

Letter to the Editor



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Conflict of Interest

The authors have no financial conflicts of interest.

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Allergy to carminic acid: *in vitro* evidence of involvement of protein-binding hapten

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ABSTRACT

We previously described a rare case of anaphylaxis presumably induced by carminic acid in cochineal dye used as a food additive. In this study, highly pure carminic acid was added to an albumin-containing buffer at various concentrations, followed by serial dilution. Varying the mixing ratio of carminic acid and albumin affected the extent of histamine release from passively sensitized basophils. Similar basophil histamine release occurred with carminic acid-globulin solutions. These results provide experimental evidence indicating that basophil activation is dependent on hapten (carminic acid) and carrier (protein) interaction.

Keywords: Anaphylaxis; Basophils; Cochineal dye; Hapten-carrier; Histamine release

INTRODUCTION

We recently reported an anaphylactic patient who developed a systemic allergic reaction following ingestion of a drink containing cochineal dye worldwide used as a food additive [1]. A basophil activation test (BAT) analyzing surface expression of activation marker CD203c demonstrated a positive result for high-purity carminic acid, but not for a protein sample prepared from *Coccus cacti*. Thus, carminic acid was thought to be the allergen eliciting the anaphylactic episode. However, there remained a small possibility that interference protein(s) in carminic acid, although at the trace concentration, was responsible for the BAT results, since previous reports by others had indicated protein(s) in cochineal dye (for example, CC38K) as the main candidate allergen, rather than carminic acid [2]. In this study, we further explored the role of carminic acid as the anaphylaxis-inducing allergen in our patient by focusing on the protein-binding property of carminic acid.

CASE REPORT

We previously reported a case of anaphylaxis induced by ingestion of a cochineal dye-containing drink [1]. In brief, a 39-year-old female was referred for evaluation of allergic reactions following daily ingestion of a bottled supplement. The reactions occurred 3 times during menstruation, at 1-month intervals. The first 2 episodes were mild. However, in the

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last episode, she went into anaphylactic shock. BAT analysis showed positive results for high-purity (98%) carminic acid, and the patient's basophils showed a positive BAT response to a relatively low concentration of carminic acid when using blood drawn during menstruation. Serum specific IgE to cochineal dye was 19.2 UA/mL (ImmunoCAP, Pharmacia Diagnostics AB, Uppsala, Sweden).

We performed additional experiments to analyze whether carminic acid, which has protein-binding ability, was responsible for eliciting the allergic responses. The aforementioned high-purity carminic acid was dissolved in water (stock concentration 10 mg/mL) and then diluted with a PIPES buffer containing 0.03% human serum albumin (HSA) to give various concentrations (Fig. 1A; a to c). Then the solutions were serially diluted using the same HSA-containing PIPES buffer. The only difference among a, b, and c was the initial ratio of carminic acid to albumin. Fig. 1A presents a schematic representation of the interaction between carminic acid and albumin. In parallel, carminic acid was serially diluted in PIPES buffer without HSA (Fig. 1A; d). Then all of the serially diluted samples were mixed with PIPES buffer containing 0.03% HSA and basophils that had been passively sensitized with the patient's serum. Histamine release from the basophils was assessed (Fig. 1B). Interestingly, 4 sample sets, a to d, failed to give identical results, indicating that the initial ratio of carminic acid to albumin affected the extent of histamine release. The results demonstrate that binding of carminic acid to albumin takes place immediately. Albumin mixed initially with lower concentrations of carminic acid more efficiently induced histamine release from basophils. The percent histamine release was even higher when carminic

