

Clinical Communications

The basophil activation test identified carminic acid as an allergen inducing anaphylaxis

Naoya Sugimoto, MD^a, Masao Yamaguchi, MD, PhD^a,
Yusuke Tanaka, MD^a, Yuko Nakase, MD^a,
Hiroyuki Nagase, MD, PhD^a, Hiroshi Akiyama, PhD^b, and
Ken Ohta, MD, PhD^{a,c}

Clinical Implication

- The basophil activation test was useful because it clearly showed that carminic acid, and not a contaminating protein, was the eliciting allergen in a rare case of carminic acid–induced anaphylaxis.

TO THE EDITOR:

In the autumn of 2011, a 39-year-old Japanese woman was referred for evaluation of allergic reactions after ingestion of a commercial bottled supplement that contained vitamin Bs and C and fruit flavors. The reactions occurred 3 times, at 1-month intervals. The first 2 episodes were mild and consisted of an itchy throat and localized hives on her lower extremities. However, after drinking the aforementioned supplement every day for 3 months, she manifested more severe symptoms of anaphylactic shock, with an itchy throat, generalized urticaria, diarrhea, and low blood pressure. Interestingly, each of the allergic reactions was associated with her menstrual period; the episodes occurred within 3 days before or after the beginning day of menstruation. Skin prick tests were negative for the supplement. An intradermal test showed that a 10-fold dilution of the solution was an irritating concentration that elicited reactions in the patient and all 4 of the tested controls; lower, nonirritating concentrations showed negative intradermal results. We then tried the *in vitro* basophil activation test (BAT), which analyzes surface activation marker CD203c expression on basophils. Importantly, the drink itself and, among its constituents, only cochineal dye gave clearly positive results in BAT with the use of the patient's blood, but not nonallergic subjects' blood. BAT was highly sensitive and selective, because obvious induction of basophil surface CD203c was observed with 10- to 640-fold dilutions of the supplement, the corresponding dose of cochineal dye and its main constituent, carminic acid (purity 97%), but not with a protein extracted from *Coccus cacti* (Figure 1).¹ *In vitro* sensitization experiments showed that carminic acid–induced basophil activation was IgE-mediated, because passive sensitization of a normal donor's basophils by the patient's serum² was completely blocked by the presence of an anti-IgE antibody, omalizumab (Figure 2).

On the basis of these results, the patient was strongly discouraged from consuming drinks and foods that contained cochineal dye. She has subsequently experienced no further anaphylactic episodes. Although confirmatory challenge was

unable to be performed to prove causation because the patient refused to undergo this test, on the basis of her BAT and the absence of anaphylaxis when she avoided carminic acid, we believe that carminic acid, in association with her menstrual cycle, was responsible for her anaphylaxis.

Cochineal dye is usually regarded as a highly safe material, and it is widely used as a food additive and cosmetic ingredient. To date, type I allergy due to the dye, including anaphylaxis, urticarial, and occupational asthma, has been reported only occasionally.^{1,3,4} Allergy to cochineal dye is generally because of a hypersensitivity reaction to a contaminating protein (ie, CC38K, a phospholipase),¹ whereas allergy to the main compound, carminic acid, is rare. Carminic acid is a relatively small molecule (molecular weight 492) that is thought to exhibit allergenicity as a protein-bound hapten. Although skin tests are usually sensitive and useful for allergen determination, they sometimes cause nonspecific skin stimulation. This limits the reliability of the tests, as in our present patient. Here, we were impressed by the finding that BAT accurately identified the allergen. That is, the test clearly showed that the patient's own basophils, but not those of nonallergic donors, responded to highly pure carminic acid but not to a protein extracted from *Coccus cacti*. Furthermore, the patient's basophils showed an obvious response to the allergen in BAT during menstruation, compared with the rest of the menstrual cycle (Figure 1, E); this result was in clear contrast to the absence of any menstruation-related enhancement of a response to polyclonal anti-IgE antibody. On the basis of these results, we believe that an *in vitro* BAT with the use of peripheral blood would be highly useful for analyzing cochineal dye-induced allergy. At present, we do not know the exact mechanism(s) underlying menstruation-associated changes in the basophil response; overnight culture of our patient's cells with estradiol or progesterone at 1 μ mol/L failed to modulate the response to carminic acid.

