

EDITORIAL REVIEW

Allergy to *Hevea* latex

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Latex is the cytoplasm of laticiferous cells found in over 2000 species of plants [1]. The cytoplasm is milky white because of numerous granules containing terpenoids—usually with a high content of polyisoprene; this may either be in the all-*cis* form (rubber, Fig. 1) or all-*trans* (gutta). Up until the middle of this century many different plants were being used as commercial sources of rubber, including guayule (a low, bushy, desert plant from Mexico) and kok-saghyz (a Russian dandelion); now rubber is produced exclusively from a single species—the para rubber tree, *Hevea brasiliensis*—imported to Malaya and Ceylon from the Amazon Basin via Kew Gardens in 1876–7. In *Hevea* the laticiferous cells form rings of inter-connecting ducts in the bark, so their cytoplasm can be tapped by cutting through the outer layers: the latex flows upwards into the incision for an hour or two; the flow slows and finally stops as the latex coagulates within the cut ends of the laticiferous ducts [2].

The raw *Hevea* latex typically contains 33% rubber in the form of particles with a modal diameter of 400 nm. The hydrophobic rubber hydrocarbon centres are surrounded by a half unit membrane and associated proteins, including the rubber elongation factor that was presumably involved along with cytoplasmic prenyl transferase in the synthesis of the rubber in the particle [3]. The remaining 67% of latex is the rest of the cytoplasm including nuclei, mitochondria, and various other organelles including the lutoids—specialized lysosome-like vacuoles [4]. The tendency of latex to coagulate is due to the effect of citrate and phosphatases released from the fragile lutoids neutralizing the negative charge on the rubber particles, allowing them to adhere to each other.

The reason so many different species of plant go to the lengths of synthesizing rubber remains mysterious. It seems not to be structurally useful, it is not a food store, nor is it obviously useful in defence, though *Hevea* latex contains hevein and hevamine in the lutoids—the former a chitin-binding protein with antifungal activity [5], the latter a chitinase with lysozyme activity which may also be antifungal [6].

Most of the latex is processed at the site of production [1,2] by coagulating the colloidal rubber with acetic or formic acid, and forming it into sheets, crepe, blocks or granules which are then manufactured into rubber goods such as tyres, shoe soles, tubing and so on. This process eliminates most of the cytoplas-

mic proteins before manufacture. About 10% of *Hevea* latex is kept in the colloidal form, with ammonia added as an anti-coagulant/preservative and the rubber particles concentrated by centrifugation to around 60% dry rubber content. The latex concentrate thus contains rubber particles suspended in ammoniated cytoplasm (often with additional preservatives such as tetramethylthiuram disulphide)—and is kept in this form for at least 3–4 weeks to mature before manufacture into gloves, condoms, catheters, elastic bands, foam or other latex goods [7]. During manufacture, various other chemicals may be added such as vulcanizers (4,4'-dithiomorpholine), accelerators (e.g. tetramethylthiuram disulphide, mercaptomixtures, benzothiazoles, etc.), stabilizers and antioxidants (thiocarbamates, paraphenylene diamine and so on) some of which may be the cause of contact (type IV) sensitization.

Latex rubber gloves are made by dipping a hand-shaped former into the latex concentrate and drying, curing and leaching the film adhering to the former. The exact details of the manufacturing process will determine how much protein is trapped in the rubber. The surface of latex is self-adhesive and must be coated or dusted with talc, or in the case of surgical gloves, corn starch powder to stop the surfaces sticking together. Peptides from the *Hevea* proteins trapped during manufacture then can migrate out of the latex and become bound to the starch particles [8]. Sweat or other body secretions can leach out the peptides, which are then absorbed through intact skin or mucosae, or introduced into the body during surgery, or inhaled on the dust particles.

The amount of protein and allergen that is readily extractable from medical rubber gloves varies over 3000-fold [8,9]; powdered gloves commonly have far more available allergen, with over 10 mg of extractable protein per glove in some, much of it absorbed into the starch. These starch granules are 1–3 μ m diameter and thus respirable, explaining how they could both sensitize and provoke respiratory allergic reactions. The use of powder-free gloves can prevent these allergic symptoms [10]. A number of other approaches have been used to produce gloves with reduced allergenicity—with variable success. A problem for the natural rubber industry is the small margin between the cost of natural latex products and synthetic substitutes, which means that the cost of additional steps to reduce allergenicity has to be kept low.

