
Endotoxin as a factor in adverse reactions to latex gloves

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Background: Endotoxin is an inflammatory agent made by gram negative bacteria that can irritate the skin, induce respiratory problems, fever, and shock. It is an adjuvant for both delayed hypersensitivity and IgE production and has been shown to magnify antigen specific mediator release. Since many of the clinical problems associated with natural latex products involve similar clinical sequelae, we investigated the possibility that latex gloves might be contaminated with endotoxin.

Objective: To measure the endotoxin content of a variety of natural latex gloves, investigate its distribution and origin, association with latex proteins, and determine the particle sizes associated with its release.

Methods: Endotoxin, protein, and allergen were measured using a quantitative kinetic Limulus assay, modified Lowry, and RAST inhibition, respectively. Particle size and density were determined using an Anderson multistage air sampler and CsCl₂ gradient.

Results: Endotoxin was found to be a highly significant contaminant of some latex gloves. Levels ranged from 0.09 ng to 2.8 µg/g of glove. Protein levels ranged from <25 to 1150 µg/g of glove while allergen levels ranged from <1 to 837 µg/g of glove. Endotoxin and protein eluted rapidly from the interior of the gloves tested. Greater than 70% of the endotoxin was found to be associated with particles in the <7 µm aerodynamic diameter range. The highest levels of endotoxin were found in nonsterile examination gloves with a tendency towards powdered gloves containing more endotoxin and protein. A slurry containing cross-linked dextran through which gloves were dipped revealed very high endotoxin contamination (64 µg/mL) while unused cross-linked dextran has very little associated endotoxin.

Conclusions: These data demonstrate that some natural rubber latex gloves, particularly nonsterile examination gloves, are contaminated with high amounts of endotoxin and proteins. These were found mostly on the inside of gloves and were released as very small respirable particles that were not physically associated with the powder. These findings support the hypothesis that endotoxin may be responsible for some of the tissue irritation associated with latex glove use. In addition, this material may be responsible for the enhancement of delayed and immediate hypersensitivity reactions to chemicals and proteins found in these products and offers a possible explanation for the disproportionate severity of these reactions.

Ann Allergy Asthma Immunol 1997;79:303-10.

INTRODUCTION

Lipopolysaccharides unique to the outermost wall of gram negative bacteria have very potent proinflammatory effects.^{1,2} These substances referred to as "endotoxins" for historical reasons and are continually being shed from grow-

ing bacteria and are released upon their death.³⁻⁵ Specific cellular and humoral receptors exist for endotoxins that enhance their ability to induce proinflammatory cytokines and chemokines such as TNFα, IL-6, and IL-8.⁶⁻¹² Clinical reactions to endotoxins include irritation, skin rashes, fever, rhinoconjunctivitis, respiratory congestion, asthma, and anaphylactoid shock.¹³⁻¹⁸ The symptoms observed depend upon the dosage, route of entry, molecular structure, and other poorly defined suscep-

tibility factors.¹⁹⁻²⁴ Endotoxin has also been shown to act as an adjuvant for allergic contact sensitivity to haptens¹³ and to be an adjuvant for IgE responses when inhaled with antigen.²⁵⁻²⁸ In addition, endotoxin acts synergistically with allergen resulting in a magnification of specific allergen-induced mediator release.²⁹⁻³²

Since the mid-eighties the cumulative prevalence of individuals experiencing adverse reactions to products manufactured from native latex has increased dramatically.³³ Clinically these reactions range from direct irritant effects to immunologically induced contact dermatitis, urticaria, rhinoconjunctivitis, asthma, and anaphylaxis.³⁴⁻³⁶ The immunologic activity of the chemicals and the allergenicity of proteins present in natural latex products have been studied by a number of investigators and many of their molecular properties have been elucidated.³⁷⁻⁴⁷ The reasons why these substances seem to be such potent sensitizers, with often severe consequences, has not been determined. The hypotheses advanced include the large increase in the use of natural rubber latex gloves as a barrier against infectious agents and the fact that increased demand may have introduced alterations in manufacturing processes.⁴⁸

Consideration of the manner in which these latex products are manufactured, with the resulting potential for endotoxin contamination, and the nature of the clinical reactions observed, prompted us to determine the endotoxin content of natural latex gloves. In this paper, we provide data on the occurrence, distribution, and probable source of endotoxin in commercially available latex gloves. Based on these findings we propose that the presence of endotoxin in some latex

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Received for publication December 5, 1996.

