

# IgE to Cross-Reactive Carbohydrate Determinants: Analysis of the Distribution and Appraisal of the *in vivo* and *in vitro* Reactivity

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## Key Words

Cross-reacting carbohydrate determinants · Diagnosis · Epidemiology · Glycoproteins · IgE · Skin tests

## Abstract

**Background:** IgE to cross-reacting carbohydrate determinants has already been described by several authors, but their function and distribution are still a matter of debate. In previous studies we showed how the presence of IgE to bromelain could be a useful and simple marker of the presence of IgE to carbohydrate epitopes. **Methods:** A survey of 1,831 subjects with a suspected allergic respiratory disease has been carried out by detecting IgE to bromelain. Data were analysed on the basis of demographic and allergological parameters. To find out whether a glycoprotein is capable of triggering an allergic reaction, 1,076 subjects were also skin tested with several purified molecules bearing carbohydrate side chains differing in number, composition and complexity. **Results:** An overall prevalence of 23% of positive IgE to cross-reacting carbohydrate determinants was recorded. Prevalence varied when subsets of non-allergic (5%), non-pollen-allergic (10%), and pollen-allergic (31%) subjects were considered. Prevalence further increased in subsets with multiple pollen sensitization (71%), and with a previous pollen immunotherapy course (46%),

whereas minor differences were found in gender and age distribution. Almost all the allergenic extracts recorded negative in the skin test gave a positive IgE test *in vitro*. A higher correlation was found mainly with plant-derived allergenic extracts, whereas a lower one was recorded with mites and fungi. Horseradish peroxidase was the only glycoprotein capable of exerting a positive skin test in 21% of the subjects with IgE to cross-reacting carbohydrate determinants, 80% of them having IgE to the HRP molecule. **Conclusions:** IgE to cross-reacting carbohydrate determinants are common among the allergic population and the binding to skin test negative allergenic extracts further confirms their poor biological activity. Further studies on horseradish peroxidase should be carried out to define the role of the glycan side chains in its allergenic activity.

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## Introduction

Since the earlier description of an IgE binding to cross-reactive carbohydrate determinants (CCD) in allergenic extracts [1], several studies have been performed studying the presence [2, 3] and the biological function [4–7] of the IgE to the CCD (CCD-IgE). Glycosylation has been described for several allergenic and non-allergenic mole-

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cules [8–11]. The glycan structures have been investigated [12, 13], and a heterogeneous IgE reactivity has been reported [14]. As a non-specific binding of the CCD-IgE can be excluded [15], a poor or absent allergological activity seems to characterize such determinants [5, 6]. In vitro demonstration of the glycan allergenic activity has been reported only for a native glycoprotein derivative (Ole e 1) [7] and for a synthetic neo-glycoproteins [16], whereas no in vivo activity of any purified allergenic glycoprotein has ever been reported [6]. From an allergological perspective, the most important feature of CCD is the wide distribution among plant-derived proteins [17], though similar structures can also be present in non-plant glycoproteins [1, 15]. Monoclonal and polyclonal antibodies to CCD have been used to define reactive carbohydrate epitope distribution [10, 17–21], whereas limited data are available on the prevalence and distribution of IgE reactive with CCD [6, 15, 22, 23].

The aim of the present study was to evaluate the distribution of CCD-IgE in a large cohort of subjects with allergic or allergy-like respiratory diseases, to analyse the degree of binding of CCD-IgE to several allergenic extracts, and to evaluate the impact of CCD-IgE on IgE detection of skin prick test (SPT)-negative extracts. Furthermore, the skin test reactivity to several purified high- and low-molecular-weight glycoproteins has been studied in order to find out whether any of them is capable of triggering an IgE-mediated reactivity in subjects with CCD-IgE.

## Materials and Methods

### Allergenic Extracts and Purified Molecules

A complete panel of allergenic extracts from pollen and non-pollen sources, already used in a previous study [22], was tested. Allergenic extracts from other arthropods (mosquito *Aedes communis*, honeybee venom), mammal dander and epithelia (cow, rabbit), latex, shrimps, and peanuts were added to the previous panel. Allergenic glycerinated extracts for SPT were obtained from Allergopharma (Reinbek, Germany), Bayer (Spokane, Wash., USA), and Stallergènes (Antony, France). Purified bee venom was used at 100 µg protein/ml in a 0.03% albumin:NaCl 0.9% water solution (Stallergènes). Arabic gum, guar gum, tragacanth gum, ascorbic acid oxidase, bromelain (BRO), horseradish peroxidase (HRP), and bee venom phospholipase A<sub>2</sub> were purchased from Sigma-Aldrich (Milan, Italy), and tested as previously reported [6]. Purified glycoproteins were used at 500 µg protein/ml 50% water/glycerine [6], whereas gums were tested at the highest concentration allowed by their solubility.

The following extracts were used for IgE detection: pollens (birch, timothy grass, hazel, juniper, mugwort, olive, pellitory, pine, plantain, ragweed, sycamore), arthropods (*Dermatophagoides pteronyssinus*, *Blattella germanica*, *Aedes communis*, bee venom), mammal dander and epithelia (cat, dog, horse), fungi (*Alternaria alternata*,

*Aspergillus fumigatus*, *Candida albicans*), other (latex, peanuts, shrimps, arabic gum, guar gum, tragacanth gum, HRP).

### Patients, Skin Testing and Sera

Unselected consecutive subjects (n = 4,535) presenting respiratory symptoms that suggested an allergic disease, and referred to the Allergy Unit, were enrolled. Demographical and clinical data were recorded for each patient. Patients with a clinical history of anaphylaxis were excluded from the study. Those patients who had previously received a specific immunotherapy (SIT) course were not excluded from the study. A SIT course was considered when treatment lasted for a minimum period of a year. All the treated patients received alum-adsorbed extract. For statistical purposes, only pollen-treated patients were evaluated.

