

## Specific IgE to cross-reactive carbohydrate determinants strongly affect the in vitro diagnosis of allergic diseases

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**Background:** Cross-reacting carbohydrate determinants (CCDs) are antigenic structures shared by allergenic components from taxonomically distant sources. The case history of a patient with a great discrepancy between skin test and specific IgE results led us to investigate the role of these determinants in his specific case and in an allergic population.

**Objective:** We sought to determine the role of CCDs in causing false-positive and clinically irrelevant results in in vitro tests. **Methods:** The involvement of CCDs was studied by specific IgE inhibition by using glycoproteins with a known carbohydrate structure. Direct and inhibition assays were performed by commercially available systems, in-house ELISA, and the immunoblotting technique. The binding to the periodate-oxidized carbohydrate structure of glycoproteins and allergenic extracts was also evaluated. A comparative study between skin test and specific IgE responses to the antigens studied was carried out in 428 consecutive allergic subjects.

**Results:** All the tests performed suggested that cross-reacting carbohydrate epitopes were the cause of false-positive specific IgE results in one of the commercial systems and the high reactivity in all the solid-phase in vitro tests. None of the cross-reacting carbohydrate allergens yielded a positive skin test response. Periodate treatment caused variable degrees of reduction of IgE binding to the different antigens studied, indicating that CCDs played a different role in each of them. About 41% of patients allergic to pollen had specific IgE for a glycoprotein, without a positive skin test response to the same molecule.

**Conclusions:** CCDs must be taken into account when evaluating the clinical relevance of positive results in in vitro specific IgE assays, at least in the diagnosis of patients with pollen allergy. Commercial systems should be carefully assessed for the ability to detect specific IgE for carbohydrate determinants to avoid false-positive or clinically irrelevant results. (*J Allergy Clin Immunol* 1999;103:1005-11.)

**Key words:** Allergens, allergy, carbohydrates, cross-reactions, diagnosis, epitopes, glycoproteins, IgE, skin tests

### Abbreviations used

CCD: Cross-reacting carbohydrate determinant  
sIgE: Specific IgE

Since the first assay to detect IgE antibodies was developed,<sup>1</sup> an increasing number of systems have been proposed for the routine detection of specific IgE (sIgE).<sup>2-7</sup> Specificity, sensitivity, predictive value, and clinical significance of detectable sIgE are matters of comparison among systems<sup>4,5,8,9</sup> and between systems and skin tests.<sup>5,8,10-12</sup>

Cross-reactive carbohydrate determinants (CCDs) from several allergenic sources induce sIgE,<sup>13-15</sup> and these epitopes are shared by taxonomically distant allergenic sources, such as pollens from unrelated botanical families, foods, and arthropods.<sup>14-20</sup>

Although sIgE for carbohydrate determinants are part of the sIgE detected in vitro, the influence of CCD-sIgE on commercial laboratory diagnostic tests has never been addressed.<sup>15</sup> Several carbohydrate moieties of allergenic molecules have been biochemically and immunochemically characterized,<sup>17,19-23</sup> but the role of sIgE to these epitopes in allergic diseases is still to be established, and poor biologic activity, if any, seems to characterize them.<sup>15,24</sup>

We report the case history of a patient whose allergic symptoms matched the skin test response positivity, but sIgE detection in his serum varied depending on the system used and showed a high number of positive results unrelated to the clinical findings. We studied the clinical significance of the results obtained by means of several in vitro systems and the role of CCD-sIgE in the diagnosis of his allergic disease. Furthermore, we demonstrated how CCDs could account for the discrepant behaviour of some commercial systems, ranging from false-positive diagnostic findings to clinically irrelevant results. A tentative evaluation of the prevalence of CCD-sIgE in a population complaining of allergic respiratory diseases caused by inhalant allergens was performed by detecting sIgE for an antigenic glycoprotein, bromelain, with a known glycan structure<sup>25,26</sup> but incapable of producing a positive skin prick test response in bromelain sIgE-positive patients.

