracts. We selected a panel of extracts from pollen species belonging to related and unrelated families. As expected, the highest reactivity in direct ELISA was recorded with pollen extracts from Cupressaceae species [6 and data not shown]. Interestingly, mAb 5E6 reacted with most of the tested extracts at different extents, although with some of them very low values (*Lolium perenne*, *Eucalyptus globulus*), or no reactivity at all (*Corylus avellana*, *Dactylis glomerata*) were obtained.

These data support the widespread nature of the glycidic allergenic epitope recognized by the mAb 5E6 and allowed us to confirm the important role of carbohydrate structures in the allergen cross-reactivity not only within the Cupressaceae family, but also among extracts from several unrelated pollen families [4]. Many authors found CCDs in different allergenic materials from plant sources, including foods [2,20,21], and pollens, as well as in purified pollen allergens from grass [22,23], olive [7] and *Parthenium* [24].

Allergen-specific monoclonal antibodies have been successfully used in many instances, such as characterization [25–27], standardization [28–30] and cross-reactivity [6,31,32] studies concerning allergenic extracts. MAb 5E6 represents, to our knowledge, the first mAb recognizing a carbohydrate allergenic epitope. Thus, the use of such a mAb would be regarded as a useful tool in those studies addressing the characterization of CCDs displayed by allergenic molecules and their role in the allergic sensitization.

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