

Environmental and occupational disorders

In situ localization of latex allergens in 3 different brands of latex gloves by means of immunogold field emission scanning and transmission electron microscopy

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Background: Latex proteins represent relevant allergens, particularly for those persons who are frequently exposed to latex products (eg, health care workers and patients with chronic disorders). Although several latex allergens have been characterized by biochemical and molecular biologic techniques, little information is available concerning the in situ localization of allergenic proteins in latex products.

Objective: The objective of the present study was the in situ localization of latex allergens.

Methods: Serum IgE from patients with latex allergy reacting with a broad range (5-200 kD) of latex allergens was used for the in situ localization of latex allergens. One surgical and 2 examination latex glove brands were investigated by using immunogold field emission scanning and transmission electron microscopy.

Results: Allergens were detected on the inner and outer surface of the gloves, particularly near the edges, crests, or folds of the bleb-like structures visible on the surface of the latex material at high magnifications. In ultrathin cross-sections, latex allergens were found throughout the sections.

Conclusions: Latex allergens were localized on the outer and inner surface but also in the interior of latex gloves. The occurrence of latex allergens on the surface of latex products may be related to their potential to induce local reactions and, perhaps, to sensitize individuals by means of contact. (*J Allergy Clin Immunol* 2000;105:561-9.)

Key words: Latex allergens, in situ localization, immunogold electron microscopy, latex-specific human IgE, surgical glove, examination glove

Abbreviations used

FESEM: Field emission scanning electron microscopy

TEM: Transmission electron microscopy

IgE-mediated reactions to latex have become a health problem of increasing importance.¹ Risk groups are persons frequently in contact with latex products (eg, latex gloves), such as medical staff,^{2,3} greenhouse workers,⁴ or patients with chronic disorders, such as spina bifida, who undergo multiple surgical procedures.^{5,6}

Considerable effort has been put toward the characterization of the various allergenic proteins or glycoproteins that occur in natural rubber latex and in manufactured latex products.⁷ Many latex allergens contain IgE epitopes that are cross-reactive with fruit allergens (bananas, avocados, and kiwi)⁸⁻¹⁰ or with grass and weed pollen allergens.¹¹

Although latex allergens have been well characterized by means of immunochemical and molecular biologic techniques, little is known regarding their in situ localization in the finished latex products. Immunogold electron microscopy has proven to be a valuable technique for the detection of allergens in pollen.¹² It was found that a correct in situ localization of pollen allergens crucially depends on the application of anhydrous fixation techniques, which prevent dislocation of highly mobile water-soluble allergens during the fixation process.¹²

Here we study the in situ localization of latex allergens by means of immunogold electron microscopy in surgical and examination gloves. Latex allergens were localized by field emission scanning and transmission electron microscopy by using serum IgE from individuals with latex allergy, anti-human IgE antibodies, and colloidal gold particle-conjugated tertiary antibodies.

The results of the in situ localization of allergens in latex gloves are reported, and a possible association of the surface localization of latex allergens with their capacity to elicit local symptoms of type I allergy and perhaps to sensitize through contact is discussed.

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TABLE I. Clinical and serologic characterization of patients with latex allergy (P1 and P2) and control patients (P3, N1, and N2)

Patient	Latex CAP (kUA/L)	Total IgE (kU/L)	Further sensitization, CAP (kUA/L)	Skin prick test to latex extract	Clinical symptoms
P1	3.6	72.5	Major birch pollen allergen (Bet v 1): 28.3	Positive	Dermatitis
P2	1.49	388	Birch: 24.0; timothy grass: 2.33; mugwort: 0.39; horse dander: 8.29; <i>Dermatophagoides pteronyssinus</i> : 0.62; <i>Cladosporium herbarum</i> : 0.98	Positive	Anaphylactic shock, angioedema, seasonal rhinoconjunctivitis
P3	0	204	Alder: 1.6; hazelnut: 0.9; apple: 7.26; celery: 3.08; <i>Dermatophagoides pteronyssinus</i> : 1.84	—	Seasonal rhinoconjunctivitis
N1	0	60	None	Negative	None
N2	0	40	None	Negative	None

METHODS

Latex gloves and extracts

Glove brand 1, an examination glove (Sempermed EXAM, powder: cornstarch), was obtained from Semperit Technical Products (Vienna, Austria). Glove brand 2, a surgical glove (Reference OP Anatom, powder: cornstarch) came from Hermes Meditrade (Kufstein, Austria). Glove brand 3, another examination glove (Ammatex examination, powder: mineral talc) was supplied by Ammatex manufacturing (Seremban, Malaysia). Total glove extracts were obtained by extracting cut glove pieces in distilled water containing 1 mmol/l phenylsulfonyl fluoride for 1 hour at 37°C as described.¹¹ Separate extracts from the outer or inner glove surface were prepared by extracting outer or inner surfaces of gloves that had been sealed at the proximal wrist. Extracts were centrifuged at 100g for 30 minutes, lyophilized, and redissolved in PBS.

Sera and antibodies

Sera were obtained from health care workers with latex allergy, from nonallergic health care workers (used as controls), and additionally from a patient with oral allergy syndrome without latex allergy. The clinical and serologic characterization of all sera used is given in Table I. Rabbit anti-human IgE antisera were purchased from DAKO (Glostrup, Denmark) and Nordic (Tilburg, The Netherlands), respectively. Goat anti-rabbit IgG antibodies coupled to 10 nm colloidal gold particles were obtained from BritishBioCell (Plano, Wetzlar, Germany).

