

Anaphylaxis produced by rubber glove contact. Case reports and immunological identification of the antigens involved

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Summary

A study was carried out on six subjects suffering from anaphylactic reactions including asthma, rhinoconjunctivitis, contact urticaria and hypotension following contact with rubber gloves. All of them showed skin sensitivity and serum specific IgE to various preparations of natural latex, and not to other chemicals used in glove manufacturing. Immunoblotting techniques demonstrated that at least four soluble polypeptides from natural latex had the ability to bind specific human IgE. The results suggested that natural latex proteins present in rubber gloves can cause severe specific hypersensitivity reactions.

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Introduction

Natural rubber (latex) is obtained from *Hevea brasiliensis*, a tree from the Amazonian region [1]. Its industrial application was introduced around the end of the last century. Several organic, as well as inorganic materials are used in the rubber manufacturing process, and some of them are frequently responsible for contact dermatitis [2,3]. However, natural latex sensitization is a fairly rare event [3].

Since 1979 several cases of contact urticaria attributed to rubber gloves have been described [2-10]. Although specific sensitivity to latex has already been demonstrated [10], the allergens involved in the patient's IgE-mediated response had not been identified.

Between 1979 and 1986 we have studied six subjects suffering from anaphylactic reactions, including contact urticaria, angioedema, rhinoconjunctivitis, asthma and hypotension, which occurred following the manipulation of gloves and other rubber objects. We demonstrated that these reactions were IgE mediated and soluble proteins from the natural latex were implicated.

Subjects and methods

Subjects

Subject 1 (MTB) a 26-year-old housewife with allergic rhinoconjunctivitis for 6 yr with positive skin tests to house dust mites and moulds. Since 1980 she had developed itching and an urticarial eruption with small size weals in rubber contact areas, while using rubber gloves for house-keeping or by handling rubber balloons. In 1983 an intracutaneous test with washing liquid from gloves was performed by a non-specialized practitioner, in 5 min she developed a severe general reaction with eyelid, lip and glottis oedema, dyspnoea and cyanosis, requiring emergency treatment with adrenaline, steroids, and oxygen. Since that episode, whenever she has touched rubber gloves or balloons she presented generalized urticaria, sneezing, nasal and conjunctival congestion, and dysphonia. All these symptoms appeared when somebody was handling rubber gloves close to her. She tolerated, without problems, contact with previously washed rubber gloves.

Subject 2 (AMC), a 17-year-old bricklayer with records of allergic rhinitis and food urticaria during childhood (with positive skin tests to milk, cacao and house dust mite). In 1984, a few minutes after wearing rubber gloves he developed eyelid oedema and urticaria in his hands and neck. Since this episode he has avoided rubber gloves. In

Table 1. List of substances used for *in-vivo* and *in-vitro* tests

1	Gloves washing samples
2	Natural latex: centrifuged liquid latex high ammonia feldatex in bulk, and centrifuged liquid latex latz feldatex in bulk.
3	Serum latex
4	Dialysis buffer 10 and 6
5	Flock
6	Detergents: dodecylbenzene-sulphonic neutralized with triethylenetetramine. Sodium mono-ester sulphonate from lauric and myristic alcohols.
7	Dispersants: diethyl-dithiocarbamic acid zinc salt, zinc oxide, dibutylparacresol sulphur, calcium carbamate, and alkyl sulphate.
8	Dyes: golden yellow levafix E-G, and CI reactive yellow 25.
9	Acrylic compounds.
10	Sanitized EA-1104.

1985, he presented four times with itching weal reactions in his arms while carrying rubber buckets in his work place, and once he developed generalized urticaria while entering a rubber factory.

Subject 3 (NAC), a 20-year-old female ceramics worker with allergic rhinitis and bronchial asthma, and positive skin tests to mites since childhood. In 1981 she presented, for the first time, facial angioedema and urticaria in her hands. She developed the same symptoms three more times but the last time associated with hypotension (90/65 mmHg) and dizziness immediately after using rubber gloves.

Subject 4 (FRM), a 27-year-old housewife with relapsing urticaria of unknown aetiology for 7 years. In 1979, immediately after inflating a rubber balloon she developed generalized urticaria with lip itching and oedema. Two more times she presented the same symptomatology after being in contact with rubber gloves. In 1983 she underwent general anaesthesia due to a caesarean operation, and immediately after the intubation with a rubber tube she presented generalized urticaria, cyanosis and hypotension (70/50 mmHg), requiring intensive treatment with intravenous steroids and plasma expanders. The following day her skin developed pronounced dermatographism and itching.

