

IgE-dependent reactions to urologic catheter extracts by skin testing in latex-allergic patients

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Background: Latex allergy is mediated by residual IgE-binding proteins found on latex products. While most reported reactions involve latex gloves, the allergenic potential of other latex containing devices is unknown.

Objective: To assess the allergenic potential of latex urologic catheters.

Methods: Two identical lots of urologic catheters were extracted in sterile saline and analyzed for latex protein content using sensitive ELISA and Western blot assays. The extracts were tested by skin prick testing in a population of 47 latex-allergic patients.

Results: Latex proteins were detected by ELISA assay, however, the Western Blot method was not sensitive enough to measure latex proteins in these catheter extracts. Skin prick testing using a standard latex reagent (Bencard) demonstrated the test population to be extremely sensitive to latex as 68% of the population reacted to a 1/100,000 dilution. Using undiluted catheter extracts, only 11% of these latex-allergic patients reacted to catheter lot A and 2% to catheter lot C, by prick skin test. Eight percent of the control patients had a positive latex prick skin test and none reacted to the catheter extract.

Conclusions: The data suggest that processing and leaching of these specific latex urologic catheters resulted in a very low latex allergen content. The low prevalence of skin test reactivity in a population of latex-allergic patients suggests that these catheters would be an unusual cause of allergic reactions. It is not possible, however, to determine whether latex-allergic patients could safely use latex urologic catheters.

INTRODUCTION

Latex products elicit allergic reactions in some patients.¹ Type 1 allergy is mediated by IgE antibody to residual rubber tree proteins in latex medical devices.²⁻⁷ Life threatening anaphylactic shock and death are extreme consequences of latex allergy.⁸ Atopic health care workers are an important risk group for latex allergy.^{9,10} The reason appears to be a genetic predisposition in combination with prolonged exposure primarily to powdered latex gloves. Children with spina bifida and patients who require recurrent surgical

procedures with frequent mucosal latex exposure are other risk groups for latex allergy.^{11,12} These patients undergo multiple surgical procedures and often require temporary or permanent urinary catheterization.

Over 90% of reported latex allergic reactions have been in response to powdered latex gloves or barium enema catheter tips.¹³ Although there are isolated case reports of type 1 allergic reactions to other latex devices, the allergenic potential remains unknown. Latex urologic catheters are manufactured differently than latex gloves and there have only been rare reports of allergic reactions.

Our objective was to determine whether extracts of urologic catheters elicit a reaction by prick skin testing in patients with known latex allergy. These data are important in determining the safety of latex catheter use.

MATERIALS AND METHODS

Patient Population

The patient population consisted of 47 otherwise healthy volunteers who had been diagnosed as latex allergic based on history and confirmed by positive latex prick skin testing (Bencard Laboratories, Mississauga, Ontario). The control population consisted of 26 atopic non-latex-allergic individuals (Table 1). The research protocol was approved by the Ethics Review Committee of the Ontario Allergy Society and each patient participated after informed consent.

Catheter Extraction

Three extracts (A, B, and C) were prepared in the laboratories at C R Bard, Inc. (Urological Division, Covington, GA). Two extracts (A and C) were prepared from latex catheters (BARD, Luricath Foley Catheters), one extract (B) consisted of a saline control. Extracts were prepared under sterile conditions with two different lots of urologic catheters. For each catheter extract, 30 catheters were completely submerged in extraction buffer (7.5 mL/g of latex) in a container which ensured all surfaces were in contact with the extraction medium. The extraction was carried out for two hours at 37 °C in normal saline (sterile, non-pyrogenic 0.9% NaCl solution, McGaw, Inc, Irvine CA). The extracts were sent to the investigators for skin testing and for latex protein antigen analysis. The investigators were blinded as to the composition of the extracts.

Ammoniated Latex Protein Extract

Non-compounded ammoniated latex was obtained from Guthrie Latex (Tucson, AZ). Three lots of ammoniated latex (Microtex, Unitex, Dynatex) were mixed together and poured onto a

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Table 1. Demographic Characteristics of Latex Allergic Patients and Controls

Characteristic	Latex Allergic Patients (N = 47)	Controls (N = 26)
Atopic/non-atopic	39/8	10/16
Age, years, mean (range)	34 (10-64)	43 (23-67)
Sex, M/F	4/43	6/20
No. HCWs*	41	7 (with nonspecific hand dermatitis only)
Clinical symptoms	All had clinical allergy to latex 44 (94%): local latex allergy, contact urticaria, eczema 42 (89%): inhalant latex allergy, rhinoconjunctivitis 20 (43%): anaphylaxis to latex	9 hand dermatitis 5 food allergy (urticaria/angioedema) 5 nonatopic/non-latex exposed 3 allergic rhinitis 1 each - angioedema, nonspecific rhinitis, local anaesthetic reaction, eczema