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**TABLE I.** Results of skin prick test and specific IgE detection in the reported patient

|                                        | Oct 1994 | Nov 1994 |                    | May 1997 |
|----------------------------------------|----------|----------|--------------------|----------|
| Serum sample no.                       |          | 704      | 704                |          |
| Test                                   | SPT      | sIgE     | sIgE               | SPT      |
| System                                 |          | CARLA*   | CAP†               |          |
| Units                                  |          | IU/mL    | kU <sub>A</sub> /L |          |
| Birch                                  | Negative |          | 2.8                | Negative |
| Mountain cedar                         | Negative | 6.2      | 5.8                | +++      |
| Mugwort                                | Negative | 5.3      | 7.8                | Negative |
| Olive                                  | Negative | 3.8      | 8.5                | Negative |
| Pellitory                              | +++      | 55.0     | 98.0               | +++      |
| Pine                                   | Negative |          |                    | Negative |
| Plantain                               | Negative |          | 12.5               | Negative |
| Rye grass                              | +++      | 26.0     | 99.0               | +++      |
| Sycamore                               | Negative |          | 15.3               | Negative |
| Mites                                  | Negative | 5.4      | Negative           | Negative |
| Cockroach                              |          |          |                    | Negative |
| Cat                                    | Negative | 5.2      |                    | Negative |
| <i>Alternaria alternata</i>            | Negative | 5.3      | Negative           | Negative |
| Bee venom                              |          |          |                    |          |
| Latex                                  |          |          |                    | Negative |
| Ascorbate oxidase                      |          |          |                    | Negative |
| Bromelain                              |          |          | 25.8               | Negative |
| Bee venom phospholipase A <sub>2</sub> |          |          |                    | Negative |
| No allergen                            |          | 5.3      |                    |          |

SPT, Skin prick test.

\*CARLA: Anti-IgE mAb-coated wells for serum IgE capturing. Biotinylated allergens are added, and the specific IgE-allergen binding is revealed by adding a streptavidin-conjugated horseradish peroxidase (negative cut-off value = 0.5 IU/mL).

†CAP: Solid-phase assay with covalent bound allergens reacting with serum-specific IgE. Bound IgE are revealed by a radiolabeled <sup>125</sup>I-anti-IgE (negative cut-off value = 0.35 kU<sub>A</sub>/L).

‡ELISA: Solid-phase adsorbed allergen assay reacting with serum-specific IgE. Bound IgE are revealed by peroxidase-conjugated anti-IgE (negative cut-off value = 0.2 OD).

§AlaSTAT: Liquid-phase allergen assay reacting with serum sIgE with a final in situ immobilization. Immobilized sIgE bound to the allergen are revealed by a horseradish peroxidase-conjugated anti-IgE (negative cut-off value = 0.35 kU/L).

## CASE REPORT

A 36-year-old man, who has been living in Rome since 1987, came to the Allergy Unit for the first time in October 1994. He began to have mild allergic rhinitis and conjunctivitis in spring of 1993. From April to June 1994, symptoms worsened. The patient did not report any symptoms in other periods of the year or after exposure to house dust, animal epithelia, or other allergenic sources. Skin test responses were positive for grass and pellitory. These results matched with the patient's symptomatic period. Specific IgE, detected by means of an IgE capture system assay (CARLA; RADIM, Pomezia, Italy),<sup>6</sup> was positive for all the allergens tested (Table I). A control assay was performed by the CAP system (Pharmacia & Upjohn, Uppsala, Sweden).<sup>3</sup> All the pollens tested were confirmed positive, whereas *Alternaria alternata* and *Dermatophagoides pteronyssinus* sIgE were undetectable (Table I). In both systems higher values were recorded for allergens causing positive skin test responses, whereas all the other sIgE-positive allergens were confirmed negative by repeating skin tests with extract preparations of the same allergen from several companies.