Subject 5 (RAP), a 33-year-old female polisher with a clinical history of allergic rhinitis and bronchial asthma since her youth, with a positive skin test to *Dermatophagoides pteronyssinus* extract. She underwent specific immunotherapy and was asymptomatic from this for several years. In 1982 and 1983 she presented several episodes of facial oedema and asthma after blowing rubber balloons. She also complained of generalized itching while using rubber contraceptives.

Subject 6 (PFR), a 29-year-old woman, employed in a chemical factory with records of allergic rhinitis. In 1985 she had presented generalized urticaria, facial angio-

edema, conjunctival congestion and rhinitis while using rubber gloves. These symptoms were repeated every time she wore rubber gloves and because of that she stopped using them. Later she experienced similar symptoms on arriving in a laboratory where rubber gloves were being used, even before touching them.

All the subjects could avoid the responses simply by washing the rubber gloves or balloons thoroughly with water before using them.

Substances used for in-vivo and in-vitro tests

To establish the component responsible for the allergic immediate reaction, the substances listed in Table 1 were tested.

Two samples of rubber tree (*Hevea brasiliensis*) latex from Malaysia were supplied by Guzman (Madrid, Spain). The two sources of material were identical except for the preservatives added: latex latz contained ammonia, tetramethylthiuram disulphide and zinc oxide, and latex high ammonia only contained 0.7% ammonia.

Rubber gloves and the substances used in their manufacture (6–10 in Table 1) were provided by Gomaitex (Hernani, Spain), and Ibartex (Alsasua, Spain).

Extracts

- 1 Gloves washing samples. Pieces of rubber (5 cm²) from gloves in different manufacturing phases were cut out and left to soak for 30 min in 5 ml of phosphate-buffered saline (PBS). After removing the rubber, samples were filtered with No. 1 Whatman paper.
- 2 Natural latex. One millilitre of both latex high ammonia and latex latz was diluted in 9 ml of PBS.
- 3 Serum latex. For the extraction of soluble components, 6 g of undiluted natural latex high ammonia were dissolved in distilled water (30% v/v). Acetic acid was

added stepwise until two phases were separated: the rubber precipitate, and the aqueous phase (serum latex) containing the soluble proteins [1,11,12]. This supernatant was neutralized with NaOH and then adjusted with 18 mM phosphate buffer. Two samples of 6 ml were dialysed against 250 ml of PBS for 6 hr using two different dialysis membranes with a cutoff of 10 and 6 kD respectively (Spectrapor). Dialysed aliquots were freeze-dried and resuspended in 2 ml of PBS.

4. Dialysis buffer 10 and 6 kD. The dialysing PBS from each one of the dialyses was concentrated in aliquots, freeze-dried and resuspended in distilled water. These materials were designated as dialysis buffer 10 and dialysis buffer 6, respectively.
5. Flock. Five milligrams of flock, the powder that coats the inner side of gloves, which consists of cotton, calcium stearate and a stilbene derivative, were mixed with 1 ml of PBS. It was stirred for 30 min and filtered with No. 1 Whatman paper.

Consecutive dilutions (from 1/10 000 to 1/1 (v/v)) in PBS of all these substances were used to perform prick tests. The rest of the substances listed in Table 1 were tested at 1% w/v in PBS, except the non-hydrosoluble ones that were tested as pure materials.

Prick tests

Skin-prick tests (SPT) were performed with the 'Allergy Pricker' (Dome-Hollister, Madrid, Spain) [13], in both forearms by the same technician. The negative control was PBS, and the positive one, histamine hydrochloride (1 mg/ml) corresponding to 3+.

Skin-prick tests were carried out with all substances indicated in Table 1. As control groups, 30 healthy people also exposed to rubber gloves, and 10 atopic patients with non-related sensitizations were prick tested with the same substances.

Provocation tests

Two pieces of a rubber glove provided by each subject were cut and one of them was thoroughly washed with water. Both pieces (the washed and the unwashed one) were slid on the volar side of the forearm for 15 min. The reaction was read at the moment the rubber piece was taken off.

Patch tests

All the patients were tested with 25 common contactants (European standard series) including the rubber chemicals thiuram mix, mercapto mix and PPD mix, the last ones diluted in vaseline, 1% (v/v). After valoration of a possible immediate reaction within the first 15 min the contactants were left in place for 48 hr and then read.