Accepted for publication in revised form June 3, 1997.

gloves is an important factor in the etiology of the clinical reactions to these products.

METHODS

Extracts

Natural latex gloves and synthetic gloves ($n = 23$) obtained from a variety of different manufacturers were cut into 0.5 cm² pieces, weighed, and placed in depyrogenated borosilicate test tubes. Glassware was depyrogenated by heating in an oven held at 180 °C for four hours. Extracts were prepared from 0.4 g of each glove by vigorously shaking for one hour in 2 mL pyrogen-free water (BioWhittaker, Inc., Walkersville, MD). This was followed by centrifugation at 3,000 g for 20 minutes and the supernate was recovered. Fifty-microliter aliquots were removed and diluted in pyrogen-free water and assayed immediately. A fresh starch extract was prepared from unused cross-linked dextran powder (Grain Processing Corporation, Muscatine, IA) by extracting at a 1:5 ratio (wt/vol) in pyrogen-free water for one hour with shaking and centrifugation at 3,000 g for 20 minutes. A sample of cornstarch slurry from a latex glove production line was collected in Malaysia and shipped frozen to the laboratory (a gift from Russell Thompson, TNI, Hartland, WI). An extract was prepared from this material by centrifuging the thawed slurry at 3,000 g for 20 minutes.

Endotoxin

Endotoxin was measured using the kinetic chromogenic quantitative *Limulus* amoebocyte lysate assay which is nonreactive to glucans (BioWhittaker, Inc., Walkersville, MD). Aliquots of the extracts were serially diluted in 10-fold increments with pyrogen free water. A 100- μ L aliquot of each of these dilutions was mixed with 100 μ L of freshly prepared *Limulus* amoebocyte lysate containing chromogenic substrate in a pyrogen-free microtiter plate (Dynatech Corporation, Chantilly, VA). The microtiter plates were kept at 37 °C and the color development was monitored every 15 seconds

with the Dynatech MR5000 microtiter plate reading spectrophotometer. The calculation program in this instrument determines the time interval to reach 0.03 absorbance and this was compared with a standard curve covering the range of 5 ng/mL to 0.5 pg/mL. The standards were linear over this 5-log range on a log-log plot. Unknowns were calculated by linear interpolation by the software. All dilutions were assayed in duplicate and a parallel dilution was spiked with 50 pg endotoxin to assess any enhancement or inhibition of activity by any of the extracts. Only those dilutions that did not exhibit enhancement or inhibition and which were parallel to the standard curve were used. Values shown are averages from the same glove assayed on three different occasions. The coefficient of variation for these assays averaged 7.6%. To convert endotoxin activity to equivalent mass units of the EC6 reference standard (US Pharmacopeia), a factor of 10 EU/ng was used.

Protein

A modification of the Lowry protein determination method was used to determine protein in the glove extracts. The method (American Society for Testing Materials 5712-95) was recently adopted by the American Society for Testing Materials and is currently the method approved by the US Food and Drug Administration for determining protein content in gloves and other medical devices derived from latex. The method incorporates several protein precipitating reagents to circumvent the interference of the Lowry assay by the various chemicals used in the manufacture of these products. The method utilized ovalbumin as a standard at a range of 5 μ g/mL to 640 μ g/mL. Since the gloves were extracted at a ratio of 1:5 (wt/vol), the resulting detection limit was 25 μ g/g of glove.

Allergen Content

The quantity of latex allergen was determined by RAST inhibition using the method previously described⁴⁹ except that for this study a non-ammoniated

latex extract was used as the reference extract or standard. This extract was prepared from *H. brasiliensis* latex shipped frozen from Malaysia as described by Tomazic et al.⁵⁰ The protein content was established by the American Society for Testing Materials modified Lowry method. Dilutions of this standard were made in phosphate buffered saline containing 1 mg/mL human serum albumin. The serum pool for the RAST inhibition was prepared from 42 sera known to have high concentrations of latex-specific IgE. For comparison purposes, a reference latex extract from the FDA (E-8) was determined to contain 1.2 mg/mL of latex allergen and 2.8 mg/mL of protein by the American Society for Testing Materials modified Lowry method. The useful limit of detection of the RAST inhibition assay in these studies was estimated to be 0.03 μ g/mL and for quantitation to be 0.060 μ g/mL.

