

Letter to the Editor

Casein-related anaphylaxis after use of an Everlast kickboxing glove

To the Editor:

Food allergen exposure can occur in the most unusual places. We report the case of a 20-year-old woman with a lifelong severe allergy to cow's milk products involving episodes of anaphylaxis who experienced a near-fatal anaphylactic reaction within 15 minutes after donning and exercising with a pair of new Everlast kickboxing gloves (Model 2772; Everlast, New York, NY). She recognized the onset of anaphylaxis, but because she had not eaten for the whole day before the class and thus had no potential exposure to milk, she did not have her epinephrine autoinjectors accessible. 911 was called, and an extraordinary emergency medical technician effort quickly resulted in intubation and cardiopulmonary resuscitation after a cardiac arrest that lasted about 3 minutes. She was administered a hypothermia protocol at a tertiary care hospital and emerged awakened 36 hours later with what has been a full recovery.

The initial suspicion was that she had reacted to a component in her newly purchased kickboxing gloves. The material data safety sheet from the manufacturer indicated rubber components in the glove but no milk products. It was unclear from the material data safety sheet whether rubber components were natural or synthetic rubber, which raised suspicion of a possible natural rubber latex allergy.

Results of the patient's IgE antibody serology to natural rubber latex and cow's milk components are presented in Table I. During hospitalization, latex-specific IgE measurements performed in a ViraCor-IBT Laboratories (Lee's Summit, Mo)–developed RIA were weakly positive. This suggested that the anaphylactogenic substance might have been natural rubber latex. However, a second blood specimen collected 3 weeks later produced negative results (<0.1 kU_A/L) by using ImmunoCAP (Thermo Fisher Scientific–Phadia, Waltham, Mass) at both the Johns Hopkins Dermatology, Allergy and Clinical Immunology Reference Laboratory (Baltimore, Md) and ViraCor-IBT Laboratories. No explanation was provided by ViraCor-IBT Laboratories as to why the first specimen was weakly positive with their

laboratory-developed RIA and the second specimen was negative with RIA and ImmunoCAP. Moreover, a high level of anti-cow's milk IgE was consistent with the patient's previous IgE anti-milk serology. Thus suspicion as to the offending allergen in the glove switched from natural rubber latex to cow's milk.

The product manager of Everlast reported no knowledge of natural rubber latex or cow's milk proteins in the model 2772 Everlast glove, which was manufactured in China. Only the glove lining was known to be coated with Ultra Fresh NM-V2 (Thomson Research Associates, Toronto, Ontario, Canada) as an antibacterial agent (R. Stewart, personal communication, Everlast Worldwide).

Because of the lack of detailed composition information from Everlast, we systematically analyzed the model 2772 kickboxing glove by using destructive extraction testing with the goal of obtaining evidence of an allergenic protein. Table II presents the total protein results of the extractions prepared from 5 distinct material components of the glove.

Following the American Society for Testing Materials D7242 Standard for extraction of natural rubber latex proteins from medical gloves,¹ each material was extracted over 24 hours at room temperature by using a ratio of 5 mL of physiologic buffered saline per gram of material. All 5 glove materials had detectable protein, as determined by means of Bio-Rad analysis (Bio-Rad Laboratories, Hercules, Calif), with the fabric, mesh, polystyrene, and rubber components containing lower levels (Table II). Extraction of the thick, spongy speckled padding throughout the glove released the most protein (0.45 mg of protein per milliliter, unconcentrated). This padding extract was subsequently concentrated with an Amicon concentrator (Millipore, Temecula, Calif) fitted with a 1-kDa cutoff filter. The concentrate emanated an intense organic odor that could have been the active ingredient in Ultra Fresh (5-chloro-2-[2,4-dichlorophenoxy]phenol) or another unspecified chemical.

