

LETTER TO THE EDITOR

Application of the basophil activation test in the analysis of allergic transfusion reactions

Allergic reactions, which are mediated by mast cells and/or basophils, are the most common adverse effects associated with transfusion, particularly with use of platelet concentrates (PCs). In general, the activation of mast cells/basophils and their subsequent histamine release are mediated via two pathways: the allergen-dependent and allergen-independent pathways. In the latter, biologic response modifiers (BRMs), such as bacterial components, activated complement components, cytokines and chemokines directly activate mast cells/basophils. Although allergens in blood components may include food or medications ingested by the donor immediately before blood collection, such specific allergens have not been identified. Indeed, the only allergens identified in blood components are plasma proteins including IgA, haptoglobin and C4; transfusion to patients who are deficient in those plasma proteins reportedly causes serious allergic reactions. Besides such cases with plasma protein deficiency, allergic reactions after PC transfusion are likely to be elicited mainly via the allergen-independent pathway by BRMs that are present or accumulated in PCs, although the roles of BRMs in this pathway remain largely unknown (Cognasse *et al.*, 2006; Blumberg *et al.*, 2006; Wakamoto *et al.*, 2003). In addition, because allergic transfusion reactions are diagnosed based on symptoms, it is often difficult to determine whether the transfused PCs are causative or whether the reactions are, in actuality, allergic in nature. These difficulties are partly because of the lack of an assay system with which activation of mast cells/basophils can be assessed. Fortunately, however, a simple test called the basophil activation test (BAT) has recently been developed for managing allergic diseases. In the test, whole blood is incubated with an allergen, and subsequent basophil activation is assessed using flow cytometry based on upregulation of cell degranulation/activation markers, CD63 or CD203c (Boumiza *et al.*, 2005; Bühring *et al.*, 1999). In this study, using

PC supernatants (PC-SNs) from nine cases with allergic transfusion and 12 cases without transfusion reaction, we assessed whether the BAT can be applied to transfusion medicine to examine the mechanisms of transfusion reactions and the causative relationship between allergic reactions and transfusion. Because patients' blood was not available for a basophil source and hence we used blood samples obtained from unrelated healthy persons, we focused on the allergen-independent direct activation pathway.

PCs were collected by apheresis by standard procedures (Wakamoto *et al.*, 2003). PCs, which were reported to be associated with allergic reactions ($n = 9$), were collected and PC-SNs (A1~9) were separated from platelets within 24 h of their use by centrifuging at $18\ 600 \times g$ for 10 min at room temperature and stored at -40°C . No antibodies against plasma proteins including albumin, α_1 -acid glycoprotein, α_1 -antitrypsin, Gc-globulin, α_2 -HS-glycoprotein, ceruloplasmin, haptoglobin, transferrin, fibrinogen, IgG, IgM, IgA, C3, C4 and C9 were detected in either the PCs associated with allergic reactions or the patients' sera (Wakamoto *et al.*, 2003). PC-SNs A1, 2, 5, 6, 7 and 8 were from urticaric cases, and PC-SNs A3, 4 and 9 were from anaphylactic cases. The AABB classification system was used to classify the transfusion reactions. Other information of the allergic reaction cases is summarized in Table 1. No antibodies were detected against HLA and HPA in any PC-SNs with allergic reactions, and no antibodies were detected against HPA in any patients with allergic reactions. Although HLA Class I antibodies were detected in patients 2, 3, 5 and 7 with allergic reactions, HLA-matched PCs were transfused to these patients. Therefore, these antibodies were not considered to be responsible for the allergic reactions.

PCs, which did not induce transfusion reactions ($n = 12$), were also collected, and the cell-free supernatants (B1~12) were separated.

BAT was performed as follows. One hundred microlitres of heparinized whole blood taken from the healthy laboratory persons were incubated with 5 μL of PC-SNs (blood : sample = 20: 1; this mimics a clinical setting in which a patient with 5-L blood receives

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Table 1. Profile of allergic reaction cases

PC-SN	PCs								Patients				
	BAT	Type of reaction	Timing of reaction* (min)	Abs against				Blood group	Abs against				
				HLA‡	HPA	Plasma proteins	Age†		HLA	HLA‡	HPA	Plasma proteins	Blood group
4	+	Anaphylactic	90	–	–	–	1	A		–	–	–	A
8	+	Urticarial	30	–	–	–	3	A		–	–	–	A
9	+	Anaphylactic	10	–	–	–	2	B		–	–	–	B
1	–	Urticarial	60	–	–	–	3	A		–	–	–	A
2	–	Urticarial	30	–	–	–	2	O	Matched§	+\$	–	–	O
3	–	Anaphylactic	30	–	–	–	2	A¶	Matched§	+\$	–	–	B
5	–	Urticarial	60	–	–	–	2	O	Matched§	+\$	–	–	O
6	–	Urticarial	60	–	–	–	2	O		–	–	–	O
7	–	Urticarial	30	–	–	–	1	B	Matched§	+\$	–	–	B

*After the start of transfusion.

†1, within 24 h after collection; 2, more than 24 h, but within 48 h after collection; 3, more than 48 h, but within 72 h after collection.

‡HLA Class I.

§HLA-matched PCs were transfused.

¶ABO-mismatched PC was transfused.

