

Latex allergy from glove powder – an unintended risk with the switch from talc to cornstarch?

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The present study aimed to investigate to what extent glove powders of different origins and brands bind and release latex allergens. One mineral talc and five cornstarch powders were used. The allergenic material was sap of *Hevea brasiliensis*. The powders were incubated together with the natural rubber latex sap, and four series of experiments were performed. The talc showed the highest tendency to bind isotope-labeled proteins. When incubated with a latex allergen solution, it reduced free allergen from 100 to 2 units/ml compared to a reduction to 70–98 units/ml for the cornstarch powders. In contrast to the nonstable binding of allergen to cornstarch, the binding to talc was irreversible. The allergen bound to talc was allergenically intact and, when incubated with serum, reduced the concentration of free IgE antibody to latex to 10% of the initial level. Mineral talc had a high capacity to bind latex allergens firmly. In contrast, cornstarch captured the latex allergen, but the binding seemed less pronounced and was unstable. The replacement of talc by cornstarch as glove-donning powder has coincided with the rapid increase in latex allergy. Mineral talc is heavy and only transiently airborne. Could this difference between talc and cornstarch in latex-allergen-binding pattern and the tendency of the latter to be airborne play an important role in the bioavailability of latex allergen and thus in the epidemic of latex sensitization?

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Formerly considered innocuous, natural rubber latex (NRL) was revealed during the 1980s to be a common occupational IgE sensitizer, mainly in hospital environments. Several factors are thought to have contributed to the epidemic rise of latex sensitization (1–4), including the increased use of latex medical gloves and the use of brands with high amounts of latex allergen (LA), together with more general awareness of the condition and the development of diagnostic tools.

How far different types of LA exposure induce IgE sensitization is insufficiently understood, but exposure to airborne, latex-contaminated glove powder is thought to be a major route for primary sensitization. Cornstarch (CS) used as glove-donning powder acts as an LA carrier (5, 6), and latex-contaminated glove powder provokes adverse allergic reactions in sensitized patients (7–11) and acts as a “latex-aeroallergen” in operating theaters (12, 13).

Initially, in the manufacture of medical examination and surgical gloves, mineral talc (MT) was used as donning powder. After observation of granulomas in surgical wounds supposed to be provoked by MT, CS has, since the 1940s, replaced MT as donning powder in surgical gloves (14, 15). For medical examination gloves, the corresponding switch from MT to CS occurred considerably later, mainly during the 1980s, when medical examination gloves also became widely used in the prevention of blood-borne infections. Thus, during the 1980s, in addition to the switch from MT to CS, the growing market for medical examination gloves led to new producers and variability of quality.

We have previously shown that the common procedure of wet-powdering gloves leads to a glove powder that is contaminated with LA (16). Whether differences in LA binding to CS and MT have a significant effect on the bioavailability and sensitizing capacity of the LA has not, to our

knowledge, been analyzed. Our aim was to investigate to what extent glove powders of different origins bind and release LA. The stability of protein and allergen attachment to the powders was a main issue.

Material and methods

Design of experiments

Four series of experiments were performed to study the protein and LA-binding capacity of common glove powders and MT.

Series 1: quantification of isotope-labeled protein tracers bound to powders – isotope tracer binding

Powders of different qualities were incubated with latex sap in which isotope-labeled protein tracer was included. The residual radioactivity after washing was measured as a percentage of the total isotope activity.

The dry powders CS1 to CS5 and MT (Table 1), 0.1 g of each, were added to two series of six polystyrene tubes. To each tube in the first set of six was added a mixture of 1 ml of a 10% latex solution in physiologic saline and 3 µl of an isotope-labeled low-molecular-weight (LMW) protein. To each tube in the second set of six was added a mixture of 1 ml of a 10% latex solution in physiologic saline and 200 µl of an isotope-labeled high molecular weight (HMW) protein.

The powder suspensions were incubated for 5 min at room temperature (19–21°C) under vertical rotation before centrifugation at 1000 g for 2 min. The supernatants and the powder pellets were separated. The radioactivity of the pellets was measured in a gamma counter (1260 Multigamma II, LKB, Wallac).