To study the possible presence of cow's milk proteins and, more specifically, casein in the glove, we performed an ImmunoCAP inhibition² with the original extractions of all 5 components, first using a milk allergosorbent and in a second analysis using a casein allergosorbent. Each extract was premixed 1:1 vol/vol with

TABLE I. Summary of IgE antibody serology results

Specimen date	IgE antibody specificity	IgE antibody result	Positive or negative	Laboratory
10/29/2013	Ammoniated latex extract RIA	6.8 U/mL	Positive	ViraCor-IBT Laboratories
10/29/2013	Buffered latex (NAL) RIA	16.8 U/mL	Positive	ViraCor-IBT Laboratories
10/29/2013	Glove extract RIA	36.5 U/mL	Positive	ViraCor-IBT Laboratories
11/20/2013	Ammoniated latex extract RIA	<0.5 U/mL	Negative	ViraCor-IBT Laboratories
11/20/2013	Buffered latex (NAL) RIA	<0.5 U/mL	Negative	ViraCor-IBT Laboratories
11/20/2013	Glove extract RIA	<0.5 U/mL	Negative	ViraCor-IBT Laboratories
11/20/2013	<i>Hevea brasiliensis</i> latex*	<0.1 kU _A /L	Negative	ViraCor-IBT Laboratories
11/20/2013	Hev b 5–enriched <i>Hevea brasiliensis</i> latex*	<0.1 kU _A /L	Negative	JHU DACI
11/20/2013	Cow's milk*	172 kU _A /L	Positive	JHU DACI
11/20/2013	Casein*	245 kU _A /L	Positive	JHU DACI
11/20/2013	α -Lactalbumin*	12.7 kU _A /L	Positive	JHU DACI
11/20/2013	β -Lactoglobulin*	10.9 kU _A /L	Positive	JHU DACI
11/20/2013	BSA*	<0.5 kU _A /L	Negative	JHU DACI

JHU DACI, Johns Hopkins University, Dermatology, Allergy and Clinical Immunology Reference Laboratory, Baltimore, Md; NAL, nonammoniated latex.

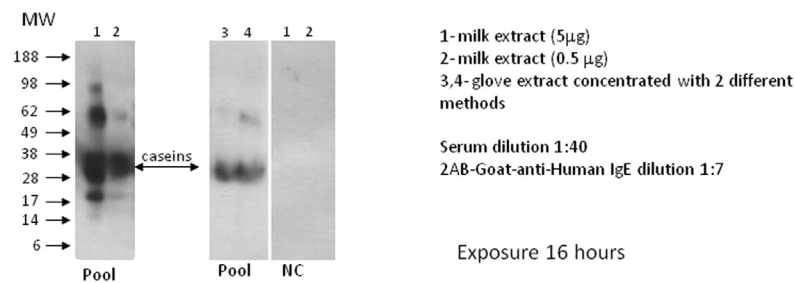
*Analysis performed by using the Thermo Fisher Scientific–Phadia ImmunoCAP.

TABLE II. Summary of ImmunoCAP inhibition analysis of Everlast glove component extracts

Glove extract description	Protein content (ng/mL)	Dilution of extract	Serum	Inhibition milk ImmunoCAP (%)	Inhibition casein ImmunoCAP (%)
Buffer (no protein control)	<50	1:1	IgE anti-milk pool	<0.1%, Reference	<0.1%, Reference
Outer gray mesh in the palm	71	1:1	IgE anti-milk pool	7.1%	NA
Gray fabric covering in contact with the hand	82	1:1	IgE anti-milk pool	2.4%	NA
Rigid finger polystyrene pad	80	1:1	IgE anti-milk pool	7.1%	NA
Black rubber backing attached to speckled pad	108	1:1	IgE anti-milk pool	4.8%	NA
Finger-palm speckled padding	452	1:1	IgE anti-milk pool	8.7%	NA
Finger-palm speckled padding	904	1:2	IgE anti-milk pool	17.2%	16.5%
Finger-palm speckled padding	4.5×10^6	1:10	IgE anti-milk pool	35.9%	27.2%
Effluent from Amicon, <500 Da	<50	Undiluted	IgE anti-milk pool	2.3%	–13%
Buffer (no protein control)	<50	Undiluted	Patient	<0.1%	NA
Finger-palm speckled padding	4.5×10^6	1:10	Patient	42.5%	28.8%

NA, Not available.

