Cow's milk casein, a hidden allergen in natural rubber latex gloves

Leea Ylitalo, MD,[a] Soili Mäkinen-Kiljunen, PhD,[b] Kristiina Turjanmaa, MD,[a,d] Timo Palosuo, MD,[c] and Timo Reunala, MD,[a,b,d] *Tampere and Helsinki, Finland*

Background: We have previously noted that one natural rubber latex (NRL) glove brand used for skin prick testing in the diagnosis of NRL allergy contained cow's milk casein.
Objective: We sought to examine whether other commonly marketed NRL glove brands contain cow's milk casein.
Methods: The casein content of 30 NRL glove extracts (1:5 wt/vol) was measured by RAST inhibition and by rocket immunoelectrophoresis and rocket radioimmunoelectrophoresis by using casein-specific rabbit antiserum, a serum pool from patients with cow's milk allergy, and radiolabeled anti-human IgE. The NRL allergen content was measured by ELISA inhibition.
Results: The casein content of the glove used in NRL allergy screening (Triflex, Baxter) was rather high (ie, about 400 μg/g of glove). Its total protein content was 1000 μg/g of glove. Rocket radioimmunoelectrophoresis detected distinct amounts of casein in 8, minimal amounts in 7, and no casein in the remaining 15 NRL glove brands.
Conclusions: Several brands of NRL gloves were found to contain casein, implying that extracts prepared from such gloves can cause false-positive skin prick test reactions when diagnosing NRL allergy. The use of casein as a stabilizer in glove manufacture without appropriate labeling should be stopped because it can also cause contact urticaria syndrome in individuals with cow's milk allergy. (J Allergy Clin Immunol 1999;104:177-80.)

Key words: *Latex gloves, cow's milk allergy, casein, latex allergy testing*

Abbreviations used
NRL: Natural rubber latex
RIE: Rocket immunoelectrophoresis
RRIE: Rocket radioimmunoelectrophoresis

Surgical gloves and many other rubber products are made of latex derived from the rubber tree *(Hevea brasiliensis)*. Many chemicals (eg, accelerators and antioxidants) are added to natural rubber latex (NRL) during the glove manufacturing process.[1,2] The chemicals, mainly thiurams, are responsible for type IV hypersensitivity reactions in persons using gloves.[3,4] Other compounds, including cow's milk casein, are sometimes added during the manufacture to improve, for example, the physical properties of the gloves.[2,5] NRL proteins eluting from the gloves are increasingly reported to cause immediate type I hypersensitivity reactions.[6,7] Because of the lack of standardized, commercial NRL allergens, NRL glove eluates have been used for over 10 years in skin prick testing to diagnose NRL allergy.[8-10] Moreover, a use test, which involves applying a piece of NRL glove directly onto wetted skin, has been performed to confirm the diagnosis.[7,9,11] Here we report that several brands of NRL gloves contain cow's milk casein, which may cause false-positive skin prick and use test responses in patients screened for NRL allergy.

METHODS

Patients

From 1992 to 1995, we screened 3269 children for NRL allergy by skin prick testing with a NRL glove eluate (Triflex; Baxter, Valencia, Calif; 1:5 wt/vol incubated in physiologic saline). Fifty children with positive results were reexamined to confirm the diagnosis of NRL allergy by using 3 diagnostic criteria: a positive skin prick test response to NRL allergens, latex RAST results, and use test responses with Triflex glove.[12] The use test was done as previously described.[12] Briefly, it was started with a finger piece of NRL glove placed on a wetted finger for 15 minutes. The test response was regarded as positive if 2 or more wheals were seen on the contact area. If the response was negative, the challenge was continued with a piece of glove placed around the wrist for 15 minutes. Thirty-three (1.0%) of the 3629 children were confirmed to have latex allergy by latex RASTs and latex glove use tests. Five children did not fulfill these criteria, although they all had contact urticaria during the use test. They had no symptoms from NRL products. All 5 children had atopic dermatitis, 3 of them also had asthma, and all these children were allergic to cow's milk. Therefore we decided to examine NRL gloves for cow's milk allergens.

