

Sensitization due to Gum Arabic (*Acacia senegal*): The Cause of Occupational Allergic Asthma or Crossreaction to Carbohydrates?

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Key Words

Gum arabic · Immunoblotting · Occupational allergens

Abstract

Background: A pharmaceutical industry worker was exposed to dust of gum arabic in the tablet coating plant and complained of work-related shortness of breath, chest tightness, runny nose, itching and redness of the eyes. This case was investigated for allergy to gum arabic and compared with a control group. The aim of the study was to identify the IgE-binding components responsible for the work-related symptoms. **Methods:** Skin prick tests (SPTs) and specific IgE (slgE) measurements with environmental and occupational allergens, spirometry and a specific bronchial challenge with gum arabic were performed. One hundred and nineteen control subjects underwent SPT with gum arabic and 43 controls were tested for slgE. Crossreactivity between gum arabic and horse radish peroxidase was investigated by IgE CAP inhibition. A combined procedure of immunoblotting and periodate treatment was applied to identify the epitope nature of gum arabic. **Results:** Allergy to gum arabic was shown by SPT, presence of slgE and a positive bronchial challenge with gum arabic. Sensitization to gum arabic was demonstrated by SPT or slgE in 7 and 5 controls, respectively. The results of inhibition with horse radish peroxidase, immunoblotting and periodate treatment suggest that gum

arabic slgE of the patient and 1 SPT-positive control subject were directed to the polypeptide chains of gum arabic. In contrast, gum arabic slgE of the other controls reacted to carbohydrate components. **Conclusions:** Sensitization to gum arabic carbohydrate structures occurs casually in atopic patients with pollen sensitization without obvious exposure to gum arabic. This study suggests that allergy to gum arabic is mediated preferentially by IgE antibodies directed to polypeptide chains of gum arabic.

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Introduction

Gum arabic exuded from the stems and branches of *Acacia senegal* has wide industrial uses as a stabilizer, thickening agent and emulsifier, mainly in the food industry, but also in the textile, pottery, lithography, cosmetics and pharmaceutical industries. It is a complex and variable mixture of arabinogalactan oligosaccharides, polysaccharides and a glycoprotein with a high-molecular-weight polysaccharide attached to a hydroxyproline-rich polypeptide backbone that accounts for only 2% of the molecular weight [1]. Although a first case of occupational sensitization to gum arabic in a candy factory was presented already in 1933 [2], and several reports on printers' asthma [3–6] and 1 case of contact dermatitis in

a pottery worker [7] were published, the offending allergens are not yet identified. Due to the high carbohydrate content of gum arabic, carbohydrate specific IgE (sIgE) was expected to be involved in allergies to gum arabic which were recently demonstrated in a chocolate confectioner with cough and dyspnea by Fötisch et al. [8]. However, sIgE to carbohydrates can be highly crossreactive and frequently occurs in patients with polyvalent food and pollen sensitization or in bee venom-allergic patients [9, 10]. sIgE to gum arabic may occur without airborne exposure to this allergenic source and without clinical significance [11, 12]. When a pharmaceutical worker with gum arabic exposure was examined for occupational allergy, it was the aim to investigate the role of crossreacting carbohydrate determinants (CCD) in his case in comparison with a control group in detail.

Patients and Methods

Case History

A 30-year-old male pharmaceutical industry worker was admitted for a medical opinion in 2002. Since April 1992, he was exposed to dust from a variety of drugs and additives in the tablet coating plant. Workplace-related shortness of breath, chest tightness, runny nose, itching, swelling, redness of the eyes and redness of the face and neck started in 1994 and worsened during the last few years. Symptoms occurred mainly during weighing gum arabic or talc in spite of wearing paper masks. Cromolyn and short-acting β -agonists were prescribed from 1994, topical steroids and long-acting β -agonists from 2000. The patient was otherwise healthy and in a stable condition without recent exacerbations. Medication was withheld for 1 day prior to the examinations. The patient as well as controls gave informed consent for the tests.