SDS-PAGE and IgE immunoblotting

Approximately 100 µg/cm latex glove extract was separated by preparative 12.5% SDS-PAGE.¹³ Proteins were transferred to nitrocellulose by using electroblotting.¹⁴ Nitrocellulose strips were cut from the membranes and blocked for 5 minutes twice and for 30 minutes once in buffer A (50 mmol/L Naphosphate [pH 7.5], 0.5% vol/vol Tween-20, 0.5% wt/vol BSA, and 0.05% wt/vol Na₂S₂O₃). Strips were then incubated overnight at 4°C at a 1:10 concentration in buffer A–diluted serum from patients with latex allergy (patients P1 and P2),

in diluted serum from control individuals (patients P3, N1, and N2), or with buffer alone. Strips were washed, and bound IgE was detected at a 1:10 concentration in buffer A–diluted iodine 125–labeled anti-human IgE antibodies (RAST; Pharmacia, Uppsala, Sweden). After washing in buffer A, strips were exposed to Kodak XOMAT films and intensifying screens (Kodak, Heidelberg, Germany).

Fixation, cryosectioning, and labeling for electron microscopy

Small pieces (approximately 4 × 6 mm in size) were dissected from the thumb of each of the 3 latex glove brands and fixed in acrolein vapor (Riedel-deHaen, Seelze, Germany) for 7 days. Ultrathin cryosections were cut on a Reichert FCS cryoultramicrotome (Leica, Bensheim, Germany). Fixed pieces of latex gloves or ultrathin cryosections (approximately 100 nm thick) of latex gloves on nickel grids were incubated at ambient temperatures with the following solutions (PBS buffer, pH 7.4; TRIS-BSA buffer, pH 8.2): (1) 5% BSA in PBS for 15 minutes, (2) undiluted serum from a patient with latex allergy (patients P1 and P2) for 2 hours, (3) PBS 3× for 5 minutes, (4) rabbit anti-human IgE antibodies diluted 1:20 in 0.1 mol/L NaCl plus 0.015 mol/L Na₂S₂O₃ for 2 hours, (5) PBS 3× for 5 minutes, (6) goat anti-rabbit IgG antibodies coupled to 10 nm colloidal gold particles diluted 1:20 in TRIS-BSA for 1 hour, (7) TRIS-BSA 2× for 5 minutes, and (8) distilled water 1× for 5 minutes.

Controls were as follows: (1) serum from the patient with latex allergy (patient P1 or P2) was omitted; (2) serum from an individual with latex allergy (patient P1 or P2) was replaced by serum from a nonallergic individual (patient N1 or N2); and (3) glove samples were incubated with the gold-coupled antibody alone.

Dehydration, drying, and coating for FESEM

Samples were dehydrated in ethanol. Some specimens were then critical point–dried from liquid CO₂, and others were air-dried from absolute ethanol. The samples were mounted on specimen stubs and coated with a platinum-carbon film of about 1.5 nm in thickness in a BAF 300 apparatus (Balzers, Principality of Liechtenstein).

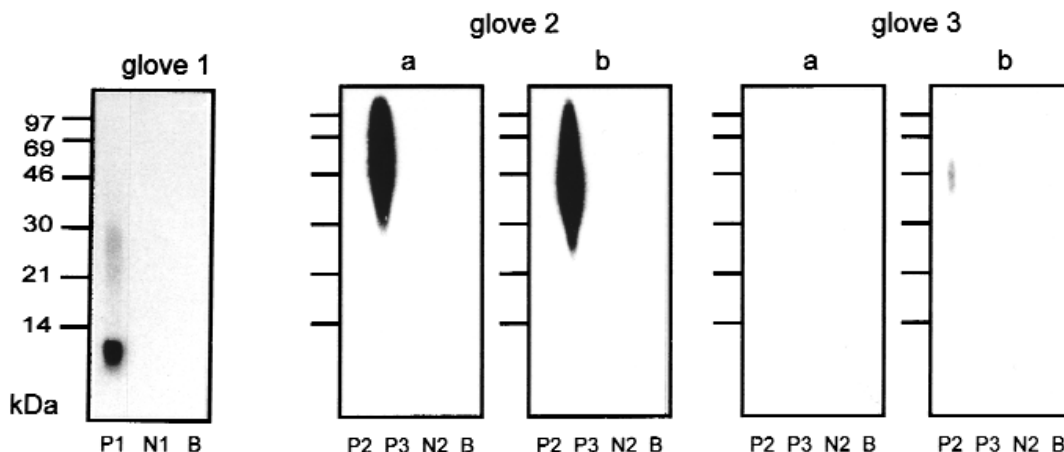


FIG 1. Allergen profile detected by sera from individuals with latex allergy in nitrocellulose-blotted latex extract. Total extract from glove 1 and extracts from the inner (a) and outer (b) surfaces of gloves 2 and 3 were separated by SDS-PAGE, blotted onto nitrocellulose, and incubated with sera from patients with latex allergy (P1, P2), serum from an allergic patient without latex allergy (P3), sera from nonatopic individuals (N1, N2), or buffer without addition of serum (B). Bound IgE antibodies were detected with iodine 125-labeled anti-human IgE. Molecular weights are indicated in kilodaltons in the left margin.

Electron microscopy and statistical evaluation

Field emission scanning electron microscopy (FESEM) was performed at room temperature on an in-lens field emission scanning electron microscope S-5000 (Hitachi Ltd, Japan) with secondary electrons and backscattered electrons at 10 kV. Transmission electron microscopy (TEM) was carried out on a Hitachi H500 transmission electron microscope (Hitachi Ltd, Japan) operated at 125 kV. Micrographs were taken by using Agfapan 100 films (FESEM) and Agfa-Gevaert 23D56 films (TEM). For quantitative evaluation, approximately 30 backscattered electron micrographs were recorded for each experiment at a magnification of $\times 30,000$. The number of gold particles in each backscatter electron micrograph was related to its corresponding sample area of $3.8455 \mu\text{m}^2$, giving the local mean labeling density.

