Extractable latex allergens and proteins in disposable medical gloves and other rubber products

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Background: *IgE-mediated sensitization to rubber proteins is being reported with increasing frequency in health care workers. To explore the relative importance of various sources of allergen exposure, we measured the total rubber allergen and protein levels in extracts of disposable rubber gloves and compared the allergen levels with those in extracts of other medical and consumer rubber products.*

Methods: *Rubber allergens were measured by inhibition immunoassay with a rubber glove extract as the solid-phase allergen and pooled plasma from five rubber-sensitized health care workers as the IgE antibody source. Proteins were measured by Ninhydrin assay.*

Results: *Among 71 lots of gloves tested, the extractable allergen and protein levels were significantly correlated and were appreciably higher in powdered gloves than in powder-free gloves. Allergen levels varied 3000-fold among gloves from different manufacturers and were higher in examination gloves than in surgical or chemotherapy gloves. Measurable allergen was found in 11 of 24 lots of "hypoallergenic" gloves tested. Allergen levels in toy balloons were comparable to those in powdered gloves; much lower allergen levels were measured in condoms and anesthesia rebreathing bags.*

Conclusions: *The allergen content of disposable rubber gloves varies widely and is higher in powdered gloves than in powder-free gloves and higher in examination gloves than in surgical gloves. Hypoallergenic gloves may contain substantial amounts of IgE-binding proteins. Gloves and toy balloons appear to be more important sources of rubber allergens than the other rubber products tested. (J ALLERGY CLIN IMMUNOL 1994;93:836-42.)*

Key words: *Latex, rubber, allergens, proteins, disposable gloves*

The use of disposable latex (rubber) gloves by medical and paramedical personnel has increased markedly in the past several years, in large part because of the acquired immunodeficiency syndrome epidemic and subsequent recommendation for universal precautions for protection from potentially infectious body fluids.[1] This increased usage has been paralleled by an increased number of reports of contact or inhalant sensitization to latex among health care workers, in whom IgE antibodies have been demonstrated by skin tests or immunoassays to proteins in raw latex or rubber gloves.[2-7] If the residual rubber protein levels in finished products could be lowered, the allergenicity of the products might also be reduced.

The Center for Devices and Radiological Health of the U. S. Food and Drug Administration (FDA) is responsible for regulating the manufacture of disposable rubber gloves for medical use. Because of concerns that rubber proteins may be allergenic, the Center for Devices and Radiological Health has encouraged rubber medical device manufacturers to collect data on

Abbreviations used
AU: Allergy unit
FDA: U. S. Food and Drug Administration

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TABLE I. Extractable allergen and protein levels in disposable nonsterile latex gloves

Brand/lot no.	Hypoallergenic designation	Allergen (AU/ml)	Protein (μg/ml)
Powdered examination gloves			
Aladan/NA*	No	<5	27
Ansell Conform/202405805†	No	126	76
Ansell Conform/207411005†	No	126	124
Ansell Exam-Tex/(L)AOB012†	No	22	181
Baxter Professional/K0N016X‡	No	20	609
Burrows/D6A23111§	No	1,220	1,140
China Nat. Meds. & Health Prod./NA‖	No	9	57
C.T. International/BT1084¶	No	1,940	152
C.T. International/1733¶	No	10,100	530
Linc Royal/NA#	No	12	20
Safeskin/AL1276**	Yes	12,800	345
Savacare/920331††	No	14,500	701
Savacare/921109††	No	16,300	613
Bodyguards/90061632‡‡	No	4,330	872
Bodyguards Ultra Light/00073145‡‡	No	662	484
Powder-free examination gloves			
C.T. International/1686¶	No	<5	<15
C.T. International/1800¶	No	12	<15
Regent Biogel/92098§§	Yes	<5	<15
Regent Biogel/92032§§	Yes	7	<15
Safeskin/2089-CP**	Yes	10	14
Savacare/05975††	No	146	19
Savacare/921216††	No	151	29
Chemotherapy gloves			
Ansell EP/205259704†	No	<5	<15
USCP ChemoBloc/OK2J339‖ ‖	No	<5	101
USCP ChemoBloc/OK2H350‖ ‖	No	<5	109

NA, Not available.
*Aladan Corp., Norcross, Ga.
†Ansell, Inc., Dothan, Ala.
‡Baxter Healthcare Corp., Valencia, Calif.
§The Burrows Co., Wheeling, Ill.
‖China National Medicines and Health Products Import and Export Corp., Nanjing, China.
¶C. T. International, San Luis Obispo, Calif.
#Linc Industries, Redondo Beach, Calif.
**Safeskin Corp., Boca Raton, Fla.
††Preventive Care, Inc., Eagan, Minn.
‡‡T. K. Glove Product Co. Ltd., Huntington Beach, Calif.
§§Regent Hospital Products Ltd., Greenville, S.C.
‖ ‖U. S. Clinical Products, Richardson, Texas.

