

ORIGINAL PAPER

Clinical utility of the basophil activation test for analysis of allergic transfusion reactions: a pilot study

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Vox Sanguinis

Abstract

Background and Objectives In previous studies, we demonstrated that the basophil-activating effects of supernatants found in residual-transfused platelet concentrates (PC-SNs) on whole blood basophils in cases of allergic transfusion reactions (ATRs) could be assessed by the basophil activation test (BAT) in terms of allergen/IgE dependency. However, in these studies, the basophils were derived from third-party healthy volunteers. In this study, we performed BAT using patients' own blood basophils to analyse ATRs.

Materials and Methods The BAT was performed in two cases of severe ATRs using residual PC-SNs and the patients' own basophils in the presence and absence of dasatinib, an inhibitor of IgE-mediated basophil activation.

Results In both cases, PC-SNs exhibited basophil-activating activity against the patients' basophils, but not against basophils from third-party healthy volunteers. In addition, basophil activation was inhibited in the presence of dasatinib, indicating that the basophils were activated in an allergen/IgE-dependent manner. Of note, the basophils in Case 2, but not in Case 1, were activated by PC-SNs from some unrelated non-haemolytic transfusion reaction cases.

Conclusion This pilot study indicates that the BAT may be useful in clarifying the causal relationship between ATRs and transfused blood as well as in elucidating the mechanisms behind ATRs considering the allergen/IgE-dependent pathway.

Key words: allergic transfusion reactions, anaphylaxis, basophil activation test.

Received: 8 July 2016,
revised 11 October 2016,
accepted 12 October 2016

Introduction

Allergic transfusion reactions (ATRs) are the most common adverse reactions occurring during the transfusion of blood components, especially platelet concentrates (PCs). The incidence of ATRs during PC transfusions ranges between 1% and 4% [1–3]. Most reactions are mild and involve cutaneous manifestations such as urticaria, rash, pruritus and flushing. Severe ATRs, including life-threatening anaphylaxis, occur in rare instances. Basophils and/or mast cells are key effector cells in immediate-type

allergic reactions, and are believed to mediate ATRs via two pathways: the allergen/IgE-dependent and -independent pathways. Although some reports suggest that plasma proteins such as IgA [4], haptoglobin [5] and C4 [6] can act as allergens for recipients with plasma protein deficiencies and antibodies, such cases are rarely identified [7]. Biological response modifiers (BRMs) that accumulate in blood components during storage are thought to elicit the alternative allergen/IgE-independent pathway, but the exact mechanisms remain unclear [8–13]. Therefore, the mechanisms involved in most ATRs are not understood.

ATRs are usually diagnosed based on symptoms and their time of onset (immediate or after transfusion). To confirm the causative relationship between the reaction and the transfusion, the tryptase test is useful [14, 15]. However, it is often difficult to obtain adequate patient serum

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samples for a tryptase test because the half-life of tryptase, once released into the circulation, is only 2 h [16]. To make up for the shortcomings of the tryptase test, the direct quantification of basophil activation by flow cytometry (known as the basophil activation test, or the BAT) has been recently applied to transfusion medicine [8]. The clinical utility of the BAT is due to the unique ability of basophils to degranulate via either the allergen/IgE-dependent or -independent pathway [17–19]. In this test, the basophil-activating ability of supernatants found in residual transfused blood to whole blood basophils in ATR cases can be assessed in the presence or absence of dasatinib, which was found to interact with and block several FcεRI downstream targets, including Btk, and hence act as an inhibitor of IgE-mediated basophil activation [18, 19]. However, in our previous two reports on the BAT [18, 20], whole blood basophils were obtained from third-party healthy volunteers, not from the patients themselves. In addition, the BAT has just started to be used in transfusion medicine; therefore, its usefulness is still being established.

In this study, we performed the BAT to analyze two cases of severe ATRs using residual-transfused PC supernatants (PC-SNs) and the patients' own blood basophils. The aim of this study was to investigate whether the BAT can be applied to clinical transfusion medicine to assess the causative relationship between the adverse reaction and the transfusion and, if possible, to reveal the mechanisms behind ATRs.