Particle Sizing

The relative size (ie, aerodynamic diameter) of the particles suspended in air when ten gloves were snapped in an enclosed room (25 m³) was determined by analyzing samples collected with an Anderson multistage air sampler (Grasby/Anderson Inc, Atlanta, GA). Prior to use this device was depyrogenated by soaking the stages overnight in 0.3 N NaOH. The glass 100-mm Petri plates were depyrogenated by heating at 180 °C for four hours. Each plate was filled with 20 mL depyrogenated water and vacuum was applied for 15 minutes at 28.3 L/min (total air volume sampled was 0.424.5 m³). Aliquots of the 20 mL of water in the individual plates (ie, stages) were centrifuged and the supernates were assayed for endotoxin by the *Limulus* amoebocyte lysate method described above. These samples were assayed prior to and immediately following the application of vacuum to the Anderson sampler. A microscopic analysis of the resulting particles in the different stages of the Anderson was performed with cytopsin (Shandon Inc, Pittsburgh, PA) preparations from each stage.

Density Studies

Particles created from snapping five pairs of gloves in a small room (25 m³) were collected in a cyclone sampler (Burkard Instruments, Britain) over a period of 90 minutes. This sample was suspended in 0.5 mL depyrogenated water and layered over a 60% wt/vol solution of CsCl₂. The dextran particles were allowing to sediment for 30 minutes at which time an aliquot was removed for endotoxin testing. The results were compared with a 10-μL sample of the suspension assayed prior to layering over the CsCl₂. Assay results were corrected for dilution and recoveries were compared.

Kinetics of Release

Endotoxin-containing gloves were either left normal or turned inside out and each was filled with 100 mL depyrogenated water. They were then attached to a 500-mL flask and placed on a rocker platform at 100 rpm. Samples (0.5 mL) were taken at times indicated and assayed for protein and endotoxin as above.

RESULTS

The results of the endotoxin, protein, and allergen assays for the 21 different gloves are listed in Table 1. The protein content of latex gloves varied over a large range, from below the assay detection limit (25 μg/g) to 1150 μg/g of glove. The average protein content for latex examination gloves was 460 μg/g compared with 74.7 μg/g of glove for latex surgical gloves (Table 2). The average protein content for powdered latex gloves was 444 μg/g of glove while that for nonpowdered latex gloves was 47 μg/g. One of the nonlatex control gloves (vinyl) demonstrated a high level of protein as measured by the modified Lowry test while the other was negative. The latex allergen content in commercially available latex gloves also varied over a wide range from below the detection limits of the assay (1 μg/g) to 858 μg/g of glove. The average allergen content for latex examination gloves was 335 μg/g of glove while latex surgical gloves averaged much lower at 27 μg/g of

glove. Powdered latex gloves contained on average 313 μg/g of glove while nonpowdered latex gloves averaged 25 μg/g of glove. Neither of the non-latex gloves demonstrated any detectable latex allergen. The endotoxin content of the latex gloves ranged from 0.09 to 2800 ng/g of glove. As with the protein and allergen measurements, there was a strong correlation of endotoxin levels with the type of glove. Examination gloves were contaminated with the highest amounts of endotoxin and averaged 404 ng/g while surgical gloves averaged a much lower endotoxin content at 3 ng/g of glove. The endotoxin content was generally higher in powdered latex gloves which averaged 338 ng/g of glove as compared with nonpowdered latex gloves which averaged 78 ng/g of glove. One powdered nonlatex control glove demonstrated 4.0 ng/g of glove while the

other was below the detection limits of the assay (0.5 pg/g of glove). On average examination gloves contained higher levels of protein, allergen, and endotoxin and this trend was apparent whether they were powdered or not. There were exceptions to this finding as one of the powdered examination latex gloves was very low in endotoxin content and two nonpowdered examination gloves were high in endotoxin content. The endotoxin content of the slurry and the unused dextran powder were found to be 64000 ng/mL and 0.3 ng/mL, respectively (Table 1).

The rate of endotoxin and allergen release into an aqueous buffer from latex gloves was very rapid (Fig 1). The majority of these materials was released in the first two minutes of incubation after which slowly increasing amounts were seen. When gloves were turned inside-out and these ex-

Table 1. Allergen, Protein, and Endotoxin Content of Surgical and Examination Gloves

	Glove Type		Allergen,* μg/g	Protein,† μg/g	Endotoxin,‡ ng/g
	Powder	Surgical/Exam			
1	P	S	88	150	0.09
2	P	E	21.9	145	0.4
3	NP	S	1.4	130	0.45
4	NP	S	1.5	<25	0.92
5	NP	S	<1	<25	3.80
6	P	S	32	90	3.80
7	P	S	66	85	5.80
8	P	S	2.7	68	6.00
9	P	E	214	360	23.0
10	NP	E	74.5	55	37.3
11	P	E	837	735	82.3
12	P	E	635	685	87.3
13	P	E	26.4	100	147.1
14	P	E	858	700	201.0
15	NP	E	3.1	45	210.5
16	NP	E	68.6	50	216.5
17	P	E	686	840	337.0
18	P	E	44	655	662.5
19	P	E	553	1150	2837.5
Vinyl	NP	S	<1	675	<0.0005
Neoprene	P	S	<1	25	4.0
Dry powder				50	0.32
Liquid slurry				405	64000

NP, nonpowdered; P, powdered; S, surgical glove; and E, examination glove.

