

LETTER TO THE EDITOR

Anaphylactic Transfusion Reaction in Homozygous Haptoglobin Deficiency Detected by CD203c Expression on Basophils

To the Editor: It has been reported that the incidence of anaphthoglobulinemia who are homozygous for *Hp^{del}* is very rare in Africans or European-Africans. However, it is relatively high incidence in East Asian; 1/4,000 in Japan, 1/1,500 in Korea, and 1/1,000 in China [1]. Some patients with anaphthoglobulinemia develop anaphylactic or allergic transfusion reactions related to antihaptoglobin (Hp) antibodies [2]. However, if anti-Hp IgE antibody is negative, it is difficult to elucidate an anaphylactic reaction. Flow cytometric basophil activating test (BAT) based on upregulation of the cell degradation/activation marker CD203c has been developed for monitoring the clinical course of patients with allergic diseases [3,4]. Here, we describe that BAT was a useful tool for diagnosing anaphylactic transfusion reaction associated with anti-Hp antibody.

This case was an 8-year-old Japanese boy with acute lymphoblastic leukemia receiving intensive chemotherapy required transfusion. He was transfused with 14 units of leukocyte-reduced red cell concentrates (RCCs) and 150 units of leukocyte-reduced platelet concentrates for chemotherapy-induced anemia and thrombocytopenia without any transfusion-related reactions. However, immediately after starting the next infusion of RCCs, he developed dyspnea, urticaria, and hypotension. Screening tests for nonhemolytic transfusion reactions (NHTRs) were performed at the Japanese Red Cross. Serum analysis by nephelometry revealed Hp deficiency and their PCR method [1] showed complete absence of the Hp gene. He was diagnosed as having anaphthoglobulinemia due to homozygous allelic *Hp^{del}*. Although anti-Hp IgG antibody was detected by ELISA and Western blotting, IgE antibody against Hp was not detected by ELISA or the IgE crosslinking-induced luciferase expression (EXILE) test [5].

To address the mechanism of this anaphylactic reaction, we performed BAT. As a control, a healthy adult and five age-matched children with severe egg allergy who had histories of food-induced anaphylaxis were examined. A commercial kit (Allergen kit; Beckman Coulter, Fullerton, CA) was used for quantifying basophil CD203c expression [4]. Upregulation of CD203c on basophils was determined using a threshold that was defined by the fluorescence of unstimulated cells and expressed as CD203c high % [6]. CD203c high % was 23% for the patient and $4.38 \pm 0.53\%$ for the control, respectively (Table I). Haptoglobin induced enhancement of CD203c expression in the patient, but not in the control.

Since basophils express high-affinity IgE receptors, it is reasonable to assume that the haptoglobin-induced basophil activation observed in the patient was mediated by haptoglobin-specific IgE bound to basophils. We did not detect antihaptoglobin IgE by the ELISA or EXILE methods, but it is possible that the serum IgE concentration was too low to be detected, whereas the amount of cell-bound IgE was sufficient to induce anaphylaxis.

Another possibility is that haptoglobin-specific IgG induced basophil activation. An IgG-mediated systemic anaphylactic reaction involving FcγRs, basophils, and platelet-activating factor was reported in a murine system [7], although it remains

TABLE I. The Basophil Activation Test (BAT) Using Flow Cytometry Based on CD203c Up-Regulated by Haptoglobin

	CD203c high % on basophils		
	Negative control	Positive control	Target
Subject	No stimulation	Anti-IgE	Haptoglobin 1:100
Index patient	3.6	80.5	23.0
Control person	4.9	91.9	5.0
Food allergy Pt 1	3.9	93.1	8.5
Food allergy Pt 2	4.9	30.5	6.8
Food allergy Pt 3	4.0	60.9	2.0
Food allergy Pt 4	3.8	97.0	5.1
Food allergy Pt 5	4.8	55.2	4.6

Flow cytometric analysis of haptoglobin-induced CD203c expression on basophils of a patient with haptoglobin deficiency and from age-matched children with food allergy and health adult donor as a control. EDTA-containing whole blood was incubated with haptoglobin at 1:100 dilution for 15 minutes. Anti-IgE antibody as a positive control and PBS as a negative control were used as stimulators. Basophils were detected on the basis of forward and side scatter characteristics and as the CD3-negative, Chemoattractant Receptor-homologous molecule expressed on Th2 cells (CRTH2)-positive population. Upregulation of CD203c was determined using a threshold that was defined by the fluorescence of unstimulated cells (negative control) and expressed as CD203c high %.

unclear whether such an IgG-mediated pathway also exists in humans.

Our findings indicate that BAT may be useful for assessing the cause of NHTRs, especially anaphylactic reactions via high-affinity IgE receptors on basophils, leading to development of type I hypersensitivity response when no IgE can be detected in serum.

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