Patients underwent skin prick testing, with a standardized procedure and recording [6, 22], using all the allergenic extracts listed above. Following the SPT, sera were obtained from 1,831 patients who consented to blood sampling for an in vitro diagnostic procedure. Sera were stored at –20°C until required.

Informed consent for skin testing and blood sampling was obtained by patients or caregivers during the allergy consultation.

### IgE Detection

Total IgE was determined in all the sera (Radim, Pomezia, Italy). Allergen-specific IgE were detected by the CAP system following the manufacturer's instructions (Pharmacia, Uppsala, Sweden). Values  $\geq 0.4$  kU<sub>A</sub>/l were considered positive. As there is not a single test to detect CCD-IgE [14, 16, 23, 24], discrepancy of the results between a positive in vitro test to BRO – a glycoprotein bearing carbohydrate moieties recognized by IgE – and a negative SPT to the same glycoprotein was assumed to be indicative of the presence of CCD-IgE [6, 22]. Previous studies showed how BRO rarely acts, if ever, as an allergenic molecule causing allergic diseases [22]. IgE detection was performed on the largest random samples of sera recorded negative in the SPT to the same allergenic extract. Extracts and tested sera are listed in table 1 and figures 4–6.

### Data and Statistics

Statistical evaluations were carried out on subsets of patients matching for demographical and allergological criteria. Data were stored by means of customized databases and retrieved by means of study-tailored query programs. Statistical analyses were carried out using the Prism package (Graphpad Software, San Diego, Calif., USA). The Fisher's exact test, the Mann-Whitney test, and the Spearman's correlation test were applied where appropriate. Statistical significance was accepted at  $p < 0.05$ .

## Results

### Prevalence of CCD-IgE

SPT with BRO was performed in 4,535 subjects. A positive reaction was recorded in 4 subjects (0.08%), all having specific IgE to BRO (range 2–20.7 kU<sub>A</sub>/l), and reporting an oral allergy syndrome to pineapple.

Sera from 1,831 subjects were available. The overall prevalence of CCD-IgE, defined by the detection of IgE to

Table 1. Analysis of the IgE reactivity to allergenic extracts of CCD-IgE-negative and -positive sera; subjects were selected on the basis of a negative SPT to the same extract (prevalence and correlation studies)

| Allergenic extracts | Prevalence   |       |              |       |                      | Correlation |                             |                      |
|---------------------|--------------|-------|--------------|-------|----------------------|-------------|-----------------------------|----------------------|
|                     | CCD-negative |       | CCD-positive |       |                      |             |                             |                      |
|                     | n            | % pos | n            | % pos | p value <sup>A</sup> | n           | r <sub>s</sub> <sup>B</sup> | p value <sup>B</sup> |
| Birch               | 216          | 12    | 187          | 63    | <0.0001              | 403         | 0.59                        | <0.0001              |
| Grass               | 571          | 5     | 101          | 53    | <0.0001              | 672         | 0.56                        | <0.0001              |
| Hazel               | 234          | 3     | 118          | 43    | <0.0001              | 352         | 0.63                        | <0.0001              |
| Juniper             | 381          | 8     | 158          | 51    | <0.0001              | 539         | 0.53                        | <0.0001              |
| Mugwort             | 205          | 17    | 190          | 72    | <0.0001              | 395         | 0.63                        | <0.0001              |
| Olive               | 460          | 16    | 206          | 74    | <0.0001              | 666         | 0.65                        | <0.0001              |
| Pellitory           | 433          | 20    | 260          | 77    | <0.0001              | 693         | 0.62                        | <0.0001              |
| Pine                | 164          | 10    | 222          | 49    | <0.0001              | 386         | 0.59                        | <0.0001              |
| Plantain            | 68           | 7     | 56           | 73    | <0.0001              | 124         | 0.77                        | <0.0001              |
| Ragweed             | 167          | 27    | 205          | 77    | <0.0001              | 372         | 0.57                        | <0.0001              |
| Sycamore            | 119          | 13    | 67           | 78    | <0.0001              | 186         | 0.74                        | <0.0001              |
| Mites               | 638          | 3     | 182          | 30    | <0.0001              | 820         | 0.42                        | <0.0001              |
| Cockroach           | 440          | 8     | 256          | 51    | <0.0001              | 696         | 0.57                        | <0.0001              |
| Mosquito            | 128          | 15    | 105          | 41    | <0.0001              | 233         | 0.34                        | <0.0001              |
| Bee venom           | 188          | 10    | 258          | 45    | <0.0001              | 446         | 0.54                        | <0.0001              |
| Cat                 | 396          | 3     | 205          | 37    | <0.0001              | 601         | 0.44                        | <0.0001              |
| Dog                 | 137          | 5     | 108          | 37    | <0.0001              | 245         | 0.47                        | <0.0001              |
| Horse               | 67           | 15    | 93           | 49    | <0.0001              | 160         | 0.44                        | <0.0001              |
| Alternaria          | 497          | 5     | 280          | 34    | <0.0001              | 777         | 0.45                        | <0.0001              |
| Aspergillus         | 254          | 5     | 171          | 32    | <0.0001              | 425         | 0.38                        | <0.0001              |
| Candida             | 432          | 6     | 217          | 41    | <0.0001              | 649         | 0.48                        | <0.0001              |
| Latex               | 210          | 19    | 281          | 66    | <0.0001              | 491         | 0.57                        | <0.0001              |
| Peanuts             | 268          | 15    | 161          | 68    | <0.0001              | 429         | 0.65                        | <0.0001              |
| Shrimps             | 198          | 19    | 154          | 45    | <0.0001              | 352         | 0.35                        | <0.0001              |

CCD-IgE were detected on the basis of positive IgE to bromelain and a negative skin prick test to the same molecule.

