

Immunoglobulin (Ig)G antibodies against IgE identified by basophil activation test as the putative causative agent of a serious allergic transfusion reaction: potential utility of the test as a new safety measure for allergic transfusion reactions

Kazuta Yasui, Nobuki Matsuyama, Takafumi Kimura, Yoshihiro Fujimura, and Fumiya Hirayama

BACKGROUND: In most cases of allergic transfusion reactions (ATRs), the causative agents have not been identified and the mechanisms are largely unknown, with a few exceptions. The basophil activation test (BAT) was recently introduced in the field of transfusion to investigate the causal relationships between ATRs and transfusion, as well as the mechanisms behind them.

STUDY DESIGN AND METHODS: The BAT was used to screen the residual supernatants (SNs) of 43 blood components associated with serious ATRs for those that can activate basophils of many healthy volunteers. The SNs were then fractionated by centrifugal ultrafiltration and protein G column chromatography and each separated fraction was reexamined by the BAT.

RESULTS: Of the 43 such blood components, one activated basophils from 19 of 21 healthy volunteers. In the blood component, the IgG antibody against IgE was identified as a putative causative agent.

CONCLUSION: Blood donors who possessed the IgG antibody against IgE may be dangerous to transfusion recipients. The BAT would be useful in identifying such high-risk blood donors, when it is used to screen the blood components associated with serious ATRs for residual SNs that can activate the basophils of many healthy volunteers.

Blood transfusion has many clinical benefits and can be lifesaving but, at the same time, carries considerable risk. Hemovigilance systems manage transfusion risk in many countries, and one of the main purposes of hemovigilance is to pursue better safety measures for transfusion. To avoid serious risks of transfusion that could affect a wide range of patients, several safety measures including questionnaires for blood donors and tests for several infectious diseases have been implemented. On the other hand, while allergic transfusion reactions (ATRs) are the most common reactions and some of them are serious,^{1,2} safety measures to prevent ATRs are not implemented.

Allergic transfusion reactions are mediated by mast cells and/or basophils. In general, the activation of mast cells/basophils and subsequent histamine release are mediated by two pathways, the allergen/immunoglobulin (Ig)E-dependent and -independent pathways.^{3,4} The allergen/IgE-dependent pathway typically occurs after aggregation if the high-affinity FcεRI on the cell surface in response to the cell surface binding of IgE in complex with several specific allergens, such as plasma proteins,^{1,2,5-8} chemical allergens,^{9,10} and food allergens.¹¹⁻¹³ The causative agents, such as

ABBREVIATIONS: AHlgE = anti-human immunoglobulin E; ATR(s) = allergic transfusion reaction(s); BAT = basophil activation test; FCM = flow cytometry; PC(s) = platelet concentrate(s); SN(s) = supernatant(s).

From the Japanese Red Cross Kinki Block Blood Center, Ibaraki, Osaka, Japan.

Address reprint request to: Kazuta Yasui, PhD, Japanese Red Cross Kinki Block Blood Center, 7-5-17, Saitoasagi, Ibaraki, Osaka 567-0085, Japan; e-mail: kazuta-yasui@kk.bbc.jrc.or.jp.

Received for publication March 4, 2018; revision received June 5, 2018; and accepted June 18, 2018.

doi:10.1111/trf.14878

© 2018 AABB

TRANSFUSION 2018;99:999;1-9

myelomatous oligomeric IgE,¹⁴ antibody against IgE,¹⁵ and soluble platelet proteins,¹⁶ were also reported, but these are rare. The alternative allergen/IgE-independent pathway that underlies ATR is likewise largely unknown and the antibody against CD36 and the biologic response modifiers, such as inflammatory cytokines and chemokines, are present and/or accumulate in blood components, have only been speculated as the causative agents of ATRs.^{2,17–21} Therefore, most of the causative agents have not been identified, and the mechanisms of ATRs are largely unknown with a few exceptions. One of the reasons for this may be the absence of a standard assay system for investigating these processes.

The basophil activation test (BAT) was developed as a simple blood test for diagnosing allergic reactions to substances such as foods, inhalants, medicines, and venom.^{22–24} A small sample from the patient is incubated with the suspected allergen. In sensitized patients, basophils are activated and release chemical mediators from their granules. During this process, specific molecules known as activation markers (CD63 and CD203c) are present on the membrane of basophils.²⁵ These markers allow the identification of activated basophils by flow cytometry (FCM). In the past decade, the BAT was also applied to transfusion medicine.^{2,9,10,13,26–31} In previous studies, we demonstrated that the BAT, performed using patient blood and the supernatants (SNs) of transfused blood components, was able to elucidate not only the causative relationship between ATRs and the transfusion but also the mechanisms behind ATRs.²⁵

The Japanese Red Cross Society performs a hemovigilance, which is similar to the “SHOT” in the United Kingdom, focusing on the relatively serious adverse effects. In this study, using the BAT, we screened 43 residual blood components, the SNs of 28 platelet concentrates (PCs), and 15 fresh-frozen plasma (FFP) samples that were reported to be associated with serious ATRs at the hospitals in the Kinki area, to determine whether any of these can activate the basophils of several healthy volunteers. We identified one FFP as having the capacity to activate basophils of several healthy donors and subsequently demonstrated that this FFP contained IgG antibodies against IgE as the putative

causative agent. Although we did not review this donor’s several other products to understand if the donor was indeed clinically dangerous, a previous study performed by Abe and colleagues¹⁵ independently demonstrated in an ATR case the presence of IgG antibodies against IgE as the putative causative agent and that the blood components of the donor elicited anaphylactic shock in at least two patients. Therefore, testing to determine whether the residual transfused product of ATR cases can activate basophils of healthy subjects using the BAT may emerge as a new safety measure for ATRs. This testing could additionally identify blood donors or blood products that are likely to cause ATRs in other patients. In some cases, we may need to consider deferring blood donations from particular donors.