Skin prick tests and RASTs

All 5 children were skin prick tested with a Triflex glove eluate and a standardized commercial NRL allergen (Stallergènes skin prick test reagent, Antony, France),[13] as well as with cow's milk

From [a]the Department of Dermatology, Tampere University Hospital, Tampere; [b]the Hospital for Skin and Allergic Diseases, Helsinki University Central Hospital, Helsinki; [c]National Public Health Institute, Helsinki; and [d]Medical School of the University of Tampere, Tampere.
Supported by the Tampere University Hospital Research Fund and Helsinki University Central Hospital.
Received for publication Nov 2, 1998; revised Mar 3, 1999; accepted for publication Mar 16, 1999.
Reprint requests: Leea Ylitalo, MD, Department of Dermatology, Tampere University Hospital, PO Box 2000, FIN-33521 Tampere, Finland.
Copyright © 1999 by Mosby, Inc.
0091-6749/99 $8.00 + 0 **1/1/98638**

TABLE I. Laboratory results in 5 children with cow's milk allergy

Sex/Age (y)	SPT glove eluate*	NRL allergen†	Cow's milk	RAST (kU/L) Latex	RAST (kU/L) Cow's milk	RAST (kU/L) Casein	Use test‡	Cow's milk challenge/ symptoms
F/2.4	3 (6)	0	10 (5)	<0.4	>400	>100	+/CU	+/CU
M/13.1	4 (6)	0	9 (6)	<0.4	22.1	24.2	+/CU	+/CU
M/2.6	3 (5)	0	15 (5)	<0.4	80.2	88.2	+/CU	+/CU
M/1.2	4 (5)	0	7 (5)	<0.4	20.3	29.8	+/CU	+/CU,FO
M/1.9	3 (4)	0	10 (5)	<0.4	3.0	20.5	+/CU	+/CU,FO

SPT, Skin prick test wheal diameter measured in millimeters (histamine dihydrochloride); *CU,* contact urticaria; *FO,* facial and lip edema.
*Triflex glove eluate (1:5 wt/vol) in screening.
†Stallergènes, SA.
‡Triflex glove.

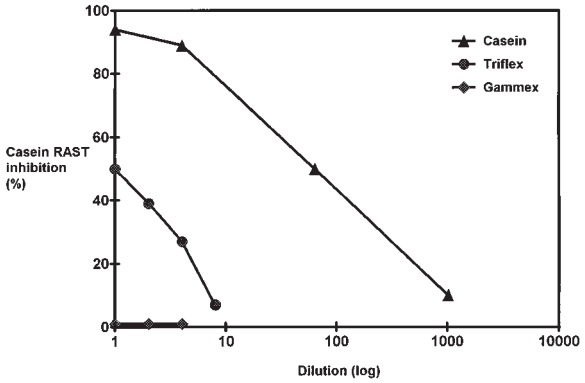

FIG 1. Cow's milk casein allergenicity in 2 NRL gloves measured by casein RAST inhibition. Triflex (glove eluate) distinctly inhibited the binding of IgE antibodies to casein (50%), whereas no inhibition was demonstrated with Gammex (glove eluate). Cow's milk casein (5 mg/mL) was used as the standard.

allergen (Allergologisk Laboratorium a/s, ALK, Hørsholm, Denmark). Serum IgE antibodies to latex,[12] cow's milk, and casein were measured by commercial CAP RAST (Pharmacia & Upjohn, Uppsala, Sweden) as previously described.

Casein content of NRL gloves

RAST inhibition. Casein in the Triflex (Baxter, lot 06 92L12DPGN) gloves was measured as allergen activity by using glove eluate (1:5 wt/vol) as inhibitor and casein RAST discs (f78, Pharmacia) as solid-phase allergens. A serum pool from the 5 children was used as the source of casein IgE antibodies. Dilutions of α-casein (Sigma C-7891), ranging from 5 μg/mL to 5000 μg/mL, were used as standards. A Gammex (Ansell, lot 9294 407) glove was used as a control glove because it is known for its low casein[14] and low NRL allergen (10 allergen units/g of glove) content.[15] Four 2-fold dilutions were made from the glove eluates. Nonammoniated NRL (protein content 9.6 mg/mL) served as a negative control. The relative casein allergen activity was calculated by the parallel line method.

Immunoelectrophoresis. Casein in the Triflex and Gammex glove eluates was also measured as precipitable protein in rocket immunoelectrophoresis (RIE)[16] by using rabbit IgG antibodies to casein (2.28 μL/cm^2; Pharmacia & Upjohn, Uppsala, Sweden). Fifteen microliters of α-casein (from 0.3 to 1250 μg/mL) was used as the standard, and 2-fold dilutions from the gloves were applied to gel wells. The casein content was calculated from the height of the rocket on the plate after protein staining with Coomassie Brilliant Blue stain.