the extractable proteins in their products. Some medical gloves are presently labeled as being "hypoallergenic." However, this claim is based on modified Draize testing, which is not an appropriate measure of the ability of the product to induce a human IgE antibody response. Indeed, the hypoallergenic claim may put IgE-sensitized users at risk for a serious adverse reaction. The Center for Devices and Radiological Health has recently announced its intent to prohibit use of the term *hypoallergenic* on packages of medical gloves.

The aims of the present study were to investigate the variability of extractable latex allergen and protein levels in a large sample of disposable medical gloves and to compare these allergen levels with those in extracts of other selected medical and consumer rubber products.

METHODS

Quantitation of latex allergens by inhibition immunoassay

Solid-phase allergen. A solid-phase allergen was prepared by extracting latex gloves (Bodyguards; T. K. Glove Product Co. Ltd., Huntington Beach, Calif.) 1:5 wt/vol in phosphate-buffered saline (pH 7.4) for 1 hour at 37° C and then overnight at 4° C. The resulting

TABLE II. Extractable allergen and protein levels in disposable sterile latex surgical gloves

Brand/lot no.	Hypoallergenic designation	Allergen (AU/ml)	Protein (μg/ml)
Powdered			
Ansell Medi-Grip/27-1335 (L)N1DO18*	No	<5	<15
Ansell Orthopedic/(L)N0A007*	No	<5	<15
Ansell Orthopedic/(L)N0A008*	No	<5	<15
Ansell Sensi-Derm/1828*	Yes	<5	<15
Ansell Sensi-Derm/1682*	Yes	<5	<15
Ansell Sensi-Touch/204105605*	No	141	29
Triflex Sterile/PGSOA075†	No	5,810	246
Triflex Orthopedic/K2H236†	No	906	131
Triflex Orthopedic/K1P146†	No	233	52
Ultraderm/M1B211†	SL	5	23
B-D Eudermic/0207131OZ‡	Yes	<5	<15
B-D Eudermic/02071345G‡	Yes	<5	<15
B-D Integron/02071336Q‡	No	57	89
B-D Integron/02072006U‡	No	27	49
HPI Sensi-Grip/A041008S§	Yes	<5	<15
HPI White Latex/A081007S§	Yes	<5	<15
HPI White Latex/A031004S§	Yes	<5	<15
J&J Maxxus Orthopaedic/04122145667‖	No	8	1,850
J&J Microtouch XP/22601760377‖	No	47	225
J&J Neutralon/5370‖	Yes	32	494
Plastic Materials of Puerto Rico/8312-01¶	No	<5	<15
Professional Medical Prod. Brown Milled/A032008S#	Yes	15	94
Professional Medical Prod. Brown Milled/A042011S#	Yes	9	93
Semper-Med/XR0A4**	Yes	<5	180
Smith & Nephew Perry Dermaguard Plus/709315Y††	Yes	481	238
Smith & Nephew Perry Dermaguard Plus/707243J††	Yes	403	390
Smith & Nephew Perry Dermaguard Plus/711751X††	Yes	1,690	342
Smith & Nephew Perry Dermaguard Plus/711250S††	Yes	505	159
Smith & Nephew Perry Duotex/701-623Y††	No	776	466
Smith & Nephew Perry Orthopaedic/710344J††	No	717	112
Smith & Nephew Perry Orthopaedic/712651J††	No	606	73
Smith & Nephew Perry Brown Latex/315-884T††	No	1,260	439
Smith & Nephew Perry White Latex/TS0066††	No	1,950	480
Smith & Nephew Perry White Latex/TS0067††	No	1,660	468
Sterling Rubber/3903058‡‡	No	12,100	437
Tagum Rubber Industries/42F6688§§	No	224	54
Travenol Ultraderm/M9C157‖ ‖	AL	18	287

SL, "For hands sensitive to latex"; *AL*, "Specially formulated for hands allergic to latex".
*Ansell, Inc., Dothan, Ala.
†Baxter Healthcare Corp., Valencia, Calif.
‡Becton-Dickinson & Co., Acute Care, Franklin Lakes, N.J.
§HPI, Incorporated, Fayette, Ala.
‖Johnson & Johnson Medical, Inc., Arlington, Texas.
¶Plastic Materials of Puerto Rico, Loiza, P.R.
#Professional Medical Products, Inc., Greenwood, S.C.
**Semper-Med, Austria.
††Smith & Nephew Perry, Massillon, Ohio.
‡‡Sterling Rubber Inc., Fergus, Ontario, Canada.
§§Tagum Rubber Industries Ltd. Herzliya, Israel.
‖ ‖Travenol Laboratories, Inc., Deerfield, Ill.
¶¶Regent Hospital Products Ltd., Greeville, S.C.