Statistics: <sup>A</sup>Fisher's exact test; <sup>B</sup>Spearman's correlation test.

BRO in patients with a negative SPT to the same molecule, was 23% (fig. 1A). Grouping patients on the basis of their SPT reactivity revealed remarkable differences in the prevalence of CCD-IgE. The majority of the CCD-IgE-positive sera were found among patients with at least one positive SPT to a pollen species (fig. 1A). An increasing prevalence of CCD-IgE among the pollen-allergic subsets was found. The trend was related to the number of positive SPT to pollens. The highest prevalence value was among patients with sensitization to more than five different pollen species (71%). Though rare, 18 patients having CCD-IgE were found among non-allergic subjects. None of these patients reported any respiratory symptom clearly related with any allergenic extract tested positive in the in vitro assay. Figure 1B shows dot-plot values considering the SPT-defined subsets. Mean values did not

differ statistically and a wide dispersion of IgE values was recorded in all the subsets.

CCD-IgE were slightly but significantly more prevalent among male patients (fig. 2A). The mean age and distribution of CCD-IgE-positive patients differ from the negative group, the former being younger than the latter. Nevertheless, CCD-IgE-positive subjects can be found in a wide age range (fig. 2B). CCD-IgE were detected in sera from patients as young as 2 years of age. Further analysis of the age distribution showed no difference between positive and negative CCD-IgE subjects within the paediatric age group (fig. 2C).

Median values and distribution of total IgE were statistically different between positive and negative CCD-IgE subsets, with higher values among subjects with CCD-IgE (fig. 3A). Nevertheless, the wide distribution of total IgE

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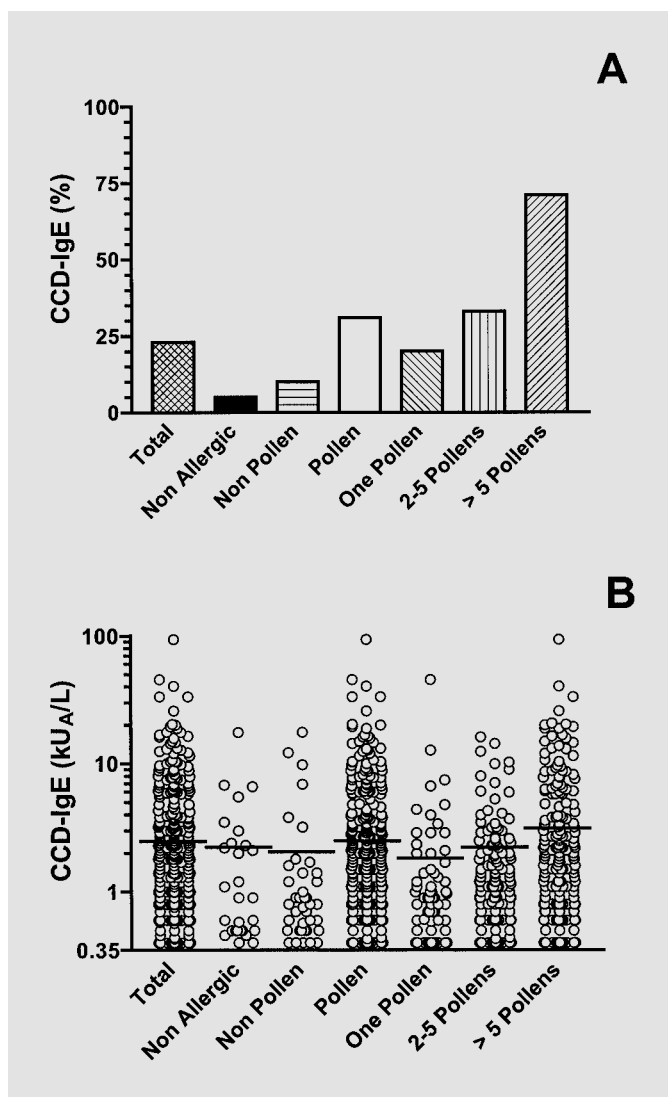
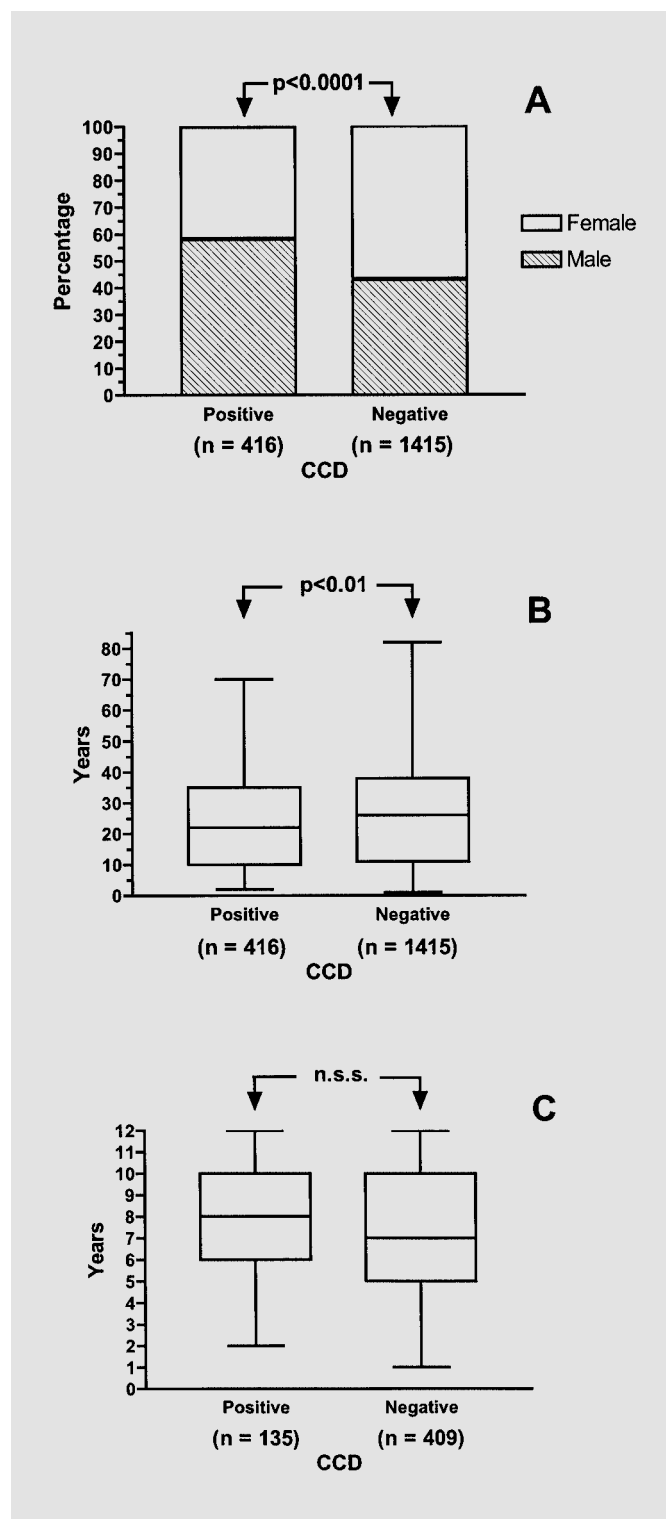