Recent progress in the pathogenesis of allergies has strongly implicated not only mast cells but also basophils as being critically involved in several allergic responses.⁵ Flow cytometric assessment of surface markers, including CD203c,⁶ has enabled precise determination of the activation status of basophils. In regard to carminic acid-related allergy, our present results suggest that basophils are highly sensitive to this allergen and that the cells might behave as a key initiator/effector *in vivo*. Thus, future analyses of the basophil activation status during clinical anaphylaxis will be important for elucidating the involvement of this cell type in this allergic reaction.

^aDivision of Respiratory Medicine and Allergology, Department of Medicine, Teikyo University School of Medicine, Tokyo, Japan

^bNational Institute of Health Sciences, Tokyo, Japan

^cNational Hospital Organization Tokyo National Hospital, Tokyo, Japan

Supported by grants from the Ministry of Health, Labor and Welfare, and the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Conflicts of interest: K. Ohta has received research support from Novartis. The other authors declare that they have no relevant conflicts of interest.

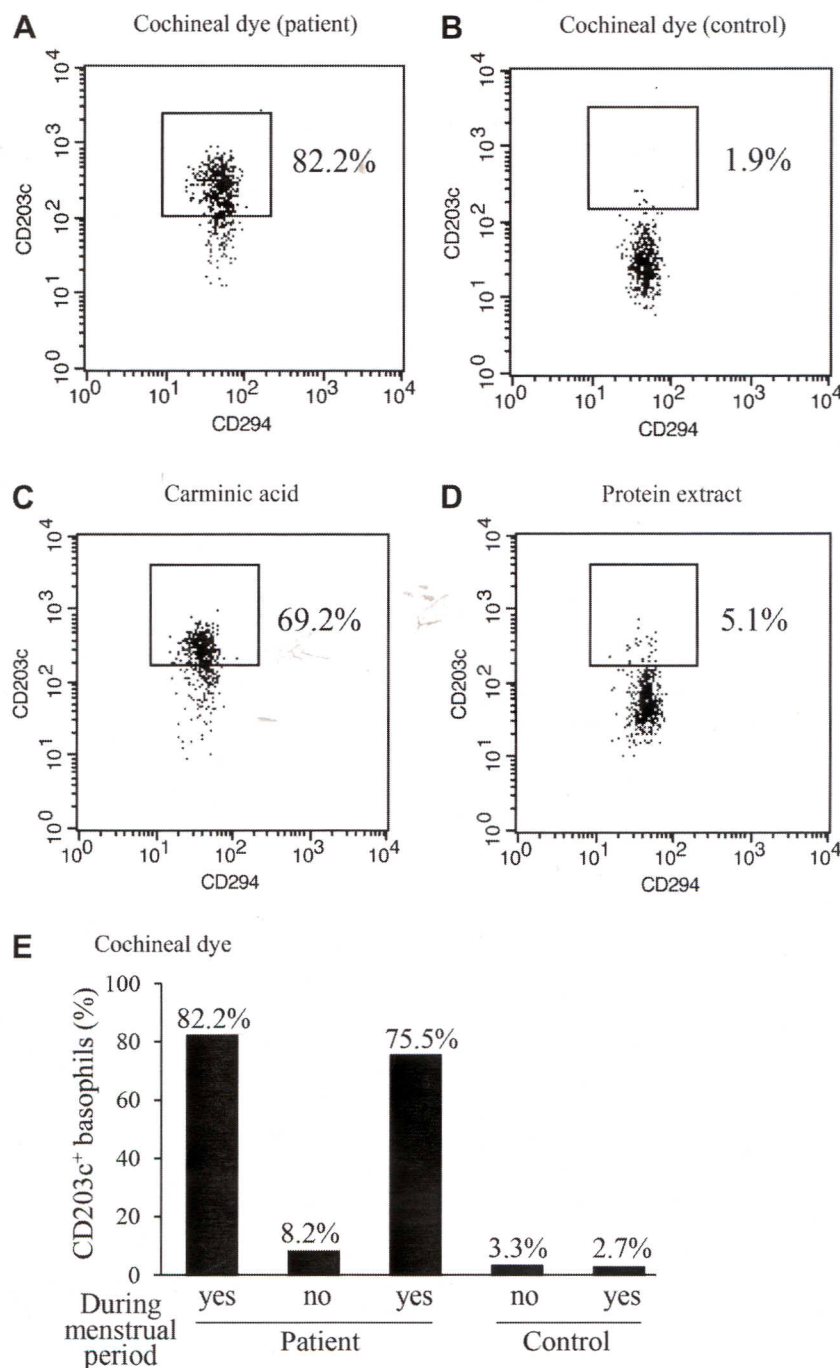


FIGURE 1. Cochineal dye and carminic acid gave positive results in BAT. Whole blood samples from the patient (**A**, **C**, **E**) and nonallergic donors (**B**, **E**; representative results are shown) were incubated with the indicated substances. With the use of flow cytometry, basophils were identified as CD3⁻ CD294⁺ cells, and the percentages of CD203c⁺ basophils after incubation with 40-fold dilutions of the substances are indicated. **C**, The tested concentration of carminic acid was 0.59 μ g/mL. **E**, Data obtained at different times during 2 menstrual cycles are presented.

Received for publication October 30, 2012; revised January 9, 2013; accepted for publication January 11, 2013.