The patient's symptoms were controlled by oral antihistamines, and he came again to the Allergy Unit in May 1997. He reported an earlier beginning of his allergic symptoms, which had started late in February. Skin tests were repeated, and a new positive result to juniper/cypress extract was recorded. The earlier beginning of the symptoms matched with the winter pollinating period of the cypress and closely related species (eg, junipers and cedars).<sup>8</sup> The detection of sIgE was repeated by using the CAP system for a wide panel of allergens on a new blood sample (Table I). High values were record-

ed again for allergens causing positive skin test responses, including juniper/cypress, but other sIgE-positive test responses were recorded for negative skin test response-eliciting pollen and some nonpollen allergens. Mild conjunctival and nasal symptoms, which ended in mid-June, were controlled with antihistamines and topical steroids.

Because a wide cross-reactivity caused by clinically irrelevant CCDs seemed to account for the diagnostic findings, the patient gave his informed consent to perform some in vivo and in vitro studies.

## METHODS

### Patients and sera

A single serum sample from the reported patient was available in 1994, whereas several samples used in this study were collected during 1997. Sera from 428 consecutive allergic patients seen at the Allergy Unit were collected during their first allergy consultation from 1997 to 1998. Patients were divided into 2 groups according to positive/negative skin test results to at least one pollen extract. Sera were stored at -20°C until used. Informed consent was obtained from all patients.

### Allergens and glycoproteins

Glycerinated allergenic extracts from several companies (Alk-Abello, Milan, Italy; Allergopharma-Bracco, Milan, Italy; and Bayer, Milan, Italy) were used in routine and control skin tests. Ascorbate oxidase, bromelain, and bee venom phospholipase A<sub>2</sub>,

| June 1997          |          | July 1997          | Dec 1997 | Dec 1997 |
|--------------------|----------|--------------------|----------|----------|
| 1046               | 1046     | 1103               |          | 1225     |
| sIgE               | sIgE     | sIgE               | SPT      | sIgE     |
| CAP†               | ELISA‡   | CAP†               |          | AlaSTAT§ |
| kU <sub>A</sub> /L | OD       | kU <sub>A</sub> /L |          | kU/L     |
| 8.3                |          | 4.3                | Negative | 2.5      |
| 54.6               | 0.55     | 25.0               | +++      | 23.3     |
| 8.9                | 1.65     | 8.9                | Negative | 2.3      |
| 9.2                | 0.57     | 11.7               | Negative | 4.9      |
| 66.8               | 2.80     | 68.4               | +++      | 71.0     |
| 2.4                |          | 2.4                | Negative |          |
| 4.1                |          | 12.4               | Negative | 6.0      |
| 51.9               | 2.45     | 76.6               | +++      | 76.6     |
| 11.9               | 0.78     | 11.9               | Negative | 4.5      |
| Negative           | Negative | Negative           | Negative | Negative |
| 2.9                | Negative | 1.3                | Negative |          |
| 0.8                |          | 2.1                | Negative | Negative |
| Negative           |          |                    | Negative |          |
| 1.7                |          | 1.1                |          |          |
| 5.1                | 0.20     | 3.7                | Negative |          |
|                    | 2.23     |                    | Negative |          |
| 14.9               | 2.96     | 19.7               | Negative |          |
|                    | 0.72     |                    | Negative |          |

the glycoprotein structures of which are known,<sup>13,25-27</sup> were purchased from Sigma-Aldrich (Milan, Italy). Concentrations of 5, 50, and 500 µg of protein/mL in 50% water/glycerin solutions of the 3 glycoproteins were prepared for the skin tests. Allergenic extracts from mountain cedar (*Juniperus ashei*), olive (*Olea europaea*), and pellitory (*Parietaria judaica*) were prepared as reported elsewhere.<sup>28-30</sup> Rye grass (*Lolium perenne*) extract was purchased from Bayer (Spokane, Wash). Mite (*D pteronyssinus*) extract was supplied by Lofarma (Milan, Italy). The noncommercial extracts and the glycoproteins were tested according to the European Pharmacopoeia Monograph on Allergen Products before they were used in human beings.<sup>31</sup> Skin prick tests were performed as reported elsewhere.<sup>8</sup>