The following washing procedure was repeated eight times to wash out the proteins reversibly bound to the powder. In the pellets, the first supernatant was replaced by an equal volume of 0.15 M saline, approximately 10 times the volume of the pellets. After vigorous manual shaking, this new mixture was centrifuged at 1000 g for 2 min. The supernatant was carefully removed and the remaining radioactivity of the powder pellet measured.

Series 2: indirect measurement of LA binding to and LA eluting from powders – LA capture

Six different powders were incubated in latex sap, filtered, dried, and rinsed. The amounts of LA in eluates were measured by an immunologic method, EAI inhibition. Amounts of 1 g each of the

powders CS1 to CS5 and MT (Table 1) were added to a tube containing 10 ml of a 10% latex solution in 0.15 M saline. The total LA content of the initial LA solution was measured by EAI assay and was given an arbitrary value of 100 U/ml. The latex-powder mixture was incubated under vertical rotation for 30 min at room temperature (19–21°C) and then filtered through filter paper type IF (Munktell, Sweden). This first filter eluate (eluate 1) was removed and stored at –20°C pending analyses. The powder pellet left on the filter paper was dried in an incubator at 37°C for 24 h. To release the water-soluble LA reversibly bound to the powder pellet, the dried pellet was incubated under vertical rotation with 10 ml 0.15 M saline for 30 min, followed by filtration as described above. The second eluate (eluate 2) was stored at –20°C pending analyses. All experiments were performed twice. The LA content of the two eluates was measured by the EAI assay.

Series 3: LA binding to powders measured by latex-specific IgE antibodies – LA binding

After incubation with latex sap, the different powders were used as LA solid phases in methods identifying latex-specific IgE antibodies.

The six dry powder pellets from the second eluate of the LA capture experiment were resuspended in 10 ml 0.15 M saline and used as follows. To 1 ml of each powder suspension was added 100 µl of a serum containing NRL-specific IgE antibodies, and the solution was incubated for 3 h at room temperature (19–21°C). To a second, control series, 1 ml of the same powder suspensions was mixed with 100 µl serum sample from a non-latex-allergic patient with an IgE concentration of 1000 kU/l. After incubation, the samples from the two series were washed four times with 10 ml 0.15 M saline by repeated agitation and centrifugation. A volume of 100 µl ¹²⁵I-labeled anti-human IgE (Pharmacia & Upjohn Diagnostics, Uppsala, Sweden) was added to each sample and incubated overnight at room temperature (19–21°C). The samples were again washed and centrifuged four times with saline as described above. The radioactivity of the powder pellet was then measured in a gamma-counter (1260 Multigamma II LKB, Wallac) and expressed in radioactive counts/min (cpm).

Series 4: LA binding to MT measured by IgE antibodies specific to latex – MT LA binding

In this experiment, the capacity of LA-loaded MT to remove LA-specific IgE antibodies from patient sera was measured. A serum sample with IgE antibodies specific to LA was dispensed in four

Table 1. Characteristics of powders examined

No	Powder	Characteristics		Trade name	Manufacturer
MT	Mineral talc	SiO ₂ : 46%, Al ₂ O ₃ : 0.4% Fe ₂ O ₃ : 1.1%, FeO: 3% CaO: 2.2%, MgO: 32%	pH 8.5	Talc FM20®	Herkules Kemiska AB
CS1	Cornstarch	Virgin	pH 5	Melojel®	Johnson & Johnson
CS2		Epichlorhydrine-treated	pH 6	Vulca 90®	Johnson & Johnson
CS3		Epichlorhydrine-treated	pH 10	Absorbo HP® (Biosorb)®	Johnson & Johnson
CS4		Epichlorhydrine-treated	pH 10.6	Emsorb®	Not known
CS5		Epichlorhydrine-treated	pH 10	Agenasorb®	Agrana Stärke GmbH
		MgO < 2%			
		MgO < 2%			

tubes (1 ml/tube). Untreated, virgin MT, 0.1 and 1 g, respectively, was added to tubes 1 and 2. LA-incubated MT from experiment 3, 0.1 and 1 g, respectively, was added to tubes 3 and 4. After incubation overnight at room temperature (19–21°C), the samples were centrifuged at 1000 g for 5 min. The concentration of IgE antibodies remaining in the supernatants of the four tubes was measured by Pharmacia CAP System FEIA (Pharmacia & Upjohn Diagnostics, Uppsala, Sweden) k82 latex ImmunoCAP according to the manufacturer’s recommendation and compared to that of the initial serum sample without MT. The results were expressed in fluorescence units (FU).