* RAST inhibition method.

† Protein determined by the American Society for Testing Materials (ASTM) standard method D5712-95.

‡ Endotoxin by kinetic Limulus Amebocyte Lysate method (average of three determinations on same glove).

periments were repeated, only a small amount of protein was seen and no endotoxin was detected. These results indicate that both the endotoxin and protein are loosely attached to the inside of the gloves tested.

When powdered gloves were snapped in an enclosed room and the airborne particles were collected and analyzed, it was found that 73% of the endotoxin was associated with particles less than 7 μm aerodynamic diameter (Fig 2). Twenty-seven percent was associated with larger particles found in the first stage of the Anderson collector. The cross-linked dextran particles were only found in the first stage as their size ranged from 20 to 70 μm . Microscopic examination of air samples (Samplair) from the room during these experiments revealed mainly two types of particles. The first appeared to be typical cross-linked dextran particles and the second were much smaller and uniform in appearance and size.

The cross-linked dextran particles were found to be quite dense (>1.79 g/mL) and sedimented very quickly through 60% CsCl_2 without centrifugation. When a suspension of air sampled with the cyclone from a room in which gloves were snapped was layered over 60% CsCl_2 and the powder allowed to sediment, the endotoxin was entirely recovered from the supernatant.

DISCUSSION

This study demonstrates that natural latex gloves contain variable and in some cases very high amounts of endotoxin. Powdered gloves had an average of 338 ng of endotoxin per gram of glove. Nonpowdered gloves had roughly a quarter of that amount. Nonsterile examination gloves had an average of 135 times more endotoxin than did sterile surgical gloves. Although exceptions were observed, protein and allergen levels were higher in the powdered gloves and lower in the surgical gloves examined. No allergen was eluted from nonlatex gloves and a very small amount of endotoxin was detected in the one neoprene glove which was lightly powdered.

Table 2. Comparison of Powdered Versus Nonpowdered and Surgical Versus Examination Gloves

	Mean Values from Gloves Tested		
	Protein, $\mu\text{g/g}$	Allergen, $\mu\text{g/g}$	Endotoxin, ng/g
Powdered latex gloves (P)	444	313	338
Nonpowdered gloves (NP)	47	25	78
Ratio (P/NP) =	9.5	12.6	4.3
Latex examination gloves (E)	460	335	404
Latex surgical gloves (S)	75	27	3.0
Ratio (E/S) =	6.2	12.3	135

Data and abbreviations from Table 1.

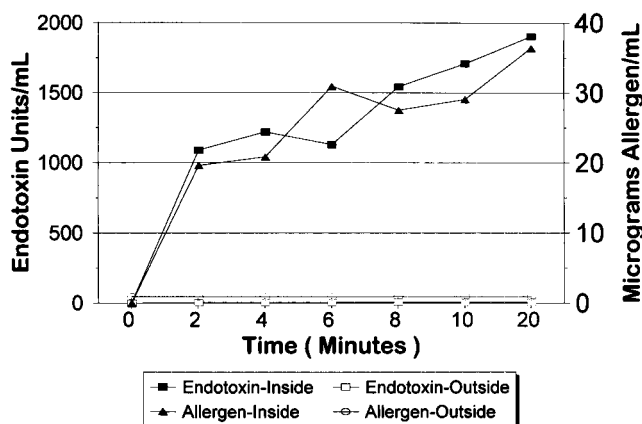


Figure 1. Kinetics of release from a latex glove: endotoxin and latex allergen. Gloves were filled with pyrogen-free water and mixed on a rocker. At the times indicated, samples were removed for analysis of endotoxin and allergen.

Both endotoxin and protein were released rapidly into an aqueous medium from the gloves tested. The similarity of the kinetics of release of both suggest that they may be associated. Levels of both endotoxin and protein increased at diminishing rates over the 20-minute observation period indicating some additional solubilization over time. Surprisingly, all the endotoxin and most of the protein were released from the interior of three different gloves tested. This would strongly suggest that the contamination with the protein and endotoxin occurred during manufacture while the gloves were on their molds in the inside-out configuration.