extract was concentrated 20-fold by pressure filtration through an Amicon YM-2 membrane (Amicon Inc., Beverly, Mass.) (final protein content = 8.9 mg/ml). The concentrated extract was coupled to derivatized polyacrylamide beads containing N-hydroxysuccinimide carboxylate ester groups (Matrix Pel 102, Amicon Inc.), washed extensively, and resuspended at 0.4 mg/ml in assay buffer.[8]

TABLE II—cont'd

Brand/lot no.	Hypoallergenic designation	Allergen (AU/ml)	Protein (μg/ml)
Powder-Free			
Ansell No Powder/1849*	No	<5	<15
Ansell No Powder/1952*	No	<5	<15
Regent Biogel M/923002¶¶	Yes	<5	<15
Regent Biogel/912134¶¶	Yes	7	<15
Regent Biogel/913029¶¶	Yes	<5	<15
Safeskin/32126030##	Yes	<5	<15
Safeskin/32053030##	Yes	<5	<15
Smith & Nephew Perry Natural/712046B††	No	35	43
Smith & Nephew Perry Natural/712044A††	No	61	46

Latex-specific IgE antibodies. Mayo Medical Center employees who presented to the Division of Allergic Diseases or Department of Dermatology with clinical histories suggesting inhalant or contact allergy to latex underwent puncture skin tests with extracts from a variety of rubber gloves.[4] Persons with significant (>4 mm diameter) skin test reactions to any of the glove extracts were asked to provide sera for quantitation of latex allergens. Defibrinated plasmas from five such individuals were pooled and used as the source of IgE antibodies in the immunoassay.

Analytes. We tested 71 lots of medical gloves (one sample per lot) from 22 different manufacturers; in 21 cases two or more different lots of the same style glove were tested. Gloves and other rubber-containing medical devices for testing were those in routine use at Mayo Medical Center in Rochester, Minnesota or at the U. S. Navy Hospital in San Diego, California in late 1991 and 1992. We made no effort to procure a specific type of glove or to select gloves from a particular manufacturer; instead we gathered samples from gloves already in stock at each institution. These and other rubber products purchased locally were cut into 1 to 2 cm pieces and extracted 1:5 wt/vol as described above in phosphate-buffered saline.

Performance of inhibition immunoassay. The assay was performed by mixing solid-phase allergen (0.5 ml), latex-specific IgE antibodies (1:5 dilution, 0.1 ml), assay buffer (0.35 ml), and several threefold dilutions of standard or test analytes (0.5 ml) overnight at room temperature. The solid-phase complex was then isolated by centrifugation, washed twice, and resuspended with assay buffer (0.9 ml) and affinity chromatography–purified iodine 125–labeled anti-human IgE (20 ng, 0.1 ml). After identical incubation and washing steps were performed, the solid-phase complex was counted in an automatic gamma spectrometer.[8] A raw latex preparation provided by the Center for Biologics Evaluation and Research of the FDA (lot E5) was used as the standard in the assay and was assigned an arbitrary potency of 100,000 allergy units (AU) per milliliter. The latex allergen contents of the various analytes were calculated by interpolation from this standard curve. The sensitivity of the assay was 5 AU/ml. In each assay we included an extract of a glove with low allergen content (approximately 15 AU/ml) as an internal standard; the interassay coefficient of variation for this sample was 21%.

Data analysis. Inhibition immunoassay results were analyzed by a computerized version of the parallel-line assay developed by the Center for Biologics Evaluation and Research of the FDA.[9] All assays met four criteria for validity: (1) minimum of 4 data points on the inhibition curve, (2) data points that bracketed the 50% inhibition point, (3) linear correlation coefficient of greater than 0.90 for each inhibition curve, and (4) slopes of inhibition curves and standard curve that were not statistically different as determined by Student's paired t test at $p < 0.01$.