Material

Powders. The origin and some properties of the MT and the different CS used are listed in Table 1.

Latex allergens. The NRL used was sap of *Hevea brasiliensis* collected in Thailand directly from the trees into 0.15 M saline containing 0.02% NaN₃ without ammonia. When ultracentrifuged at 100 000 g for 60 min, the material separated into three phases of which the middle one, the serum phase (C-serum, cytosol), was isolated and used. The protein concentration of the preparation used was 1.8 mg/ml, measured by the modified Lowry method (17).

The allergenic proteins were characterized by SDS–PAGE and immunoblotting using sera from patients clinically allergic to latex. The latex sap included the major LAs in a pattern corresponding well to previously presented immunoblot studies (18, 19).

Isotope-labeled protein tracers: nonlatex proteins. Two non-latex-related proteins were ¹²⁵I-labeled and used as model substances for how LA of corresponding sizes may be captured by the powder.

The LMW marker was β-lactoglobulin, 20 kDa, at a protein concentration of 10 µg/ml (30 ng/sample). The chloramine T iodination method (20) was used to label the LMW proteins to a high specific activity (0.8 MBq/µg). The HMW marker, 150 kDa, was rabbit IgG with anti-human-IgE specificity (Pharmacia & Upjohn Diagnostics, Uppsala, Sweden), used at a concentration of 30 ng/sample (0.3 MBq/µg).

Serum. Four sera with IgE antibodies specific to NRL and clinically documented allergy to NRL were used. The patients were selected as being positive in SDS–PAGE and IgE immunoblotting to all major LA components. One serum sample with monoclonal IgE with no known antibody activity was diluted in normal serum to 1000 kU/l and used as negative control.

Methods

Latex IgE antibody inhibition test (EAI assay). RAST inhibition was used to measure the allergen concentration of latex solutions (16, 21). In this method, LA block latex IgE antibodies, resulting in a reduction in the level of remaining free IgE antibodies, as measured by the Pharmacia CAP System (Pharmacia & Upjohn Diagnostics, Uppsala, Sweden).

The serum pool was incubated with five serial dilutions of each latex extract, followed by a standardized amount of IgE antibodies to latex. Good parallelism between different samples was obtained. The allergen concentration was determined by comparing the results to an LA reference at 50% inhibition, and was expressed in units/ml (U/ml). The LA reference consisted of sap of *H. brasiliensis* without ammonia, diluted in 0.15 M saline and given an arbitrary value 1000 U/ml. With this method, 0.03 U/ml (1 SD=0.007 U/ml) of LA was accurately quantified (50% inhibition) and detected down to 0.02 U/ml (30% inhibition).

Results

Isotope tracer binding

After the first two washings, the percentage of bound isotope activity remained stable. MT showed the highest tendency to bind the two isotope-labeled proteins. After seven washings, the remaining activity of the LMW isotope was 45–60% for CS1, CS3, and MT, with MT binding most isotope. CS2, CS4, and CS5 showed a remaining activity of 2–8% (Fig. 1).

Corresponding figures for the HMW isotope were generally lower. MT showed remaining activity of 20% and CS1 to CS5 less than 2% (Fig. 2).

LA capture

The remaining concentrations of LA expressed in EAI units/ml, in a saline solution after 30-min incubation with the five different powders, are shown in Fig. 3A. After incubation with MT, the concentration of LA was reduced from 100 to 2 units/ml. The corresponding mean reduction in LA levels in solutions incubated with different CS qualities varied between 2 and 30 units/ml (Fig. 3A).