RESULTS

Allergen profiles detected by sera used for in situ localization of latex allergens

The different sera used for the in situ localization of latex allergens (Table I) showed the following reactivities in nitrocellulose-blotted aqueous extracts of latex gloves. Serum P1 reacted strongly with a moiety of 5 to 10 kd and weakly between 20 and 30 kd (Fig 1, *glove 1*, P1). The serum from a nonatopic individual (N1), although containing comparable levels of total serum IgE failed to display IgE reactivity (Fig 1, *glove 1*, N1). Serum P2 showed strong IgE reactivity with high molecular weight latex allergens ranging from 30 to 200 kd in extracts obtained from the inner and outer surfaces of glove 2 and weaker IgE binding to a 46-kd band in the extract prepared from the inner side of glove 3 (Fig 1, *gloves 2* and 3, P2). Serum P3 did not exhibit IgE binding to blotted glove extracts (Fig 1, *gloves 1*, 2, and 3, P3). Likewise, no reactivity was observed when blotted extracts from gloves 2 and 3 were incubated with a serum from another nonatopic individual (Fig 1, *gloves 2* and 3, N2) or

when buffer without addition of serum was used (Fig 1, *gloves 1*, 2, and 3, B).

Survey of localization experiments

Serum P1 was used for allergen localization in glove 1 (Fig 2, inner surface). Serum P2 was used also in glove 1 (Fig 3, inner surface) and in gloves 2 and 3 (Figs 4 and 5, inner and outer surfaces). Serum P2 was also used for localizing allergens in ultrathin sections of glove 2 (Fig 6). Serum N1, showing similar amounts of total IgE antibodies to P1, was used for a control in glove 1 (Fig 2), whereas N2 was used in all other experiments (Figs 3, 4, 5, and 6). A statistical evaluation of the labeling density was performed in glove 1 (inner surface) by using sera P1/N1 and P2/N2 (Fig 7).

In situ localization of latex allergens by FESEM

We chose glove 1 (inner surface) for carrying out a detailed analysis of allergen mapping on different morphologic surface sites of the latex material and combined it with a statistical evaluation. The second examination glove (glove 3) and the surgical glove (glove 2) were used for an overall inspection of gold particle binding.

At low magnification (Fig 2, A), the inner surface of latex glove 1 is reminiscent of cracked soil, presenting many irregularly formed *clods* (x) separated from one another by *ditches* (arrowheads). No powder particles are visible. As shown in previous experiments, this morphology was not influenced by fixation because in all gloves investigated, unfixed samples showed essentially the same morphologic structures as the fixed ones. Fixation, however, was found to be crucial for detection of water-soluble allergens. Fig 2, B, shows an area, such as that indicated by the rectangle in Fig 2, A, at a higher magnification. Beside the ditch, several minor fissures

