

IgE-mediated allergic reactions to fruit gums and investigation of cross-reactivity between gelatine and modified gelatine-containing products

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Summary

A 32-year-old female reacted with a contact urticaria syndrome after eating 'gummy bears' (fruit gums). The reaction began in the oral mucosa and led to treatment on an in-patient basis. RAST measurements with allergen discs produced with gelatine and gelatine-containing products (among them 'gummy bears') demonstrated the presence of IgE antibodies in the serum of this patient. Proteins with molecular weights in the range of 40–120 kD were identified as the allergens in gelatine by using Western blot analysis. RAST inhibition showed cross-reactivity between gelatine, gelatine-containing products and the modified gelatine used in some plasma substitutes. Allergic reactions towards coloured fruit candies and 'gummy bears' may result from an IgE-mediated hypersensitivity towards gelatine. The long-known anaphylactoid reactions towards gelatine-containing plasma substitutes may, at least in part, be of an allergic nature.

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Introduction

'Gummy bears' (fruit gums) are often used as typical examples for the provocation of allergy-like intolerance reactions towards food dyes [4]. Beside these food dyes, 'gummy bears' and other sweets/foods like liquorice, marshmallows, corned beef, brawn, fruit yogurt, instant whipped cream, instant pudding, juices, etc., contain gelatine. The amount of gelatine of the whole weight is 12% in 'gummy bears' and 30–50% in brawn. For atopic individuals gelatine can act as an allergen and can induce specific IgE antibodies [2]. Also modified gelatine used in some plasma substitutes caused histamine release from blood basophils [6].

In the present study RAST measurements were done with several gelatine and modified gelatine allergen discs using the serum of a patient who showed a contact urticaria syndrome after chewing 'gummy bears'. Furthermore cross-reactivity was examined between gelatine and modified gelatine-containing products.

Case report

Several minutes after chewing 'gummy bears' a 32-year-old patient (W) developed a contact urticaria syndrome beginning in the oral mucosa and severe enough to make in-patient treatment necessary. She is atopic with an occasional atopic eczema in the bend of the knees and a slightly elevated total serum IgE of 142 U/ml (Phadezym-PRIST). The 'gummy bears' were composed of gelatine and starch syrup. They contained citric acid, polyoxyethylene glycerine monostearate, tartrazine, cochineal red and patent blue V. Scratch testing with the four variously coloured 'gummy bears' resulted in positive immediate reactions with weals and erythema corresponding to the histamine reaction. Similarly positive was the scratch test with gelatine (in a suspension in physiologic saline), while 20 control subjects showed a negative test. Scratch testing with tartrazine, cochineal red, patent blue V and polyoxyethylene glycerine monostearate was negative. An oral provocation test with 50 mg tartrazine led to no reaction. At a later date an intradermal test was performed with Haemacel®35, a colloidal plasma substitute based on gelatine. The test led to a positive immediate reaction comparable to the histamine reaction up to a dilution of 1:10 000.

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Materials and methods

RAST

The serum of the patient W and a non-allergic control pool serum ($n=112$) were measured by RAST (Pharmacia, Freiburg, FRG) using several gelatine and modified gelatine allergen discs produced according to the prescription of Ceska *et al.* [1] and Maasch *et al.* [3]. The following gelatine-containing extracts were coupled to CNBr-activated paper discs: so-called Pharma-gelatine (a blend of bovine and swine gelatines), gelatine derived from beef bone, bovine split-skin preparations and from pig skin (all produced by Deutsche Gelatine-Fabriken Stoess & Co., Eberbach, FRG), white and red 'gummy bears' (Haribo, Bonn, FRG), so-called 'Katzenpfötchen' (liquorice) candies (Katjes Fassin GmbH, Emmerich, FRG). The plasma substitutes had the following characteristics: Haemaccel®35 (Behringwerke AG, Frankfurt, FRG): polypeptides of bovine gelatine bridged with hexamethylene diisocyanate with an average molecular weight of 35 kD; Gelifundol®S (Biotest, Hanau, FRG): oxypolygelatine (bovine), average molecular weight 30 kD.

For the preparation of the allergen discs 1 µg protein [8] was used for coupling for each disc. After the coupling the amount of protein coupled on each disc was measured. For non-specific binding the allergen discs were measured with the non-allergic control pool serum ($n=112$).

RAST-inhibition

To determine the cross-reactivity between the nine gelatine-containing products, they were examined by RAST inhibition [7] using the Pharma-gelatine allergen discs and the serum of patient W (diluted 1:3). Each extract was calibrated to the same protein content (1 mg/ml). The specificity of the percentage inhibition was controlled with a birch pollen allergic pool serum ($n=23$, diluted 1:10) using the Pharma-gelatine allergen discs and the Pharma-gelatine extract.