Materials and methods

Patients

Two cases of transfusion-related anaphylaxis occurring between May 2014 and December 2014 at the Shizuoka Cancer Center were included in this study (Table 1). Medical histories were obtained via chart review. In Case 1, a 61-year-old woman with acute myeloid leukaemia experienced anaphylactic shock while receiving leuko-reduced ABO-identical PCs. In Case 2, a 53-year-old man with acute lymphoblastic leukaemia underwent allogeneic bone marrow transplantation from a one-antigen mismatch unrelated donor at first complete molecular remission. On day 19 after transplantation, he experienced anaphylaxis while receiving leuko-reduced ABO-identical PCs. In both cases, the patients had received prior PC transfusions without any adverse reactions.

Tryptase, plasma protein and plasma protein antibodies

Blood samples for the measurement of serum levels of tryptase were collected a few hours after the reactions.

Table 1 Patients' characteristics

	Case 1	Case 2
Age/gender	61/female	53/male
Drug/Food allergies	None	None
Inhalant allergies	None	None ^a
Disease	Acute myeloid leukaemia	B lymphoblastic leukaemia
Treatment status	Day 13 of re-induction chemotherapy	Day 19 of unrelated bone marrow transplantation
Time of anaphylaxis after starting transfusion	5 min	30 min
Symptoms	Rash, hypotension, clouding of consciousness and incontinence	Urticaria, angioedema and dyspnoea
Previous platelets transfusions (n)	1	>20
Adverse reaction observed during previous transfusion	None	None

^aThe bone marrow donor had a pollen allergy.

Tryptase level was examined by the ImmunoCAP tryptase kit (Phadia, Uppsala Sweden). No antibodies against plasma proteins were detected in the patients' sera, including IgA, haptoglobin (Hp), complement C4, C9, α 2-macroglobulin or ceruloplasmin, human leukocyte antigen (HLA) class I, HLA class II or human platelet antigens (HPAs). In addition, detailed laboratory analyses ruled out the possibilities of deficiencies in IgA, Hp, C4, α 2-macroglobulin or ceruloplasmin, allergies to drugs or latex or transfusion-related acute lung injury. All of the above evaluations, except for allergies to drugs, were performed by the Japanese Red Cross Society Blood Service Headquarters, whereas allergies to drug were tested in the hospital. The BAT was assessed by the Japanese Red Cross Kinki Block Blood Center. This study was approved by the Institutional Review Board at Shizuoka Cancer Center (28-J35-28-1-3).

PCs associated with ATRs

All PCs in this study, both leuko-reduced and irradiated, were prepared from single donors using apheresis. The residual PC products, which induced an ATR in Case 1, had been kept in the hospital at room temperature (around 24°C) for 4 days, and the product was subsequently frozen at –40°C before the PC-SNs were separated from the platelets, although the PC-SNs should have been separated and frozen as soon as possible after the onset of the ATR. At the time of the BAT, the stored PC product

was quickly thawed and the cell-free PC-SNs were separated via centrifugation at $3000 \times g$ for 30 min at 4°C [17, 20]. In Case 2, the collection of the residual PC and the preparation of cell-free PC-SNs via centrifugation at $3000 \times g$ for 30 min at 4°C [18] were performed immediately after the occurrence of the ATR, and the PC-SNs were stored at -40°C . PC-SNs were also similarly prepared using PCs that had caused non-haemolytic transfusion reactions (NHTRs), both ATRs and non-ATRs, in other patients. Those samples were utilized as control NHTR PC-SNs. A summary of these control PCs is described in Table 2.

Preparation of peripheral whole blood

Heparinized peripheral whole blood was collected from the patients and healthy laboratory personnel (as a

control). These samples were used as a source of basophils for the *in vitro* basophil activation assay.

Reagents

Purified mouse anti-human IgE monoclonal antibody (AHlgE) was obtained from Beckman Coulter (Fullerton, CA, USA). FITC-conjugated mouse anti-HLA-DR MoAb and PE-conjugated mouse anti-CD203c MoAb were also purchased from Beckman Coulter. PE-Cy5-conjugated mouse anti-CD123 MoAb and FACS lysing solution were purchased from BD Biosciences (San Jose, CA, USA). Recombinant human (rh) IL-3 was purchased from AbD Serotec (Oxford, UK). Dasatinib was obtained from Trout Research Chemicals (Toronto, ON, Canada). Dimethyl sulphoxide (DMSO) was obtained from Thermo Fisher Scientific Inc. (Waltham, MA, USA).