MATERIALS AND METHODS

PCs and FFPs associated with ATRs

We screened 43 residual blood components, the 28 PC-SNs, and 15 FFP samples associated with serious ATRs, to determine whether any one of them can activate basophils of multiple healthy volunteers. The clinical symptoms of the 43 blood components are summarized in Table 1. Twenty-eight PCs associated with ATRs, both leukoreduced and irradiated, were prepared from single donors using apheresis. Fifteen FFPs with ATRs were also leukoreduced and prepared from single donors. The residual PC products, which were temporarily stored at 4°C after the occurrence of the ATR, were collected from the hospital, and cell-free PC-SNs were separated via centrifugation at 3000 × *g* for 30 minutes at 4°C and stored at –40°C.¹³ The residual FFPs were also collected from the hospital and stored at –40°C.

Reagents

Purified mouse anti-human IgE (AHlgE) monoclonal antibody (MoAb) was obtained from Beckman Coulter. Fluorescein isothiocyanate (FITC)-conjugated mouse anti-HLA-DR and phycoerythrin (PE)-conjugated mouse anti-CD203c MoAbs were also purchased from Beckman Coulter.

TABLE 1. Summarized profile of 43 ATR blood components

Blood components	Symptoms	Number of cases	
		Total	BAT positive
PC	Anaphylactic shock	8	2*
	Hypotension	7	1*
	Anaphylaxis	13	0
FFP	Anaphylactic shock	7	1†
	Hypotension	2	0
	Anaphylaxis	6	0

* The residual PC-SN-activated basophils in one of five healthy volunteers’ blood samples.
† The residual FFP-activated basophils in four of five healthy volunteers’ blood samples. This FFP was named as FFP-ATR1.

PE-Cy5-conjugated mouse anti-CD123 MoAb and FACS lysis solution were purchased from BD Biosciences. PE-conjugated mouse AHlgE MoAb was purchased from BioLegend. Allophycocyanin (APC)-conjugated mouse anti-human IgG MoAb was purchased from BD Biosciences. Chimeric human IgE anti-nitrophenylacetyl was obtained from Bio-Rad. Dasatinib was obtained from Trout Research Chemicals. Hydroxyethyl starch was purchased from NIPRO (HES40). Heparin sodium and CaCl₂ were purchased from Mochida Pharmaceutical Co. Ltd. and Nacalai Tesque, respectively. Human serum albumin was obtained from Wako Pure Chemical Industries.

Using BAT to screen for ATR blood components reactive to basophils in multiple subjects

To screen for ATR blood components that can activate basophils of multiple healthy volunteers, whole blood samples of five healthy volunteers were used as source of target basophils. For ATR blood components that activated two or more healthy volunteers' basophils, whole blood samples of another 16 healthy volunteers were used to confirm reactivity. To determine the activation pathway of basophils, the whole blood was incubated in the presence or absence of dasatinib (3 μ mol/L), which inhibits IgE/IgE receptor-triggered basophil activation, at 37°C for 30 minutes, and then incubated with the residual blood components at 37°C for an additional 30 minutes.^{13,30} For evaluation of basophil activation of our healthy laboratory personnel, we used the BAT that we previously reported.^{13,30}

Identification of the causative agent that activated multiple healthy volunteers' basophils

To separate the causative agents included in the residual blood component named FFP-ATR1 (Table 1), which activated basophils from multiple healthy volunteers, the FFP was fractionated by centrifugal ultrafiltration and column chromatography (protein G or A). The centrifugal ultrafiltration kits were obtained from Merck Millipore (Amicon Ultracel-3k, -10k, -30k, -50k, and -100k). The centrifugal column chromatography kits were obtained from GL Sciences (Monospin-ProG and -ProA). Target fractions of the FFP-ATR1 were obtained using these columns in accordance with the manufacturers' protocols. Both bound fractions and unbound fractions were collected. The unbound fractions of ultrafiltration and the bound or unbound fractions of protein G and A were incubated with whole blood cells for 30 minutes at 37°C and the extent of basophil CD203c expression was analyzed by FCM. In some experiments, several concentrations of chimeric human IgE mixed with the residual ATR product for 5 minutes at 4°C before performing the BAT to assess the nature of causative agent.

Evaluation of the binding capacity of IgG autoantibody against human IgE using basophils whose surface IgE, bound on Fc ϵ R, was replaced with IgE originated from other healthy subjects

Replacement of IgE bound on basophils was performed with the two-step procedure that has previously been reported.³¹ The plasma used for replacement of receptor-bound IgE was derived from 11 representative healthy laboratory personnel, who showed different reactivity of basophils for the FFP-ATR1. We defined basophils in the cell pellet, whose own receptor-bound IgE was replaced by third-party IgE, as "quasi-basophils."