Quantitation of protein

Protein was assayed by a modified Ninhydrin method established by the Center for Biologics Evaluation and Research.[10] Standards or test analytes containing 1 to 15 μg protein (0.1 ml) were hydrolyzed overnight at 95° C in screw-topped tubes containing 10 mol/L NaOH (0.1 ml). Tubes were then cooled, uncapped, incubated at 75° C for 30 minutes to drive off free ammonia, and placed in an ice water bath. The pH was then lowered to 5.0 by addition of 12 mol/L HCl (0.125 ml), after which freshly prepared Ninhydrin reagent (0.6 ml) was added with mixing; the capped tubes were incubated in a boiling water bath for 20 minutes, then cooled again. After mixing, 0.5 ml from each tube was transferred to small glass tubes and diluted in 1:1 water:1-propanol (0.5 ml); the absorbance was read at 570 nm. Bovine serum albumin was used as the assay standard. The sensitivity of the assay was 15 μg/ml.

RESULTS

The extractable allergen and protein contents of the 25 lots of nonsterile medical gloves tested are shown in Table I. Allergen levels in the 15 lots of powdered examination gloves from 10 different manufacturers varied by more than 3000-fold. In general, much less allergen and protein could be extracted from powder-free examination gloves or from chemotherapy gloves than from powdered examination gloves. In the latter group allergen

TABLE III. Extractable latex allergens in other medical and consumer rubber items

Item/manufacturer	Allergen (AU/ml)
Medical products	
Trojan ribbed condom/Carter Products (New York, N.Y.)	50
Anesthesia breathing circuit rebreathing bag/Intertech Resources, Inc. (Lincolnshire, Ill.)	50
Intravenous T-connector/Medex, Inc. (Hilliard, Ohio)	<5
Intravenous tubing/Gemini 20 (Imed Corp., San Diego, Calif.)	<5
Nasopharyngeal air-way/Porges Corp. (Fairfield, N.J.)	<5
Consumer products	
Rubber balloon/Betta Products, Inc. (Chatsworth, Calif.)	4700
Baby pacifier/Playtex Inc., Family Products, Div. (Stamford, Conn.)	<5
Ezy infant care ortho nipples/Apothecary Products, Inc. (Minneapolis, Minn.)	<5
Evenflo wide base nipples/Evenflo Products Co. (Ravenno, Ohio)	<5
Evenflo water nipples/Evenflo Products Co. (Ravenna, Ohio)	<5

and protein levels were significantly correlated by regression analysis ($r = 0.60$; $p < 0.02$).

The extractable allergen and protein contents of the 46 lots of sterile surgical gloves tested are shown in Table II. There was a wider range of extractable allergen levels measured among gloves from different manufacturers (<5 to 16,000 AU/ml, approximately 3000-fold) than that measured among gloves from the same manufacturer (range = twofold to 1000-fold). As was the case with the nonsterile gloves tested, the extractable allergen and protein levels were appreciably higher in the 37 lots of powdered gloves than in the nine lots of powder-free gloves. However, the allergen and protein levels in powdered surgical gloves were not significantly correlated ($r = 0.26$; $p > 0.10$). These data were skewed by one lot of gloves that was low in allergen content but high in extractable protein. It was subsequently learned that exogenous (nonlatex) protein was added to this type of glove during manufacture. If data from this glove were omitted, the correlation between the allergen and protein contents of the surgical gloves became highly significant ($r = 0.69$; $p = 0.0001$).

Overall, the extractable allergen in gloves labeled as being hypoallergenic tended to be less than extractable allergen in gloves without the hypoallergenic designation. However, 11 of the 24 lots of hypoallergenic gloves contained measurable amounts of rubber allergens.

As anticipated, there was no measurable latex allergen in two lots of gloves made from synthetic materials (Dermaprene; Ansell, Inc., Dothan, Ala., and Sensi-Care; Becton-Dickinson and Co., Becton-Dickinson Div., Rutherford, N.J.) or in bulk cornstarch powder kindly provided by one of the glove manufacturers (data not shown).

Extractable allergen levels in other medical and consumer products are shown in Table III. Allergen could be quantitated in toy balloons, a rubber condom, and the rebreathing bag from a disposable anesthesia breathing circuit. No extractable allergen could be measured in rubber components in intravenous tubing, a disposable airway, baby bottle nipples, or a baby pacifier.

DISCUSSION

The increase in reported cases of latex allergy is probably due to several factors, including a real increase in the incidence of latex allergy; increased recognition of latex allergy by clinicians; and increased awareness of latex allergy by latex-exposed health care workers, who now may be less apt to self-diagnose and self-treat their problems and more inclined to seek allergy consultation. The relative importance of these factors is completely unknown; there are no reliable data from large-scale epidemiologic studies of latex allergy.