Table 2 Profile of control platelet concentrates derived from other adverse reaction cases

	No.	Symptom ^a	Type of reaction	Increase of tryptase ^b	Timing of reaction ^c (min)	Pcs age ^{d,e}
Case 1	1	ATR	Anaphylactic shock	Yes	60	2
	2	ATR	Anaphylactic shock	Yes	5	3
	3	ATR	Allergic reaction	Yes	30	3
	4	Non-ATR	Transfusion-associated dyspnoea	NA	1440	3
	5	Non-ATR	Febrile non-haemolytic transfusion reaction	NA	660	3
Case 2	1	ATR	Anaphylactic shock	Yes	40	2
	2	ATR	Anaphylactic shock	No	15	2
	3	ATR	Allergic reaction	NA	75	2
	4	ATR	Anaphylaxis	NA	40	2
	5	ATR	Hypotensive transfusion reaction	No	35	2
	6	ATR	Anaphylaxis	No	25	1
	7	ATR	Allergic reaction	No	60	3
	8	ATR	Hypotensive transfusion reaction	NA	4	2
	9	ATR	Allergic reaction	NA	120	2
	10	ATR	Anaphylactic shock	No	60	2
	11	ATR	Anaphylaxis	No	35	2
	12	ATR	Anaphylactic shock	NA	90	2
	13	Non-ATR	Hypertension, dyspnoea, tachycardia	No	60	3
	14	Non-ATR	Febrile non-haemolytic transfusion reaction	NA	660	3
	15	Non-ATR	Febrile non-haemolytic transfusion reaction	NA	60	3
	16	Non-ATR	Transfusion-associated dyspnoea	NA	210	2
	17	Non-ATR	Transfusion-associated dyspnoea	NA	10	3
	18	Non-ATR	Febrile non-haemolytic transfusion reaction	NA	60	3
	19	Non-ATR	Transfusion-associated dyspnoea	No	54	2
	20	Non-ATR	Tachycardia	NA	15	3
	21	Non-ATR	Hypertension, dyspnoea, tachycardia	NA	30	2
	22	Non-ATR	Transfusion-associated dyspnoea	NA	1440	3
	23	Non-ATR	Febrile non-haemolytic transfusion reaction	NA	70	3

^aATR: includes allergic reactions, anaphylaxis, anaphylactic shock, hypotensive reactions; non-ATR: Other

^bNA: not available

^cAfter the start of transfusion

^dNone of the PC-SNs possessed antibodies against HLA class I or class II.

^e1, 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.

In vitro BAT

Aliquots of whole blood (100 µl) collected from patients and healthy laboratory personnel (control) were incubated with 5 µl of PC-SNs, derived from PCs with or without ATRs, at 37°C for 30 min; the blood/sample ratio was set to 20:1 to mimic a clinical setting in which a patient with 5 l of blood receives 250 ml of PCs. After incubation, the mixtures were washed with phosphate-buffered saline (PBS) containing 0.5% bovine serum albumin (BSA) and 10 mM ethylenediaminetetraacetic acid (EDTA; PBS/BSA/EDTA). The extent of CD203c expression on basophils from the whole blood samples was analyzed via flow cytometry (FCM) (FACSCanto II, BD Biosciences) as described below. To inhibit IgE/IgE receptor-triggered basophil activation, whole blood was pre-incubated in the presence or absence of dasatinib (3 µM) at 37°C for 30 min [18, 19] and subsequently incubated with PC-SNs at 37°C for an additional 30 min. Dasatinib was first dissolved in a small amount of DMSO and then diluted with PBS. When dasatinib was used in the BAT assay, the final dilution of DMSO was calculated as 13 300-fold. DMSO was similarly diluted in the BAT assay when used as a negative control of dasatinib.