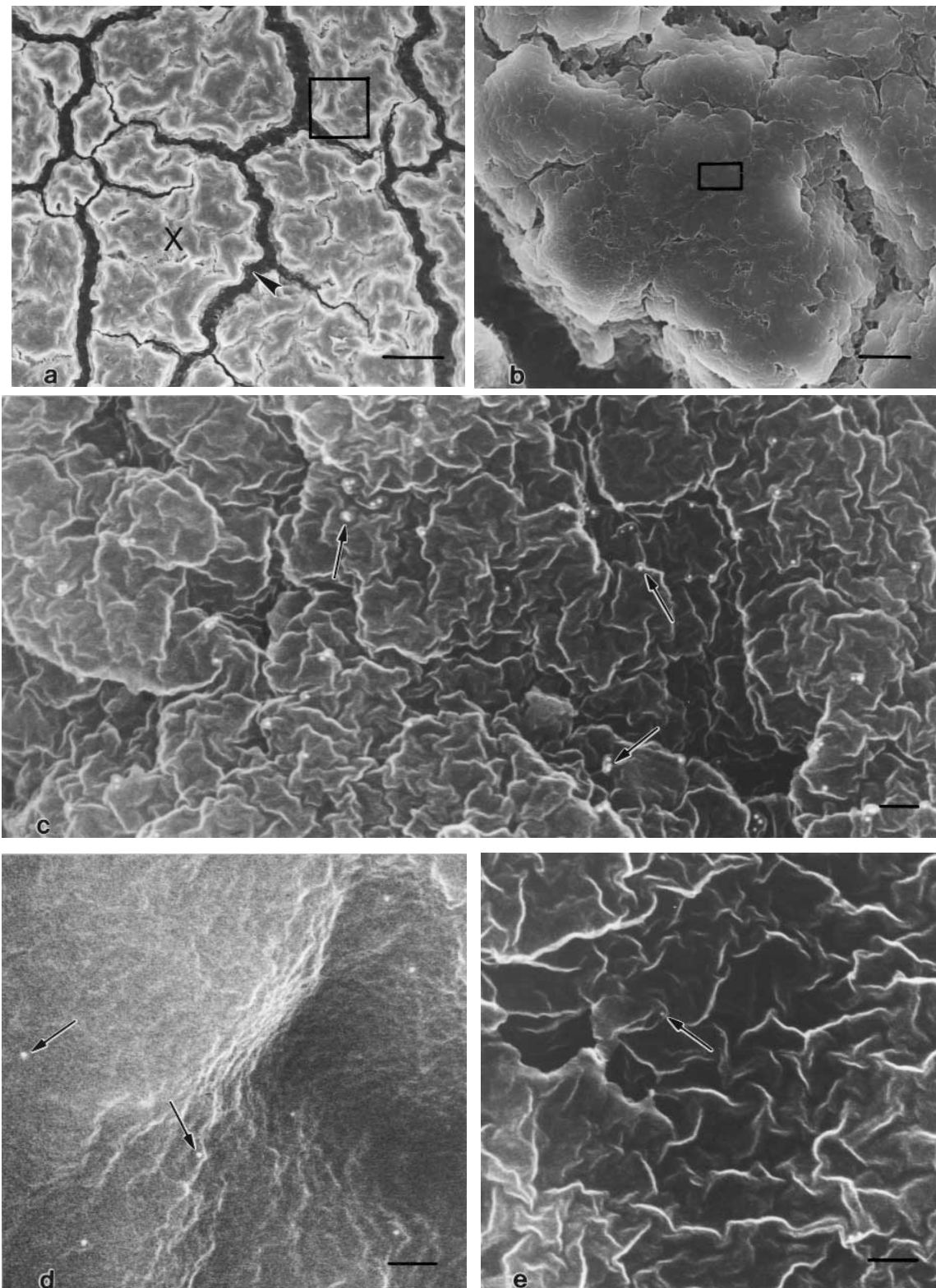


FIG 2. FESEM micrographs of the inside of latex glove 1 (examination brand) after immunogold labeling with serum P1 and control serum N1, respectively, and critical point drying. **a**, Survey of the surface. Flat clods (x) are separated by deep ditches (black arrowheads) (magnification, $\times 520$; bar = $20\ \mu\text{m}$). **b**, Medium magnification of an area, such as that indicated by the rectangle in panel **a** (magnification, $\times 4500$; bar = $2\ \mu\text{m}$). **c**, High magnification of an area, such as that indicated by the rectangle in panel **b** after immunogold labeling for latex allergens. Gold particles appear as white dots (arrows) (magnification, $\times 66,000$; bar = $0.1\ \mu\text{m}$). **d**, Bottom of a ditch (magnification, $\times 78,500$; bar = $0.1\ \mu\text{m}$). **e**, Control experiment (magnification, $\times 80,000$; bar = $0.1\ \mu\text{m}$).

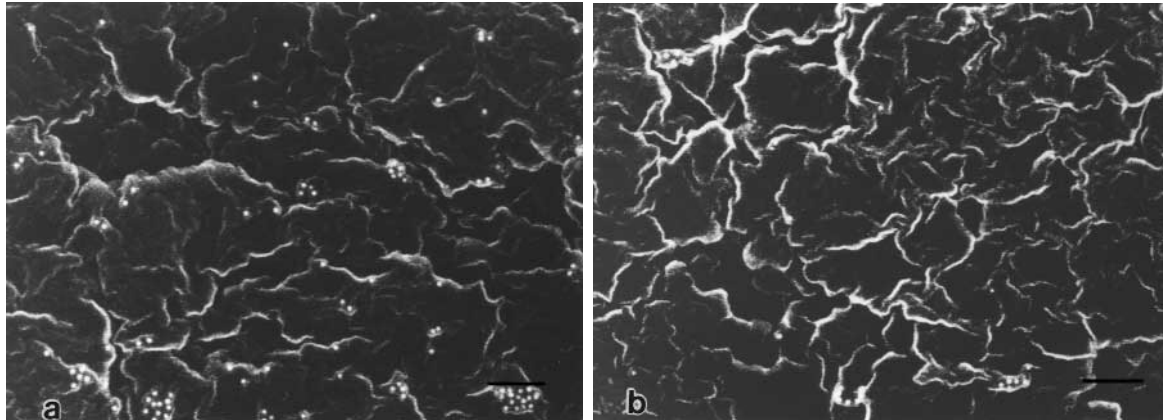


FIG 3. FESEM micrographs of the inside of latex glove 1 (examination brand) after immunogold labeling with serum P2 and control serum N2, respectively, and air drying from ethanol. Although there is intense labeling of the clods in the test experiment (**a**; magnification, $\times 76,000$; bar = $0.1\ \mu\text{m}$), the control shows few gold particles (**b**; magnification, $\times 80,000$; bar = $0.1\ \mu\text{m}$).