Casein allergens in the 30 brands of NRL glove eluates (28 surgical and examination gloves and 2 household gloves; 1/5 wt/vol) widely marketed in 1994[17] were examined by rocket radioimmunoelectrophoresis (RRIE).[16] For this, RIE was run as described above, and then, instead of protein staining, the plate was consecutively incubated with a pool of 50 sera from patients allergic to milk and radiolabeled anti-human IgE (Pharmacia & Upjohn). IgE binding to casein allergens was visualized by autoradiography. NRL and cow's milk skin test allergens were used as negative and positive controls, respectively.

Total protein measurement and NRL allergen content

The total protein content of the Triflex glove eluate was measured by the method of Lowry, as previously described.[18] The NRL allergen content measured by ELISA inhibition, as previously described, was 1400 allergen units/mL in the glove eluate (ie, 7000 allergen units/g of glove).[17]

RESULTS

Skin prick tests and RASTs

Skin prick test responses with a commercial NRL allergen and latex RAST results were negative in all 5 children, although they had positive skin prick test responses to Triflex glove eluate (Table I). Skin prick test responses with cow's milk allergen and RAST results to cow's milk and casein were clearly positive in all 5 children (Table I). All children showed immediate symptoms (contact urticaria and lip and facial edema) to cow's milk in open challenge.

Casein content of gloves

In casein RAST, a distinct inhibition was observed with the Triflex, but not with the Gammex, glove eluate (Fig 1). The NRL control preparation did not inhibit the casein RAST. The casein content calculated from the standard was 75 μg/mL (ie, 375 μg/g) for the Triflex and less than 1 μg/mL (ie, 5 μg/g) for the Gammex glove.

In RIE for casein antigens, a faint staining of rockets was demonstrated with the Triflex, but no staining was seen with the Gammex glove (results not shown). The casein content calculated from the standard was 78 μg/mL (ie, 390 μg/g) for the Triflex and less than 2 μg/mL (ie, 10 μg/g) for the Gammex glove.

In RRIE for casein allergens, a distinct binding of IgE

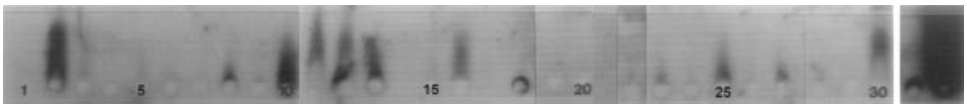

FIG 2. Casein allergens in 30 NRL gloves demonstrated by RRIE. Gloves examined were *(1)* Lic examination; *(2)* Triflex (Baxter); *(3)* Ultraderm (Baxter); *(4)* Medical Exam, no powder (Ansell); *(5)* Conform Sterile (Ansell); *(6)* Nutex (Ansell); *(7)* Medical, no powder (Ansell); *(8)* Conform nonsterile (Ansell); *(9)* Gammex (Ansell); *(10)* Bextmold (Bextmold); *(11)* Micro-Touch (J&J); *(12)* Neutralon (J&J); *(13)* Sempermed NS (Semperit); *(14)* Sempermed Ultra (Semperit); *(15)* Sempermed Classic (Semperit); *(16)* Digitil HP (Wuhlin-Soplamed); *(17)* Digitact (Wuhlin-Soplamed); *(18)* Glads (Mölnlycke); *(19)* Prolax (Prod del latex); *(20)* Biogel (Regent); *(21)* Household (Ansell); *(22)* Household (Vasco de C); *(23)* Safeskin E (Safeskin); *(24)* Safeskin NP (Safeskin); *(25)* Perry White latex (S&N); *(26)* Medigrip (Ansell); *(27)* SensiTouch (Ansell); *(28)* SensiDerm (Ansell); *(29)* Ansell EP (Ansell); and *(30)* Flexam E (Baxter). Controls were *(31)* NRL and *(32)* cow's milk skin test allergen. A distinct binding of IgE antibodies was demonstrated in *2, 10, 11, 12, 13, 16, 25*, and *30*; a faint binding was shown in *3, 5, 8, 15, 23, 27*, and *28*.

antibodies was demonstrated in the Triflex gloves and in 7 other glove brands (Fig 2). The heights of the rockets were approximately of the same order of magnitude (11 to 17 mm) in all of these gloves. In addition, 7 other glove brands showed a faint IgE binding (rocket heights of 1 to 5 mm), but the remaining 15 glove brands, including the Gammex glove, were negative for casein in RRIE.