Cite this article as: Sugimoto N, Yamaguchi M, Tanaka Y, Nakase Y, Nagase H, Akiyama H, et al. The basophil activation test identified carminic acid as an allergen inducing anaphylaxis. *J Allergy Clin Immunol: In Practice* 2013;1:197-9. <http://dx.doi.org/10.1016/j.jaip.2013.01.001>.

Corresponding author: Masao Yamaguchi, MD, PhD, Division of Respiratory Medicine and Allergy, Department of Medicine, Teikyo University School of Medicine, 2-11-1 Kaga, Itabashi-ku, Tokyo 173-8605, Japan. E-mail: myama@med.teikyo-u.ac.jp. 2213-2198/\$36.00

© 2013 American Academy of Allergy, Asthma & Immunology
<http://dx.doi.org/10.1016/j.jaip.2013.01.001>

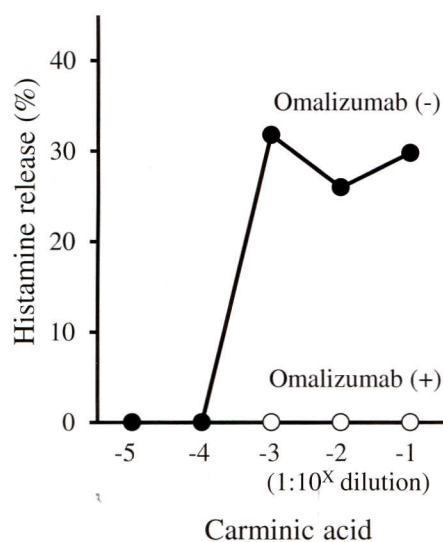


FIGURE 2. Blockade of passive sensitization of basophils by omalizumab. Basophils from a nonallergic donor were treated with IgE-eluting lactate buffer, pH 3.7, and then incubated in the presence of the patient's serum, with and without omalizumab at 10 µg/mL. The highest concentration, that is, 10-fold dilution, of carminic acid was 2.36 µg/mL.

REFERENCES

- Ohgiya Y, Arakawa F, Akiyama H, Yoshioka Y, Hayashi Y, Sakai S, et al. Molecular cloning, expression, and characterization of a major 38-kd cochlear allergen. *J Allergy Clin Immunol* 2009;123:1157-62.
- Suzukawa M, Yamaguchi M, Komiya A, Kimura M, Nito T, Yamamoto K. Ortho-phthalaldehyde-induced anaphylaxis after laryngoscopy. *J Allergy Clin Immunol* 2006;117:1500-1.
- Kagi MK, Wuthrich B, Johansson SG. Campari-Orange anaphylaxis due to carmine allergy. *Lancet* 1994;344:60-1.
- Quirce S, Cuevas M, Olaguibel JM, Tabar AI. Occupational asthma and immunologic responses induced by inhaled carmine among employees at a factory making natural dyes. *J Allergy Clin Immunol* 1994;93:44-52.
- Karasuyama H, Mukai K, Obata K, Tsujimura Y, Wada T. Nonredundant roles of basophils in immunity. *Ann Rev Immunol* 2011;29:45-69.
- de Weck AL, Sanz ML, Gamboa PM, Aberer W, Bienvenu J, Blanca M, et al. Diagnostic tests based on human basophils: more potentials and perspectives than pitfalls. *Int Arch Allergy Immunol* 2008;146:177-89.