Flow cytometry

Following agent-mediated activation as described above, the whole blood samples were incubated on ice for 15 min with 10 µL of FITC-conjugated anti-HLA-DR MoAb, PE-conjugated anti-CD203c MoAb and PE-Cy5-

conjugated anti-CD123 MoAb. After washing the samples with PBS/BSA/EDTA, the cells were treated with FACS lysing solution and subjected to FCM. HLA-DR-negative and CD123-positive cells in the FSC vs. SSC blast cell window were identified as basophils. CD203c was used as a basophil activation marker. To calculate the percentage of CD203c-positive basophils in each panel whole blood sample, a cursor was placed on the CD203c histograms at the point at which 5% of the basophils were CD203c positive without stimulation. When the percentage of CD203c-positive basophils exceeded 20%, the PC-SN was categorized as BAT positive. A representative result of flow cytometric analysis is presented in Fig. 1.

Results

Serum tryptase levels and clinical outcomes

Both patients recovered from the ATR episodes within 1 h of resuscitation with corticosteroids and adrenaline. The patients' serum levels of tryptase were within normal limits: 6.1 µg/l and 3.5 µg/l (normal range, <9.0 µg/l). The delay in the blood sampling might have led to the negative tryptase test results. After the ART episodes, they received washed PCs with premedication of antihistamine and hydrocortisone and consequently developed no ATR afterwards.

Basophil activation test

We analysed Case 1 1 month after the ATR episode, at which point the patient had fully recovered from

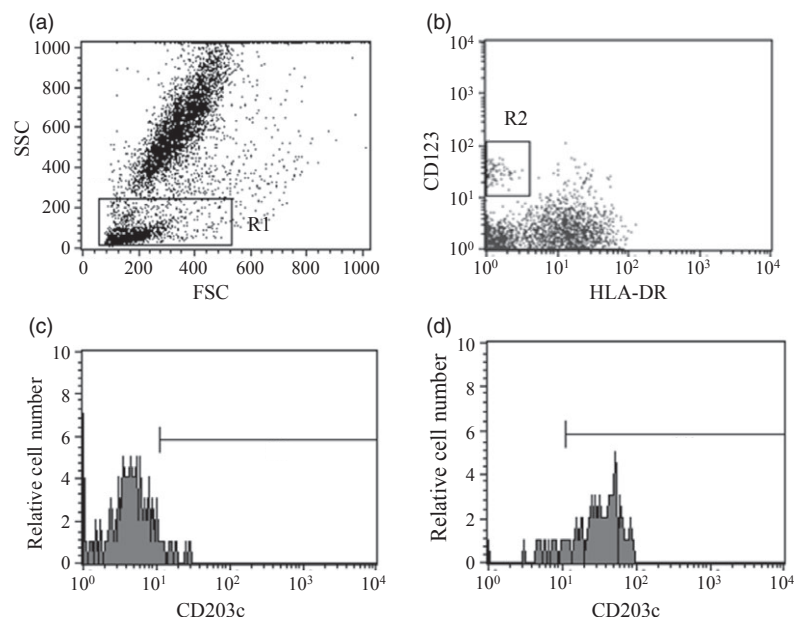


Fig. 1 Gates utilized to determine basophils and the cursor position to determine activated basophils. (a and b) Cells in the regions R1 (a) and R2 (b) were regarded as basophils. (c and d) WB was stimulated in the absence (c) or the presence (d) of AHlgE. The position of the cursor was set so that 5% of the basophils without stimulation were classified as 203c positive.

chemotherapy-induced myelosuppression. The PC-SN that had been derived from residual PCs activated basophils in the patient's blood sample (Fig. 2a). Conversely, basophils found in two whole blood samples taken from healthy volunteers were not activated by the PC-SN. In addition, dasatinib inhibited basophil activation elicited by the PC-SN in the patient's blood sample, indicating that basophil activation had occurred via the IgE-dependent pathway. On the other hand, none of the five control NHTR PC-SNs (three ATRs and two non-ATRs) (Table 2) activated the patient's basophils (Fig. 2b). These results suggested that this patient's basophils were exclusively activated by the PC-SN from the residual product that had caused her anaphylactic shock.