* HCWs = laboratory, hospital, dental health care workers.

large glass plate, spread thinly with a glass rod and air dried overnight to produce a thin film. The film was cut into small pieces and extracted in carbonate buffer (pH 9.6) overnight at 37°C with continuous agitation. The extract was centrifuged (1000 g, 30 minutes), dialyzed for three days at 4°C to remove impurities and lyophilized. The residue was reconstituted to 1 mg/mL in phosphate buffered saline (pH 7.4) for a latex protein-specific indirect ELISA assay and Western Blot analysis, and, reconstituted to 1 mg/mL in phosphate buffered saline/50% glycerol for skin testing.

Skin Testing

Skin prick tests were done on the volar aspect of the forearm at least 2.5 cm apart using a drop of test solution and the epidermis beneath the drop pricked with a 25-gauge needle. The needle was wiped clean between skin tests. Skin prick test sites were wiped clean after 15 minutes and the wheal and flare reactions were carefully measured. Positive wheal (>5 mm of the negative control) and flare reactions were outlined, transferred onto transparent tape and recorded as a permanent record. To avoid adverse reactions and to assess the degree of patient sensitivity, skin prick testing with latex containing extracts was done using serial dilutions. A dilution of 1/100,000 was the initial concentration with continued testing using subsequent tenfold lower dilutions (higher concentrations of extracts) until a positive skin test reaction was obtained. Allergenic components used for skin prick testing

included the standard latex reagent, Bencard ammoniated latex; ammoniated latex, 1 mg/mL, determined by ELISA assay; three latex urologic catheter extracts; and eight commercially available food allergen extracts (1/10 - banana, avocado, kiwi, pineapple, tomato and potato; 1/20 - chestnut and milk). Histamine (1 mg/mL) was used as a positive control and diluent (human serum albumen, 0.3 mg/mL in 0.9% saline) and normal saline were negative controls.

Latex ELISA for Antigenic Protein

A latex protein-specific, indirect ELISA technique was used to quantify the latex protein content in the extracts.¹⁴ Latex protein antigens from a standard preparation (prepared from ammoniated latex) or the catheter extracts were adsorbed to the wells of a 96-well ELISA plate by incubating twofold serial dilutions of the solutions in the wells for four hours. Next, non-specific binding of protein was blocked using an excess of a non-immunoreactive protein, bovine serum albumin (Fraction V, Sigma Chemical Co). Third, a specific, rabbit anti-latex antiserum (1/5000 dilution) was allowed to react with the plastic-bound latex proteins. Fourth, the specifically bound rabbit anti-latex antibodies were reacted with a 1/1000 dilution of goat anti-rabbit IgG conjugated with horseradish peroxidase (Sigma). Finally, a colored reaction product is produced by incubating in 10 mg/mL of the horseradish peroxidase substrate *o*-phenylenediamine. Protein content is calculated using a parallel line assay

by comparing the unknowns to the standard.

Western Blot

Latex proteins were visualized by Western blot as previously described.¹⁵ In brief, latex proteins were separated by SDS-PAGE on 15% polyacrylamide gels. The separated proteins were transferred to nitrocellulose (Scheicher and Schuell, Keene, NH) using the buffer system of Towbin.¹⁶ The membranes were blocked for one hour with 100 mg/mL bovine serum albumin, and incubated overnight at 37 °C with a 1/1000 dilution of rabbit anti-latex antiserum. The blots were washed three times and reacted for one hour at 37 °C with 1/5000 dilution of alkaline-phosphatase labelled anti-rabbit IgG (Promega, Madison, WS). After final washing to remove non-reacted anti-rabbit IgG, protein bands were visualized using nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate (Promega).

RESULTS

Protein Testing

Latex ELISA for antigenic protein is a sensitive and specific assay for latex protein antigens with a detection limit of 31 ng/mL (0.23 µg/g catheter).¹⁴ The catheter extracts were analyzed using twofold serial dilutions starting with undiluted extract solution. Table 2 shows the values for the catheter extracts and the standard solutions for skin testing. The catheter extracts (A and C) were found to have levels of latex proteins near the lower limits of

Table 2. Latex Antigenic Protein Levels of Skin Testing Reagents

Test Solution	Latex Protein Level
Bencard latex	7700
Amm. Ltx protein	1000
Extract A	0.049* (0.4 µg/g)
Extract B (saline)	below detection
Extract C	0.036* (0.3 µg/g)

Latex protein levels are reported in µg/mL as determined by the latex ELISA for antigenic protein assay.