Comparison of the binding amount of third-party IgE on quasi-basophils and that of IgG autoantibody against human IgE to the IgE on quasi-basophils was carried out as follows. 1) To measure the amount of replaced receptor-bound IgE, the IgE bound to aliquots of 2.5×10^5 peripheral blood mononuclear cells (PBMCs) containing quasi-basophils were analyzed after the cells were stained with FITC-conjugated anti-HLA-DR MoAb, PE-Cy5-conjugated anti-CD123 MoAb, and PE-conjugated AHlgE MoAb by FCM. 2) To evaluate the binding capacity of the IgG autoantibody against human IgE, aliquots of 2.5×10^5 PBMCs containing quasi-basophils were treated with the FFP-ATR1 followed by FITC-conjugated anti-HLA-DR MoAb, PE-Cy5-conjugated anti-CD123 MoAb, and APC-conjugated AHlgG MoAb. The IgG against human IgE bound to quasi-basophils was analyzed via FCM.

Enzyme-linked immunosorbent assay

The concentrations of IgE antibody in the test samples were measured by an enzyme-linked immunosorbent assay kit (BD cytometric bead array, BD Biosciences) according to the manufacturer's instructions.

Research ethics

This research project was approved by the ethics committee of the Japanese Red Cross Society Blood Service Headquarters (#2015-48).

Statistical analysis

We used a Wilcoxon signed-ranks test with the Bonferroni correction to determine statistical significance. A p value of less than 0.01 was considered statistically significant. The Spearman's correlation test was used to estimate correlation. A p value of less than 0.01 was considered statistically significant, indicating a correlative relationship.

RESULTS

One residual blood component from 43 ATR cases activated basophils from multiple healthy volunteers in vitro

We screened 43 residual blood components associated with ATRs, the 28 PC-SNs, and 15 FFP samples, to determine whether any one of them can activate basophils of multiple healthy volunteers. Of those blood components, one FFP named as FFP-ATR1, associated with anaphylactic shock, activated basophils of four of the five whole blood samples, and two PC-SNs with anaphylactic shock activated basophils of one of the five whole blood samples. The rest of the 43 components did not activate any basophils (Table 1). Since the residual quantity of the two PC-SNs was very small, we focused on FFP-ATR1 hereafter. The donor of the FFP-ATR1 had a record of 12 times of blood donation. The FFP-ATR1 was prepared from his sixth donation and caused anaphylactic shock to the recipient. There was no other record of transfusion reactions caused by his blood, although only 2% to 3% percent of the whole numbers of reactions are reported to blood centers in Japan. The mechanism of basophil activation in response to the FFP-ATR1 was further investigated using basophils of the four subjects who tested positive to the FFP-ATR1 described. The basophil activation was inhibited by dasatinib in all the four subjects, indicating that basophil activation occurred via IgE-dependent pathway (data not shown). The basophil activation was observed in one of the four healthy volunteer sample, even when the FFP was diluted 1000 times (data not shown).

Identification of the causative agent in the FFP-ATR1

Considering the results of ultrafiltration experiments which indicated the molecular weight of the causative agent was higher than 100 kDa (data not shown) and the report by Abe and coworkers,¹⁵ in which IgG autoantibody against IgE contained in blood components were reported as a causative factor for anaphylactic transfusion reactions, we next attempted to explore the possibility that IgG autoantibody to IgE is the causative agent in the FFP-ATR1 using protein G column chromatography and protein A column chromatography; the IgG fractions (absorbed fractions) induced the basophil activation, while protein G- and protein A-unbound fractions did not (Fig. 1A). To confirm the presence of IgG antibody against IgE in the FFP-ATR1, we incubated responder basophils with the FFP-ATR1 treated with several concentrations of recombinant human IgE. As shown in Fig. 1B, basophil activation was clearly inhibited by recombinant human IgE in a dose-dependent manner. These results strongly suggested that the IgG autoantibody against IgE, present in the FFP-ATR1, caused activation of basophils of the four healthy subjects. We now named the