The amount of LA released into a second eluate from dried incubated powders ranged from <0.1 units/ml for the MT and 2–5 units/ml for the CS qualities (Fig. 3B).

LA binding

The specific and unspecific IgE-binding capacity of powders incubated with LA and thereafter rinsed was generally low for starch-based powders (Table 2). Only one powder (CS1) showed a higher binding of anti-IgE when the patient serum contained IgE antibodies to LA. High percentages of latex-specific and unspecific IgE were bound to LA-incubated MT; approximately 17% in both cases (Table 2).

MT LA binding

When a serum with a known level of specific IgE antibodies was incubated with virgin and LA-incubated MT, the latter reduced the concentration of specific IgE antibodies to latex to approx. 10% of the original level. Addition of virgin MT without LA did not bind any significant amounts of IgE antibodies to LA (Fig. 4).

Discussion

In diagnosing IgE-mediated allergy by skin prick testing, it is a common experience that allergenic,

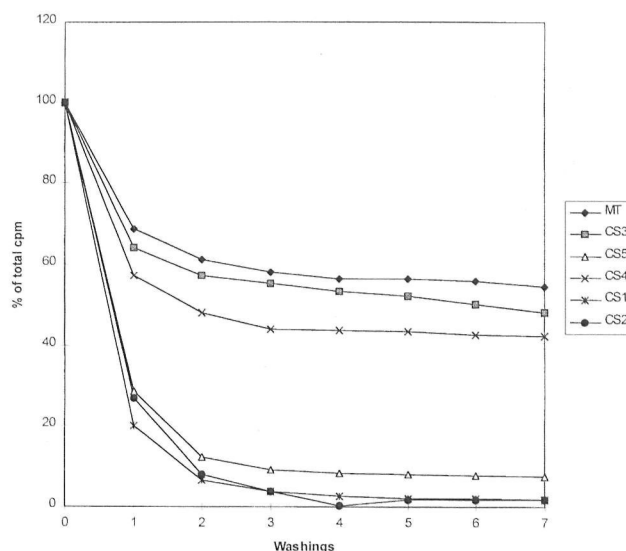


Fig. 1. Residual LMW isotope activity in different powders after washing, expressed as percentage of total initial counts/min (cpm).

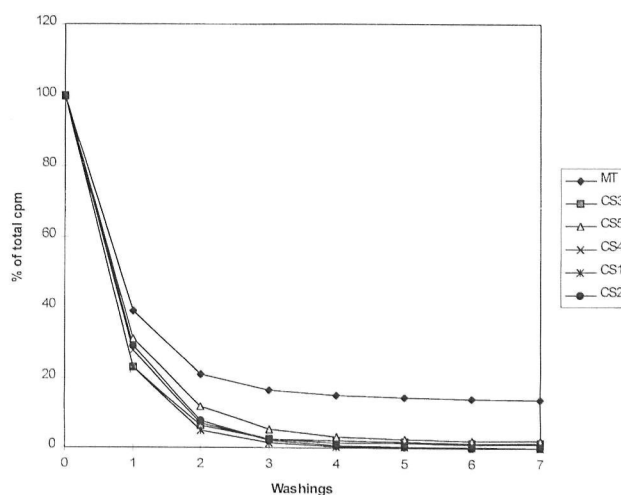


Fig. 2. Residual HMW isotope activity in different powders after washing, expressed as percentage of total initial counts/min (cpm).

water-soluble proteins at low concentrations must be kept in albumin diluent to prevent their adherence to plastic and glass surfaces. This knowledge has also been used for practical purposes such as adherence of proteins, e.g., antibodies, to glass beads in different laboratory methods (22). The main results in our series of experiments indicate that also the surface of MT can attract proteins in general as well as allergenic proteins in a corresponding way. We also found that different brands of glove-donning CS behaved differently among themselves but especially differently from MT in binding and releasing of LA.