* Estimated latex protein levels were near the detection limit of 0.03 µg/mL (0.2 µg/g of catheter).

the ELISA assay (0.2 µg/g catheter). Extract B (saline negative control solution) was as expected, below detection.

Western blot analysis identifies protein bands using specific antiserum and characterizes the proteins based on molecular size. As shown in Figure 1, ammoniated latex proteins are a mixture of polypeptides which range in molecular size from less than 10 kDa to greater than 150 kDa. By serial dilution analysis, it was determined that the limit of detection of the Western blot was 0.63 µg (158 µg/g catheter) of latex protein (Fig 1). When catheter extracts A and C were analyzed by

Western blot analysis (Fig 2) no protein bands were observed. Similar negative findings were obtained when the immunoblot was performed using pooled sera from latex-allergic patients (data not shown).

Skin Testing

Skin prick test may be a better indicator of the latex protein sensitivity limit. Skin testing was done in patients who were clinically very allergic to latex (Table 3). The results of the skin testing in non-latex-allergic control patients are as follows. The histamine reaction was positive in all 26 control subjects. None of the control subjects reacted to the saline or albumin control solutions. None of the non-latex-sensitive subjects reacted to the catheter extracts demonstrating that there was no irritant or nonspecific effect. One control subject reacted to the Bencard latex skin testing reagent, and another control subject reacted to ammoniated latex protein; both of these individuals had food allergies.

Latex-allergic patients have a wide range of sensitivity to latex, therefore, in an effort to determine the individual sensitivity, each patient was skin tested by end point titration. The skin prick

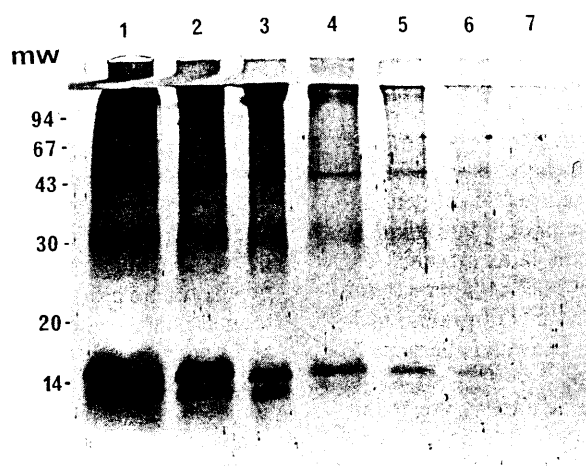


Figure 1. Calibration of the anti-latex protein Western blot. Ammoniated latex proteins (AL) were separated by SDS-PAGE and transferred to a nitrocellulose membrane. The membrane was then reacted with the rabbit anti-latex antiserum and AP-labeled anti-rabbit IgG as the second antibody to reveal the latex proteins. The total amount of latex protein loaded in each well was: 1 = 20 µg; 2 = 10 µg, 3 = 5 µg, 4 = 2.5 µg; 5 = 1.25 µg; 6 = 0.63 µg; and 7 = 0.31 µg. The molecular weight is indicated in the left margin.

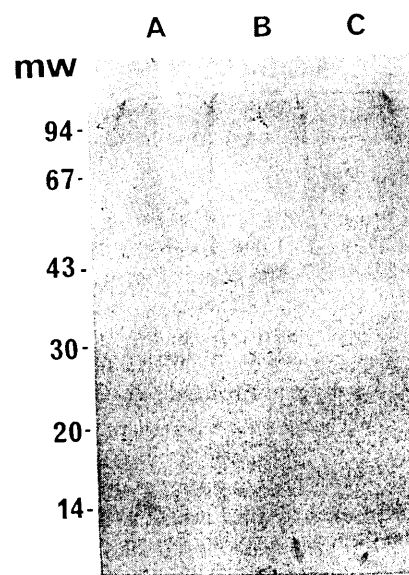


Figure 2. Anti-latex protein Western blot analysis of catheter extracts. Extracts A, B, and C (30 µL each) were separated by SDS-PAGE and transferred to nitrocellulose. The membrane was then reacted with rabbit anti-latex antiserum and AP-labeled anti-rabbit IgG as the second antibody to visualize latex proteins. The molecular weight is indicated in the left margin.

test results for the 47 latex-sensitive patients are presented in Table 3. Patient sensitivity was assessed by the dilution of latex extract at which they first reacted. The population of latex-allergic patients was highly sensitive to latex as 68% of the population reacted to 1/100,000 of the Bencard skin prick test reagent representing approximately 77 ng/mL latex protein.