Total protein content of the Triflex glove

The Triflex glove had a total protein content of 1000 μg/g of glove.

DISCUSSION

It is well known that NRL allergen levels may vary considerably between different glove brands and that the reactions of patients allergic to NRL to gloves are dependent on the allergen amount present in the gloves.[17,19-21] This study showed that a hidden allergen, casein, identified in several glove eluates, could be responsible for the positive skin prick and use test responses in the children allergic to cow's milk in this study. It is not commonly known that cow's milk casein can be added as a stabilizer during glove manufacture.[2,5] In this study we examined the casein content by RRIE in 30 widely marketed brands of NRL gloves and found casein in 8 of these brands. These included the Triflex glove, which has been used because of its high NRL allergen content[18,19] in skin prick and use tests when screening and diagnosing NRL allergy. The casein results in RRIE could be confirmed by RAST inhibition, and a mean casein content of 77 μg/mL (ie, about 400 μg/g of glove) was obtained for the Triflex glove, a finding that is in accordance with our preliminary report.[14] The casein content of this glove was about half of the total protein content (1000 μg/g). This finding suggests that a total protein content is not a good measurement for NRL allergen content of gloves. A specific immunologic assay should be used for that purpose.

NRL glove eluates have been widely used in skin prick testing because a commercial and standardized NRL reagent has been available in European countries for only a short time,[13] and the NRL glove use test has been performed to confirm the diagnosis of NRL allergy.[6,12,22] Our study pointed out that standardized skin prick test reagents should be recommended in the screening and diagnosis of NRL allergy. When a use test is needed to confirm the allergy, it is mandatory to know that the test gloves do not contain casein. Otherwise false-positive skin prick and use test reactions may occur because cow's milk allergy is quite common among children and may also, although rarely, be present in adults.[23-25]

In this study we showed that several brands of NRL gloves contain casein, and use tests demonstrated that a casein-containing glove can cause contact urticaria in children allergic to cow's milk. Previously we reported the case of an adult patient with cow's milk allergy who had immediate symptoms when using casein-containing NRL gloves.[14] The children in our study had not been operated on or exposed to surgical or other NRL gloves. It seems evident that if children or adults allergic to cow's milk are exposed to casein-containing NRL gloves in the health care setting or at home, they may be at risk of having at least local allergic reactions. The greatest risk may occur during surgery, when casein elutes directly to mucous membranes from the surgeon's gloves. Mild allergic reactions, either from casein or NRL, may easily pass unnoticed, but systemic reactions would alert physicians to suspect the gloves and especially NRL allergy. If allergologic examinations fail to confirm NRL allergy, it seems relevant to test the patients for cow's milk allergy. To our knowledge, no systemic reactions have yet been attributed to casein eluting from the gloves, but there is one report of anaphylaxis from milk protein containing napkin rash ointment.[26] Casein could, in addition to NRL gloves, be added also to dummies and balloons. We preliminarily examined 2 rubber dummies and 2 balloons, but found no evidence of casein by the RAST inhibition method (S. Mäkinen-Kiljunen, unpublished observation). To avoid unexpected allergic reactions among persons with cow's milk allergy, casein should be labeled if it is used as an additive in NRL or other products.

In conclusion, casein was found, in several brands of NRL gloves, to be an additional allergen that can cause contact urticaria in persons with cow's milk allergy. If the use of casein as a stabilizer in glove manufacture is continued, there is a need to label packages appropriately. Standardized NRL reagents should be used for screening and diagnosing NRL allergy.