Western blot

Utilizing Western blot analysis [5, slightly modified], the relative molecular weights of the proteins acting as allergens in the Pharma-gelatine were determined. To perform the Western blot, 100 µg of protein of the Pharma-gelatine was separated via SDS-PAGE according to molecular weight. The proteins were transferred from the gel to a nitrocellulose (NC) membrane (Schleicher & Schüll, Dassel, FRG) using electrotransfer. The NC membrane was incubated with the serum of patient W (diluted 1:10) overnight at room temperature, then washed and incubated again overnight at room temperature with ^{125}I labelled anti-IgE (Pharmacia). Following incubation the NC membrane was washed, dried and autoradiographs using Kodak X-O-mat AR films at -20°C over 7 and 14 days were performed. The specific IgEs in the patient's serum were thus visualized as variously strong bands on an X-ray film. The determina-

Table 1. RAST class measurements of the serum of patient W and a non-allergic control pool serum using various gelatine and modified gelatine allergen discs

Serum	Allergen discs	RAST class	Percentage binding
Reference system			
Reference A	Reference	4	29.0
Reference B	Reference	3	13.6
Reference C	Reference	2	3.6
Reference D	Reference	1	2.3
Test			
Patient W	Pharma-gelatine	3	14.8
Patient W	Gelatine from bovine bone	2	10.0
Patient W	Gelatine from bovine split-skin preparation	2	9.0
Patient W	Gelatine from pig skin	2	11.2
Patient W	White 'gummy bears'	2	7.2
Patient W	Red 'gummy bears'	2	7.5
Patient W	'Katzenpfötchen' (liquorice)	2	9.6
Patient W	Haemaccel	2	10.0
Patient W	Gelifundol	2	11.7
Non-allergic control pool serum	Measured with all allergen discs	0	0.5-0.7

tion of the molecular weights was done by comparison with a reference protein mixture (Pharmacia). The non-allergic pool serum ($n=112$) was used as a negative control.

Results

Allergen discs—RAST

The extracts used for the preparations of the gelatine and modified gelatine allergen discs may contain substances which may influence the modified Lowry protein determination, therefore the amounts of protein coupled on each disc should be considered as approximate values. The protein values coupled on each disc were in the range of 0.10–0.24 μg . RAST measurements performed with the serum of patient W showed that the allergen discs were of acceptable quality.

RAST class 3 (14.8% binding) was measured with the serum of patient W with the Pharma-gelatine allergen discs. RAST class 2 was measured with the other gelatine and modified gelatine (Haemacel, Gelifundol) allergen discs. The only differences found were in the percentage-binding which varied for RAST class 2 from 7.2 to 11.7. The non-allergic control pool serum showed RAST class 0 (0.5–0.7% binding) with the various allergen discs, i.e. the positive RAST measurements with the various gelatine and modified gelatine allergen discs with serum W did not depend on non-specific bindings (Table 1).

RAST inhibition

To determine cross-reactivity, the nine gelatine-containing extracts were measured against Pharma-gelatine (a

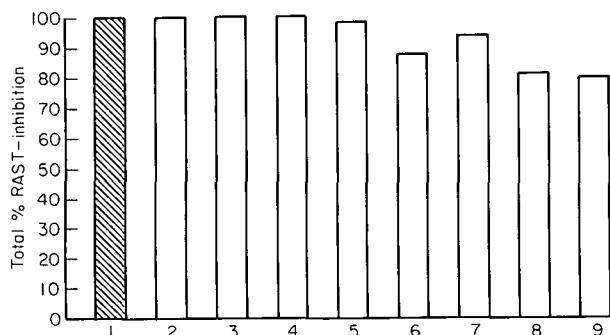


Fig. 1. RAST-inhibition measurements of the nine gelatine-containing products using Pharma-gelatine allergen discs and the serum of patient W. (▨) Homologous system; (□) Heterologous system. (1) Pharma-gelatine, (2) red 'gummy bears', (3) white 'gummy bears', (4) 'Katzenpfötchen' (liquorice), (5) Haemacel, (6) Gelifundol, (7) gelatine from pig skin, (8) gelatine from bovine bone, (9) gelatine from bovine split-skin preparation.

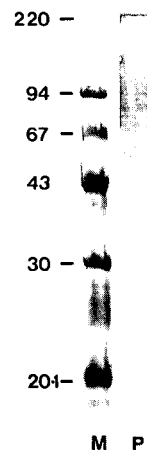


Fig. 2. Western blot of Pharma-gelatine (P); M, reference protein mixture (marker for molecular weights from 20.1–220 kD); time of autoradiography: 14 days.

blend of bovine and swine gelatine) by RAST inhibition using the Pharma-gelatine allergen discs. Figure 1 shows 80–100% of total % RAST inhibition for all extracts. With the birch pollen allergic pool serum no inhibition was measured (not shown).