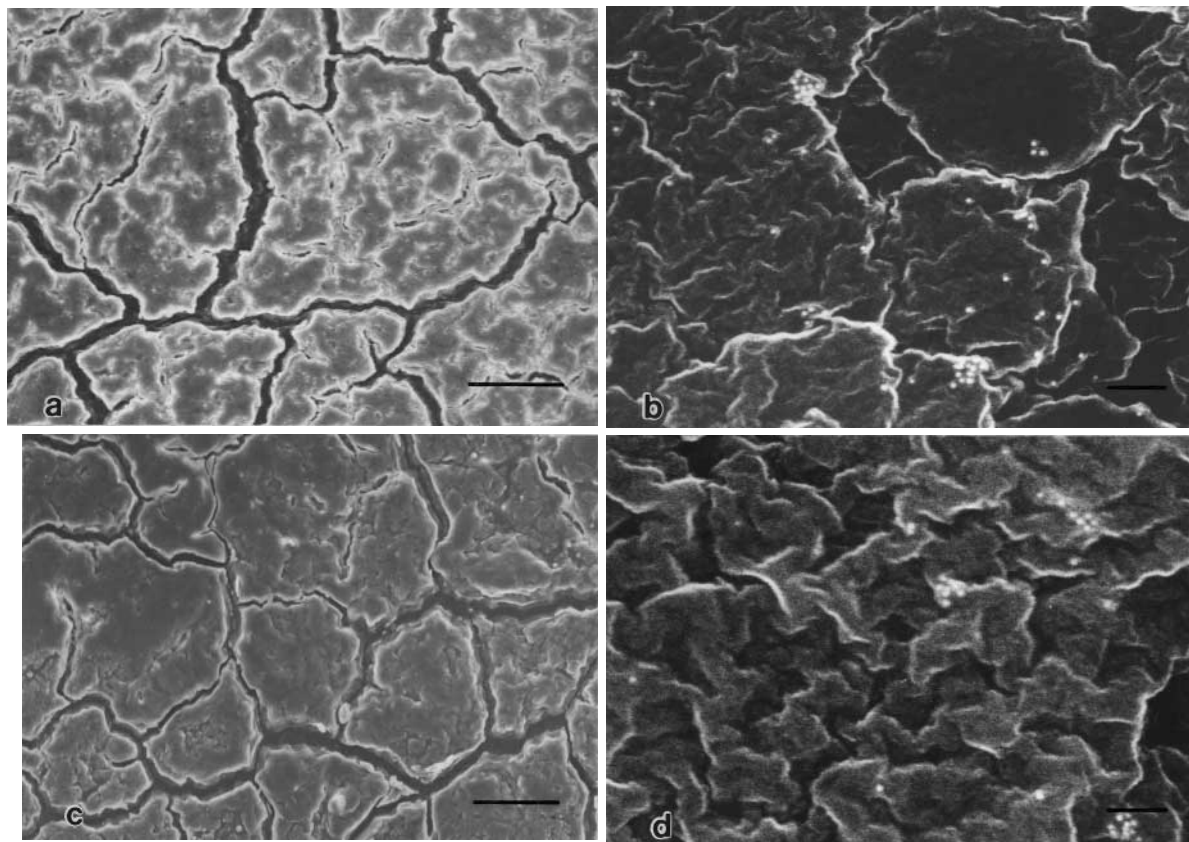


FIG 4. FESEM micrographs of latex glove 2 (surgical brand) after immunogold labeling with serum P2 and air-drying from ethanol. Panels **a** and **b** show the inner side of the glove, and panels **c** and **d** show the outer side of the glove. Panels **a** and **c** show the morphologic survey of surface structure, and panels **b** and **d** show high-magnification micrographs of a clod showing gold particle distribution (magnification: **a**, $\times 625$; **b**, $\times 75,000$; **c**, $\times 575$; **d**, $\times 80,000$; bar = $20\ \mu\text{m}$ (**a** and **c**) and $0.1\ \mu\text{m}$ (**b** and **d**)).

and crevices are visible. At a still higher magnification (Fig 2, C), the seemingly smooth areas between the crevices (indicated by the rectangle in Fig 2, B) reveal a highly structured morphology (lots of smaller and bigger irregularly shaped bleb-like structures).

After immunogold labeling by using serum P1, gold particles are found single or in doublets and triplets on the surface of the blebs, preferentially near edges, crests, or folds (Fig 2, C, *bright dots*). In contrast to this finding, the ditches (Fig 2, A) present a comparatively smooth

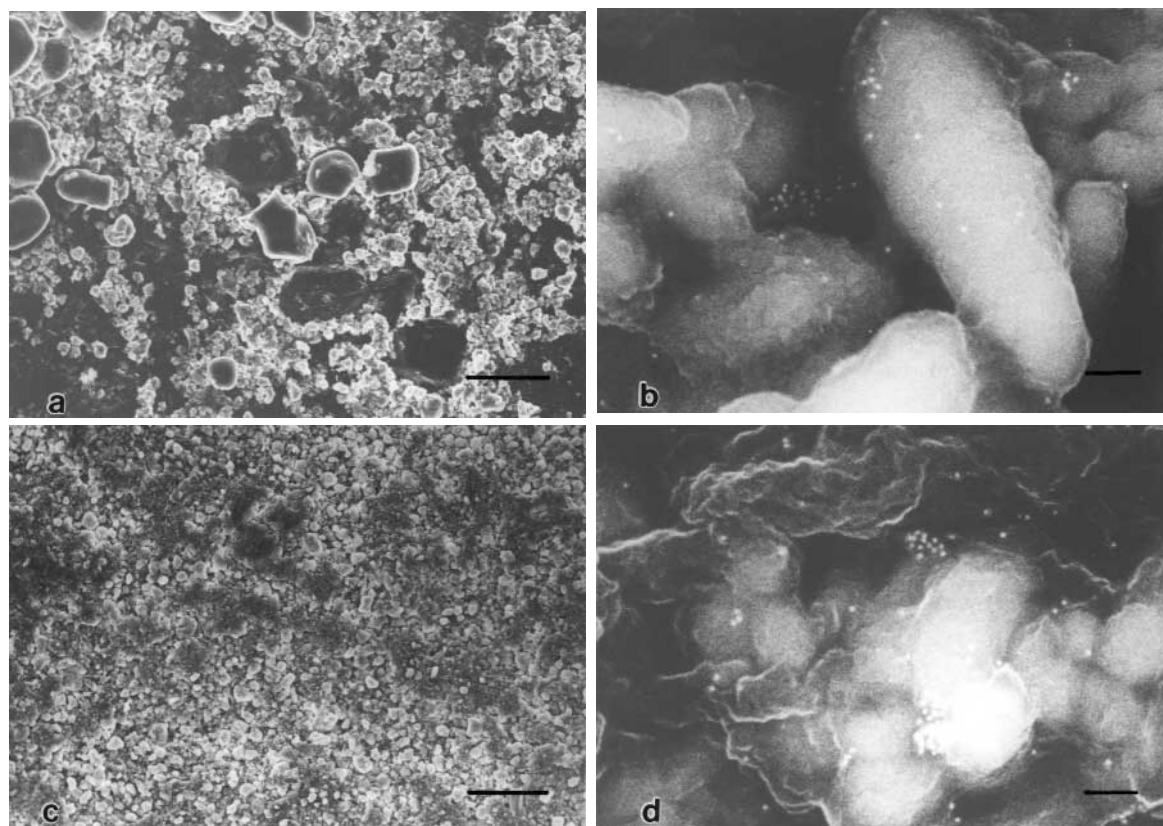


FIG 5. FESEM micrographs of latex glove 3 (examination brand) after immunogold labeling with serum P2 and air-drying from ethanol. Panels **a** and **b** show the inner side of the glove, and panels **c** and **d** show the outer side of the glove. Panels **a** and **c** show the morphologic survey of surface structure, and panels **b** and **d** present high-magnification micrographs of a clod showing distribution of gold particles (magnification: **a**, $\times 545$; **b**, $\times 75,000$; **c**, $\times 535$; **d**, $\times 75,000$; bar = 20 μm (**a** and **c**) and 0.1 μm (**b** and **d**)).

aspect (Fig 2, D). Gold particles are distributed randomly over the surface.