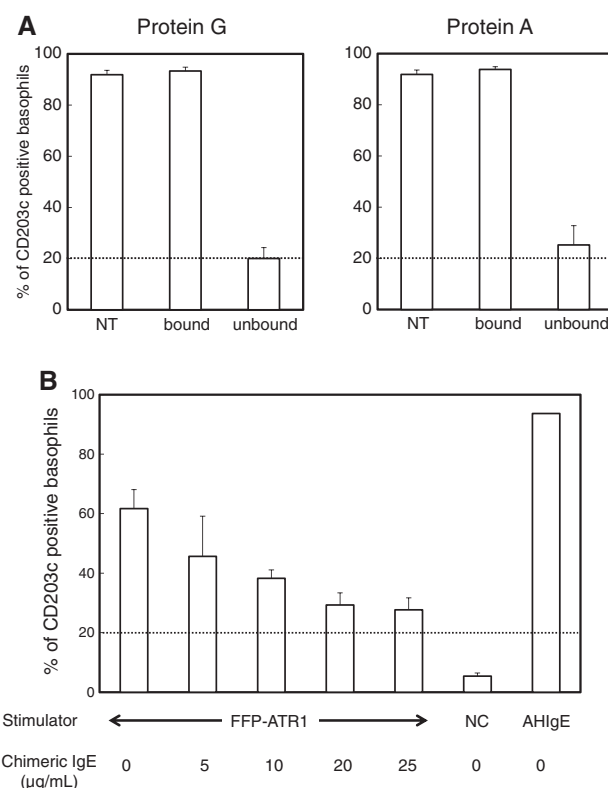


Fig. 1. Basophil activation ability of each fraction separated from the FFP-ATR1. The basophil activation ability was estimated by the BAT. (A) The FFP-ATR1 was fractionated by protein G and protein A column chromatography. The panel whole blood was stimulated with the original FFP-ATR1, bound fractions, or unbound fractions. The whole blood panels were then stained with anti-HLA-DR, anti-CD123, and anti-CD203c MoAbs. HLA-DR-negative CD123-positive cells in the FSC versus SSC blast cell window were identified as basophils, and CD203c expression was determined. Bars show the mean percentage of CD203c-positive basophils. When the percentage of CD203c-positive basophils exceeds 20% (dotted line), the PC-SNs were categorized as BAT-positive. The results are representative of two experiments. (B) The panel whole blood #2 was treated with various concentrations of recombinant chimeric human IgE (0 to 25 μg/mL), and then the cells were stimulated with the FFP-ATR1, PBS as the negative control (NC), or AHIgE MoAb as the positive control.

IgG autoantibody against IgE present in the FFP-ATR1 as “anti-IgE in FFP-ATR1.”

Characterization of the anti-IgE in FFP-ATR1

In the course of the screening test, the FFP-ATR1 activated basophils from the whole blood of four out of five healthy volunteers. It is therefore possible that the FFP-ATR1 possessed different activation abilities to different healthy donor basophils. To clarify whether the anti-IgE in FFP-ATR1 has such abilities, we performed the BAT using basophils from

an additional 16 healthy subjects, in addition to the above five healthy volunteers. We observed various reactivity patterns in healthy subject basophils to the anti-IgE in FFP-ATR1 (Fig. 2A). We then compared the extent of basophil activation of the individual healthy subjects to the anti-IgE in FFP-ATR1 with that to AHlgE MoAb. As shown in Fig. 2B,

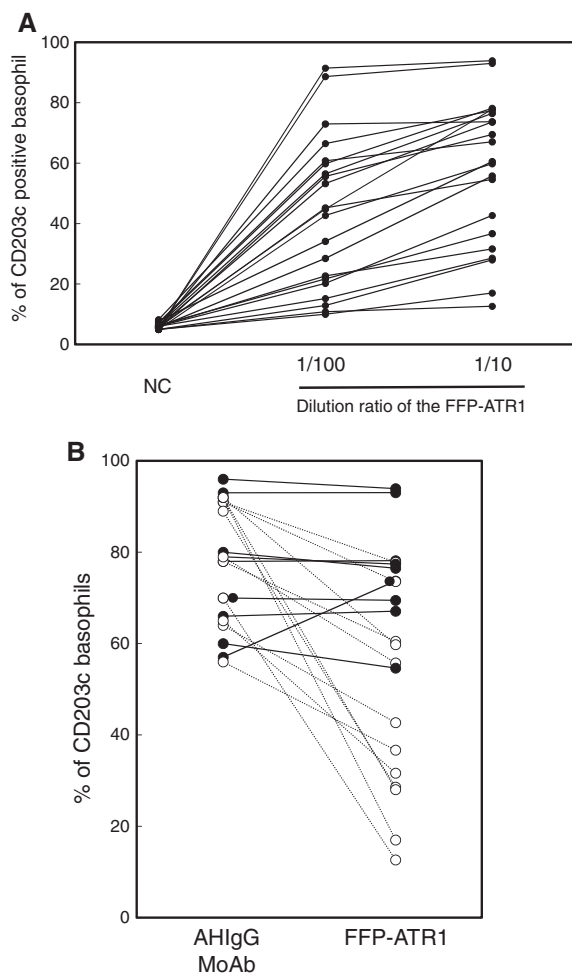


Fig. 2. Various basophil activation responses of healthy subjects to FFP-ATR1. The basophil activation ability of healthy subjects to the FFP-ATR1 was evaluated by the BAT. (A) The whole blood samples derived from 21 healthy volunteers were stimulated with the FFP-ATR1 at two concentrations (1/10 or 1/100 dilution) or PBS as negative control. (B) The whole blood samples derived from 21 healthy volunteers were stimulated with either AHlgE MoAb at the concentrations (500 $\mu\text{g/mL}$) or the FFP-ATR1 at the dilution ratio of 1/10, so that the maximum basophil activation was obtained in both stimulations. (●) Percentage of CD203c-positive basophils after the stimulation with the FFP-ATR1 was higher than, or comparable with, those obtained after the stimulation with AHlgE MoAb. (○) Percentage of CD203c obtained after FFP-ATR1 was lower than that obtained after AHlgE MoAb by more than 10%. We classified the former and the latter groups as the high-responder and low-responder groups, respectively.