We have documented a variation of greater than 3000-fold in latex allergen levels in disposable rubber gloves from several manufacturers. Appreciably more allergen could be extracted from powdered latex gloves than from powder-free latex gloves. In addition, sterile gloves and chemotherapy gloves as a group had lower allergen levels than examination gloves. Cornstarch used to powder gloves has rarely been found to be allergenic by itself,[11] although latex allergens may be absorbed by the powder after contact with the glove.[12, 13] The processes used to manufacture surgical gloves and chemotherapy gloves differ

from those used to manufacture examination gloves, and the processes used to manufacture powder-free gloves differ from those used to manufacture powdered gloves. For example, powder-free gloves often undergo a chlorination washing process that powdered gloves do not[14]; residual rubber proteins may be extracted or denatured during this process. The exact processes used by the various glove manufacturers are considered proprietary.

Because most investigators have found that rubber allergens are native rubber proteins,[15-17] it is reasonable to assume that a reduction in residual rubber proteins in finished rubber products would be accompanied by a reduction in allergenicity. However, to the extent that exogenous proteins are added to liquid latex during glove manufacture, extractable protein measurements may not correlate well with allergenicity.

With the exception of toy balloons, the extractable latex allergen levels from the nonmedical products tested were much less than those of rubber gloves. Allergen levels in the four unused baby bottle nipples and pacifiers tested were below the level of detection, and it is likely that any extractable allergen would be further reduced by washing or boiling these items before use. Rubber items such as vial stoppers and baby bottle nipples are examples of extruded or compression-molded dry products, whereas gloves and condoms are dipped products produced from liquid latex. We postulate that dry molded rubber products contain lower residual latex protein levels or that the proteins are less easily extracted from these products.

It has been recommended that latex-free operating rooms be used for latex-sensitive persons scheduled to undergo surgery. Our data suggest that disposable powdered rubber gloves contribute greater quantities of latex allergen to the surgical suite environment than other rubber-containing items commonly found in operating rooms. The FDA has recently announced to manufacturers its intent to require all medical devices that contain natural rubber latex and that come directly or indirectly in contact with the body to state on the principal display panel: "*This product contains natural rubber latex.*" Furthermore, the FDA intends to prohibit the use of terms such as *hypoallergenic* on latex-containing medical devices. However, no timetable for implementation of these changes has been published. Although some lots of hypoallergenic gloves tested in our series contained relatively low levels of latex allergen, we consider both changes in labeling proposed by the FDA to be timely, justified, and helpful to both latex-sensitized patients and health care workers. However, allergists and sensitized health care workers must keep in mind that gloves are worn for their barrier properties and that vinyl and other synthetic gloves may not confer the same level of protection as rubber gloves.[18]

We reemphasize that the latex allergen values reported were from gloves being used in late 1991 and throughout 1992. It is possible that disposable gloves being manufactured and used in 1993 and 1994 may contain different quantities of extractable allergen. Prospective studies are under way to investigate long-term trends in the allergen content of disposable medical gloves. Nevertheless, on the basis of the results of these and other studies, Mayo Medical Center will begin to purchase only lower allergen latex examination gloves and surgical gloves. The use of high allergen gloves is being phased out, initially in areas of the medical center where latex-sensitized persons are working. Sequential measurements of latex aeroallergen levels are being obtained to monitor the effects of the use of only low allergen gloves.[19]

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Effect of allergen avoidance in infancy on allergic manifestations at age two years

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Background: *One hundred twenty children, identified before birth as being at high risk for atopy, were prenatally assigned to prophylactic or control groups.*
Methods: *The infants in the prophylactic group either received breast milk from mohters on an exclusion diet or an extensively hydrolyzed formula. Their bedrooms and living rooms were treated repeatedly with an acaricide, and they used polyvinyl-covered mattresses with vented head areas. The infants in the control group were fed conventionally, and no environmental control was recommended.*
Results: *A significant advantage, first demonstrated at 1 year of age, persists for children in the prophylactic group. They have less of any allergy or eczema, but the reduced prevalence of asthma is no longer significant. Only three children in the prophylactic group had positive skin prick test results compared with 16 in the control group, suggesting a significant reduction in sensitization.*
Conclusion: *A dual approach to allergen avoidance, focusing on foods and aeroallergens, appears to be beneficial in selected high-risk infants. Avoidance of potent allergens in early life increases the threshold for sensitization in these high-risk infants. Whether sensitization has been avoided or merely deferred has yet to be proved. (J ALLERGY CLIN IMMUNOL 1994;93:842-6.)*

Key words: *Allergy, prevention, asthma, eczema, food intolerance, skin prick tests*

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Abbreviations used
HDM: House dust mite
SPT: Skin prick test

A number of studies have shown that certain manifestations of IgE-mediated food allergy may be lessened by carefully controlling the diet of the newborn infant. This may be done by offering the infant a formula modified to provide minimal