Western blot

The Western blot performed with so-called Pharma-gelatine shows that most specific IgE affinities (allergens) possessed a molecular weight in the range of 40–120 kD (Fig. 2). In the Western blot of the non-allergic control pool serum using the Pharma-gelatine no allergen bands could be seen (not shown).

Discussion

Gelatine is a hydrocolloid which, besides water and mineral salts, is composed of 85% protein. It is a derivate of animal collagen. Starting materials are pig skin as well as bovine split-skin preparations and bones. For humans, gelatine is thus an altered, foreign protein. It is obviously capable of inducing specific IgE antibody production via the mucosa of the digestive tract in atopic individuals [2].

In the present study serum was obtained from patient W 6 months following the reaction for detailed immunological examinations. Using RAST with allergen discs produced with several gelatine varieties and gelatine-containing products RAST class 3 (14.8% binding) was measured with the Pharma-gelatine allergen discs and RAST class 2 (7.2–11.2% binding) with the other gelatine allergen discs. With the non-allergic control pool serum 0.5–0.7% binding was measured, so that the positive

RAST results with the serum of patient W could not be attributed to non-specific binding.

RAST inhibition measurements verified the suspected cross-reactivity of the gelatine-containing products. Using Pharma-gelatine allergen discs and the serum of patient W, 80–100% of total RAST inhibition was measured for the nine gelatine-containing products. The extracts thus possess common allergens. Using Western blot analysis, the proteins, which are at the same time the allergens, could be identified as having molecular weights in the range of 40–120 kD.

Considering the anaphylactoid reactions which have been known for many years towards gelatine-containing colloidal plasma substitutes, it seemed appropriate to test the serum of patient W to see if the IgE antibodies would react with such a substitute. This was clearly the case. The binding rate was virtually the same as that measured for the various nutritional gelatines (10.0 and 11.7% binding). Almost 100% inhibition occurred using the plasma substitute Haemaccel and ~90% inhibition using Gelifundol in RAST inhibition.

In the production of colloidal plasma substitutes the gelatine is first broken and then re-bridged. The allergenic structures apparently are retained. Post observations have always suggested that the anaphylactoid reactions towards gelatine-containing plasma substitutes may reflect an immediate-type allergy. In several of these cases positive intradermal tests producing weals and erythemas were obtained while testing with highly diluted plasma substitutes; histamine release from blood basophils has also been observed [6].

The clinical significance of the case presented here is that an immediate-type hypersensitivity towards gelatine must be considered in allergy-like reactions to food containing gelatine as a binding agent. This also holds true for reactions towards 'gummy bears' (fruit gums) and other chewable candies; these are most often coloured with azo-dyes, and the blame for intolerances has up to

now always been placed upon these dyes. The results obtained with the modified gelatine of colloidal plasma substitutes appear even more important. The identification reactions, to our knowledge the first, towards gelatine-containing plasma substitutes are of an allergic nature. The sensitization in some cases may be induced in part through gelatines in food.

Acknowledgments

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References

- 1 Ceska M, Eriksson R, Varga JM. Radioimmunosorbent assay of allergens. *J Allergy Clin Immunol* 1972; 49:1–9.
- 2 Kleinhans D. Speisegelatine: Allergen in Gummibärchen. *Allergologie* 1987; 10:242–4.
- 3 Maasch HJ, Wihl JA, Schultze-Werninghaus G, Geissler W, Wahl R. A manufacturer's criteria for in-house reference preparations for RAST-inhibition. *Ann Allergy* 1987; 58:29–33.
- 4 Schulz K-H. Lebensmittelzusatzstoffe: Vorkommen und allergologische Bedeutung. 19. Fortbildungstagung des Ärzteverbandes Deutscher Allergologen e.V., 2.6–4.6.1988, Bad Soden, F.R.G. *Allergologie* 1989; in press.
- 5 Tovey ER, Baldo BA. Characterization of allergens by protein blotting. *Electrophoresis* 1987; 9:452–62.
- 6 Vervloet D, Senft M, Dugue P, Arnaud A, Charpin J. Anaphylactic reactions to modified fluid gelatines. *J Allergy Clin Immunol* 1983; 71:535–40.
- 7 Wahl R, Maasch HJ, Geissler W. Comparison of three variations of the radioallergosorbent test-inhibition assay for measuring allergenic activity of grass pollen extracts. *Anal Biochem* 1983; 134:189–94.
- 8 Wahl R, Geissler W, Maasch HJ. Comparison of classical Lowry, modified Lowry and a dye-binding assay for the estimation of protein in allergen extracts and influence of different parameters on the modified Lowry assay. *Biol Chem Hoppe-Seyler* 1985; 366:979–84.

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