Control Patients

One hundred and nineteen consecutive patients from our outpatient clinic underwent skin prick tests (SPTs) with gum arabic and environmental allergens. Thirty-six of these subjects with total IgE ≥ 100 kU/l or at least 1 positive SPT were tested for sIgE to gum arabic. Additionally, 7 sera of known highly atopic patients without occupational exposure to gum arabic were selected to complete the control group for in vitro tests.

SPTs and sIgE

SPT [13] was performed with gum arabic (1% w/v, protein concentration 40 μ g/ml; material from the patient's workplace) and a panel of environmental allergens: house dust mites (*Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*; ALK, Copenhagen, Denmark), animal dander (dog, horse, cat; ALK), bird feathers (Allergopharma, Reinbek, Germany), moulds (*Alternaria tenuis*, *Aspergillus fumigatus*, *Cladosporium herbarum*; ALK), three different tree pollen mixtures (I: hazel, birch, alder; II: ash, willow, cottonwood; III: oak and beech), herb pollen (HAL, Düsseldorf, Germany), grass pollen (ALK), latex, and lycopodium.

The latter allergens were prepared in our laboratory (0.01 and 0.05 mg/ml). A wheal diameter ≥ 2 mm was considered positive.

sIgE determination was performed with the Pharmacia UniCAP100 system (Uppsala, Sweden) according to the manufacturer's protocol with gum arabic ImmunoCAP (f297), horse radish peroxidase (HRP; o400), natural rubber latex (k82), *Ficus benjamina* (k81), *D. pteronyssinus* (d1), *D. farinae* (d2), cat and dog dander (e1, e2) and tree pollen mixtures (tx5, tx6). Values ≥ 0.35 kU/l were considered positive. IgE inhibition experiments were performed with gum arabic ImmunoCAP as solid phase and with 100 μ g HRP (Sigma, Taufkirchen, Germany) in 10 μ l phosphate-buffered saline (PBS) added to 50 μ l serum as inhibitor. As control without inhibition 10 μ l PBS was added.

Lung Function and Challenge Tests

Spirometry and methacholine testing were performed as described recently [14–16]. Bronchial challenge with gum arabic was performed with a dosimeter (APSPRO, Viasys, Würzburg, Germany) and a DeVilbiss 646-nebulizer (Viasys; output 900 mg/min). Nebulizations were performed for 0.6 s during each of 10 slow total lung capacity breaths with doubling concentrations up to 10 mg gum arabic/ml (maximal cumulative dose 0.45 mg; this corresponds to a maximal protein dose of 1.8 μ g/ml).

Electrophoresis, Blotting and Periodate Oxidation

Gum arabic (5 g) from the patient's workplace was extracted with 200 ml 0.1 M sodium carbonate buffer, pH 9.6, at 60°C according to Fötisch et al. [8] and freeze-dried. The lyophilisate was dissolved in distilled water in a concentration of 50 mg/ml (w/v) containing a protein concentration of 120 μ g/ml according to Bradford [17]. For SDS-PAGE, 300 μ l were mixed with 100 μ l fourfold concentrated Laemmli sample buffer [18] and electrophoretically separated under reducing conditions in a precast NuPAGE Bis-Tris gel (Invitrogen, Groningen, The Netherlands). The separated proteins were transferred by semidry blotting to polyvinylidene fluoride membrane (Millipore, Germany) [19]. Blotted membrane strips were stained by Coomassie brilliant blue R-250 or blocked with 1% BSA and 1% polyvinylpyrrolidone-40 in TBS buffer (15 mM Tris, 150 mM NaCl, 1 mM EDTA, pH 7.8) over night for immunostaining. Immunoreactive carbohydrate components of proteins were inactivated by periodate acid oxidation [20]. Blotted membrane strips were blocked with 4% BSA in TBS over night, washed and oxidized with 50 mM periodate acid in 50 mM sodium acetate (pH 4.5) in the dark for 2 h. The treatment was stopped with 1% glycerol. To control deglycosylation, blot strips were incubated with peroxidase-labeled jackbean lectin (concanavalin A, L6397, Sigma, Deisenhofen, Germany) in substrate buffer (50 mM Tris/HCl, pH 7.4, 150 mM NaCl, 1 mM CaCl_2 , 1 mM MnCl_2) at a concentration of 15 μ g/ml for 1 h. Enzymatic staining with 4-chloronaphthol (C6788, Sigma) was performed according to the manufacturer's instructions.