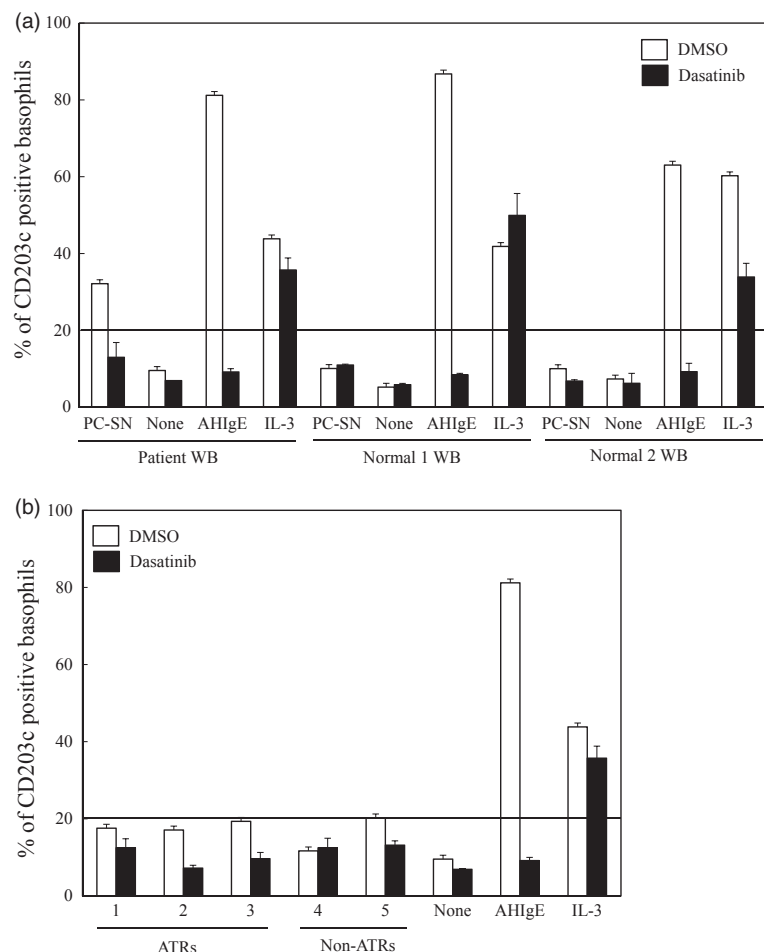
In Case 2, the BAT was performed 2 weeks after the transfusion-related anaphylaxis episode. Basophil activation was elicited in the patient's blood sample using the PC-SN derived from the residual product that had caused his anaphylaxis (Fig. 3a). This basophil activation was inhibited in the presence of dasatinib. The PC-SN did not

activate basophils in two samples obtained from third-party healthy volunteers. Basophil activation decreased in the patient sample when the PC-SN was diluted 100–200 times; however, the activation was still observed at a dilution ratio of 1:200 (Fig. 3b). We also investigated basophil activation in 23 control NHTR PC-SNs (Table 2). Control samples #1–12 involved ATR cases, whereas samples #13–23 were obtained from non-ATR cases that included fever, dyspnoea or hypertension. None of these 23 NHTR PC-SNs activated basophils in samples from two healthy volunteers (data not shown). In contrast, the basophils of this patient were activated by 12 of 23 PC-SNs (8 ATR and 4 non-ATR) in the absence, but not in the presence, of dasatinib (Fig. 3c).

Discussion

In our previous studies, we showed that the BAT could be used as a functional test in transfusion medicine to assess the causal relationship between ATRs and transfusions,

Fig. 2 Basophil activation ability of PC-SNs for Case 1 and other NHTR cases. AHlgE was used as a positive control, the stimulation ability of which is inhibited by Dasatinib, and IL-3 was used as another positive control, the stimulation ability of which is not inhibited by Dasatinib. a) WB of Case 1 (Patient) and two healthy volunteers (Normal 1 and Normal 2) were pre-incubated in the absence (square) or presence (black square) of dasatinib, and were subsequently incubated with Case 1 PC-SN. The WB samples were then stained with anti-HLA-DR, anti-CD123 and anti-CD203c MoAbs. HLA-DR-negative CD123-positive cells in the FSC vs. SSC blast cell window were identified as basophils, and CD203c expression was determined. AHlgE: anti-human IgE MoAb, IL-3: recombinant human IL-3. When the percentage of CD203c-positive basophils exceeded 20% (solid line), the PC-SNs were categorized as BAT positive. Case 1 PC-SN exclusively activated Case 1 basophils, and the activation was inhibited by dasatinib. b) WB of Case 1 was pre-incubated in the presence or absence of dasatinib and subsequently incubated with five control NHTR PC-SNs (three ATRs and two non-ATRs). Basophil activation was not observed in any control NHTR PC-SNs.