the former varied more than the latter. In addition, it appeared that there were two groups of healthy subjects; the basophils of the first one elicited comparable response both to the anti-IgE in FFP-ATR1 and AHlgE, while those of the second one showed lower activation response to the anti-IgE in FFP-ATR1 than to AHlgE. We called the subjects “high responders” when the extent of basophil activation induced by the anti-IgE in FFP-ATR1 was not less than 90% of that by AHlgE. We considered the remaining subjects “low responders.” We considered two possible causes of the difference between the high and the low responders. One is that IgE concentration may affect the extent of the anti-IgE in FFP-ATR1 induced basophil activation, as reported by Abe and colleagues.¹⁵ The other one is that the binding ability of the anti-IgE in FFP-ATR1 to IgE may differ between the two groups. In regard to the first explanation, a negative correlation was observed between the basophil reactivity and the IgE concentrations of individual whole blood; the basophil activation was not observed in subjects with high IgE concentration (data not shown). Because the correlation was low (dilution ratio 1/10, $R^2 = 0.36$, $p = 0.01$, and dilution ratio 1/100, $R^2 = 0.43$, $p < 0.01$), we presumed that the second explanation is also likely; the anti-IgE in FFP-ATR1 may bind the IgE of various subjects differently. To be more precise, the anti-IgE in FFP-ATR1 may bind IgE from the low-responder subjects less than the IgE from the high-responder subjects, leading to lower basophils activation to the low responder than in the higher responder.

To explore the possibility above, we utilized the passive immune BAT system.³¹ We prepared two groups of quasi-basophils; they have the same source of basophils, but their replaced IgE was different. Each type of quasi-basophils possessed IgE derived from either the high- or the low-responder subjects. The outline of above experiment is shown in Fig. 3A. We compared the two groups of quasi-basophils in the amount of replaced IgE bound on their cell surfaces (Fig. 3B) and the binding ability of the anti-IgE in FFP-ATR1 to the replaced IgE (Fig. 3C). While there was no difference in the amount of reconstituted cell surface IgE between the two groups of basophils, the amount of the anti-IgE in FFP-ATR1 bound on the IgE-replaced quasi-basophils was quite different; the anti-IgE in FFP-ATR1 was found to be bound more on quasi-basophils replaced with IgE derived from the high responder subjects than on those with IgE from the low-responder subjects. These results indicated that the difference of basophil activation resulted from the different binding ability of the anti-IgE in FFP-ATR1 to IgE derived from different subjects.

DISCUSSION

Identification of causative agent in the FFP-ATR1 by the BAT

We demonstrated that one (FFP-ATR1) of the 43 blood components with serious ATR possesses a capacity to