For immunostaining, NaIO_4 -treated or untreated blot strips were incubated overnight with patients' sera at room temperature and washed three times with TBST (TBS + 0.05% Tween-20). Bound IgE was detected after 2 h incubation with anti-human IgE conjugated with alkaline phosphatase (Sigma) diluted 1:1,000 in TBST with 2% BSA by NBT/BCIP (0.03% *p*-nitroblue tetrazolium, 0.015% 5-bromo-4-chloro-3-indolyl-phosphate) as a substrate.

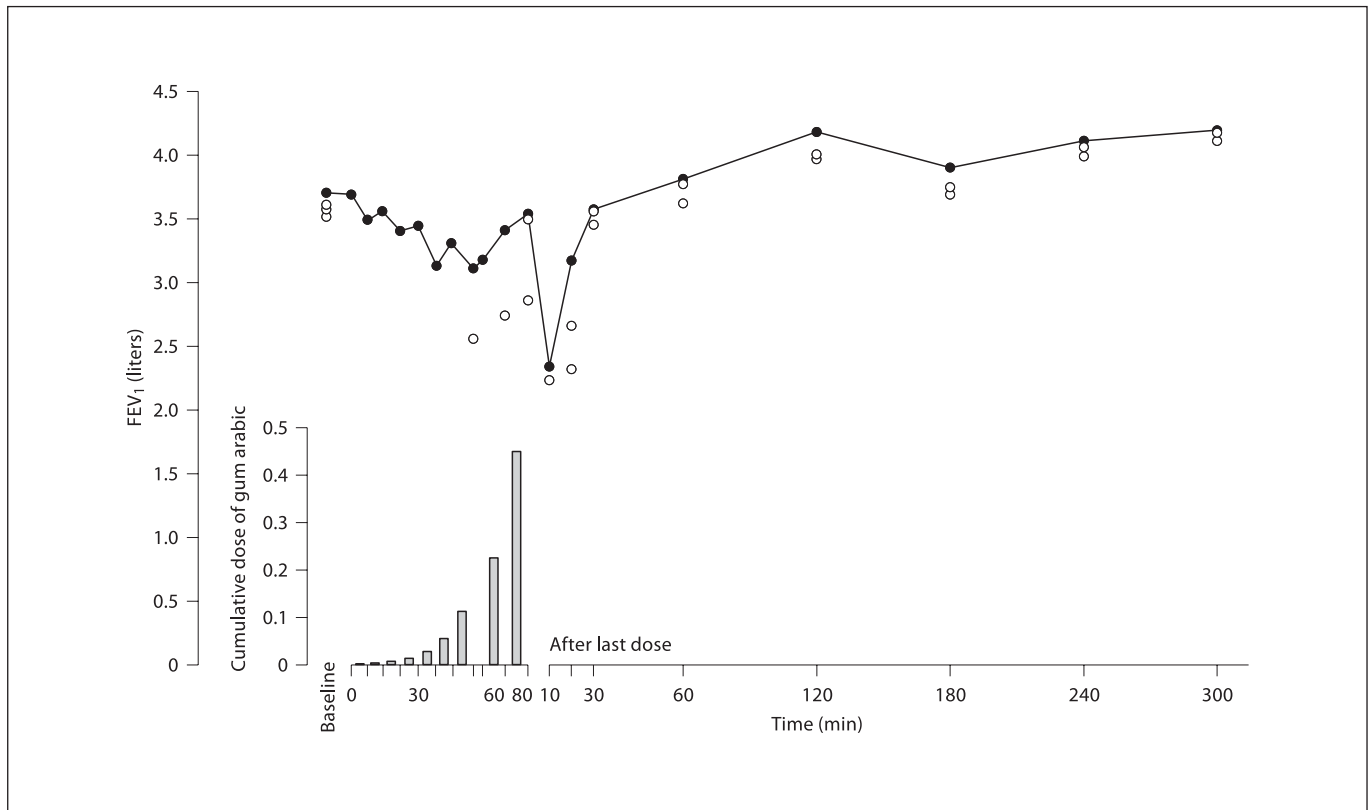


Fig. 1. The patient (No. 1; table 1) underwent a bronchial provocation test with a gum arabic extract. The line represents the best FEV₁ measurements, the open dots represent additional FEV₁ measurements.

Results

Case Examination

Clinical examination did not show any signs of eye, nose, skin, lung or airways disease. Spirometry was normal: forced expiratory volume in 1 s (FEV₁) 91% predicted, FEV₁/inspiratory vital capacity ratio 71%. There was no bronchial hyperresponsiveness to methacholine. SPT with environmental allergens showed weak reactions with wheal diameters of 4 mm to house dust mites, dog and cat dander and 2 mm to grass, herb and tree pollen. SPT with extracts of materials from the patient's workplace in PBS showed negative reactions, with the exception of gum arabic which produced a 4 mm wheal/20 mm flare with the 1% (w/v) extract and a 2 mm wheal/8 mm flare with a diluted 0.1% (w/v) extract. Total IgE was 80 kU/l. sIgE was demonstrated for house dust mites (CAP class 3) and gum arabic (CAP class 2; 1.23 kU/l). No in vitro sensitization could be shown for cat and dog dander, tree pollen, latex, HRP and *F. benjamina*.