REFERENCES

1. Hamann CP. Natural rubber latex protein sensitivity. Am J Contact Dermat 1993;4:4-21.
2. Subramaniam A. The chemistry of natural rubber latex. In: Fink J, editor. Latex allergy. Immunol Allergy Clin North Am 1995. 1-20.
3. Heese A, v Hintzenstern J, Peters K-L, Koch HU, Hornstein P. Allergic and irritant reactions to rubber gloves in medical health services. J Am Acad Dermatol 1991;25:831-9.
4. Conde-Salazar L, del-Rio E, Guimaraens D, Gonzáles Domingo A. Type IV allergy to rubber additives: A 10-year study of 686 cases. J Am Acad Dermatol 1993;29:176-80.
5. Blackley DC. In: Higher polymer latices: their significance and technology. Volume I. London: Maclaren & Sons; 1966. p. 119-20.
6. Turjanmaa K, Alenius H, Mäkinen-Kiljunen S, Reunala T, Palosuo T. Natural rubber latex allergy. Allergy 1996;51:593-602.
7. Warshaw E. Latex allergy. J Am Acad Dermatol 1998;39:1-24.
8. Vandenplas O, Delwiche J-P, Evrard G, et al. Prevalence of occupational asthma due to latex among hospital personnel. Am J Respir Crit Care Med 1995;151:54-60.
9. Turjanmaa K, Mäkinen-Kiljunen S, Reunala T, et al. Natural rubber latex allergy—the European experience. In: Fink J, editor. Latex allergy. Philadelphia: Saunders; 1995. p. 71-88.
10. Liss G, Sussman G, Deal K, et al. Latex allergy: epidemiological study of 1351 hospital workers. Occup Environ Med 1997;54:335-42
11. Sussman G, Beezhold D. Allergy to latex rubber. Ann Intern Med 1995;122:43-46.
12. Ylitalo L, Turjanmaa K, Palosuo T, Reunala T. Natural rubber latex allergy in children who had not undergone surgery and children who had undergone multiple operations. J Allergy Clin Immunol 1997;100:606-12.
13. Turjanmaa K, Palosuo T, Alenius H, et al. Latex allergy diagnosis: *in vivo* and *in vitro* standardization of a natural rubber latex extract. Allergy 1997;52:41-50.
14. Mäkinen-Kiljunen S, Reunala T, Turjanmaa K, Cacioli P. Is cow's milk casein an allergen in latex-rubber gloves? [letter]. Lancet 1993;342:863-4.
15. Palosuo T, Turjanmaa K, Reunala T, Mäkinen-Kiljunen S, Alenius H. Allergen content of latex gloves used in 1994-1995 in health care in Finland. Results of renewed market survey in 1995. Publications of the National Agency for Medicines 1996;2:1-7.
16. Mäkinen-Kiljunen S, Turjanmaa K, Palosuo T, Reunala T. Characterization of latex antigens and allergens in surgical gloves and natural rubber by immunoelectrophoretic methods. J Allergy Clin Immunol 1992;90:230-5.
17. Palosuo T, Mäkinen-Kiljunen S, Alenius H, Reunala T, Yip E, Turjanmaa K. Measurement of natural rubber latex allergen levels in medical gloves by allergen specific IgE-ELISA inhibition, RAST inhibition, and skin prick test. Allergy 1998;53:59-67.
18. Alenius H, Mäkinen-Kiljunen S, Turjanmaa K, Palosuo T, Reunala T. Allergen and protein content of latex gloves. Ann Allergy 1994;73:315-20.
19. Turjanmaa K, Laurila K, Mäkinen-Kiljunen S, Reunala T. Rubber contact urticaria. Allergenic properties of 19 brands of latex gloves. Contact Dermatitis 1988;19:362-7.
20. Yunginger JW, Jones RT, Fransway AF, Kelso JM, Warner MA, Hunt LW. Extractable latex allergens and proteins in disposable medical gloves and other rubber products. J Allergy Clin Immunol 1994;93:836-42.
21. Beezhold D, Pugh B, Liss G, Sussman G. Correlation of protein levels with skin prick test reactions in patients allergic to latex. J Allergy Clin Immunol 1996;98:1097-102.
22. Liebke C, Niggeman B, Wahn U. Sensitivity and allergy to latex in atopic and nonatopic children. Pediatr Allergy Immunol 1996;7:103-7.
23. Schrander JJP, van den Bogart JPH, Forget PP, Schrander-Stumpel CTRM, Kuijten RH, Kester ADM. Cow's milk protein intolerance in infants under 1 year of age: a prospective epidemiological study. Eur J Pediatr 1993;152:640-4.
24. Isolauri E, Turjanmaa K. Combined skin prick and patch testing enhances identification of food allergy in infants with atopic dermatitis. J Allergy Clin Immunol 1996;97:9-15.
25. Sampson HA. Food Allergy. JAMA 1997;278:1888-94.
26. Lecks H. Anaphylaxis from milk protein in diaper ointment [letter]. JAMA 1980;244:1560.