Fig. 1. Prevalence (A) and values (B) of CCD-IgE in the entire population ( $n = 1,831$ ) and in the SPT-defined subsets (non-allergic,  $n = 367$ ; non-pollen,  $n = 274$ ; pollen,  $n = 1,190$ ; 1 pollen,  $n = 354$ ; 2–5 pollens,  $n = 533$ ; >5 pollens,  $n = 303$ ). CCD-IgE were detected on the basis of positive IgE to bromelain and a negative skin prick test to the same molecule.

Fig. 2. A Gender distribution in the CCD-IgE-positive and -negative subsets ( $n = 1,831$ ). Statistics: Fisher's exact test. B Age distribution in the CCD-IgE-positive and -negative subsets; entire cohort ( $n = 1,831$ ). Statistics: Mann-Whitney test. C Age distribution in the CCD-IgE-positive and -negative subsets; paediatric cohort ( $n = 544$ ). Statistics: Mann-Whitney test.



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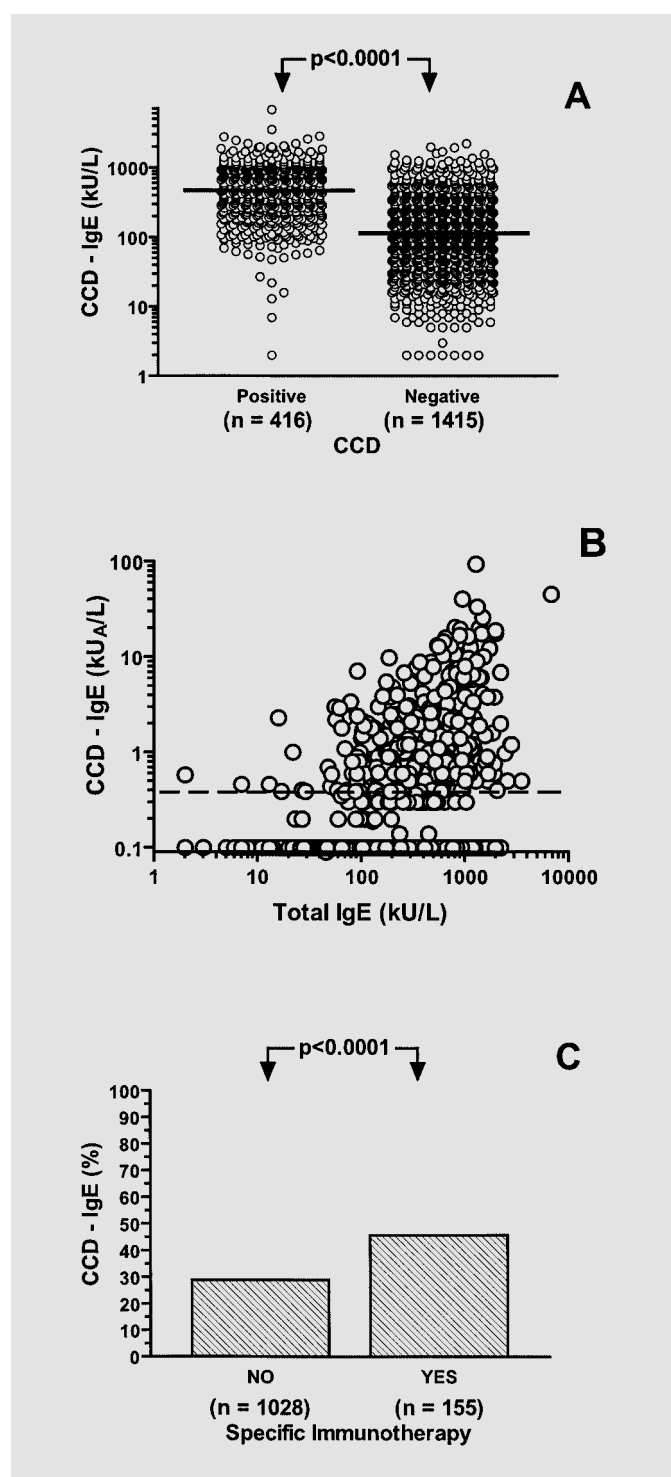


