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Demonstration of Carbohydrate-Specific Immunoglobulin G4 Antibodies in Sera of Patients Receiving Grass Pollen Immunotherapy

Key Words

Carbohydrate epitopes
Immunoglobulin G₄
Lol p XI

Abstract

From a group of 92 patients receiving grass pollen immunotherapy, and selected on grounds of high IgG₄ titers against *Lol p* I, sera were tested for IgG₄ antibodies against the glycosylated grass pollen allergen *Lol p* XI. In 72 of 92 cases IgG₄ antibodies were demonstrated. The N-glycan of *Lol p* XI was earlier shown to be an epitope for IgE antibodies. In the present study it was demonstrated that the carbohydrate structure is also recognized by IgG₄ antibodies, illustrating that IgG₄ responses are not immuno-restricted to peptide epitopes, as was suggested in several reports.

Introduction

Carbohydrate structures are often regarded as antigens that elicit isotype-restricted antibody responses: in humans predominantly T-cell-independent IgM and IgG₂ responses [1–3]. Polysaccharides that are not attached to peptide structures will usually be incapable of inducing the antigen-specific T-cell responses that are necessary for switching B cells to isotypes like IgE and IgG₄. Glycoproteins, in which relatively small carbohydrate groups are linked to immunogenic peptide structures, could, however, provide T-cell help for class switching of carbohydrate-specific IgM-bearing B cells.

For IgE antibodies, recognition of carbohydrate epitopes was demonstrated earlier [4]. For IgG₄ antibodies it was suggested that there is a restriction to peptide epitopes. For example, in contrast to the strong IgG₄ responses observed in parasite-infected patients in general, phosphocholine-

containing polysaccharides from filarial parasites or group-A carbohydrate from *Streptococcus pyogenes* were shown to lack the capacity to induce IgG₄ antibodies in these patients [3]. These carbohydrate structures, however, belong to the T-cell-independent antigens. These observations should not be generalized to all carbohydrate epitopes, especially those on T-cell-dependent antigens. In this study we investigated whether anti-carbohydrate IgG₄ could be demonstrated in sera from grass pollen-allergic patients undergoing immunotherapy. After prolonged allergen immunotherapy, predominantly IgG antibodies of the IgG₄ subclass are induced [5]. Grass pollen extracts used in this treatment contain several T-cell-dependent glycosylated antigens. We recently purified and characterized one of these antigens, the major allergen *Lol p* XI [6]. This allergen contains an IgE-binding carbohydrate determinant. This was demonstrated by the finding that IgE binding was hardly affected by protease-digestion (proteinase K) of the al-

Received:
March 9, 1994
Accepted after revision:
November 1, 1994

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1018–2438/95/1062–0146
\$8.00/0

lergen, but almost completely disappeared after chemical deglycosylation (trifluoromethanesulfonic acid, TFMS). *Lol p* XI was used as a model glycoprotein to investigate whether carbohydrate-specific IgG₄ antibodies are induced during immunotherapy.

Materials and Methods

Grass pollen allergen, *Lolium perenne* pollen, was purchased from Diephuis Laboratories (Groningen, The Netherlands). Pollen extract was made as described elsewhere [7]. The major allergen *Lol p* XI was purified from the extract by means of monoclonal antibody affinity purification and gelfiltration, as previously reported [6]. Radiolabeling with ¹²⁵I of *Lol p* XI (20 µg) was performed by the chloramine T method.

L. perenne extract was protease digested with proteinase K (Boehringer Mannheim, Germany). Deglycosylation was performed with TFMS. Both procedures are described elsewhere [6]. The efficiency and specificity of these treatments were monitored with monoclonal antibodies directed to carbohydrate determinants and directed to peptide epitopes [6]. It was shown that the protease digestion resulted in a more than 1,000-fold enrichment for carbohydrate determinants (>95% recovery), whereas the TFMS treatment gave a similar shift in favor of peptide epitopes (also with >95% recovery).

Protease digested *L. perenne* extract, originating from 5 mg lyophilized extract, was coupled to 1 g CNBr-activated Sepharose (Pharmacia, Uppsala, Sweden) for application in an IgG₄-binding assay.

All sera were from grass pollen-allergic patients receiving immunotherapy. A selection was made from hundreds of sera submitted for routine diagnostic allergy tests, on grounds of a high IgG₄ titer against *Lol p* I (>10% binding with 1.25 µl serum in an allergen-binding assay with ¹²⁵I-labeled *Lol p* I).

The radio-allergosorbent test (RAST) was performed as described earlier [4]. In short, Sepharose-coupled proteinase K-treated *L. perenne* extract (0.5 mg/test) was incubated overnight with 1.25 µl serum in a final volume of 300 µl phosphate-buffered saline/0.3% bovine serum albumin/0.1% Tween 20 (PBS-AT). After washing, bound antibodies were detected with ¹²⁵I-labeled mouse monoclonal anti-human IgG₄ (CLB: MH 164-1). Results are expressed as percent binding of total counts added, results of <2% were regarded as negative.