### Specific IgE detection, immunoblotting, and inhibition

The detection of sIgE on coded sera was performed by an experienced operator according to manufacturers' instructions on the following systems. The first system was AlaSTAT (DPC, Los Angeles, Calif),<sup>2</sup> which is a system based on the reaction of the liquid-phase allergen with serum sIgE followed by a final in situ immobilization on a solid-phase; immobilized sIgE are revealed by a horseradish peroxidase-conjugated anti-IgE. The second system used was CAP (Pharmacia & Upjohn, Uppsala, Sweden),<sup>3</sup> which is a solid-phase assay in which covalent bound allergens react with serum sIgE; bound IgE are revealed by a radiolabeled <sup>125</sup>I-anti-IgE. Finally, CARLA (RADIM, Pomezia, Italy),<sup>6</sup> which is a system that uses wells coated with anti-IgE mAb for serum IgE capturing, was used;

biotinylated allergens are then added, and the sIgE-allergen complex is revealed by streptavidin-conjugated horseradish peroxidase. In-house ELISA (performed with the allergen adsorbed onto the solid-phase) and immunoblotting were performed as reported elsewhere.<sup>30</sup> Inhibition with optimal inhibitor concentrations, defined by preliminary experiments and reported in the Results section, were performed as described elsewhere.<sup>30</sup>

### Deglycosylation

For the deglycosylation treatment, we used a 10 mmol/L concentration of sodium *m*-periodate (NaIO<sub>4</sub>), as described elsewhere.<sup>32</sup> The treatment was performed on the antigens, showing higher ELISA OD values (ascorbate oxidase, bromelain, and rye grass, olive, and pellitory extracts). Antigens in control wells (mock control) were treated as above, excluding *m*-periodate.

## RESULTS

Table I shows the diagnostic results of the reported patient for each allergen tested by in vivo or in vitro methods. In the case of discrepant results compared with sIgE, skin tests were performed with at least 3 allergenic extracts from different manufacturers. Because the results were identical for positive and negative test responses, they are reported in a single column. High levels of glycoprotein sIgE were detected, but the skin tests performed with the highest concentration of the same

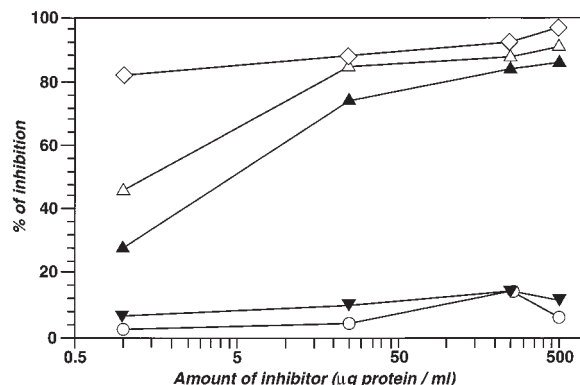


**FIG 1.** Specific IgE inhibition performed on the IgE capture system (CARLA) by using a buffer solution instead of an allergen and ascorbate oxidase (open diamonds), bromelain (open triangles), bee venom phospholipase A<sub>2</sub> (open circles), olive extract (filled triangles), and mite extract (filled inverted triangle) as inhibitors (serum 1103). Mean value of 2 noninhibited determinations = 7.62 IU/mL; negative cut-off value = 0.5 IU/mL.

molecules resulted in negative responses. Negative skin test results suggested that part of the detected sIgE recognized *in vivo* inactive epitopes on the glycoproteins and on some allergenic extracts.