Upon gently snapping inverted gloves, we found that the majority (ie, 73%) of the endotoxin could be found in fully respirable airborne particles less than 7 μm in aerodynamic diam-

eter. This again suggests that the endotoxin is not associated with the cross-linked dextran particles which are much larger (20 to 70 μm in diameter). In addition, the endotoxin found in the upper stage of the Anderson sampler (27%) may be an overestimate because of the trapping effects of fluid medium in the first stage. Protein was not measured in these Anderson sampling experiments. The fact that both endotoxin and protein elute with similar kinetics in aqueous medium suggests that they may be associated with each other as an aerosol. This conclusion is somewhat contradictory to the findings of Tomazic et al who provided data that the cross-linked dextran particles were carriers of allergenic proteins.⁵¹ Upon close inspection, their report suggests that only a small fraction of the protein (1% to 11%) and specific IgE binding (6%) is associated with the

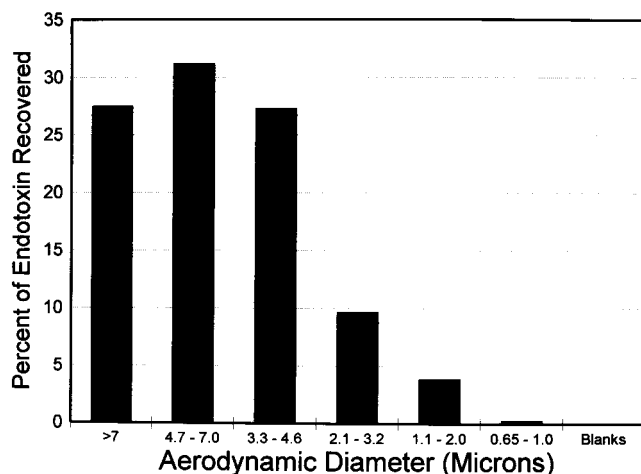


Figure 2. Size distribution of endotoxin in air. Gloves were snapped in a 25-m³ room and air samples were collected with the Anderson multistage sampler. An aliquot from each stage was assayed for endotoxin content.

cross-linked dextran particles. Since practically all the cross-linked dextran particles are too large to be fully respired, it is difficult to understand how these could reach tissues and effect allergen-specific mediator release and/or sensitization. The density of the cross-linked dextran particles we studied was shown to be very high (>1.79 g/mL). Powder of this density and size would not be expected to remain airborne for a long period of time and as such would not be likely to contribute to sensitization. This procedure also separated the endotoxin from the cross-linked dextran indicating again that the endotoxin is not closely associated with the cross-linked dextran particles.

What is the possible origin of this endotoxin? Since practically all of the endotoxin and most of the protein were found on the inner surface of the gloves, it is probable that they were passively absorbed from the dipping slurries through which the gloves were put prior to their removal from their molds. These slurries are kept at elevated temperatures, can be highly malodorous, and contain both protein and cross-linked dextran which should provide an excellent growth medium for microbes. In support of this, the slurry sample that we tested contained a high amount of endotoxin which is probably

an underestimate since the slurry had been frozen and thawed, a treatment that greatly reduces measurable endotoxin activity.⁵² Furthermore, there was only a small amount of endotoxin detected in the unused cross-linked dextran particles. It is reasonable to anticipate that the amount of endotoxin and protein in the slurry baths would increase over time during manufacture and thus with the size of a particular lot. This could result not only in lot-to-lot variations, as documented by Yunginger et al.,⁵³ but also in a time-dependent increase in both endotoxin and protein during the manufacture of a single lot. As demand for gloves increased, it is likely that the number of gloves/lot also increased. In addition, with the rapid increase in demand for latex gloves, more of the production was shifted to overseas sites, particularly Malaysia, where much of the latex is harvested. It is possible that the tropical environmental conditions at these sites favors higher levels of bacterial growth and thus more endotoxin in the slurries. The conversion from talc to cross-linked dextran powder, which occurred in the mid-80s (close to when problems with latex products began to accelerate) would also be expected to favor bacterial growth. Finally, the shortened time between harvest and use in production would be

expected to allow for less breakdown of proteins and endotoxin by the action of various proteases and ammonia.