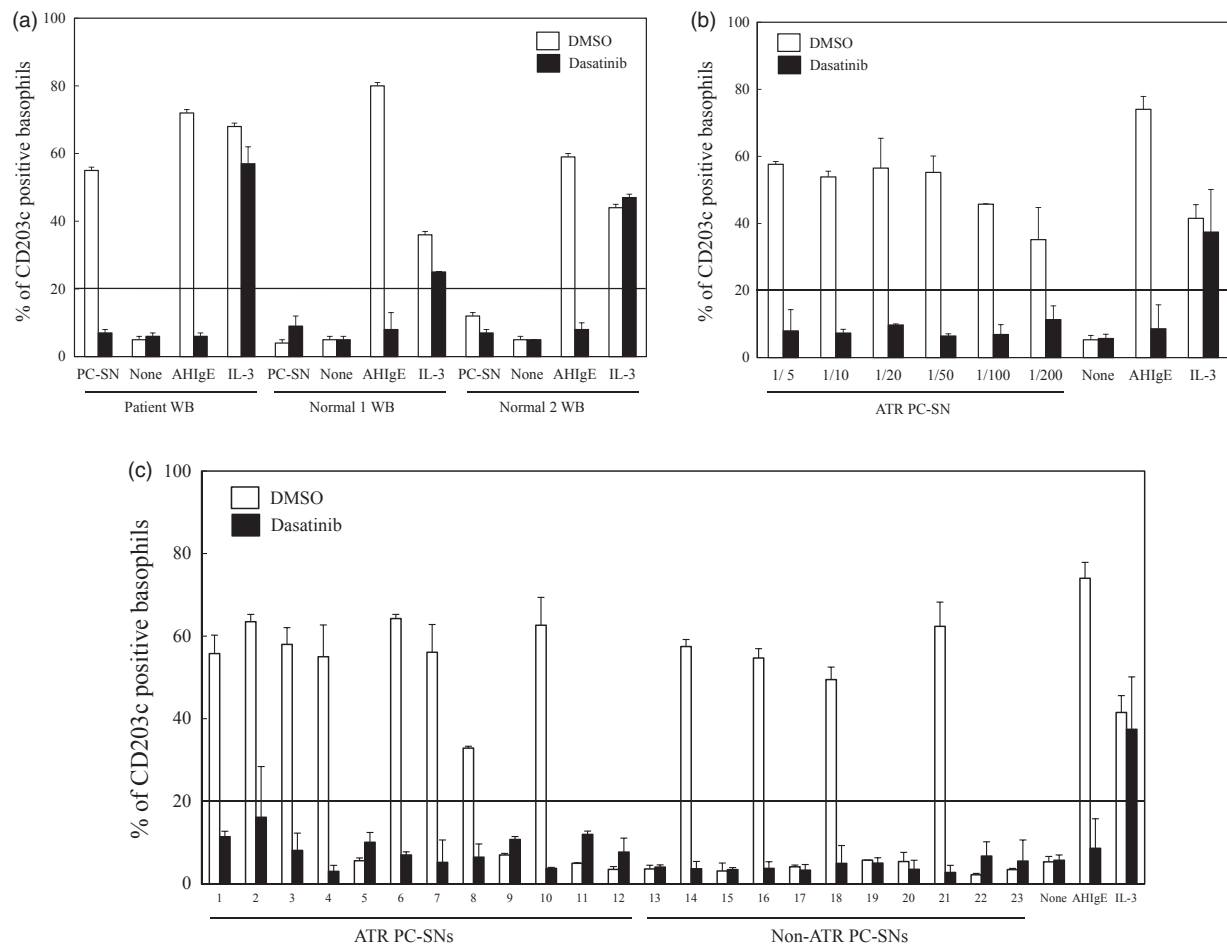


Fig. 3 a) Case 2 PC-SN exclusively activated Case 2 basophils; activation was inhibited by dasatinib. b) Whole blood of Case 2 was pre-treated with dasatinib or DMSO, and then the cells were stimulated with Case 2 PC-SN at several dilution ratios (1:5, 1:10, 1:20, 1:100 or 1:200). c) Whole blood of Case 2 was pre-treated with dasatinib or DMSO, and then the cells were stimulated with 23 control NHTR PC-SNs.