Areas for gold particle counting were chosen from the clods (Fig 2, C) and from the bottom of the ditches (Fig 2, D). In control specimens only a few gold particles are found in all sites analyzed (ie, clods and ditches). The control micrograph in Fig 2, E, shows the typical surface of a clod after incubation with control serum N1. Quantitative data were collected from this site only.

The shown findings (Fig 2) were confirmed by further examination of glove 1 with serum IgE from a different patient with latex allergy (P2) and another control serum (N2). The labeling pattern and a control are shown in Fig 3, A and B.

For both experiments (Figs 2 and 3), the results of the statistical evaluation are presented in Fig 7. The mean densities of the 2 morphologic sites (clods and ditches) showed large differences. The highest densities of about 18 gold particles and 30 gold particles, respectively, were found on the clods (clod, P1 and P2) followed by the ditches with about 5 and 13 gold particles, respectively (ditch, P1 and P2). In contrast to this, the controls (clods) were characterized by a mean density of approximately 2.5 gold particles and 5 gold particles, respectively (control, N1 and

N2). By using the Kruskal-Wallis test (χ^2 approximation), significant differences ($P < .01$) were found between the labeling density on clods, ditches, and control after incubation both with sera P1/N1 and sera P2/N2.

The morphology of glove 2 (surgical glove) much resembles that of examination glove 1 (Fig 4, A and C). After incubation with antiserum P2, gold particles are found at the inner and outer surfaces on the numerous bleb-like structures. Again, gold particles are preferably associated with edges and crests (Fig 4, B and D). Incubation with control serum N2 and other controls showed almost no nonspecifically bound gold particles (micrographs not shown).

The outer and inner surfaces of glove 3 are almost completely covered with talc particles, leaving only sparse bare areas in between. On the outer surface, talc particles are smaller and more homogeneous than on the inner surface, where large crystals appear (Fig 5, A and C). Gold particles (incubation with serum P2) are associated with the talc particles (Fig 5, C and D). However, there are also allergens observed on the bare surface (micrograph not shown). Incubation with serum N2 and other controls rendered samples nearly devoid of gold markers (micrographs not shown).