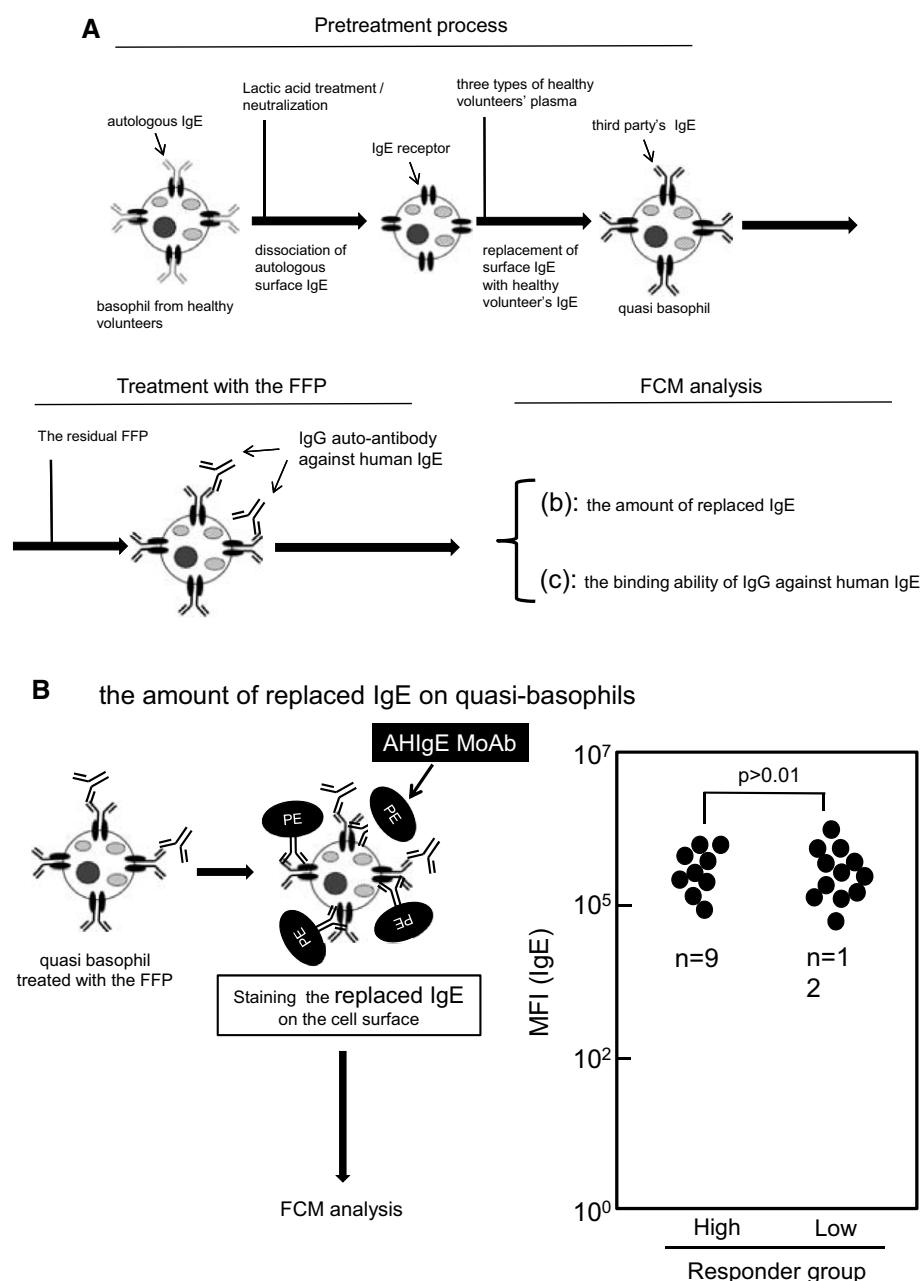


Fig. 3. Difference in the presence of IgE derived from healthy subjects on the surface of IgE-depleted basophils and in the binding ability of the anti-IgE in FFP-ATR1 to the IgE of healthy subjects bound on quasi-basophils. (A) The outline of the experiment: basophils were obtained from a healthy volunteer. After removing the IgE from the basophil surface, IgE was reconstituted using sera obtained from the 21 healthy volunteers analyzed in Fig. 3A, through Figs. 2, 14 and seven of which were classified as the low and high responders, respectively, in Fig. 4. (B) The amount of replaced IgE was evaluated by FCM analysis using AHlgE MoAb. (C) The binding ability of IgG autoantibody against human IgE was evaluated using AHlgG MoAb.

activate basophils of a wide range of healthy subjects. We further revealed that the causative agent in the FFP-ATR1 was IgG class autoanti-IgE antibody (Fig. 1). This study was the second to demonstrate the presence of an IgG autoantibody against IgE as the causative agent of ATR from blood components.¹⁴ IgG autoantibodies against IgE are found in patients with various diseases, such as allergic

disorders,^{32–36} systemic lupus erythematosus,³⁷ parasite infection,³⁸ systemic sclerosis,³⁹ Crohn's disease,⁴⁰ and visceral leishmaniasis.⁴¹ Such autoantibodies are also detected in normal individuals, as reported in some studies.^{32,40} Although the presence of such a pathogenetic auto anti-IgE antibody in blood components may be rare, it is important to keep in mind that the IgG autoantibody against IgE might

C the binding ability of IgG against human IgE on quasi-basophils

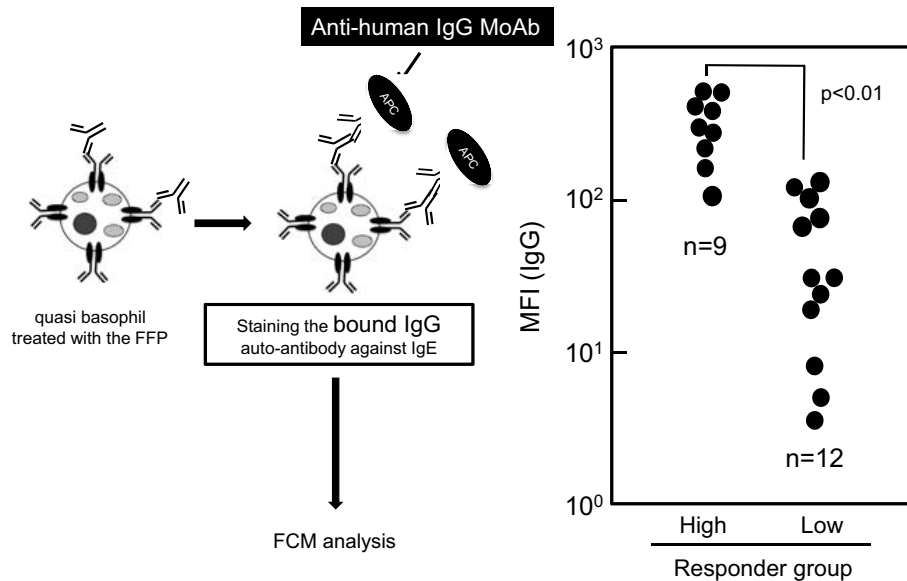


Fig. 3. (Continued)

be present in blood components, when the mechanisms of ATR are analyzed.

It is noteworthy that there was a big difference among healthy subjects in the magnitude of basophil activation elicited by the IgG autoantibody against IgE present in the FFP-ATR1. Although the overall correlation was low, we observed a negative correlation between the magnitude of basophil activation by the anti-IgE in FFP-ATR1 and plasma (or serum) IgE concentrations. The basophil activation was not observed in subjects with high IgE concentrations. In this regard, Abe and coworkers¹⁴ demonstrated the importance of IgE concentration; the degradation of cord blood mononuclear cells (CBMCs) was observed when IgE was not too high and not too low. Therefore, IgE concentration may be a key factor affecting basophil activation by anti-IgE autoantibody. The reason why there is such a subtle difference between the results of the study by Abe and colleagues and ours is unknown, although it could occur because of differences in the concentration of anti-IgE autoantibody. We prefer another possible explanation: that magnitude differences in basophil activation among healthy subjects may be due to the different binding abilities of the IgG autoantibody against IgE to IgE from different healthy subjects, as shown in Fig. 2B and Fig. 3C. One interpretation may be that the IgG autoantibody recognizes the polymorphism of human IgE.