Because streptavidin-conjugated horseradish peroxidase, used as tracer in the CARLA capture system, is an enzymatic glycoprotein,<sup>33</sup> an sIgE assay was performed by adding a buffer solution to the system instead of an allergen, so as to assess whether the peroxidase would bind directly to serum IgE. The assay yielded a positive result (Table I), which was confirmed by repeating the test with different samples of the patient serum (serum 1046 = 7.69 IU/mL; serum 1225 = 24.2 IU/mL). The test was further repeated with serum samples from 3 subjects with negative skin test responses to ascorbate oxidase, bromelain, and phospholipase A<sub>2</sub> and high levels of sIgE to the same molecules, as detected by ELISA (data not shown). These results suggested a specific binding between IgE, captured by the anti-IgE coated on the wells, and an antigenic determinant on the horseradish peroxidase. In fact, a nonspecific binding was ruled out by negative sIgE results obtained by testing high total IgE allergic sera on allergens eliciting negative skin test responses (data not shown). The total IgE of the reported patient ranged from 831 and 1392 U/mL. An inhibition assay of the sIgE binding to the horseradish peroxidase tracer, which acted as an allergen in the system, was performed by preincubating the serum with increasing concentrations of ascorbate oxidase, bromelain, phospholipase A<sub>2</sub>, and mite and olive extracts; results are shown in Fig 1. An almost complete inhibition was obtained with ascorbate oxidase, bromelain, and olive extract, whereas phospholipase A<sub>2</sub> and mite extract were unable to inhibit the binding to the tracer. These results suggested that common glycan antigenic determinants, which are recognized by the same sIgE and present in some glycoproteins with different protein backbones or in some components of allergenic extracts, may influence the results

**TABLE II.** Specific IgE inhibition performed by using 500 μg protein/mL glycoprotein solutions as inhibitors and patient-specific IgE CAP system-positive allergenic extracts as antigens (serum 1103)

|                | No inhibitor (kU <sub>A</sub> /L)* | ASC (%) inhibition | BRO (%) inhibition | PLA (%) inhibition |
|----------------|------------------------------------|--------------------|--------------------|--------------------|
| Mountain cedar | 25                                 | 12.4               | 14.4               | 0.8                |
| Pellitory      | 68.4                               | 0                  | 8.6                | 0                  |
| Rye grass      | 76.6                               | 8.2                | 25                 | 0                  |
| Birch          | 4.3                                | 84                 | 58                 | 9.3                |
| Mugwort        | 8.9                                | 80                 | 76                 | 12                 |
| Olive          | 11.7                               | 72                 | 71                 | 9.4                |
| Pine           | 2.4                                | 83                 | 54                 | 0                  |
| Plantain       | 12.4                               | 81                 | 78                 | 16.9               |
| Sycamore       | 11.9                               | 84                 | 81                 | 14                 |
| Latex          | 3.7                                | 65                 | 38                 | 13.5               |
| Bee venom      | 1.1                                | 54                 | 64                 | 27                 |
| Cockroach      | 1.3                                | 31                 | 0                  | 13                 |
| Cat            | 2.1                                | 29                 | 0                  | 38                 |

ASC, Ascorbate oxidase; BRO, bromelain; PLA, bee venom phospholipase A<sub>2</sub>.

\*Negative cut-off value = 0.35 kU<sub>A</sub>/L.

obtained by sIgE detection methods by using the anti-IgE capturing technique.

On the basis of these results, we investigated the specificity of the IgE binding obtained with the serum of the reported patient by CAP. Specific IgE inhibition was performed by using sIgE-positive allergens as antigens and glycoproteins, at a concentration of 500 μg of protein/mL, as inhibitors (Table II). Allergens eliciting positive skin test responses were only slightly inhibited by glycoproteins, whereas sIgE to allergens eliciting negative skin test responses were strongly inhibited by at least 2 glycoproteins. These results suggested that, in the case of allergens eliciting positive skin test responses, epitopes other than carbohydrate moieties were involved in the IgE binding. A second inhibition test was performed by using bromelain as antigen (the only glycoprotein available in CAP, code Rk202); inhibitors were glycoproteins and some allergenic extracts (Fig 2). Increasing concentrations of ascorbate oxidase and allergenic extracts caused inhibition curves comparable to the homologous bromelain inhibition, whereas phospholipase A<sub>2</sub> was confirmed to be a poor inhibitor.