Fig. 3. A Total IgE distribution in the CCD-IgE-positive and -negative subsets (n = 1,831). Statistics: Mann-Whitney test. B Total IgE and CCD-IgE correlation (n = 1,831). Dashed line indicates negative cut-off. Statistics: Spearman's correlation test. Statistically no significant correlation was found. C CCD-IgE among subjects with a previous pollen specific immunotherapy (SIT) course (n = 1,183). Statistics: Fisher's exact test.

values in both the subsets, and the presence of sera with high total IgE and negative CCD-IgE excluded a correlation between the two parameters (fig. 3B). A statistically significant difference was found when comparing prevalence of positive CCD-IgE sera in the SIT-treated group with patients who never received such treatment (fig. 3C).

#### *Comparative Reactivity of CCD-IgE and Allergenic Extracts*

Table 1 shows comparative prevalence of positive IgE detection to several allergenic extracts in CCD-IgE-positive and -negative subsets. IgE detection was performed on sera from patients recorded SPT-negative to the same allergenic extract. All the allergenic extracts considered showed significant differences between the two subsets, though the highest values were obtained in almost all the plant-derived extracts, and lowest values were recorded for mites, *Aspergillus* and shrimps. Correlation between values of CCD-IgE and IgE detected for each allergenic extract is reported. As for prevalence, variable degrees of correlation were found between IgE to each allergenic extract and CCD-IgE. None of the patients with high levels of IgE to peanuts without a positive SPT to the same allergenic extract reported adverse reaction when consuming such food.

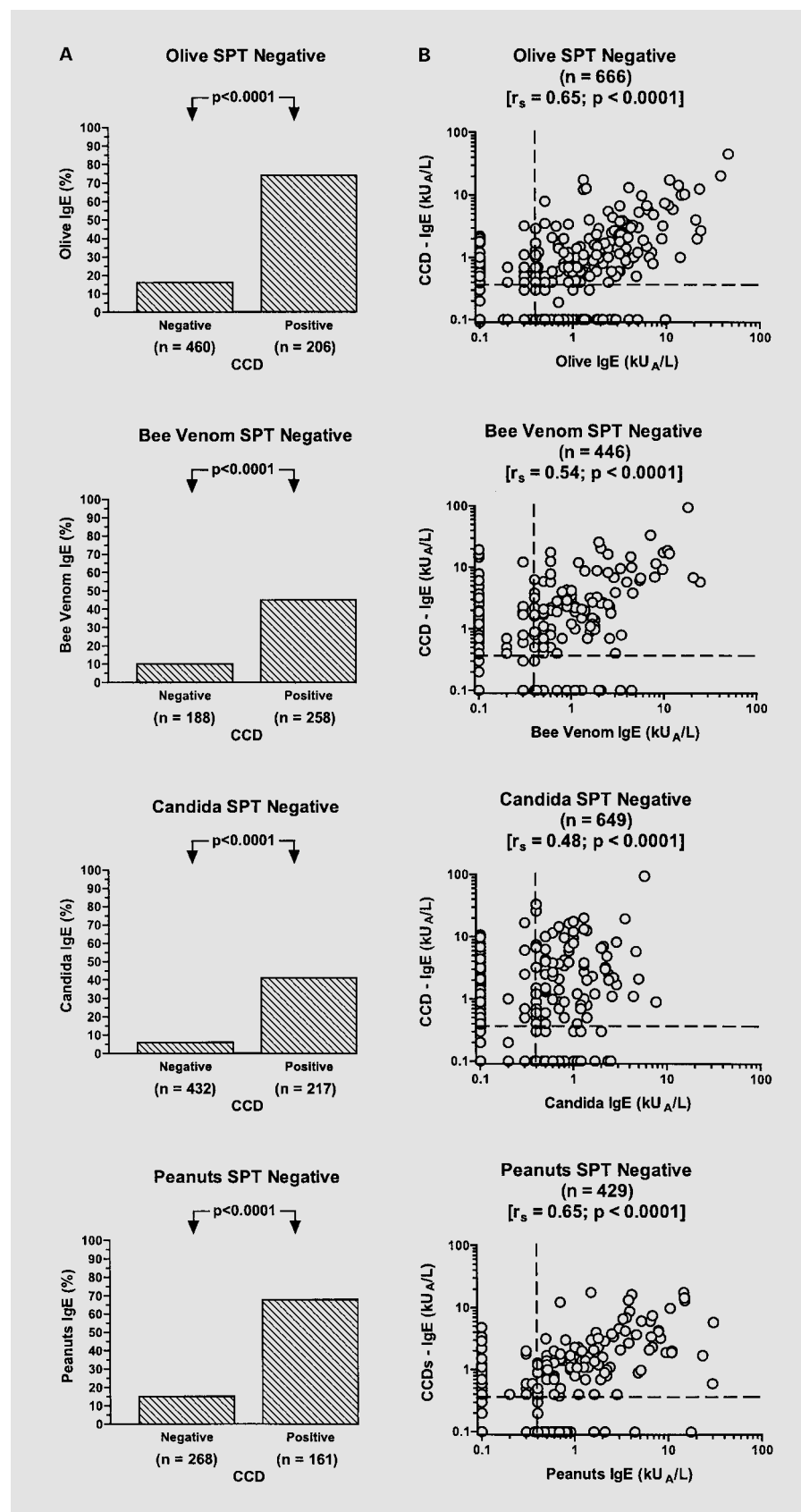
Figure 4 shows the same data as in table 1 for four selected allergenic extracts (fig. 4A: comparative prevalence; fig. 4B: correlation test).