“Two-hit” mechanism in ATR mimicking that in transfusion-related acute lung injury

Savage et al. reported that an allergic predisposition in transfusion recipients was strongly associated with ATR

risk.⁴² In addition, Azuma et al. demonstrated the ability to induce Ca^{2+} influx into cultured mast cells in the pre-transfusion sera of patients who had transfusion reactions.⁴³ These reports suggested that a “two-hit” mechanism may be common in ATRs; the first hit is allergic predisposition or priming of a patient’s mast cell/basophils, whereas the second hit is the action of the bioactive substances in the blood to be transfused.⁴⁴ The antibody against IgE could be a second hit, like how the HLA antibody acts in transfusion-related acute lung injury.

Limitations of our study and future perspectives

One major limitation of this study was that we performed the BAT between the FFP-R1 and the basophils of healthy subjects, instead of between the FFP-R1 and the basophils from the index patient. However, because of the retrospective nature of this study, we could not obtain whole blood from the index patient. To avoid these drawbacks, future prospective studies should first perform BAT with the causative blood product and the patient to confirm the causative relationship between the transfusion and the ATR and then carry out BAT between the ATR blood product and unrelated patients, in addition to the BAT between the ATR product and healthy subjects, to examine whether basophils of healthy subjects can be used in place of unrelated patient basophils to screen for potentially risky blood products.

Future perspective for safety measures for ATR

Causative agents have not been identified in most ATR cases. In this study, we identified the IgG antibody against IgE as one of the causative agents, demonstrating that the

BAT is useful in investigation of the causative relationship between ATRs and transfusion and the mechanisms behind them.^{13,30} We hope that the BAT facilitates identifying causative agents in ATR.

We used whole blood of healthy subjects as basophil source and demonstrated that the IgG antibody against IgE activated basophils. Considering the possible “two-hit” mechanism, in which patients’ basophils are somewhat primed, it is likely that patients’ basophils would show higher responses to the IgG antibodies against IgE than those from healthy subjects. From this reason, we believe that the BAT between ATR products and basophils of healthy subjects would provide a new tool to screen with a certain level of sensitivity for high-risk products and/or donors that could cause ATRs to other patients, although it may not be so specific because the specificity of the BAT as the new tool screens high-risk products and/or donors remains to be clarified in the future.

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

REFERENCES

1. Sander SG, Mallory D, Malamut D, et al. IgA anaphylactic transfusion reactions. *Transfus Med Rev* 1995;1:1-8.
2. Lambin P, Le Pennec PY, Hauptmann G, et al. Adverse transfusion reactions associated with a precipitating anti-C4 antibody of anti-Rodger specificity. *Vox Sang* 1984;47:242-9.
3. Savage WJ, Tobian AAR, Savage JH, et al. Scratching the surface of allergic transfusion reactions. *Transfusion* 2013;53:1361-71.
4. Hirayama F. Current understanding of allergic transfusion reactions: incidence, pathogenesis, laboratory tests, prevention and treatment. *Br J Haematol* 2013;160:434-44.
5. Koda Y, Watanabe Y, Soejima M, et al. Simple PCR detection of haptoglobin gene deletion in anhaploglobinemic patients with antihaptoglobin antibody that causes anaphylactic transfusion reactions. *Blood* 2000;95:1138-43.
6. Shimada E, Tadokoro K, Watanabe Y. Anaphylactic transfusion reactions in haptoglobin-deficient patients with IgE and IgG haptoglobin antibodies. *Transfusion* 2002;42:766-73.
7. Arnold P, Schmidt MD, Howard F, et al. Anaphylactic transfusion reactions associated with anti-IgA antibody. *N Engl J Med* 1969;280:188-93.
8. Westhoff CM, Sipherd BD, Wylie DE, et al. Severe anaphylactic reactions following transfusions of platelets to a patient with anti-Ch. *Transfusion* 1992;32:576-9.
9. Dewachter P, Castro S, Nicaise-Roland P, et al. Anaphylactic reaction after methylene blue-treated plasma transfusion. *Br J Anaesth* 2011;106:687-9.
10. Nubret K, Delhoume M, Orsel I, et al. Anaphylactic shock to fresh-frozen plasma inactivated with methylene blue. *Transfusion* 2011;51:125-8.
11. Jacobs JF, Baumert JL, Brons PP, et al. Anaphylaxis from passive transfer of peanut allergen in a blood product. *N Engl J Med* 2011;364:1981-2.
12. Gao L, Sha Y, Yuan K, et al. Allergic transfusion reaction caused by the shrimp allergen of donor blood: a case report. *Transfus Apher Sci* 2014;50:68-70.
13. Matsuyama N, Yasui K, Amakishi E, et al. The IgE-dependent pathway in allergic transfusion reactions: involvement of donor blood allergens other than plasma proteins. *Int J Hematol* 2015;102:93-100.
14. Abe T, Matsumoto C, Shimada E, et al. Immunoglobulin E oligomers identified in blood components activate mast cells: relevance to anaphylactic transfusion reaction. *Transfusion* 2011;51:2327-36.
15. Abe T, Shimada E, Takahashi M, et al. Antibody against immunoglobulin E contained in blood components as causative factor for anaphylactic transfusion reactions. *Transfusion* 2014;54:1953-60.
16. Lee DD, Muskaj I, Savage W. Platelet proteins cause basophil histamine release through an immunoglobulin-dependent mechanism. *Transfusion* 2017;57:1709-16.
17. Wadhwa M, Seghatchian MJ, Lubenko A, et al. Cytokine levels in platelet concentrates: quantitation by bioassays and immunoassays. *Br J Haematol* 1996;93:225-34.
18. Edvardsen L, Taaning E, Mynster T, et al. Bioactive substances in buffy-coat-derived platelet pools stored in platelet-additive solutions. *Br J Haematol* 1998;103:445-8.
19. Phipps RP, Kaufmann J, Blumberg N. Platelet derived CD154 (CD40 ligand) and febrile responses to transfusion. *Lancet* 2001;357:2023-4.
20. Wakamoto S, Fujihara M, Kuzuma K, et al. Biologic activity of RANTES in apheresis PLT concentrates and its involvement in nonhemolytic transfusion reactions. *Transfusion* 2003;43:1038-46.
21. Garraud O, Hamzeh-Cognasse H, Cognasse F. Platelets and cytokines: how and why? *Transfus Clin Biol* 2012;19:104-8.
22. MacGlashan DW Jr. Basophil activation testing. *J Allergy Clin Immunol* 2013;132:777-87.
23. McGowan EC, Saini S. Update on the performance and application of basophil activation tests. *Curr Allergy Asthma Rep* 2013;13:101-9.
24. Hoffmann HJ, Santos AF, Mayorga C, et al. The clinical utility of basophil activation testing in diagnosis and monitoring of allergic disease. *Allergy* 2015;70:1393-405.
25. Macglashan D Jr. Expression of CD203c and CD63 in human basophils: relationship to differential regulation of piecemeal and anaphylactic degranulation processes. *Clin Exp Allergy* 2010;40:1365-77.
26. Hirayama F, Yasui K, Matsuyama N, et al. Possible utility of the basophil activation test for the analysis of mechanisms involved in allergic transfusion reactions. *Transfus Med Rev* 2018;32:43-51.
27. Yasui K, Matsuyama N, Kuroishi A, et al. Mitochondrial damage-associated molecular patterns as potential proinflammatory mediators in post-platelet transfusion adverse effects. *Transfusion* 2016;56:1201-12.