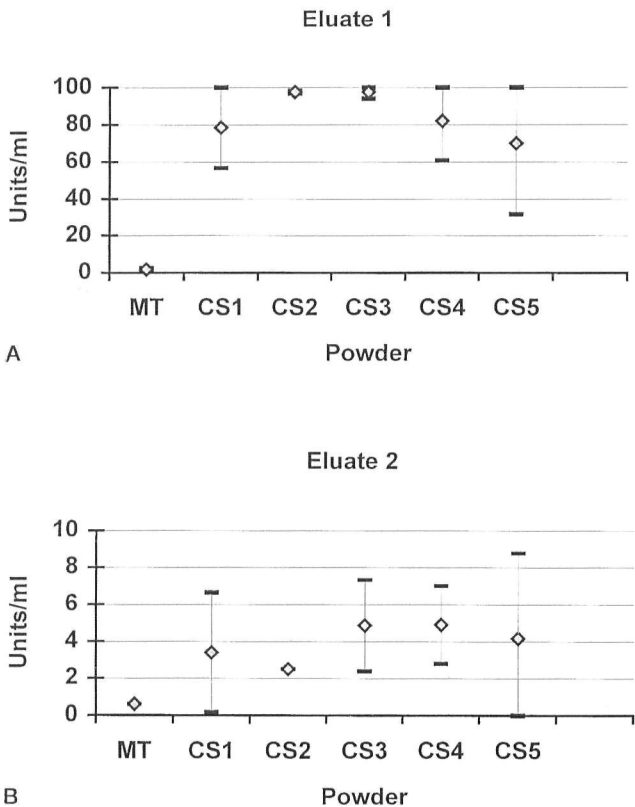


Fig. 3. A) Remaining levels of LA concentration (initially 100 units/ml) in saline solution after 30-min incubation with different powders, expressed in EAI units/ml (eluate 1). B) Amounts of LA carried by different dried powders and released into second water volume: LA concentration expressed in EAI units/ml (eluate 2). Diamonds represent mean value and bars include $\pm$ standard deviation (SD) in two runs.

Latex proteins, including the allergenic proteins, may be chemically bound, or just adsorbed to different donning powders, such as MT and CS. The process may be reversible or irreversible. Proteins of different molecular sizes may also show different binding patterns.

MT shows irregular form with dimensions extending down to submicrometer sizes in contrast to CS, which is spheric with a diameter of approximately 15 μ m (23). MT consists of hydrous magnesium silicate, calcium magnesium carbonate, calcium magnesium silicate, and related minerals in various proportions. CS, on the other hand, consists of a mixture of amylose and amylopectose with the polymer chains cross-linked by 1,3-dietheryglycerin groups. To improve the lubricating properties of CS used in glove donning, it may be treated chemically; e.g., with etherification and epichlorohydrine.

As the first step to elucidate the protein binding to powders, we used a quantitative method to measure how much two non-latex isotope-labeled proteins of various molecular sizes bound to virgin starch of different commercial CS qualities and to

Table 2. IgE-binding capacity of powder incubated with LA and then rinsed. Serum with IgE antibodies to LA was compared with control serum from nonallergic subject. This serum was spiked with myeloma IgE to 1000 kU/l. Results were expressed in percent cpm of added radiolabeled anti-IgE tracer activity (%T)

LA-loaded powder	Serum with IgE Ab specific to latex % T	IgE-spiked control serum % T
MT	17.3	17.4
CS1	1.7	0.3
CS2	0.6	0.3
CS3	0.7	1.5
CS4	3.0	3.8
CS5	0.4	0.3

MT. The molecular sizes of LA are in the range 4–100 kDa (18), the major allergens being of 14–36 kDa. In our studies, we chose a LMW protein of 20 kDa and a HMW protein of 150 kDa. The LMW protein showed a higher tendency than the HMW to bind to several of the powders tested. For both tracers, the bound activity remained after two washings, and showed the highest binding to MT. Since we do not know whether the tracer proteins behave in the same way as allergenic proteins, no definitive conclusion can be drawn on the condition for LA binding to powders. However, from our data, it can be expected that different LA behave differently.