After the bronchial challenge with gum arabic, the patient complained of chest tightness. A 36% decrease in FEV₁ (from 3,690 to 2,340 ml PBS) was documented 10 min after the maximal dose (fig. 1).

The patient was re-examined in September 2005. He reported improvement in his airway symptoms, but was still taking topical steroids and long-acting β -agonists. Lung function and methacholine testing were normal; sensitization to gum arabic as assessed by SPT and sIgE had disappeared.

Sensitization to Gum Arabic and HRP

Thirty-seven of the 119 controls had at least 1 positive SPT to common allergens, and 7 of the 119 controls also showed a reaction to gum arabic (1% w/v) with a small wheal (2 mm), in only 2 cases with flares (2 and 3 mm). All but 1 gum arabic SPT-positive controls were polysensitized to environmental allergens. These 7 controls with positive SPT to gum arabic and an additional 29 atopic controls with negative SPT to gum arabic were tested for

Fig. 2. Binding of IgE from human sera to gum arabic separated by SDS-PAGE and transferred to polyvinylidene fluoride membrane. Protein from gum arabic was Coomassie stained (P) and carbohydrate structures were stained by concanavalin A (Ga = untreated blot strips, Gb = periodate blot strips). The sera of the patient (No. 1) and control patients (No. 2–11; controls No. 2–6 with sIgE to gum arabic, numbered as in table 1, and controls No. 7–11 without sIgE to gum arabic; 2 controls without binding are not shown) were incubated either with untreated (a) or with periodate-treated blot strips (b). As control (K), the reaction of the secondary antibody with untreated (a) or periodate-treated blot strips (b) is shown.