The results obtained with in-house ELISA and Alastat (Table I) also confirmed that sIgE detected by means of solid-phase immunoassays can recognize antigenic determinants that do not elicit a skin test response at the usual testing concentrations.

Fig 3 shows ELISA results performed on deglycosylated glycoproteins and allergenic extracts. The sIgE binding to ascorbate oxidase, bromelain, and olive extract was almost completely abolished by deglycosylation, whereas 55% rye grass reactivity and 87% pellitory reactivity was retained. This suggested that the positive ELISA results of antigens eliciting negative skin test responses could be ascribed to sIgE for carbohydrate epi-

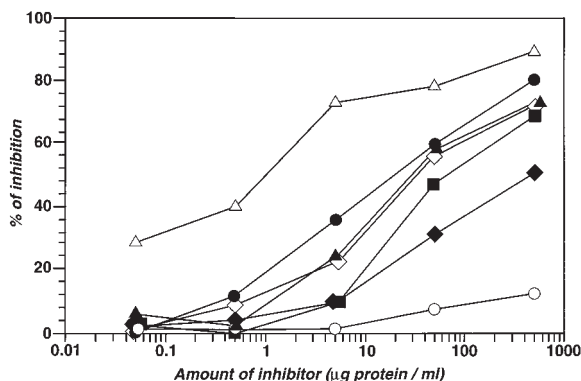


**FIG 2.** Specific IgE inhibition performed with the serum of the reported patient on CAP system by using bromelain (code Rk202) as antigen and ascorbate oxidase (open diamonds), bromelain (open triangles), bee venom phospholipase A<sub>2</sub> (open circles), rye grass extract (filled circles), mountain cedar extract (filled diamonds), olive extract (filled triangles), and pellitory (filled squares) as inhibitors (serum 1103). Mean value of 2 noninhibited determinations = 10.4 kU<sub>A</sub>/L; negative cut-off value = 0.35 kU<sub>A</sub>/L.

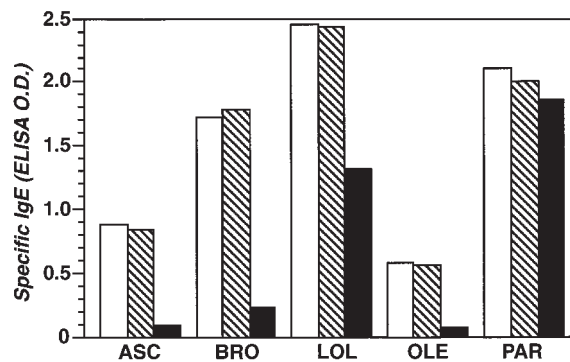


**FIG 3.** Specific IgE ELISA with deglycosylated ascorbate oxidase (ASC), bromelain (BRO), rye grass extract (LOL), olive extract (OLE), and pellitory (PAR) obtained by means of a 10 mmol/L *m*-periodate treatment. Antigen treatment in control wells (mock control) was performed following the same procedure, excluding *m*-periodate treatment (serum 1225). White bars, not treated; hatched bars, mock control; black bars, periodate treated.

topes. Rye grass partially lost its reactivity, suggesting the presence of IgE directed against a mixture of protein and carbohydrate epitopes in the rye grass extract. A slight decrease of pellitory IgE reactivity was obtained after deglycosylation, which suggests the presence of IgE mainly directed toward protein epitopes only.

Specific IgE blotting inhibition of ascorbate oxidase, bromelain, phospholipase A<sub>2</sub>, and rye grass, mountain cedar, olive, and pellitory extracts by means of ascorbate oxidase is reported in Fig 4. The heterologous inhibition of the sIgE binding to bromelain and phospholipase A<sub>2</sub> and the reduction of sIgE reactivity on several bands of the allergenic extracts confirmed the data obtained in the previous experiments. The best inhibition was obtained on blotted olive extract, whereas only a minimal inhibition was achieved on some bands of blotted pellitory. The sIgE inhibition of multiple bands by a single molecule indicates the presence of certain epitopes shared by different proteins, in the same manner as carbohydrate side chains.