IgE reactivity to gums, high-molecular-weight complex polysaccharide, was almost exclusively recorded in the CCD-IgE-positive subsets of tested sera (fig. 5A). None of the gums gave a positive SPT in any tested patients. A correlation between the IgE reactivity to polysaccharides and BRO (CCD-IgE), a low-molecular-weight glycoprotein, was found. As in the case of the allergenic extracts, several sera with CCD-IgE did not react with gums (fig. 5B). Prevalence, mean value and degree of correlation were higher for tragacanth gum compared with the other two gums.

#### *Skin Testing and IgE Detection to Purified Glycoproteins*

Three glycoproteins, with a known glycan structure, were tested in vivo in 1,076 subjects. None of the tested patients reacted to ascorbic acid oxidase. Six patients with a positive SPT to phospholipase A<sub>2</sub> turned out to have a history of local reactions after bee stings. Phospholipase A<sub>2</sub> reactors were equally distributed among CCD-IgE-positive and -negative subsets. None of the patients with

Fig. 4. A Prevalence of positive IgE detection in subjects with negative SPT to the same allergenic extract and belonging to the CCD-IgE-positive and -negative subsets. Statistics: Fisher's exact test. B Correlation between the concentrations of the CCD-IgE and those of the allergenic extract IgE in subjects with negative SPT to the same allergenic extract. Dashed lines indicate negative cut-off. Statistics: Spearman's correlation test. Allergenic extracts, tested sera and statistical results are displayed on graphs for the four selected allergenic extracts.



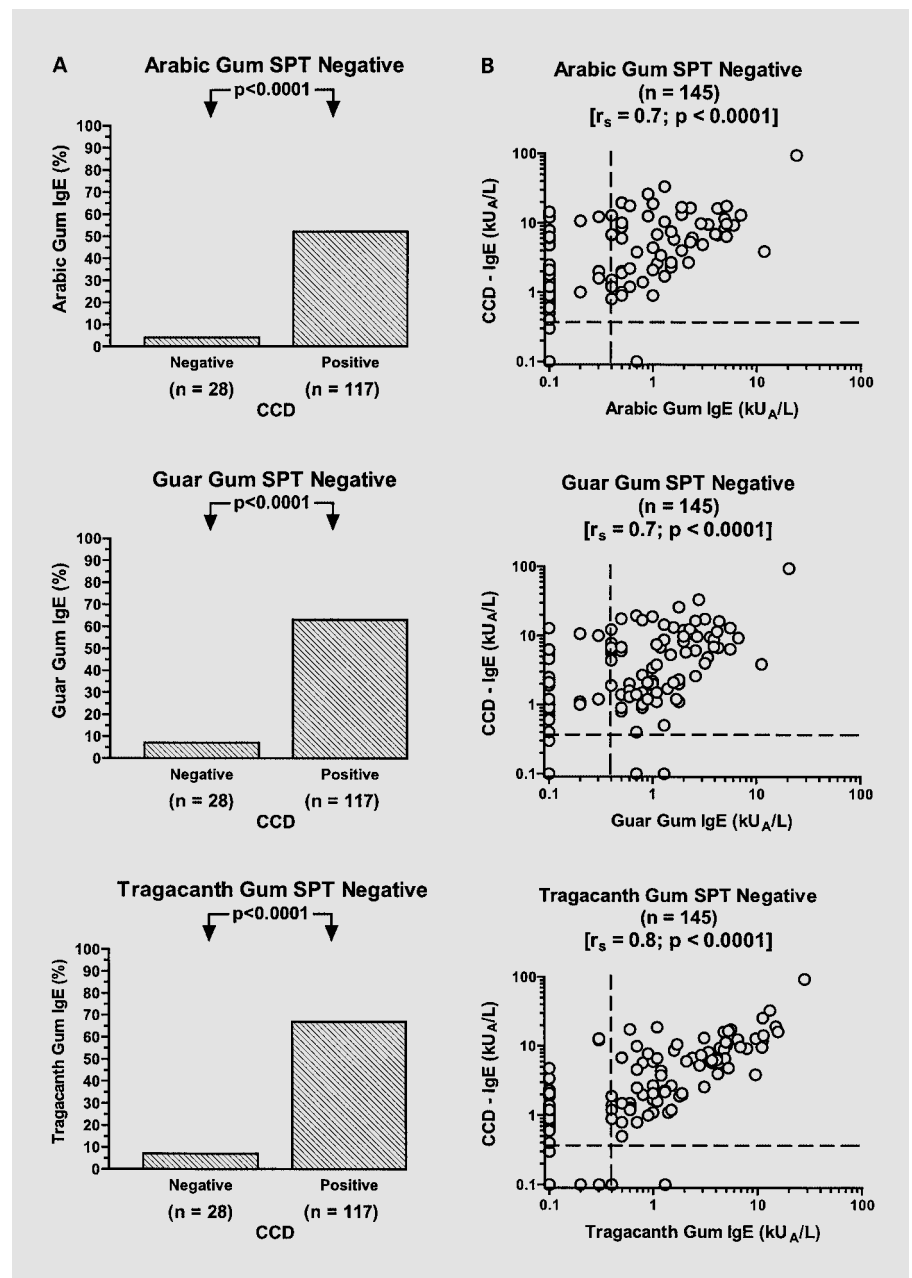
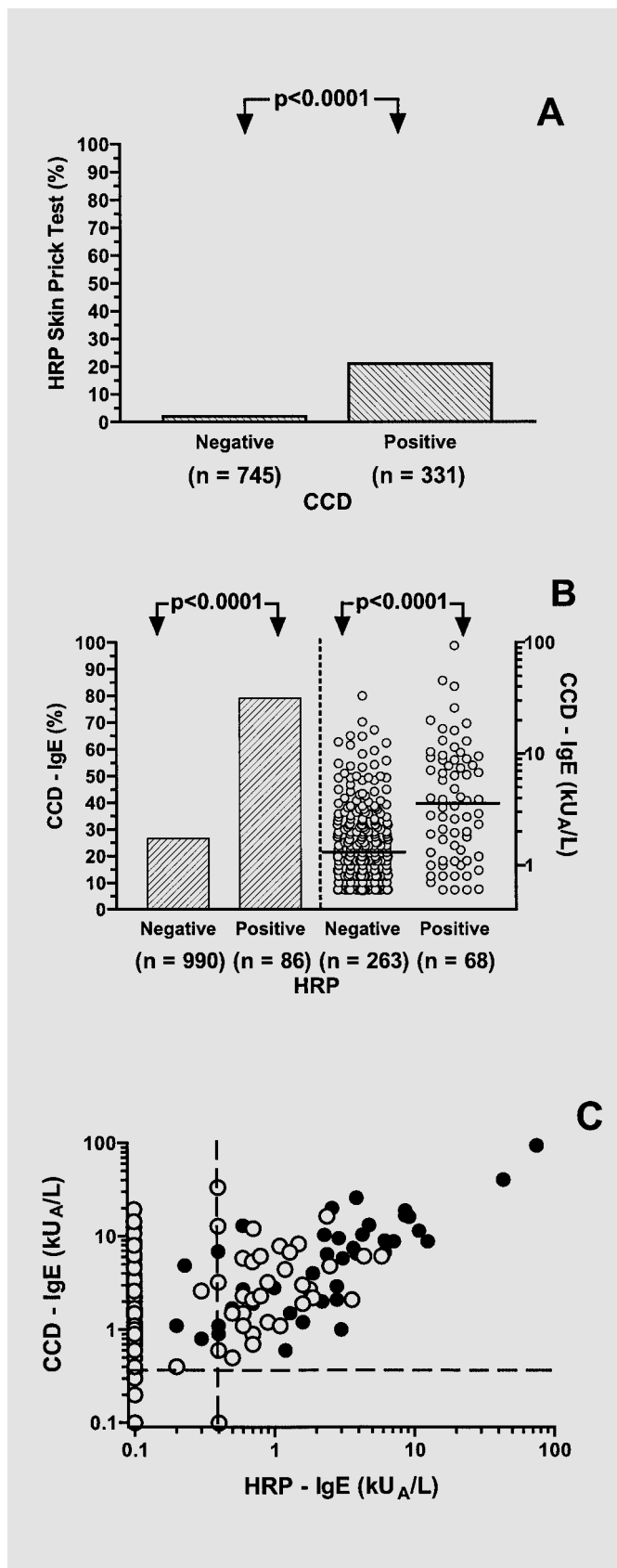