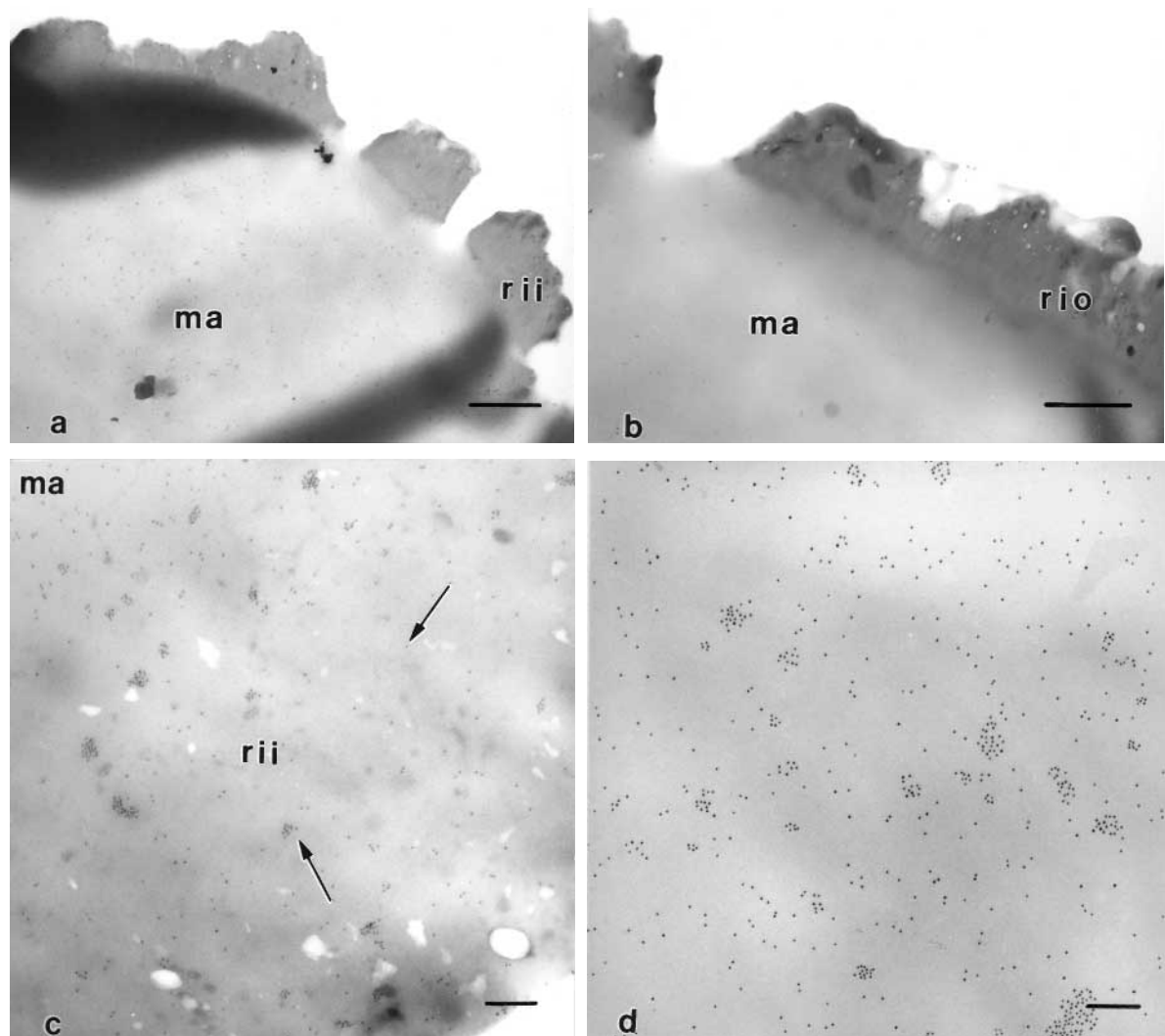


FIG 6. Cross-sections of latex glove 2 (surgical brand) after immunogold labeling for latex allergens with serum P2 in TEM. **a**, Survey of the inner rim (magnification, $\times 4600$; bar = $2\mu\text{m}$). **b**, Survey of the outer rim (magnification, $\times 4600$; bar = $2\mu\text{m}$). **c**, Detail of the inner rim. Gold particles appear as black dots (arrows) (magnification, $\times 35,000$; bar = $0.2\mu\text{m}$). **d**, detail of the matrix (magnification, $\times 35,000$; bar = $0.2\mu\text{m}$) *ma*, Matrix (middle layer); *rii*, inner rim; *rio*, outer rim.

In situ localization of latex allergens on ultrathin glove sections by TEM

Ultrathin cross-sections of glove 2 showed 2 electron-dense rims (Fig 6, A and B) enclosing a nearly structureless electron-transparent matrix (Fig 6, D). After incubation with antiserum P2, gold labeling (*black dots* and *arrows*) is found throughout the cross-section (Fig 6, C and D). Control preparations with serum N2 and other controls showed a low degree of background staining (micrographs not shown).

DISCUSSION

Latex gloves have hitherto been investigated by scanning electron microscopy to carry out morphologic safe-

ty controls for lack of homogeneity, holes,¹⁵ impermeability to herpes virus,¹⁶ or perforations and proteinaceous contamination.¹⁷ Other studies concern the safety of protective gloves in the handling of cytotoxic medicines¹⁸ or insecticides and herbicides.¹⁹ The inspection of powder on latex gloves was also a subject of scanning electron microscopic investigations.²⁰

The aim of the present study was the in situ localization of latex allergens in latex gloves. To avoid artificial translocation of allergens during aqueous fixation, specimens from 3 different gloves (examination and surgical brands) were fixed under anhydrous conditions. To detect several latex allergens, we selected 2 sera for immunogold labeling, which displayed IgE reactivity with a broad range of latex allergens (5-200 kD) and which were obtained from patients with different symptoms after

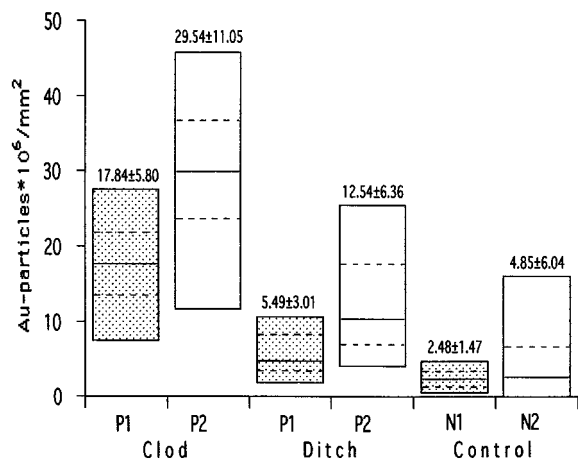


FIG 7. Statistical evaluation of immunogold localization experiments in Figs 2 and 3. Diagram of the mean densities of colloidal gold (Au) particles at different morphological sites (clods and ditches) of the inner side of latex glove 1 by using 2 different sera. Control data were obtained from the clod area only. Each enclosure 90% of the variable. The bottom and top of each box represent 5% and 95%, respectively, of the data. Inside each box, the middle line equals the median value of the data (50%), and the lower and upper dashed lines equal 25% and 75%, respectively, of the data. The mean density value $\pm$ SD is indicated on the top of each column. (dotted columns: clod P1 vs ditch P1, $P < .01$; ditch P1 vs control N1, $P < .01$; clod P1 vs control N1, $P < .01$; open columns: clod P2 vs ditch P2, $P < .01$; ditch P2 vs control N2, $P < .01$; clod P2 vs control N2, $P < .01$).

contact with latex products (dermatitis, angioedema, and anaphylactic shock).

We could show binding of latex-specific IgE antibodies to allergenic surface structures of the glove material. The highest concentration of allergens in the first examination brand (glove 1) and in the surgical brand (glove 2) was found on the outermost surface structures of the glove material (ie, on the edges and crests of the bleb-like structures covering the clods; Fig 2). The second examination brand (glove 3) showed association of latex proteins with the talc particles covering the surface, as well as with the latex surface uncovered by talc. Our findings are in good agreement with earlier data that describe the liberation of allergens from the surface of surgical gloves by using a fingerprint-immunoblot technique.²¹ Surface localization of latex proteins in latex gloves seems to be caused by the manufacturing process during which water-soluble proteins in wet natural rubber latex films are reported to migrate (bloom) with the evaporation stream to the surface when the film dries.²² Concerning the clinical importance of this finding, surface localization of latex glove allergens may be prone to induce sensitization on mucocutaneous contact in certain risk groups. Regarding the specific glove surface morphology ("cracked soil"), we presume that these structures also form during oven drying of latex films.