To investigate the significance of these findings in an allergic population, skin test and sIgE results for bromelain were studied in 428 consecutive patients with allergic respiratory disease. The skin test was performed by using 500 µg of protein/mL bromelain solution, and sIgE for bromelain was detected by CAP (values ≥0.4 kU<sub>A</sub>/L were considered positive). One hundred thirty-six of 326 (41.7%) subjects with pollinosis had sIgE for bromelain ranging from 0.4 to 33.2 kU<sub>A</sub>/L (mean ± SD: 4.02 ± 5.71 kU<sub>A</sub>/L), but none of them had a positive skin test response to bromelain. A control group of 102 subjects allergic to mites or molds had negative results to bromelain in both skin tests and sIgE measurements.

## DISCUSSION

Discrepancies between skin test and sIgE results have been recorded and interpreted in several ways.<sup>10-12</sup> In our

patient the diagnostic discrepancies could be explained by the nature of the epitope recognized by sIgE. Negative skin test results to glycoproteins and allergenic extracts containing glycoproteins, as reported in this study, have been associated with the monovalency of the carbohydrate epitope present on the molecules.<sup>15</sup> In fact, allergenic molecules bearing a single epitope recognized by sIgE are not able to cross-link 2 IgE antibodies on the mast cell surface and are thus incapable of triggering the degranulation of the effector cell.<sup>15</sup>

The presence of CCD-sIgE can also yield equivocal results in some sIgE detection systems. The CCD-sIgE present in our patient's serum determined 2 different patterns of results, depending on the detection method. The results obtained with the sIgE capture system, relative to all the allergens eliciting negative skin test responses, were actually false-positive results, possibly caused by the presence of cross-reactive sIgE that recognized the carbohydrate moiety of the horseradish peroxidase used as tracer in the system. However, streptavidin-horseradish peroxidase conjugation is obtained by means of a mild NaIO<sub>4</sub> oxidation of the peroxidase,<sup>6</sup> which, according to our explanation, should be unable to destroy all the possible carbohydrate epitopes on the horseradish peroxidase molecule.<sup>33</sup> Considering the wide distribution of CCDs among plants,<sup>34</sup> the use of horseradish peroxidase or any other plant-derived enzymatic glycoprotein tracer should be carefully evaluated for their binding to CCD-sIgE.

Systems using solid phase-coupled allergens can detect sIgE for plant- and nonplant-derived allergenic extracts already known to bear carbohydrate moieties on their components.<sup>13,15,17,19,21,23,32</sup> Because some of these *in vitro* positive results (eg, bee venom and latex) may negatively affect the patient's quality of life, their clinical significance should always be assessed. Being a provocation test easily applicable to patients, the skin test seems to be the best tool to discriminate between clini-