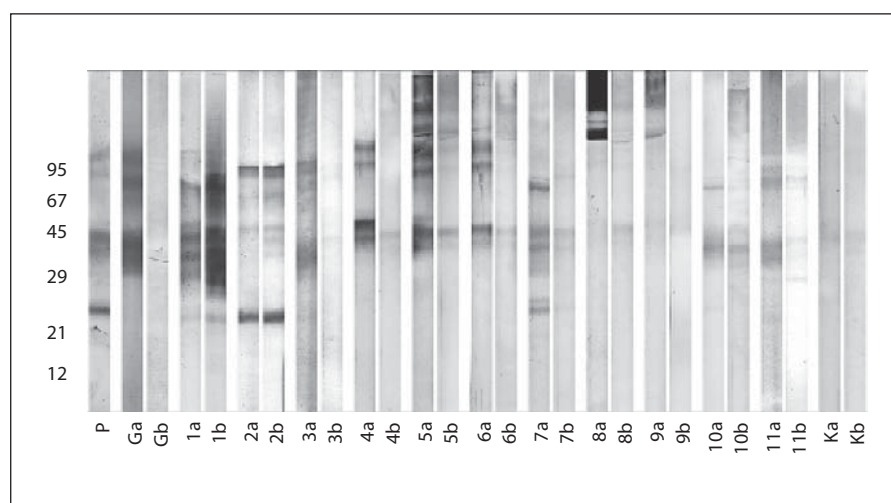


Table 1. Results of SPTs and in vitro tests for the patient (No. 1) and gum arabic-sensitized controls (No. 2–12)

No.	Profession/workplace	Total IgE kU/l	SPT positive	sIgE to gum arabic, kU/l	sIgE to HRP kU/l	sIgE to gum arabic, inhibitor HRP
1	pharmaceutical plant	80.4	G, H, A, P	1.23	<0.35	no inhibition
2	nurse for the elderly	4,145	G, P, A, L	1.27	0.41	no inhibition
3	machine operator	109	H, P, A	3.19	5.75	complete inhibition
4	steel worker	102	nt, P, H	0.77	2.11	complete inhibition
5	florist	>2,000	nt, P, A	0.94	0.87	complete inhibition
6	dental technician	>2,000	nt, P, H, A, M, B	0.83	3.14	complete inhibition
7	nurse	748	G, P, A, H, L	<0.35	<0.35	–
8	foundry mechanic	9	G, P, A, H	<0.35	<0.35	–
9	preparator	34	G	<0.35	<0.35	–
10	coal miner	87	G, P, A	<0.35	<0.35	–
11	editor	35	G, P, A, H	<0.35	<0.35	–
12	physician	46.2	G, A, H	<0.35	<0.35	–

G = Gum arabic; H = house dust mites; A = animal dander; P = grass and/or tree pollen; L = latex; nt = not tested with gum arabic; M = moulds; B = bird feather.

sIgE to gum arabic. Only 3 subjects showed sIgE to gum arabic (table 1): the patient (No. 1), 1 control with a positive SPT (No. 2; 2 mm wheal, 3 mm flare) and 1 control with a negative SPT to gum arabic (No. 3). The sera of the highly atopic subjects had sIgE to gum arabic in 3 out of 7 cases (No. 4–6; table 1). All sera with sIgE or a positive SPT to gum arabic (the patient and 11 controls) were also tested for sIgE to HRP. As sIgE values for gum arabic and HRP were obviously correlated (Pearson's coefficient of correlation $r^2 = 0.68$), inhibition experiments were performed. Crossreactivity between gum arabic and HRP

with a complete inhibition was found in 4 cases (No. 3–6; table 1).

Immunoblot Study

Coomassie-stained gum arabic extract showed five protein bands at molecular weights between 22 and 95 kDa after electrophoresis (fig. 2, lane P). By peroxidase-labeled concanavalin A staining, carbohydrate components were detected in the molecular weight range of 32–40 kDa (fig. 2, lane Ga). After oxidation with periodate acid, no binding of concanavalin A was observed

(fig. 2, lane Gb). Immunoblot analysis of gum arabic was carried out with the patient's serum and sera from 12 controls. Ten of these controls including the 5 with gum arabic-positive CAP showed IgE binding to gum arabic in the immunoblot (fig. 2). The patient's IgE binds preferentially to proteins with a molecular weight of 23, 28, 33, 39 and 65 kDa. While the patient's serum (fig. 2, lane 1a, 1b) and that of 1 control subject (fig. 2, lane 2a, 2b) reacted to periodate-treated as well as to untreated blot strips, the sera of the other control subjects showed a much stronger reaction to the untreated blot strips indicating reactivity to carbohydrate structures which were destroyed by the oxidation process.