Fig. 5. A Prevalence of positive IgE detection in subjects with negative SPT to gums and belonging to the CCD-IgE-positive and -negative subsets. Statistics: Fisher's exact test. B Correlation between the concentrations of the CCD-IgE and those of the gum-IgE in subjects with negative SPT to the same extract. Dashed lines indicate negative cut-off. Statistics: Spearman's correlation test. Extracts, tested sera and statistical results are displayed on graphs for each extract.

elevated CCD-IgE (greater than CAP class II) had a positive SPT to phospholipase A<sub>2</sub>.

Twenty-one percent of the subjects with CCD-IgE were positive to the HRP-SPT (fig. 6A). Positive HRP-SPT patients were not restricted to any of the previously indicated subsets, as they belong to the pollen, the non-pollen (2 subjects), and also, but rarely, to the non-allergic subsets (1 subject). Excepting little class III cutaneous reactivity, almost all the SPT reactions to HRP were recorded in

the class II. About 80% of the HRP-SPT-positive sera had detectable CCD-IgE (fig. 6B, left part), with median values significantly greater than sera from patients with a negative HRP-SPT (fig. 6B, right part). Though a statistical correlation was found between CCD-IgE and HRP-IgE, some CCD-IgE-positive sera did not react with HRP using the CAP system (fig. 6C). None of the sera had detectable IgE to HRP without CCD-IgE.



## Discussion

In the present study we tried to define the prevalence of CCD-IgE in a large cohort of subjects with allergy-related respiratory symptoms. Testing sera on BRO seems to be a rapid method to detect CCD-IgE as the protein *per se* rarely seems to act as an allergen. Compared with previous preliminary studies [6, 22] prevalence of CCD-IgE was better defined in the present cohort of subjects including not only pollen-allergic patients, but also those sensitized to non-pollen sources and non-allergic subjects with respiratory symptoms. The evidence of a higher prevalence of CCD-IgE within patients with plant-derived allergies is confirmed, furthermore by the increasing prevalence among patients with multiple pollen sensitization [22, 23]. Whether the CCD-IgE is an epiphenomenon of the plant-derived allergen sensitization or whether it plays an active role in the sensitization process is still to be explained. The higher prevalence of CCD-IgE among patients with multiple pollen sensitization could be in favour of the latter hypothesis, and could also suggest a role for CCD-IgE as marker of the risk of developing multiple allergies. Prospective studies, involving younger patients having CCD-IgE in either the non-allergic or the non-pollen subsets, could be an interesting model to explore such a hypothesis.