Total serum IgE

Total IgE level in the subjects sera was determined by Phadezym IgE Prist (Pharmacia Diagnostics, Uppsala, Sweden).

Specific IgE

To determine the presence of allergen-specific IgE, cyanogen bromide-activated paper discs [14] were coupled with: (i) glove washings, (ii) dialysed serum latex, and (iii) dialysis buffer 10 and 6. RAST was performed using the commercially available ¹²⁵I-labelled anti-IgE (Phadebas RAST), according to the manufacturer's instructions, and the results were expressed in arbitrary units, PRU/ml, from the Phadebas RAST Reference System (Pharmacia Diagnostics AB, Uppsala, Sweden).

Serum samples from 30 healthy and 10 atopic individuals, non-sensitized to rubber gloves, were used as controls.

Protein concentration

Protein concentration of the non-dialysed and dialysed serum latex extracts was determined according to the method of Lowry *et al.* [5] using bovine serum albumin as a standard.

Isoelectric focusing

Isoelectric focusing (IEF) was performed on polyacrylamide plates (LKB, Sweden) according to the manufacturer's instructions. Dialysed samples of serum latex containing 75 µg/ml of protein were applied to each gel, with a pH range of 3.5–9.5.

Sodium dodecylsulphate polyacrylamide gel electrophoresis

SDS-PAGE was carried out as indicated by Laemmli [16] with an acrylamide gradient concentration from 8% (top) to 15% (bottom). Two hundred micrograms of protein (serum latex extract) were applied to each gel.

Electrotransfer and immunodetection

Proteins resolved on SDS-PAGE were electro-transferred onto nitrocellulose sheets as described by Towbin *et al.* [17]. The sheets were incubated with the subjects sera pool and a normal sera pool, and then with a ¹²⁵I-labelled monoclonal antibody specific for human IgE immunoglobulin (Abelló, Madrid, Spain). Protein bands with the ability to bind specific human IgE were revealed by autoradiography.

Table 2. Results of the skin-prick tests performed in six subjects, with the soluble components of the rubber natural latex tree (*Hevea brasiliensis*) and other substances listed in Table 1, all used in the manufacturing of gloves

Subject	Gloves washing samples	Natural latex	Serum latex	Dialysis buffer		Other substances
				10 kD	6 kD	
1	a	a	b	d	—*	—
2	b	a	a	d	—	—
3	a	a	a	c	—	—
4	a	a	a	c	—	—
5	c	b	c	d	—	—
6	b	a	a	c	—	—

Consecutive dilutions giving a reaction equal to histamine chloride (1 mg/ml)=3+: a=1/10 000 b=1/1000 c=1/100 d=1/10 e=1/1.

— * =negative with all dilutions.

Table 3. Total and specific IgE (RAST) to rubber allergens in six sensitized subjects

Subject	Total IgE	Glove washing samples	Serum latex	Dialysed buffer 10 kD
1	62	2.7	2.8	0.30
2	184	3.1	3.1	0.93
3	303	4.3	9.3	4.1
4	150	6.3	8.2	2.4
5	411	0.6	0.55	0.30
6	244	n.d.	n.d.	n.d.

Total IgE is expressed in IU/ml.

Results of RAST are expressed in PRU/ml.

n.d. = not done.

Results

The six subjects gave immediate positive reaction in SPTs to all glove washing samples, and to the extracts from natural latex and serum latex. Most of the reactions were positive at dilutions of 1/10 000 or 1/1000 (3+). The SPTs were also positive with dialysis buffer 10, but negative with dialysis buffer 6. Skin-prick tests were negative for the rest of the substances (Table 2). All the SPTs performed in the control group were negative.

The provocation test by applying the unwashed piece



Fig. 1. Iso-electrofocusing of the serum latex of *Hevea brasiliensis*. Most of the bands are focused in the acidic side.

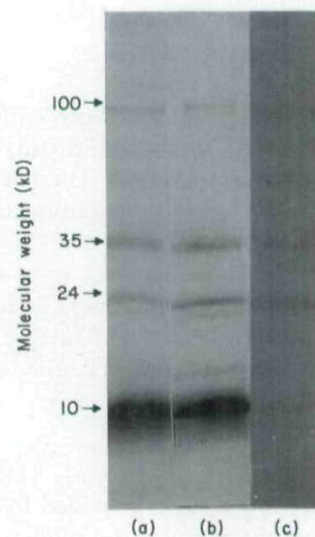


Fig. 2. (a and b) Electrotransfer and (c) immunodetection of the IgE binding proteins in the serum latex of *Hevea brasiliensis*.

of rubber gloves directly over the skin was positive in all of the subjects, eliciting itching and a weal-and-flare reaction within 15 min. The results were all negative with the washed rubber glove piece.