28. Matsuyama N, Hirayama F, Wakamoto S, et al. Application of the basophil activation test in the analysis of allergic transfusion reactions. *Transfus Med* 2009;19:274-7.
29. Iwamoto S, Yonekawa T, Azuma E, et al. Anaphylactic transfusion reaction in homozygous haptoglobin deficiency detected by CD203c expression on basophils. *Pediatr Blood Cancer* 2014;102:1160-1.
30. Okamura-Shiki I, Matsuyama N, Yasui K, et al. Clinical utility of the basophil activation test for analysis of allergic transfusion reactions: a pilot study. *Vox Sang* 2017;112:114-21.
31. Yasui K, Matsuyama N, Okamura-Shiki I, et al. Clinical utility of a passive immune basophil activation test for the analysis of allergic transfusion reactions. *Transfusion* 2017;57:2084-95.
32. Eckman JA, Hamilton RG, Gober LM, et al. Basophil phenotypes in chronic idiopathic urticaria in relation to disease activity and autoantibodies. *J Invest Dermatol* 2008;128:1956-63.
33. Smith SJ, Joses NS, Shakib F. The prevalence of IgG1 and IgG4 autoantibodies to IgE in patients with allergic and non-allergic rhinitis. *Clin Otolaryngol Allied Sci* 1995;20:540-3.
34. Nawata Y, Koike T, Yanagisawa T, et al. Anti-IgE autoantibody in patients with bronchial asthma. *Clin Exp Immunol* 1984;58:348-56.
35. Nawata Y, Koike T, Hosokawa H, et al. Anti-IgE autoantibody in patients with atopic dermatitis. *J Immunol* 1985;135:478-82.
36. Gruber BL, Baeza ML, Marchese MJ, et al. Prevalence and functional role of anti-IgE autoantibodies in urticarial syndromes. *J Invest Dermatol* 1988;90:213-7.
37. Gruber BL, Kaufman LD, Marchese MJ, et al. Anti-IgE autoantibodies in systemic lupus erythematosus. Prevalence and biologic activity. *Arthritis Rheum* 1988;31:1000-6.
38. Nawata Y, Koike T, Tsutsumi A, et al. Anti-IgE autoantibody in patients with parasite infection (in Japanese). *Arerugi* 1988;37:204-7.
39. Kaufman LD, Gruber BL, Marchese MJ, et al. Anti-IgE autoantibodies in systemic sclerosis (scleroderma). *Ann Rheum Dis* 1988;48:201-5.
40. Huber A, Genser D, Spitzauer S, et al. IgE/anti-IgE immune complexes in sera from patients with Crohn's disease do not contain food-specific IgE. *Int Arch Allergy Immunol* 1998;115:67-72.
41. Atta AM, Sousa-Atta ML, D'Oliveira A, et al. IgG anti-IgE autoantibodies in visceral leishmaniasis. *Mem Inst Oswald Cruz* 2002;97:101-3.
42. Savage WJ, Tobian AA, Savage JH, et al. Transfusion and component characteristics are not associated with allergic transfusion reactions to apheresis platelets. *Transfusion* 2015;55:296-300.
43. Azuma H, Yamaguchi M, Takahashi D, et al. Elevated Ca²⁺ influx-inducing activity toward mast cells in pretransfusion sera from patients who developed transfusion-related adverse reactions. *Transfusion* 2009;49:1754-61.
44. Silliman CC. The two-event model of transfusion-related acute lung injury. *Crit Care Med* 2006;34(5 Suppl):S124-31. ■