Aalberse and van Ree [15] clearly stated how the binding of IgE to the glycans is specific. The possible interference of high total IgE levels on the detection of CCD-IgE has been ruled out in the present study. In fact, subjects with high total IgE levels and undetectable CCD-IgE were found. A factor contributing to the presence of CCD-IgE could be a previous SIT. Moverare et al. [25] have recently suggested that the development of new IgE specificities could be due to SIT. New reactivities to panallergens (profilins and calcium-binding proteins) or to CCD have been suggested as a possible explanation. In a recent study, we recorded a higher number of patients with a previous SIT

Fig. 6. A Prevalence of positive SPT to HRP in the CCD-IgE-positive and -negative subsets (n = 1,076). Statistics: Fisher's exact test. B Left part: Prevalence of CCD-IgE in HRP-SPT-positive and -negative subsets (n = 1,076). Statistics: Fisher's exact test. Right part: Values of positive CCD-IgE in HRP-SPT-positive and -negative subsets (n = 331). Statistics: Mann-Whitney test. C Correlation between CCD-IgE and HRP-IgE (n = 353). Filled circles = HRP-SPT-positive subjects; empty circles = HRP-SPT-negative subjects. Dashed lines indicate negative cut-off. Statistics: Spearman's correlation test:  $r_s = 0.6$ ;  $p < 0.0001$ .

course among calcium-binding protein sensitized patients when compared to those sensitized to profilins [22]. In the present study a statically significant higher prevalence of CCD-IgE has been found among patients who previously received a SIT course. Prospective studies by means of molecular markers are necessary to clarify such issues [26].

Many of the known allergenic molecules are glycoproteins [8], but non-allergenic glycoproteins may be present in allergenic extracts as well. The use of monoclonal antibodies reactive to carbohydrate moiety allowed the detection of carbohydrate determinants in extracts from unrelated sources [17, 20, 21], further documenting the widespread reactivity of carbohydrate epitopes. Starting from a preliminary description of a wide CCD-IgE binding to clinically irrelevant allergenic extracts [6], we tried to define the degree of interference of CCD-IgE in the IgE detection. A panel of allergenic extracts recorded negative in the SPT was used. Results from the present study show that the presence of CCD-IgE directly correlates with positive assay for many different allergenic extracts that are unable to trigger an allergic reaction in the skin test. Plant-derived glycoproteins are indeed the most reacting ones, but the higher the concentration of CCD-IgE the more probable is the chance to detect IgE for any SPT-negative allergenic extract. Moreover, the differences recorded in the sera reactivity, and the presence of sera with CCD-IgE but not reacting with certain extracts, suggest a possible heterogeneity of the CCD-IgE [14]. The use of purified allergenic glycoproteins in comparative studies would certainly increase the information on the specificities of CCD-IgE [14]. From an immunochemical point of view, extended inhibition studies are needed to confirm the statistical correlations recorded in the present study [6]. The negative SPT, the absence of clinical reactivity to inhaled or ingested allergens seem to further confirm the poor or absent clinical relevance of CCD-IgE [5]. This was particularly evident for peanuts. Several patients with high levels of peanut IgE, well correlated with CCD-IgE, had negative SPT to the peanut extract and did tolerate the peanut ingestion. Clinical reactivity to gums is rarely reported [27]. The detection of IgE to polysaccharides without a positive SPT or a clinical reactivity would include such food industry additives among allergenic extracts that can bind CCD-IgE [15]. As the structure and composition of the complex glycan chains of polysaccharides are not completely elucidated, the reason why a high-molecular-weight carbohydrate polymer is not capable of triggering an IgE reactivity at least in subjects with high levels of CCD-IgE remains a question.

The identification of a native glycoprotein capable of inducing skin test reactivity was achieved with HRP. In fact, in the present study, 21% of the patients having CCD-IgE displayed a cutaneous reactivity to the purified HRP. None of these patients reacted to the other tested glycoproteins having a different number of glycan side chains and also different glycan structures [2], though such molecules have been shown to be CCD-IgE reactive [6, 21]. The heterogeneous distribution among the subsets of respiratory patients (e.g. one among non-allergic patients, two among the non-pollen-allergic patients) would exclude the HRP as an allergen in any of the extracts used for the screening SPT. HRP is a glycoprotein with seven N-glycan side chains, ascorbic acid oxidase has four oligosaccharide units, whereas BRO and bee venom phospholipase A<sub>2</sub> have a single glycan side chain, and they all differ in the carbohydrate composition [2]. The CCD-IgE reactivity of their carbohydrate epitopes has been clearly documented [2, 6]. A single study describing a peroxidase as a wheat flour allergen has been reported [28]. Authors showed its IgE-binding capacity, but no in vivo or in vitro triggering activity has been demonstrated for the purified glycoprotein. A histamine release activity of a purified glycoprotein has been reported only by Batanero et al. [7], by using an Ole e 1 derivative in 5 olive pollen-allergic patients. Fötisch et al. [16] reported a bovine serum albumin-BRO glycopeptide conjugate as active in the histamine release test in a single serum. CCD contributing to the allergenic activity of several food extracts has recently been reported [29, 30], but no provocative test has been performed with the purified allergenic molecules. Further biochemical, immunochemical and functional studies should be carried out by means of HRP to define the role of its CCD in the allergenic activity. Moreover, the negative SPT reactivity to HRP, as well as to all the other glycoproteins with different glycan number and/or composition, polysaccharides, and extracts containing glycoproteins, of subjects with comparable amount of CCD-IgE still remains to be explained.

In conclusion, IgE to CCD are common among the allergic population and the binding to skin-test-negative allergenic extracts further confirms their poor, if any, biological activity. CCD-IgE reactivity can be recorded for a wide range of allergenic unrelated extracts. Sera to be used in any in vitro assay employing native or recombinant glycoproteins should be carefully evaluated for the presence of CCD-IgE. The first preliminary report of a glycoprotein (HRP) capable of triggering an in vivo reactivity in a subset of subjects with CCD-IgE has been documented.

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