No immediate reactions occurred at the sites of patch testing within 15 min. Within 48 hr there was no reaction to any of the standard contactants, except in subject 4 who showed a reaction to nickel sulphate.

The protein content of the non-dialysed and dialysed serum latex was 4.5 and 2.42 mg/ml respectively.

Table 3 shows the total serum IgE, ranging from 62 U/ml in subject 1, to 411 U/ml in subject 5. Most of the subjects possessed specific IgE to allergens present in glove washings, in latex serum and in dialysis buffer. All the normal and atopic human sera used as controls produced negative results.

Figure 1 shows that serum latex contained several proteins, most of them focused in the acidic zone of the pH gradient. The polypeptide distribution, and subsequent immunodetection, proved that at least four protein bands, with approximate molecular weights of 10, 24, 35, and 100 kD displayed the ability to bind human IgE indicating their allergenic character (Fig. 2).

Discussion

Although latex has been used in industry for more than a century, contact urticaria and anaphylactic reactions caused by rubber gloves have only been described very recently. Since Nutter [3] described a case of contact urticaria elicited by rubber gloves, we have reviewed more than 27 cases of contact urticaria attributed to them [2–10], but in none of the cases was the causative agent identified. According to Forstrom [4] the allergic reaction should be produced by a chemical substance added either during the vulcanization process or already contained in the natural latex, since scarification with latex, free from flock, induces a reaction similar to that of finished rubber gloves. The negative results of the patch tests in our subjects, in agreement with other experiments [4,6,8], suggests that neither of the traditional chemicals responsible for the well-known contact dermatitis are responsible for the anaphylactic reactions. Others suggested that a component of flock could be responsible for an immunological reaction [7], in spite of the fact that all SPTs with flock were negative. Seaton [18] described a case of glove asthma due to vapours of terpenes produced by rubber gloves. The subjects we present complained initially of symptoms in the region of rubber contact, and later the symptomatology was generalized.

Meding and Fregert [6] thought that the causative component should probably be a soluble protein from natural latex, and Axelsson, Johansson and Wrangsjö [10] confirmed this hypothesis, and demonstrated an IgE-mediated mechanism.

All the symptoms in our subjects could be avoided if the rubber gloves were thoroughly washed with water, indicating that flock might be responsible and not any chemical product added to the latex in the polymerization process. However, SPTs were negative with the soluble

components of flock while it was positive with the soluble materials from different manufacturing phases of rubber gloves, including those before flock addition. Moreover, direct provocations with the unwashed rubber gloves were positive. The conclusion was that we were dealing with a soluble substance of latex which remained in the rubber after manufacturing, and which could be released spontaneously with the flock and induce symptoms by means of simple contact or through inhalation. This soluble substance appears to be a complex mixture of proteins, some of them identified by SDS-PAGE as bands of molecular weights ranging from 100 to 10 kD. The smallest one was able to pass through a 10 kD membrane filter, was positive by SPT, and bound specifically to human IgE. It is reasonable to assume that the 10 kD polypeptide could be identical to hevein, a well known latex protein [1,11,12], and could be considered as the major antigen of latex. The other three polypeptides with the ability to bind specific IgE could not be identified as α -globulins (200 kD), or as the two nucleoproteins (11.4 and 28 kD) [1,11,12] since our molecular weight determination was carried out in presence of a dissociating agent. However, the possibility of these three allergenic bands being subunits of larger molecular weight native proteins cannot be ruled out.

Five out of six subjects had histories of atopy. In the cases previously described atopy had been documented in at least 50% of patients [4,5,10].

We conclude that the reactions produced by contact with rubber gloves or by manipulation, previously classified as urticaria, greatly exceeded the cutaneous syndrome, and gave rise to real anaphylactic reactions. The mechanism is mediated by IgE and the allergens seem to be water-soluble and thermostable proteins (they are still allergenic after being at 130°C for 30 mins, in the last process of the manufacturing of rubber gloves). Normally, these proteins remained attached to the gloves' surface and can be removed by exhaustively washing the gloves with water.

We wonder why these reactions had not been described before, taking into account their severity and their clear cause-effect relationship. Technical factors in the extraction procedure of the latex, or some recent changes in the gloves' manufacturing might have been responsible for the appearance of these anaphylactic reactions not described until now.

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