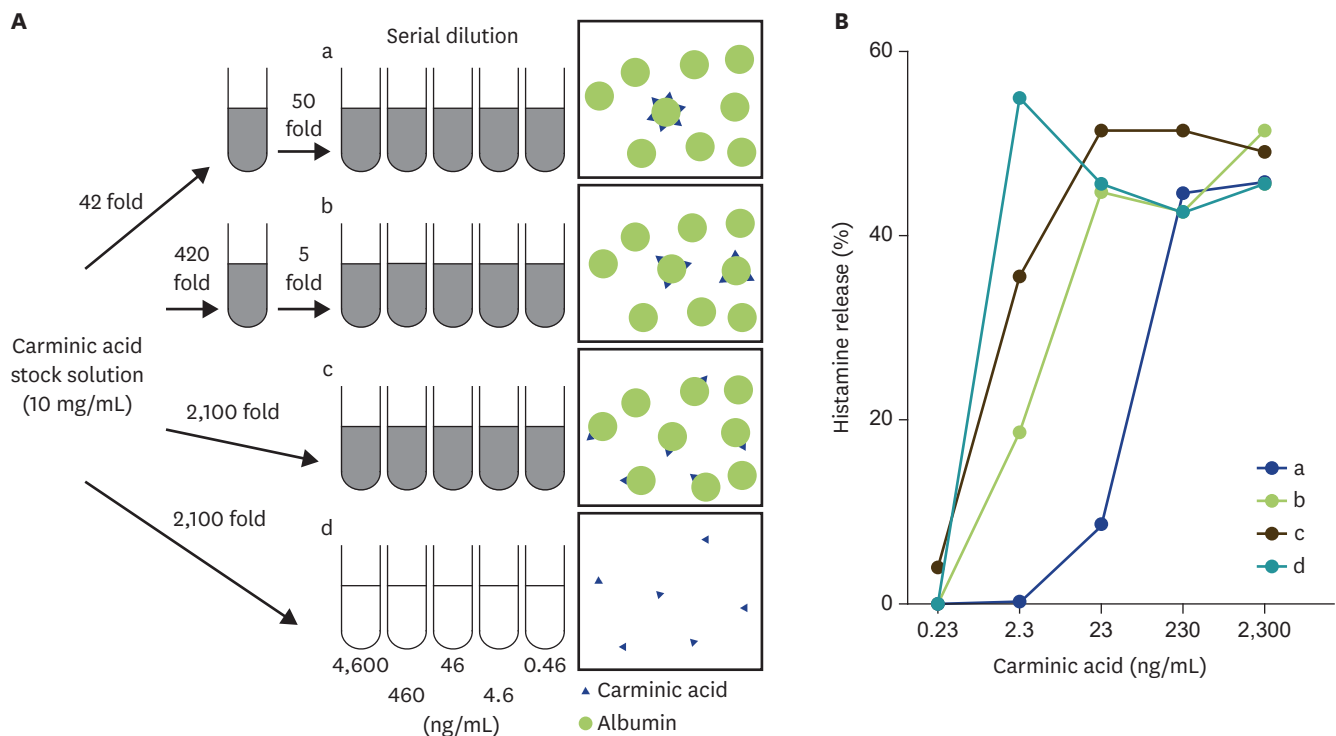


Fig. 1. The mixing ratio of carminic acid and albumin affects basophil histamine release. (A) Carminic acid stock solution (10 mg/mL) was prepared in water and then diluted to various concentrations using PIPES buffer containing 0.03% HSA (gray) (a, b, c) or PIPES buffer without HSA (white) (d). The carminic acid solutions were then serially diluted to a very low (0.46 ng/mL) concentration. On a molar basis, the concentration of carminic acid at 4,600 ng/mL (9.2 μ M) is twice of that of albumin at 0.03% (4.5 μ M). (B) Percent histamine release induced by four different sets (a to d) of the carminic acid solutions is shown. Note that the concentrations of carminic acid in diluted samples (A) were halved after addition of basophil preparations (B). Representative results are indicated, and another experiment gave similar results. The histamine release experiment was performed in duplicate, and mean percentages are shown.

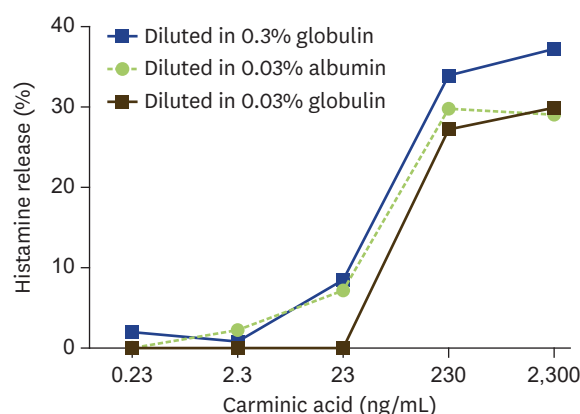


Fig. 2. Carminic acid is capable of inducing basophil histamine release when bound to albumin, and also when bound to globulin. Carminic acid solutions were prepared in a similar way to in Fig. 1A using a buffer containing 0.03% HSA, or 0.03% or 0.3% globulin and then mixed with passively sensitized basophil preparations. Histamine release experiment was performed in duplicate, and mean percentages are shown.

acid was diluted in protein-free PIPES buffer (Fig. 1A, B; d). These findings suggest that in solution carminic acid might exist as di- or multivalent aggregates, rather than monovalent molecules, since monovalent allergen-carrier conjugates are generally not good at triggering basophil activation [3, 4]. As shown in Fig. 2, carminic acid mixed in globulin (0.03% and 0.3%)-containing buffer induced basophil histamine release comparable to when mixed with albumin. Moreover, carminic acid added to denser globulin (0.3%) tended to induce a stronger basophil response, possibly suggesting that the ratio of carminic acid to globulin may also affect basophils' response. These results strongly suggest that carminic acid acts as a hapten, and that the carrier can be either albumin or another protein(s) such as globulin.