The increase in type I allergic reactions to *Hevea* proteins seems to be associated with increased exposure to goods manufactured from latex concentrates rather than the other forms of rubber, which have for a long time been associated

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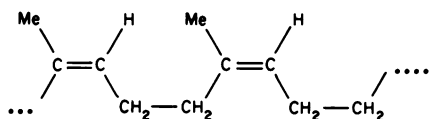


Fig. 1. The structure of rubber. Two *cis*-isoprene repeats: in *Hevea brasiliensis* rubber the molecules have a wide range of sizes, with a modal value of around 30 000 repeats.

with type IV contact sensitivity to additives such as thiurams. Though one might suppose the increase in reactions was due to the increased wearing of latex gloves due to hazards such as hepatitis B and HIV, this cannot be the only reason, as the incidence of anaphylactic sensitivity in the non-medical public also appears to have increased (though this could merely be due to increased recognition). Of 1000 volunteer blood donors 6.5% had specific IgE reactive with latex, 2.5% at a high level [11]. One possibility is that, with the increase in manufacture of latex goods in the country of origin, the storage of ammoniated latex concentrates has become much shorter, leading to less-denatured proteins in the finished article [12]. Another group where latex anaphylactic reactions have been reported is children who have multiple surgical operations—such as those with spina bifida. Rubber tappers spend every working day with their hands in contact with raw latex, with frequent minor cuts and abrasions; a recent study in Malaysia revealed positive skin prick tests to latex glove extract in one out of 42 tappers—roughly the same incidence as in the local hospital workers (three out of 119) [13].

Analysis of latex allergens has been complicated by the differences between ammoniated latex (AL) and non-ammoniated latex (NAL). There has been no standardization between studies of the length of time and conditions under which the latex has been kept following ammoniation—nor of the type of ammoniation, which may be high (0.7%) or low (0.2% with additional preservatives). Gel fractionation of NAL proteins in

one study revealed at least 30 bands, 19 of which bound IgE from latex-allergic patients [14]. Two-dimensional separation shows that the actual number of proteins in the latex is far higher and most of the bands will contain more than one protein of similar size. Conversely one protein may be present in several bands due either to polymerization or cleavage; this is supported by both analysis of the bands [15] and by the reaction of a MoAb with multiple bands [16] or amino acid composition similarity between bands [17]. The pattern of bands becomes altered and smeared to a variable extent in ammoniated latex—presumably because of denaturation and hydrolysis—but binding to specific components can still be identified. Ammoniation may unmask epitopes hidden in non-ammoniated latex [15]. Several groups [14–28] have analysed the molecular weights of allergens in latex (Table 1), but the patterns of reactivity vary greatly between the studies; as well as the large numbers of patterns, the picture is being confused by methodological differences such as the use of reduced and denatured proteins by some groups. No clear relationship has emerged between the type of symptom and allergens, but between allergens and patient group there is a suggestion that the 27-kD peptide in reduced/denatured latex may be the dominant allergen in spina bifida patients [28].

Beezhold *et al.* in their article in this issue [15] have identified peptides homologous with hevein preprotein in bands at 14 and 18 kD, and with heveamine in the 29-kD band. They also found a previously unidentified sequence in the bands at 46 and 110 kD. It remains to be proved that these peptides are in fact the allergens—as appears to be the case for the rubber elongation factor [29]. The confusion in Table 1 may be resolved when other groups are able to report on the identity rather than molecular weight of the allergens recognized by their patients. Some patients show cross-reactivity between *Hevea* latex and remotely related food allergens such as banana and chestnut [30,31]. It will be very interesting to see whether the proteins involved have sequence similarities as well as immunological cross-reactivity.

Table 1. Latex allergens reported in the literature

Reference	Sample	Method	Source and mol. wt (kD) of allergen
Carillo 1986 (18)	M	D	> 30ne
Morales 1989 (19)	O	S	10 24 35 100
Warpinski 1991 (20)	MO	S	30e 66e
Alenius 1991 (21)	M	R	11e 14ane 18n 21an 23an 26e 29ane 35an 38an 44an 53an 70n
Žacharisen 1992 (22)	M	C	≈ 30e {abstract}
Slater 1992 (23)	SB	S + R	14n(a) 20n (+ others in reduced NAL)
Jaeger 1992 (24)	MO	S	10a 14a 35–40a 67a
Chambeyron 1992 (25)	U	R	10an 15an 18an 20an 25an 30an 45an 65an 78n 100n
Mathew 1992 (26)	SB	S?	10u, 14u {abstract}
Czuppon 1993 (27)	O	CSR	58ae = tetramer of 14ae = rubber elongation factor
Alenius 1993 (28)	SBMO	R	14n 20n 27n 30n 45n 75n (+ many others)
Alenius 1994 (14)	U	R	(14 18 19 20 23 26 29 30 33 35 36 39 44 46 54 63 67 75 111)n
Slater 1994 (16)	SBM	S + R	*27n = (6 14 21 27)a
Beezhold 1994 (15)	MO	S	14n 18n 26n 29n 38n 43n 46n 55n 65–70n 90n 110n (diffuse)e

SB, Spina bifida; M, medical workers; O, others; U, unspecified; S, SDS-PAGE; R, SDS-PAGE (reduced); C, column chromatography; D, dialysis; u, unspecified; a, ammoniated; n, non-ammoniated; e, extracted from formed goods.

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