Although scanning electron microscopy localized latex allergens on the inner and outer surface of the

gloves, TEM monitored allergen distribution throughout the triple-layered (surgical) glove. Both the outer and inner rim, as well as the matrix between, were heavily labeled for latex allergens. This shows that only a fraction of total allergens is localized on the surface of the glove, probably because of migration during drying of the latex films. The fact that the two sera, although reacting with latex allergens of different molecular weights, exhibited a comparable in situ labeling pattern may be explained by the fact that during the manufacturing process different allergenic moieties from the natural latex fluid are mixed and redistributed in the finished product. In this context it is of note that so far no correlation has been found among the nature of detected latex allergens, the levels of latex-specific IgE, and the severity of clinical symptoms.²³

Because our fixation procedure did not fix cornstarch-powder particles to the glove surface, we cannot contribute to the discussion of the presence of latex allergens on cornstarch-powder particles. However, the talc-powder particles present on the third (examination) glove brand firmly adhered to the latex material during preparation. We found latex allergens associated with these talc-powder particles. A high capacity of talc particles to bind latex allergens firmly and irreversibly was reported to play an important role in the bioavailability of latex allergens in talc-powder particles.²⁴

In conclusion, we demonstrate that latex allergens are localized in the interior, as well as on the inner and outer surfaces, of latex gloves. This finding may at least in part explain the great potential of latex products to elicit allergic symptoms and perhaps to sensitize by means of contact.

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REFERENCES

- Slater JE. Latex allergy. *J Allergy Clin Immunol* 1994;94:139-49.
- Swanson MC, Bubak ME, Hunt LW, Yunginger JW, Warner MA, Reed CE. Quantification of occupational latex aeroallergens in a medical center. *J Allergy Clin Immunol* 1994;94:445-51.
- Beezhold DH, Sussman GL, Kostyal DA, Chang NS. Identification of a 46 kD latex protein allergen in health care workers. *Clin Exp Immunol* 1994;98:408-31.
- Carillo T, Blanco C, Quirarte J, Castillo R, Cuevas M, Rodriguez de Castro F. Prevalence of latex allergy among greenhouse workers. *J Allergy Clin Immunol* 1995;96:699-701.
- Slater JE, Mostello LA, Shaer C. Rubber specific IgE in children with spina bifida. *J Urol* 1991;146:578-9.
- Cahaly RJ, Slater JE. Latex hypersensitivity in children. *Curr Opin Pediatr* 1995;7:671-5.
- Breiteneder H, Scheiner O. Molecular and immunological characteristics of latex allergens. *Int Arch Allergy Immunol* 1998;116:83-92.
- Ross BD, McCullough J, Ownby DR. Partial crossreactivity between latex and banana allergens. *J Allergy Clin Immunol* 1992;90:409-10.
- Lavaud F, Prevost A, Cossart C, Guerin L, Bernard J, Kochman S. Allergy to latex, avocado, pear, and banana: evidence for a 30 kD antigen in immunoblotting. *J Allergy Clin Immunol* 1995;95:557-64.
- Beezhold DH, Sussman GL, Liss GM, Chang NS. Latex allergy can induce clinical reactions to specific foods. *Clin Exp Allergy* 1996;26:416-22.

11. Fuchs T, Spitzauer S, Vente C, et al. Natural latex, grass pollen, and weed pollen share IgE epitopes. *J Allergy Clin Immunol* 1997;100:356-64.
12. Grote M. In situ localization of pollen allergens by immunogold electron microscopy: allergens at unexpected sites. *Int Arch Allergy Immunol* 1999;118:1-5.
13. Fling SP, Gregerson DS. Peptide and protein molecular weight determination by electrophoresis using a high molarity Tris buffer system without urea. *Anal Biochem* 1986;55:83-8.
14. Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some application. *Proc Natl Acad Sci USA* 1979;76:4350-4.
15. Tucci MG, Mattioli-Belmonte M, Toschi E, et al. Structural features of latex gloves in dental practice. *Biomaterials* 1996;17:517-22.
16. Richards JM, Sydiskis RJ, Davidson WM, Josell SD, Lavine DS. Permeability of latex gloves after contact with dental materials. *Am J Orthod Dentofacial Orthop* 1993;104:224-9.
17. Nikawa H, Hamada T, Tamamoto M, Abekura H. Perforation and proteinaceous contamination of dental gloves during prothodontic treatments. *Int J Prothodont* 1994;7:559-66.
18. Dinter-Heidorn H, Carstens G. Comparative study on protective gloves for handling cytotoxic medicines: a model study with carmustine. *Pharm Weekbl Sci* 1992;14:180-4.
19. Moody RP, Nadeau B. Effect of the mosquito repellent DEET and long wave ultraviolet radiation on permeation of the herbicide 2,4- D and the insecticide DDT in natural rubber gloves. *Am Ind Hyg Assoc J* 1992;53:436-41.
20. Stein HA. Powder-free gloves for ophthalmic surgery. *J Cataract Refract Surg* 1997;23:714-7.
21. Beezhold DH, Kostyal DA, Wiseman J. The allergen transfer of protein allergens from latex gloves. *AORN J* 1994;59:605-14.
22. Yeang HY, Sunderasan E, Ghazaly HM. Latex allergy studies: extraction of natural rubber latex proteins with reference to film thickness, latex D.R.C. and protein migration behaviour. *J Nat Rubb Res* 1995;10:46-62.
23. Muguerza J, Capo C, Porri F, Jacob JL, Mege JL, Vervolet D. Latex allergy: allergen identification in Hevea brasiliensis fractions by immunoblotting. *Clin Exp Allergy* 1996;26:1177-81.
24. Lundberg M, Wrangsjö K, Johansson SGO. Latex allergy from glove powder—an unintended risk with the switch from talc to cornstarch? *Allergy* 1997;52:1222-8.

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