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**Title:** Clinical allergy : basophils, T cells, and therapeutic design

**Issue Date:** 2016-06-16
CLINICALLY RELEVANT IGE REACTIVITY AND BASOPHIL ACTIVATION TO GOAT’S MILK AFTER CUTANEOUS SENSITIZATION

ADAPTED FROM

GOAT’S CHEESE ANAPHYLAXIS FOLLOWING CUTANEOUS SENSITIZATION BY MOISTURIZER CONTAINING GOAT’S MILK

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Journal of Allergy and Clinical Immunology: In Practice 2014;2:629-30
TO THE EDITOR:

The current treatment for atopic eczema includes topical corticosteroid application during exacerbations, and regular use of emollients and moisturizers to optimally hydrate affected areas. Many creams to treat dry skin and eczema are advertised as “natural” products, but they may contain potential food allergens. For example, ingredients can include goat’s milk, cow’s milk, coconut milk or oil, oats and nut oil. Application of such food allergens to barrier-compromised skin could cause sensitization to the allergen, leading to severe reactions when the food is subsequently ingested.

A 55 year old female medical research administrator developed a generalized allergic reaction, characterized by urticaria and rapidly evolving oral and upper airway angioedema, immediately after eating two mouthfuls of a salad at a restaurant. This resulted in Emergency Department attendance and administration of intramuscular adrenaline. The salad contained zucchini, goat’s cheese, broad beans, mustard, vinegar, olive oil, basil and lemon zest. Her medical history was unremarkable, except for lifelong extensive atopic eczema and seasonal asthma. Direct questioning revealed that four months prior to the anaphylactic episode, for several weeks, she had been applying a skin moisturizer that contained goat’s milk. After an application resulted in acute erythema and itch, she subsequently ceased applying the product.

Investigational serum specific-IgE was strongly positive for goat’s milk (65.7 kU/L; ImmunoCAP, Phadia, Uppsala, Sweden) and negative for mustard. The remaining salad ingredients have been subsequently eaten without reaction, and she regularly consumes cow’s milk products. The patient confirmed ingestion of goat’s cheese, without any adverse effects, prior to the use of the moisturizer. We hypothesized that IgE mediated sensitization occurred during application of the moisturizer that contained goat’s milk to inflamed, eczematous skin, which resulted in the generalized allergic reaction when the foodstuff was subsequently encountered orally.

The basophil activation test (BAT) is an in vitro test for functional IgE reactivity. The test assesses IgE-mediated basophil activation in response to stimulation with allergen by detection of CD63 on the basophil surface by flow cytometry\(^1\). Results from this test have previously shown high correlation with clinical symptoms\(^2\). We performed a BAT using whole blood of the patient stimulated with both goat’s milk (Greer Laboratories, NC, USA) and her moisturizing product. The moisturizer was diluted at a 1:2 ratio in phosphate buffered saline (PBS; Life Technologies, NY, USA) before use. As a control, we also tested cow’s skimmed milk protein. Dose-dependent basophil activation induced by goat’s milk and moisturizing product used by the patient (Figure 1). Basophils from a non-allergic donor were also assessed, and failed to respond to any of the antigens (data not shown). Positive controls included the bacterial peptide formyl-methionyl-leucyl-phenylalanine (fMLP) and anti-IgE antibody induced activation. We also performed serum IgE immunoblotting to the moisturizer and goat’s milk. The concentration of goat’s milk within the moisturizing product was calculated to be approximately 0.44 mg/ml based on the equivalent levels of basophil activation achieved with a known concentration of goat’s milk (Figure 1).

![Figure 1. Basophil activation test.](image)

Dose-dependent basophil activation induced by goat’s milk and moisturizing product used by the patient. (●) Goat’s milk (µg/mL), (■) cow’s milk (µg/mL), (▲) moisturizing product (µL), (♦) anti-IgE, (○) fMLP, (▼) no antigen.

Antigen preparations (3µg/lane) were resolved by 4% to 12% gradient SDS-PAGE under reducing conditions and then transferred to nitrocellulose and probed with the patient’s serum at a 1:20 dilution. IgE immunoblotting revealed serum IgE binding to components in both the moisturizing product and goat’s milk (Figure 2A, lane 2 and Figure 2B, respectively), consistent with the ImmunoCAP and basophil activation test results. To verify our hypothesis that the initial sensitization developed through use of the moisturizing product we performed IgE inhibition immunoblotting\(^3\). For this, prior to probing the nitrocellulose, the patient’s serum was incubated with increasing concentrations of goat’s milk, moisturizing product or control protein keyhole limpet hemocyanin (KLH) for 1 hour. IgE binding to the moisturizing product (lane 2) is shown in Figure 2, which is completely inhibited by pre-incubation with goat’s milk (lanes 3-6) as well as the moisturizing product (lanes 7-10), which confirms that the IgE target protein within the moisturizing product contains B-cell epitopes present in goat’s milk. Controls for the IgE inhibition immunoblotting included KLH (lane 11-13), which did not cause any nonspecific inhibition of IgE binding to the moisturizing product, and a ryegrass (Greer Laboratories) immunoblot with serum from a ryegrass allergic donor in which no nonspecific inhibition of IgE binding occurred with

CAPSULE SUMMARY

We report anaphylaxis to goat’s cheese after cutaneous sensitization with a moisturizer containing goat’s milk applied to eczematous skin confirmed by specific in vitro basophil activation and inhibition serum IgE immunoblotting.
goat’s milk (Figure 2, C). Together, these results support the conclusion that an allergic response was induced by the moisturizing product, and this response could be attributed to the goat’s milk proteins within the product. The immunoblot results show strong IgE reactivity to proteins with a molecular weight between 20 and 28 kDa within the moisturizing product, which may correspond with goat β-casein (approximately 27 kDa) and γ-casein (22 kDa) and which differs from typical cow’s milk allergy, in which α-casein and whey proteins (e.g., β-lactoglobulin) are the dominant allergens4, which likely explains the lack of cross-reactivity with cow’s milk in this case.

When combined, the clinical history and in vitro immunological data clearly support the route of sensitization as being through topical application of the allergen to inflamed and compromised skin with subsequent anaphylaxis to the ingested allergen. This route of sensitization has been suggested in clinical studies of the development of wheat, oat, peanut and goat’s milk allergy5-8 with speculation in several of these studies that the suspected sensitizing product consisted of soaps and oils used to alleviate the symptoms of eczema. Murine studies demonstrate systemic sensitization after cutaneous allergen exposure9. Our data provide, to our knowledge, the first direct evidence of an immunological response in a patient to the suspected causative agent involved in the development of the allergy. We remind clinicians and patients that skin care ought to be bland, and we advocate avoidance of agents capable of sensitization, especially foods.

Figure 2. Inhibition IgE immunoblotting.
A, Inhibition of patient serum IgE binding to moisturizing product with 10-fold dilutions of inhibiting extract. Lane 1, non-allergic control serum; lane 2, no inhibitor; lanes 3-6, 0.1-100 µg/mL goat’s milk.; lanes 7-10, 0.1-100 µg/mL moisturizing product.; lanes 11-13, 1-100 µg/mL KLH. B, Patient serum IgE reactivity to goat’s milk. C, Inhibition of ryegrass allergic patient serum IgE reactivity to ryegrass. Lane 1, no serum; lane 2, no inhibitor.; lanes 3 and 4, 10,100 µg/mL ryegrass; lanes 5-8, 0.1-100 µg/mL goat’s milk.

REFERENCES