The second series of experiments aimed at elucidating how LA behave in contact with CS and MT. The LA capture experiment was designed to mimic the way glove powders are exposed to soluble LA in the wet-powdering process (16), and the ensuing drying, storage, and re-exposure to humidity upon human contact. The sensitive EAI-inhibition technique was used to measure the amounts of free LA. In this test, MT showed a remarkable capacity to withdraw LA from the LA solution. A corresponding activity was also shown by the different CS qualities but to a much lower extent. In the second part of this experiment, which mimicked LA release from dry powders of used gloves upon contact with the mucous membranes of the respiratory tract, remarkable differences were seen. Although the MT had bound a high proportion of the LA from the initial solution, it did not release LA but seemed to hold the allergen captured. In contrast, the CS bound only small amounts of the initial LA, but these were easily released into a water solution.

In the third series of experiments, it was shown that neither MT nor CS functioned as a solid phase in an IgE-detecting, RAST-like assay. MT showed a high proportion of unspecified binding, and the LA binding to CS was very unstable. A previous

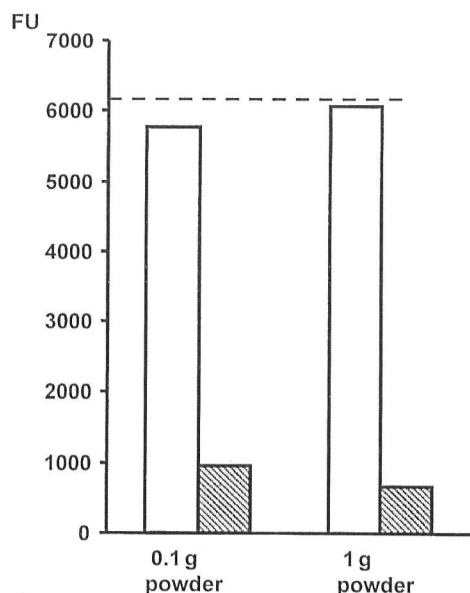


Fig. 4. Levels of free latex-specific IgE antibodies in patient serum after treatment with virgin MT (open bars) and latex-incubated MT (hatched bars) as compared to untreated serum (-----), expressed in fluorescence units (FU).

study described CS obtained directly from latex gloves as functioning as a solid-phase "allergo-sorbent" (5). In our study, these findings could not be reproduced, although CS1 had a tendency to specific binding of IgE antibody albeit at a very low level (1.7%).

In several assays for IgE antibodies such as RAST, the allergens are covalently coupled to a solid phase to avoid allergen leakage. Epichlorohydrine activation is one way to promote the binding of the proteins to the surfaces. In our study, however, even the epichlorohydrine-treated CS did not show results comparable with those described in the previous report (5).

The last series of experiments was performed to investigate how far LA adhering to MT was still antigenic and how the LA could attract and bind latex-specific IgE antibodies. Virgin MT only marginally reduced the amount of LA-specific IgE antibodies when incubated in patient serum. In sharp contrast, LA-loaded MT reduced the level of LA-specific IgE antibodies in the corresponding serum to less than 10% of the initial value. Thus, the expression of the allergenicity remains in the LA bound to MT.

Several studies have shown that the allergen content in cornstarch-powdered surgical and medical gloves is higher than in unpowdered brands (24, 25). The demonstrated allergens may come from the glove material itself, but may also be released from the powder, a possibility not studied. In glove manufacture, both the powdering method and the

choice of powder may be of crucial importance for how far the powder may act as an allergen carrier (16).

The demonstrated differences between MT and CS in their way of binding allergenic proteins are of both theoretic and practical interest. LA adheres irreversibly to MT and is no longer releasable in water solution, and this may have a crucial impact on its bioavailability. Even if the LA adsorbed to MT is antigenic/allergenic, it might not be immunogenic even if, in principle, it could be. MT, compared to CS, is also heavy and may not easily become airborne (23). These factors should affect the capacity to cause sensitization. Further studies of this issue are needed. Although of principal interest, immunogenicity, defined as initiation of an IgE antibody response, is very difficult to study due to lack of a suitable animal models. How far these findings shed light upon the epidemic rise of latex sensitization in the 1980s is an open question. Did MT act as an efficient allergen eliminator? It is interesting to remember that the increase in latex allergy is synchronous with the change from MT to CS as a donning powder in medical examination gloves.

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