A Immunolabeling of milk proteins extract and extract from kick boxing glove with serum pool of 5 highly milk allergic subjects



B Immunolabeling of milk extract and extract from kick boxing glove with patient serum

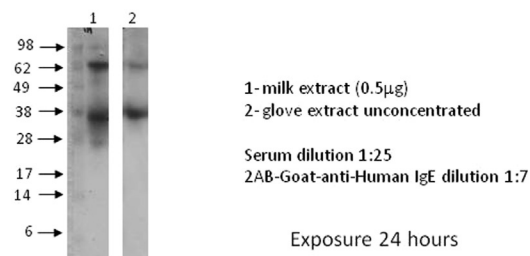


FIG 1. Western blot analysis of milk extract controls and Everlast kickboxing glove padding extract using an IgE anti-milk serum pool and an IgE anti-milk antibody–negative control serum (NC) as probes for milk proteins (A). The kickboxing glove padding extract used in Fig 1, A, was a 10-fold concentrate prepared with 2 methods. In Fig 1, B, the patient's serum was used as an IgE antibody source for detection of milk components in unconcentrated glove padding extract. MW, Molecular weight.

a pooled cow's milk specific IgE antibody-positive serum or the patient's serum. After 4 hours, IgE anti-milk or casein antibodies were detected by means of routine ImmunoCAP analysis (Johns Hopkins University, Dermatology, Allergy and Clinical Immunology Reference Laboratory, Baltimore, Md). Low levels of inhibition of less than 10% in comparison with those of a buffer sham control were seen with the 5 unconcentrated extracts. After 10-fold concentration, the speckled padding extract inhibited IgE anti-milk and casein antibodies from binding to their respective allergosorbents by 42.5% and 28.8% when using the patient's serum as an IgE antibody source. As an external control, no inhibition (<0.1%) was observed when the concentrated

speckled padding extract was preincubated with an IgE anti-peanut antibody–positive control serum and analyzed for IgE anti-peanut antibodies by using ImmunoCAP. These IgE competitive inhibition results support the conclusion that cow's milk and, more specifically, casein were present in the glove.

The padding extracts were analyzed by means of Western blot analysis (Dr H. A. Sampson's laboratory) to confirm casein in the glove.³ Fig 1, A, displays the speckled padding extract that has been separated by means of SDS-PAGE and blotted in lanes 3 and 4. An IgE anti-milk antibody–positive serum pool was used as the antibody source to detect any cow's milk proteins bound to the blot. The bands observed at 24 to 28 kDa indicated the

presence of casein in the glove extract. Analogous banding patterns are seen with the unconcentrated padding extract by using the patient's serum as an IgE anti-casein antibody source (Fig 1, B).

We believe this is the first reported case of a life-threatening allergic reaction involving a kickboxing glove. Casein has been reportedly used^{4,5} as a stabilizer or lubricant in natural rubber latex products, such as medical gloves. The lack of complete and accurate content information on the material data safety sheet for this consumer product and the lack of knowledge the company Everlast displayed about their glove and its actual contents is most concerning. Possible contributing factors to the anaphylaxis included the fact that the patient had received a manicure before joining the kickboxing group on the day of the exposure, which resulted in abrasion of the skin around the cuticles. This could have potentiated topical delivery of an allergenic molecule, such as casein. The presence of a fairly significant level of volatile organic compounds might have facilitated transdermal absorption. The level of volatile organic compounds in these gloves was sufficient to give rise to an intense unpleasant odor in the original glove and the extracts. The young woman also exercised for about 15 minutes with the new gloves before anaphylaxis, and exercise can enhance the risk of anaphylaxis. Casein appears to have been in sufficient quantities in the padding and on the glove liner surfaces of this brand new pair of Everlast kickboxing gloves such that either inhalation, adsorption through warm sweaty hands and/or lesions around the cuticles, or both was sufficient to induce systemic anaphylaxis. Natural rubber latex allergy was ruled out as an exposure concern based on the negative serologic results and the patient's unremarkable personal history with other natural rubber products. The patient has otherwise managed her milk allergy successfully by avoidance. Avoiding the use of known food allergens by manufacturers of consumer products that contact human skin or mucous membranes is critical to minimizing needless life-threatening anaphylactic events such as this in the future.

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