Discussion

sIgE reactivity to carbohydrate determinants accompanied by crossreactivity between nonhomologous proteins is frequently observed during *in vitro* experiments. The relevance of IgE binding to such 'crossreactive carbohydrate determinants' is often stated as clinically insignificant [11, 12]. Gum arabic which is mainly composed of carbohydrates was also found to bind crossreacting anticarbohydrate IgE of polysensitized patients [20]. In a survey of 1,831 patients with a suspected allergic respiratory disease, Mari et al. [21] found an overall prevalence of 23% with positive IgE to CCD. A subset of 145 patients was also tested with gum arabic by SPT and for sIgE. While no positive SPT to gum arabic was found, 42% had sIgE (≥ 0.4 kU/l) to gum arabic, all but one of them belonging to the CCD-positive group. In contrast to these findings, in our control group, SPT reactions to gum arabic were found in 7 out of 119 cases. However, the wheals were very small and only 1 SPT-positive control had sIgE to gum arabic. Out of the altogether 42 controls with increased sIgE and/or positive SPT to common allergens, 5 had sIgE to gum arabic. All 5 controls were also sensitized to pollen (positive SPT) and had sIgE to HRP. IgE binding to gum arabic ImmunoCAP was inhibited completely by HRP in 4 cases. No inhibition by HRP was obtained with the patient's serum and the serum from 1 control subject (a nurse) who was sensitized to gum arabic according to sIgE and SPT, although no occupational exposure to gum arabic was documented. Immunoblots with periodate-treated and thus deglycosylated gum arabic strips confirm that the patient and the control with the positive SPT to gum arabic had sIgE to the polypeptide structure of gum arabic, while the other controls mainly reacted to carbohydrate determinants which are

likely to be similar to the ones of HRP. The carbohydrate determinant of HRP is a paucimannosidic-type N-glycan, having only $\alpha(1,3)$ -fucose and $\beta(1,2)$ -xylose to the core $\text{Man}_3\text{GlcNAc}_2$ (MMXF³) [22].

Recently, a case of a confectioner in a chocolate plant with exposure to gum arabic powder developing cough and dyspnea was presented [8]. sIgE antibodies of this patient who had a negative SPT but a positive intradermal test to gum arabic were also mainly directed against the carbohydrate fraction of gum arabic. Periodate-treated and thus deglycosylated gum arabic showed no IgE binding in these experiments. In contrast to these findings, sIgE of the pharmaceutical industry worker presented in our case binds equally well to deglycosylated as to untreated gum arabic blot strips. The same refers to 1 control subject with a positive SPT and CAP with gum arabic. The effectiveness of the deglycosylation procedure was proven by concanavalin A staining and, additionally, by reaction of the other control sera with anticarbohydrate IgE. With the sera of the other controls, IgE binding was reduced after deglycosylation. Thus, for the allergic patient and the control with the positive SPT and CAP, the polypeptide structure of gum arabic is assumed to contain the IgE-binding epitopes.

In vivo, the patient showed a positive SPT reaction and a positive bronchial challenge with gum arabic. It is probably not by chance that the patient reacted with the polypeptide chain of gum arabic whereas all but 1 control showed IgE binding to carbohydrate structures. It remains unclear whether this control subject who was also sensitized to protein structures of gum arabic has symptoms when exposed to gum arabic. IgE binding to protein structures of gum arabic should be investigated in cases of suspected gum arabic allergy.

Although no bronchial hyperresponsiveness 1 day after the discontinuation of medication with topical steroids and long-acting β -agonists could be documented, the patient was diagnosed with occupational allergy to gum arabic and exposure cessation was recommended. On follow-up about 2 years after exposure cessation, the patient reported improvement in airway symptoms, but was still taking anti-obstructive medication on a regular basis.

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