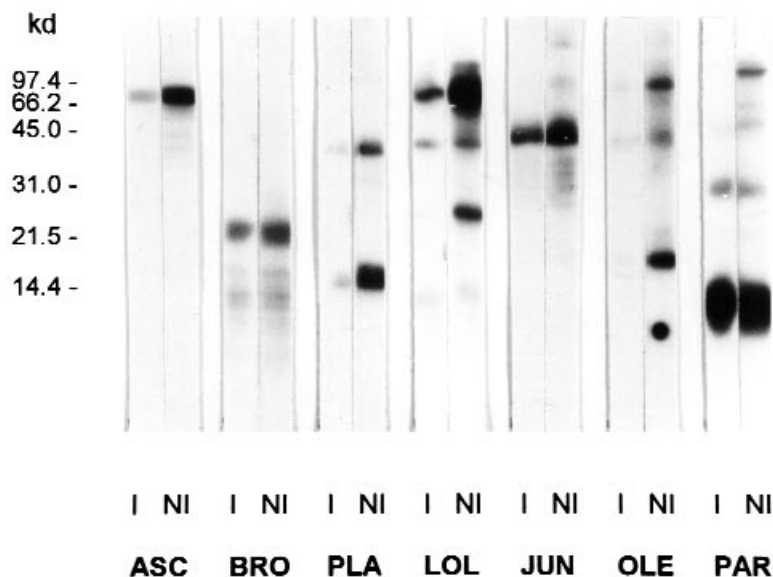


**FIG 4.** Specific IgE blotting inhibition with 500  $\mu$ g of protein/mL ascorbate oxidase using ascorbate oxidase (ASC), bromelain (BRO), bee venom phospholipase A<sub>2</sub> (PLA), rye grass (LOL), mountain cedar (JUN), olive (OLE), and pellitory (PAR) as antigens (serum 1225). Lanes I, No inhibitor; lanes NI, inhibited.

cally relevant and irrelevant allergens. In fact, bromelain has been described as an allergen able to sensitize and induce positive skin test responses, sIgE results, and symptoms in exposed workers of the pharmaceutical industry.<sup>35</sup> Although the level of sIgE to bromelain in our patient was as high as that reported by Gailhofer et al,<sup>35</sup> the authors elicited a positive skin test response with a bromelain concentration (0.1  $\mu$ g of bromelain/mL) 5000-fold lower than the one we used, which did not elicit a positive skin reaction in our patient.

Ascorbate oxidase, bromelain, horseradish peroxidase, and phospholipase A<sub>2</sub> have been described as cross-reacting glycoproteins bearing the carbohydrate moieties of allergenic molecules from rye grass<sup>20</sup> and olive.<sup>17</sup> The carbohydrate structures present in these glycoproteins are frequently present in plant- and arthropod-derived material,<sup>36</sup> and they can be recognized by mAbs raised against glycoproteins.<sup>34</sup> The data obtained in our study by inhibiting the sIgE binding to allergenic extracts eliciting negative skin test responses with ascorbate oxidase and bromelain contributes to the identification of potential CCD-bearing allergenic sources. The partial IgE-binding inhibition obtained with the phospholipase A<sub>2</sub> from bee venom on cat extract could suggest the possible recognition of cat glycans<sup>37</sup> by the CCD-sIgE. These preliminary data deserve further study.

The prevalence of CCD-sIgE seems to involve about 10% to 30% of patients allergic to pollen,<sup>15,24</sup> and the frequent presence of sIgE for some allergenic extracts (eg, bee venom)<sup>38</sup> in the atopic population could be explained by this high prevalence of glycoprotein-induced CCD-sIgE. Until now, there has been no commercially available test to directly evaluate the presence

of CCD-sIgE, although protease-digested glycoproteins could be used.<sup>15,24</sup> The combined use of negative skin test and positive sIgE results for characterized glycoproteins seems to be a good method to evaluate the presence of inactive CCD-sIgE. When these criteria were applied to our unselected consecutive patients, we recorded a 41.7% prevalence of CCD-sIgE among those subjects sensitized to at least one pollen species. The prevalence of CCD-sIgE was higher (62.4%) when patients sensitized to several pollen species were considered, whereas none of those sensitized only to mites or molds met these criteria.

The comparative use of purified or glycosylated recombinant allergens and their recombinant nonglycosylated counterparts<sup>15,39,40</sup> might increase the specificity and the sensitivity of both in vivo and in vitro tests. It would also help the allergist to discriminate between clinically relevant or irrelevant allergenic sources and to better address diagnostic and therapeutic information given to allergic patients.

In conclusion, discrepancies between negative skin test responses and positive sIgE detection can be associated with the presence of CCD-sIgE, which seem to be incapable of triggering an allergic reaction in vivo. Detection of sIgE in patients affected by pollinosis should always be compared with the skin test results. If sIgE is detected for plant allergens causing a negative skin test response, the presence of CCD-sIgE is to be suspected, and the clinical significance of the allergen can be ruled out. An overall reappraisal of commercial sIgE detection systems is undoubtedly needed.

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