Medical problems from endotoxin are diverse and have been well documented. They include irritation, rhinoconjunctivitis, pulmonary congestion and asthma, fever, malaise, and anaphylactoid shock.¹³⁻¹⁸ These nonspecific effects have been shown to involve the induction of proinflammatory cytokines such as TNF- α , IL-6, and IL-8 via specific humoral factors (LBP-lipid binding protein-a co-factor in the binding of LPS to CD-14) and cell receptors (CD-14).⁶⁻¹² These cytokines can in turn induce chemotactic factors, cellular adhesion molecules, and effector systems such as nitric oxide synthetase and platelet activating factor.^{54,55} The clinical effects of exposure to endotoxin depends upon the dosage, the route of exposure, the structure of the endotoxin encountered, and individual susceptibility factors.^{19-24,57} Specific effects of endotoxin include its activity as an immunologic adjuvant both for IgE antibodies to bystander antigens when administered through the respiratory route and also as an adjuvant for delayed hypersensitivity to chemical haptens on the skin.^{13,25-28} Both the nonspecific and specific activities of endotoxin could be playing a role in clinical problems with natural latex products and these are not necessarily mutually exclusive. This may explain some of the difficulties in diagnosing these problems as these symptoms can arise through specific or nonspecific mechanisms. This possibility is strengthened by Sullivan's finding that a number of ill defined symptoms blamed upon latex sensitization do not seem to be associated with the presence of IgE antibodies.⁵⁶ In addition to being an immunologic adjuvant, endotoxin has been shown to enhance antigen-specific mediator release from basophils.²⁸⁻³¹ The possibility exists that endotoxin can magnify these immunologically induced reactions. This in turn could explain why so many of these reactions are so severe.

Our experiments demonstrated that ten lightly snapped gloves (with high endotoxin content) in a room of 25 m³ resulted in a concentration of endotoxin in the air of 520 ng/m³. This is a highly significant level of endotoxin as illustrated by studies indicating that levels of 10 ng endotoxin/m³ and 100 ng endotoxin/m³ can significantly compromise asthmatic and normal individuals' pulmonary problems.^{57,58} The total endotoxin exposure to a single pair of highly contaminated gloves (#19) can be calculated to be around 84 µg assuming the average glove weighs 7.5 g and the endotoxin is confined to the inside of the glove. Since many pairs of these gloves are worn during a working period, the actual exposure would be much higher. The time the gloves are worn might also be a factor in estimating exposure as moisture accumulates over time and would be expected to enhance elution of both endotoxin and proteins.

An additional complication of endotoxin-induced inflammation includes the fact that other bacterial products such as peptidoglycans and teichoic acid can not only induce similar inflammation but can act to prime animals in a way that their reactions to endotoxins are more severe.¹⁹⁻²⁴ The possibility exists that these priming effects could also affect the susceptibility of different individuals at different times to exhibit symptoms upon exposure to endotoxins and allergens.

These findings strengthen the arguments that increased demand and changes in manufacturing procedures, such as the use of cross-linked dextran, the production of larger lots of gloves, and the increase use of foreign production sites are likely factors responsible for the current high incidence of sensitization to natural latex products. They also indicate that the powder is most likely relevant to this phenomenon, not as a carrier of allergens, but as a growth source for bacteria. Collectively, these processes would be expected to increase the chances of bacterial and protein contamination.

In conclusion, given their nonspecific and specific inflammatory poten-

tial, we propose that the finding of endotoxin in some latex gloves could have serious health implications. Endotoxin would be expected to be an irritant to the skin and to increase the sensitization rate to chemicals inherent in these products. Second, since endotoxin was found in small fully respirable particles, areas of heavy glove usage may represent an environmental threat from endotoxins alone. The adjuvancy properties of endotoxin through the respiratory route would be expected to increase sensitization rates. Finally, the fact that endotoxin and other bacterial products can enhance antigen-specific mediator release and other inflammatory processes may explain why many of the reactions to these products are so severe. This latter property of endotoxin may also provide an explanation for why a high number of latex skin tests are positive in individuals with negative clinical histories.^{35,59}

Our results do demonstrate that these contaminants readily elute from natural latex gloves in aqueous medium and thus can be removed by washing. The observation that several different gloves were very low in protein, allergen, and endotoxin provides evidence that this can be accomplished on a routine basis.

ACKNOWLEDGMENTS

We thank Dr. Ronald Berzofsky at Biowhittaker for providing reagents for endotoxin measurements, Dr. Timothy J Sullivan for his more than helpful editorial suggestions, and Ms Varsha DeWitt for her excellent technical assistance.

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