and to examine whether or not the reactions occurred via the allergen/IgE-dependent pathway [18, 20]. In those studies, however, blood from healthy volunteers rather than patients was used as a source of basophils, and hence a definitive conclusion regarding this relationship could not be made. To make the relationship clearer, this study used patients' own whole blood as a source of basophils. In both cases, the PC-SNs prepared from residual-transfused PCs exclusively exhibited basophil-activating activity against the patients' own basophils, and basophil activation was completely inhibited by dasatinib. These results unambiguously demonstrate a positive causal relationship among ATRs, PC transfusion and allergen/IgE-dependent basophil activation.

We also examined whether the patients' basophils were activated by other NHTR PC-SNs. None of the five NHTR PC-SNs activated Case 1 basophils. In contrast, 12 of the

23 NHTR PC-SNs activated Case 2 basophils; therefore, if these PCs had been transfused to Case 2, he might have developed an ATR. We hypothesize that a certain unknown common antigen in the PCs caused Case 2 to develop an ATR, but the exact antigen remains to be identified. Alternatively, it might be related with the fact that Case 1 had only received one transfusion before, and that Case 2 had received more than 20. In conclusion, this pilot study indicates that BAT can clarify the causal relationship between ATRs and transfused blood in light of the allergen/IgE-dependent pathway. More so, BAT may be useful in assessing the mechanisms behind ATRs.

However, this study has several limitations. First, we performed BAT in only two cases of ATR, so its sensitivity and specificity remain undetermined. In addition, there are no data about the relationship between the severity of an ATR and the level of basophil activation.

Therefore, similar but larger-scale studies are required to make a final evaluation of BAT. In such studies, it is necessary to confirm if BAT using patients' blood and supernatants from PCs transfused to them without giving symptoms tests negative.

Second, a large number of patients who receive transfusions are in myelosuppression. In such patients, we cannot immediately collect basophils to perform the BAT. In Case 1, samples could not be collected until bone marrow recovery was complete, which occurred 1 month after anaphylaxis. To conquer this drawback, we are now setting up a passive immune BAT system in which patient serum/plasma is used as the patient IgE source, residual-transfused blood is used as the allergen source and healthy volunteer donor basophils are used as the responder cell source. The fact that patients under myelosuppression receiving PCs have anaphylactic response to them raise a possibility that the response stems from the mast cell because mast cells are tissue resident and live about 3 months, whereas basophils originate from hematopoietic progenitors and circulate only for about 1-5 days.

A third limitation is related to the inappropriate collection of the residual blood product, preparation of the PC-SN and storage of the PC-SN in Case 1, which occurred because the clinicians did not understand the importance of appropriate sample preparation. Storage at room temperature for 4 days at the hospital and subsequent freezing-thawing of the residual PCs might have induced the release of some biologically active agents from platelets and thereby influenced the activation of basophils.

However, considering that the PC-SN exclusively exhibited basophil-activating activity against the patient's whole blood, and that the activation was inhibited by dasatinib, we believe that sample storage and preparation did not strongly affect the result of BAT. Based on this experience, we centrifuged the residual product of PCs immediately after anaphylaxis in Case 2, and the cell-free PC-SN was stored at -40°C . To mitigate potential related issues in the future, we need to share appropriate handling methods for residual ATR blood products with other medical institutions and inspection institutes.

This pilot study of BAT, which involved two cases of severe ATRs using residual PC-SNs and the patients' own basophils, demonstrates the usefulness of this novel test in clarifying the causal relationship between ATRs and transfused blood in light of the allergen/IgE-dependent pathway. Prospective studies that include a large number of ATR cases are warranted to confirm the clinical benefit of BAT in transfusion medicine.

Acknowledgements

We wish to thank Mr. Hirofumi Yanase and Dr. Masafumi Fukaya for their assistance in preparing this manuscript. We would like to thank Editage (www.editage.jp) for English language editing.

Conflict of interests

The authors declare no conflict of interests.

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