Reactivity to Catheter Extracts

The results of skin prick tests with the catheter extracts were as follows: catheter extract A = 5/47 (11% skin test positive) saline negative control, extract B = 1/47 (2% skin test positive), and catheter extract C = 1/47 (2% skin test positive). All of the skin test-positive reactions to catheter extracts were to undiluted solutions. All five patients who reacted to extract A were skin test positive at a 1/100,000 dilution of the Bencard latex extract. This represents reactivity to approximately 77 ng/mL based on the latex antigen content of

Table 3. Skin Test Reactivity to Catheter Extracts in Latex Allergic Patients

Extract Dilution (1/dilution)	Bencard PST reagent	Amm. Ltx	Catheter Extract A	Catheter† Extract B	Catheter Extract C
100,000	32 (68%)*	22 (47%)	0 (0%)	0 (0%)	0 (0%)
10,000	2 (4%)	6 (13%)	0 (0%)	0 (0%)	0 (0%)
1000	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
100	5 (11%)	6 (13%)	0 (0%)	0 (0%)	0 (0%)
10	1 (2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
1	7 (15%)	8 (17%)	5 (11%)	1 (2%)	1 (2%)
Totals	47/47 (100%)	42/47 (89%)	5/47 (11%)†	1/47 (2%)	1/47 (2%)

Data represent the absolute number (and percentage) of 47 latex-allergic patients with positive skin tests at the extract dilution shown.

* All skin testing was done by dilution analysis beginning with a 1/100,000 dilution. Testing was stopped when a positive skin reaction was obtained.

† All patients that reacted to the catheter extracts also reacted to the Bencard reagent at a 1/100,000 dilution representing a sensitivity limit of 77 ng/mL (0.6 µg/g catheter) based on results with Bencard SPT reagent.

‡ Control, saline solution.

the Bencard reagent. This is equivalent to 0.6 µg latex protein/g of urinary catheter. One patient reacted to both extract C and extract A. In contrast, the other four patients who reacted to catheter extract A were skin test negative to extract C. Both A and C catheter extracts were from similar latex catheters but different lot numbers. It is possible, however, that lot A had slightly more allergen than lot C. One subject reacted to extract B (saline) and one to the saline control. This most likely represents individual patient hyperresponsiveness due to the large number of skin tests.

DISCUSSION

In this study we have assessed the level of latex protein antigens and allergens in one type of urologic catheter. These catheters were qualitatively and quantitatively analyzed for latex protein by Western blot and ELISA techniques which are sensitive laboratory tests.^{14,15} The catheter extracts used in these studies have very low levels of latex protein antigens. Extracts A and C were found to have slight reactivity in the ELISA assay (0.2 µg/g) which was near the lower limit of detection. The latex protein in these extracts was below the detection limit of the less sensitive Western blot assay.

By skin testing, we confirmed that these urologic catheter extracts contain extremely low levels of latex allergens. In our control group, no skin test reac-

tivity to the catheter extracts was found demonstrating that they did not contain irritants.¹⁷ Two control patients with a negative history of latex allergy had positive skin prick test to latex skin test reagents. Both of these individuals had food allergies and positive prick skin test to banana, avocado, and chestnuts. These foods are known to contain cross-reacting allergens with latex¹⁸⁻²² and we feel this most likely represents food cross-reactivity.

Using prick skin testing, catheter extract A elicited reactions in 11% and catheter extract C in 2% of our latex-allergic patients. None of these patients reacted to a tenfold dilution and were skin prick test positive only to full strength catheter extracts. These latex allergic patients were extremely sensitive by end point titration skin testing with standard latex skin test reagents. They reacted to diluted skin test extracts containing less than 77 ng/mL of antigenic latex proteins. The data confirm that both catheter extracts contain small amounts of protein measured by ELISA assay and allergens by prick skin testing.

Latex urologic catheters are manufactured under conditions that are very different from those used for the manufacture of gloves. Urologic catheters are thicker products produced by multiple dippings that can withstand multiple rigorous washing procedures. The very low protein and allergen levels of these catheter extracts suggest that la-

tex urologic catheters manufactured under rigorous conditions with prolonged leaching followed by chlorination results in a lower allergen level. These findings support the clinical data that latex urologic catheters are an unusual route for sensitizing individuals to latex proteins or for inducing allergic reactions.¹³ This study demonstrated that medical devices made from natural rubber latex can be manufactured with a low allergen content.

In the present study it is not possible to determine whether latex-allergic patients could use latex urologic catheters. Others have reported systemic reactions as a result of bladder catheterization.²³ Latex gloves are known to differ from lot to lot and brand to brand in their levels of residual latex protein and allergens.^{14,24} This may also be true for urologic catheters. Latex devices may contain insoluble latex proteins which upon exposure to body fluids may release some allergen by enzymatic processes.²⁵ There is prolonged contact with urologic catheters and expansion of the catheter balloon may release latex allergens. Presently, latex-allergic patients should only be exposed to non-latex devices including urologic catheters whenever possible.

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