DISCUSSION

Allergy to cochineal dye has rarely been reported [2, 5-8]. It is regarded as a type I allergy. The main candidate reported as the allergen is interference protein(s) (i.e., CC38K, a phospholipase), while allergy to carminic acid has not been considered a dominant pathogenic mechanism. However, carminic acid is a relatively small molecule (molecular weight 492) that might be able to exhibit allergenicity as a protein-bound hapten.

In our previous analyses, a highly pure carminic acid preparation potently induced histamine release from basophils passively sensitized with a patient's serum. However, the possibility that interference substance(s) other than carminic acid induced the basophil histamine release could not be ruled out. Thus, in this study, we analyzed whether carminic acid really is an allergen that can induce allergic cell activation. Our present findings clearly demonstrated that a hapten molecule capable of binding to albumin is critically involved in basophil activation. The hapten molecule might be carminic acid itself, rather than a trace amount of some interference substance(s), since basophil activation occurred at nanogram-per-mL concentrations of carminic acid (Fig. 1). In addition, carminic acid can be surmised to form di- or multivalent aggregates in water, since a nanogram-per-mL concentration of carminic acid mixed with a large excess of albumin potently induced histamine release from basophils (Fig. 1B; d). The hapten-binding carrier protein could be albumin or some other protein(s), including globulin (Fig. 2). This finding is in clear contrast to formaldehyde allergy, since our preliminary analysis indicated that albumin-bound formaldehyde induced basophil

histamine release but globulin-bound formaldehyde did not (data not shown) [9]. **Fig. 1** indicates that even slight modification of the experimental procedure for handling hapten molecules might alter the results of functional assessment; we thus need to be careful in the planning of such experiments.

In view of the findings of this study, it can be concluded that carminic acid may behave as a hapten in some patients sensitive to cochineal dye. Carminic acid tends to bind strongly to proteins, making it difficult to obtain absolutely pure carminic acid in the preparation from protein-rich cochineal dye. Further studies exploring the allergenicity of carminic acid and interference proteins carried over from cochineal dye in other patients may have future scientific and clinical impact.

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REFERENCES

1. Sugimoto N, Yamaguchi M, Tanaka Y, Nakase Y, Nagase H, Akiyama H, Ohta K. The basophil activation test identified carminic acid as an allergen inducing anaphylaxis. *J Allergy Clin Immunol Pract* 2013;1:197-9.
[PUBMED](#) | [CROSSREF](#)
2. Ohgiya Y, Arakawa F, Akiyama H, Yoshioka Y, Hayashi Y, Sakai S, Ito S, Yamakawa Y, Ohgiya S, Ikezawa Z, Teshima R. Molecular cloning, expression, and characterization of a major 38-kd cochineal allergen. *J Allergy Clin Immunol* 2009;123:1157-62, 1162.e1-4.
[PUBMED](#) | [CROSSREF](#)
3. Ishizaka T, Ishizaka K. Triggering of histamine release from rat mast cells by divalent antibodies against IgE-receptors. *J Immunol* 1978;120:800-5.
[PUBMED](#)
4. Dembo M, Goldstein B, Sobotka AK, Lichtenstein LM. Histamine release due to bivalent penicilloyl haptens: control by the basophil plasma membrane. *J Immunol* 1978;121:354-8.
[PUBMED](#)
5. Kägi MK, Wüthrich B, Johansson SG. Campari-Orange anaphylaxis due to carmine allergy. *Lancet* 1994;344:60-1.
[PUBMED](#) | [CROSSREF](#)
6. 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(1 Pt 1):44-52.
[PUBMED](#) | [CROSSREF](#)
7. De Pasquale T, Buonomo A, Illuminati I, D'Alò S, Pucci S. Recurrent anaphylaxis: a case of IgE-mediated allergy to carmine red (E120). *J Investig Allergol Clin Immunol* 2015;25:440-1.
[PUBMED](#)
8. Takeo N, Nakamura M, Nakayama S, Okamoto O, Sugimoto N, Sugiura S, Sato N, Harada S, Yamaguchi M, Mitsui N, Kubota Y, Suzuki K, Terada M, Nagai A, Sowa-Osako J, Hatano Y, Akiyama H, Yagami A, Fujiwara S, Matsunaga K. Cochineal dye-induced immediate allergy: review of Japanese cases and proposed new diagnostic chart. *Allergol Int* 2018;67:496-505.
[PUBMED](#) | [CROSSREF](#)
9. Tanaka Y, Nakase Y, Yamaguchi M, Sugimoto N, Ohara K, Nagase H, Ohta K. Allergy to formaldehyde: basophil histamine-release test is useful for diagnosis. *Int Arch Allergy Immunol* 2014;164:27-9.
[PUBMED](#) | [CROSSREF](#)