250 mL of PCs) at 37°C for 30 min, and then stained with anti-HLA-DR-FITC (Beckman Coulter, Fullerton, CA, USA), anti-CD123-PE-Cy5 (BD Biosciences, San Jose, CA, USA) and anti-CD203c-PE (Beckman Coulter) at 4°C for 15 min. After lysis of RBCs by FACS lysing solution (BD Biosciences), the cell samples were subjected to flow cytometric analysis. HLA-DR-negative CD123-positive cells in the FSC vs. SSC blast cell window were identified as basophils, and CD203c expression was determined (Boumiza *et al.*, 2005; Bühring *et al.*, 1998). Recombinant human IL-3 was purchased from Pepro Tech, Rocky Hill, NJ, USA and used as a positive control at a final concentration of 20 ng/mL (Hauswirth *et al.*, 2007). To calculate CD203c-positive basophils, a cursor was placed on the CD203c histograms at the point where 5% of basophils are CD203c-positive after incubation without a stimulus. When the percentage of CD203c-positive basophils after stimulation by each PC-SN exceeds the mean + 3 SDs of 12 PC-SNs without reactions, the PC-SN was categorized as BAT-positive.

The percentages of CD203c⁺ basophils for five blood samples after stimulation by the PC-SNs were summarized in Fig. 1. Three of the nine PC-SNs with allergic reactions (33%) activated basophils in at least one of five blood samples. The PC-SN A4 activated basophils in all blood samples; while the PC-SNs A8 and A9 activated basophils in three and one of the five samples, respectively. The reason why PC-SNs A8 and A9 were BAT-positive in only a limited number of blood samples is unclear, but the difference in

responses of the basophils between the blood samples may be one of the key factors. Since there were no obvious differences between BAT-positive and BAT-negative cases with allergic reactions in the timing

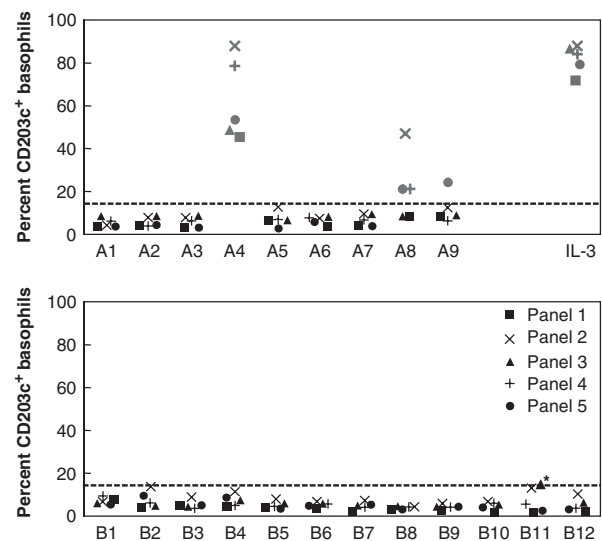


Fig. 1. BAT performed on PC sups with and without allergic transfusion reaction. To calculate CD203c-positive basophils, the cursor was set so that 5% of basophils are CD203c-positive after incubation without a stimulus. Dashed line: the mean + 3 SDs of the percentage of CD203c-positive basophils in five blood samples stimulated with 12 PC sups without reactions. *PC sup B11 is BAT-positive to blood sample 3.

of the reaction after transfusion and component age (Table 1), the reasons why six of the nine PC-SNs with allergic reaction were BAT-negative are also currently unknown. However, there are four possibilities. First, the reactions were not caused by the transfusion, but it is quite difficult to exclude this possibility. Second, the reactions were caused by the transfusion but were not allergic. Tryptase is a mast cell granule protein that is reported to be one of the good tools to diagnose allergic reactions (Schwartz *et al.*, 1987; Van der Linden *et al.*, 1992). Therefore, measurement of serum tryptase levels in patients after transfusion would test this possibility. Third, the reactions were caused by the transfusion and were allergic, but were allergen-dependent. Since unrelated blood samples were used, this study cannot evaluate the allergen-dependent pathway. BAT should have been performed simultaneously using the patients' blood to explore the allergen-dependent pathway. We are now planning such a study. The last possibility is that the BAT was not sensitive enough, although this could be overcome using a higher ratio of PC-SN to blood, which may increase the sensitivity. However, because the increased ratio does not mimic clinical settings, it may lead to misinterpretation. The ratio needs to be carefully determined in a future study.

In contrast, only one (B11) of the 12 PC-SNs without a reaction (8%) elicited basophil activation. The PC-SN was BAT-positive only to one of the five blood samples and, furthermore, the percentage of CD203c-positive basophils was close to the cutoff point (mean +3 SDs), suggesting that the PC-SN (B11) was false BAT-positive. Even if this PC-SN was really BAT-positive and the PC was transfused to other patients, allergic symptoms would have not been serious.

The upregulation of CD203c was accompanied by that of CD63, another basophil degranulation/activation marker (Boumiza *et al.*, 2005), when PC-SNs A4 and A8 were used, but CD63 was less sensitive than CD203c (data not shown). In addition, platelet activation leads to upregulation of not only P-selectin but also CD63 and to production of platelet microparticles with P-selectin and CD63 on their surface (Ruf *et al.*, 1995), and these activated platelets and microparticles can bind to basophils via their cell surface PSGL-1, a ligand of P-selectin (Yang *et al.*, 1999), misleading to a false-positive result of CD63 (unpublished data). In contrast, CD203c is expressed only by basophils and their precursors (Bühning *et al.*, 1999). Therefore, we believe that CD203c should be a better basophil activation marker than CD63, when using whole blood as basophil source.

In this study, we showed a possibility that BAT can be used in transfusion medicine as a functional test to examine the causative relationship between allergic

reactions and transfusion. However, only three of nine PC-SNs with allergic reaction were BAT-positive, and the reasons why the rest of the PC-SNs were BAT-negative are unknown. To provide evidence supporting the possibility above, we need to show more data in a future study, for example, what BRMs in PC-SNs caused basophil activation and whether those BRMs were responsible for allergic reactions.

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