IgG₄ antibodies against *Lol p* XI were measured in an allergen-binding assay with ¹²⁵I-*Lol p* XI. Sepharose-coupled monoclonal anti-human IgG₄ (0.5 mg/test) was incubated with 1.25 µl serum and 50 µl ¹²⁵I-labeled *Lol p* XI (20,000 cpm) in a final volume of 350 µl PBS-AT. Results are expressed as percent binding of total counts added (<1% was regarded negative). In a competitive RIA serum was pre-incubated for 2 h with 50 µl *L. perenne* extract (treated or untreated). Results are expressed as percent inhibition compared to an uninhibited value (PBS-AT added instead of inhibitor).

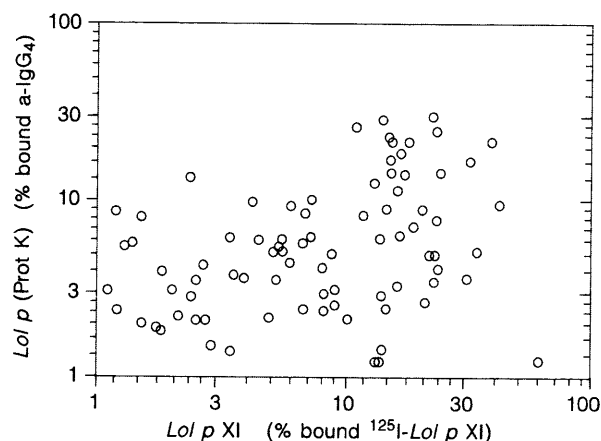


Fig. 1. Anti-*Lol p* XI and anti-carbohydrate IgG₄ responses. IgG₄ antibodies against *Lol p* XI and against *L. perenne*-derived sugar groups were measured, the former in an allergen-binding assay with ¹²⁵I-labeled *Lol p* XI, the latter in a RAST with Sepharose-coupled proteinase K-digested *L. perenne* extract. Results are shown for all sera with IgG₄ against *Lol p* XI (n = 82), and are expressed in percent binding of total counts added (¹²⁵I-labeled *Lol p* XI or ¹²⁵I-labeled monoclonal anti-human IgG₄).

Results

In an allergen-binding RIA with ¹²⁵I-labeled *Lol p* XI, 83 of 92 sera (>90%) with high IgG₄ titers against *Lol p* I demonstrated specific IgG₄ antibodies against *Lol p* XI (>1% binding with 1.25 µl serum). The mean percent binding of the positive sera was 13.7±1.3% (SE). In a binding assay with Sepharose-coupled proteinase K-digested *L. perenne* extract, specific IgG₄ against carbohydrate determinants was demonstrated in 75 of 82 *Lol p* XI-reactive sera (fig. 1.).

Fifteen sera with IgG₄ antibodies against *Lol p* XI were tested in a competitive RIA with ¹²⁵I-labeled *Lol p* XI and treated (proteinase K and TFMS) and untreated pollen extract as inhibitor. For all sera >98% inhibition was found with untreated pollen extract. In most cases the binding was only partially inhibited by the treated extracts. For all sera, the percentage inhibition, reached with protease-digested extract, plus the percentage inhibition, reached with TFMS-treated extract, added up to >95% inhibition. This is shown for 5 sera in table 1. This complementary effect was further confirmed for 6 sera by adding both TFMS-treated extract and proteinase K-treated extract simultaneously. Again, inhibition was >95%.

Table 1. Relative importance of carbohydrate and peptide determinants for IgG₄ anti-*Lol p* XI

Inhibitor	Serum No. 14	No. 17	No. 49	No. 71	No. 89
Untreated	98.8	97.9	99.3	98.6	98.1
TFMS	68.8	39.1	30.3	98.1	70.6
Proteinase K	35.0	67.2	65.7	5.1	35.3
TFMS+proteinase K	98.8	99.9	99.9	ND	99.7

In a competitive RIA with ¹²⁵I-*Lol p* XI, it is shown that inhibition of IgG₄ binding with *Lol p* extract can be explained by a combination of carbohydrate and peptide determinants. Results are given as percentages inhibition. Some sera have IgG₄ anti-*Lol p* XI that is predominantly anti-peptide (No. 14, 89, and especially 71), others have IgG₄ that is more anti-carbohydrate (No. 17 and 49). The last row illustrates that inhibition with a mixture of TFMS and proteinase K-treated pollen extract adds up to complete inhibition, as is the case with untreated extracts.

Discussion

In this study it was shown that IgG₄ antibodies reactive with *Lol p* XI are found in the majority of grass pollen immunotherapy patients with high IgG₄ titers against *Lol p* I. Responses to both allergens were not correlated, as was judged by a Spearman rank correlation ($R = 0.09$; $p = 0.4$). In inhibition assays, the IgG₄ response was shown to be directed to both the peptide backbone of *Lol p* XI and its carbohydrate group. By means of Sepharose-coupled carbohydrate determinants, derived from total pollen extract, it was demonstrated that 75 of 82 sera with IgG₄ antibodies against *Lol p* XI had anti-carbohydrate IgG₄. These findings do not support the hypotheses that IgG₄ responses are restricted to peptide-derived epitopes, as was suggested in several studies [1-3]. Carbohydrate epitopes presented to the immune system in the context of T-cell-dependent antigens are capable of inducing IgG₄ antibodies with anti-sugar specificity, as was already known for IgE antibodies [4].

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