

Recent advances in laboratory assays for nonhemolytic transfusion reactions

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Nonhemolytic transfusion reactions (NHTRs) are the most common transfusion reactions and include transfusion-related acute lung injury (TRALI) and allergic and febrile reactions. White blood cell (WBC) antibodies (Abs) against human leukocyte antigen (HLA) and human neutrophil antigen (HNA) in blood components are frequently implicated in NHTRs, especially in TRALI. Recently, we established a five-cell-lineage immunofluorescence test, a modified granulocyte immunofluorescence test, and a panel of cell lines stably expressing HNAs for efficient detection of Abs against well-known HNA-1 to HNA-5, and also Abs against neutrophil antigens other than HNA-1 to HNA-5. Using these techniques, we found that most of the non-HLA WBC Abs detected in NHTR cases were against antigens other than HNA-1 to HNA-5. In addition, using our newly established neutrophil activation test, which assesses neutrophil activation elicited by immunologic stimuli such as WBC Abs and immune complex, we found that heparin-binding protein is a potential final effector molecule that induces NHTRs, including TRALI. More recently, we reported that the basophil activation test, which was originally developed to identify allergens in the field of allergic diseases, might be useful in transfusion medicine. In this review, we summarize these new techniques and other related tests.

NONHEMOLYTIC TRANSFUSION REACTIONS AND THEIR POSTULATED MECHANISMS

Nonhemolytic transfusion reactions (NHTRs) are the most common transfusion reactions and include transfusion-related acute lung injury (TRALI), allergic reactions, febrile reactions, hypotension, bacterial sepsis, circulatory overload, and metabolic complications. TRALI is one of the most serious NHTRs and is reported as approximately 0.02%, and the mortality rate ranges from 5% to 10%.¹⁻⁴ Blood components used for transfusion typically implicated in TRALI include whole blood, red blood cells (RBCs), fresh-frozen plasma (FFP), cryoprecipitates, and platelet concentrates (PCs).^{1,2} Even a small amount of plasma in RBCs may induce TRALI.^{1,2,4,5} In most cases of TRALI, antibodies (Abs) or neutrophil-priming antigens present in the blood components induce the pulmonary reactions.⁴ Donor human leukocyte antigen (HLA) Abs have been widely reported, dating back to the first TRALI case series in 1985.⁶ In fact, HLA Class I Ab is most frequently associated with fatal cases of TRALI.⁷ Nishimura

ABBREVIATIONS: Ab(s) = antibody(-ies); BAT = basophil activation test; FCM = flow cytometry(-ic); G-IFT = granulocyte immunofluorescence test; HBP = heparin-binding protein; HLA = human leukocyte antigen; HNA = human neutrophil antigen; HPA = human platelet antigen; IFT = immunofluorescence test; NeuAT = neutrophil activation test; NHTR(s) = nonhemolytic transfusion reaction(s); PC(s) = platelet concentrate(s); PC-SN(s) = PC supernatant(s); sCD40L = soluble CD40 ligand.

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TRANSFUSION **,*,**.*.

and colleagues⁸ reported that immune complexes are mediators in the development of TRALI, while other studies have focused on the potential contributions of lipids^{9,10} or soluble CD40 ligand (sCD40L)¹¹ accumulated in stored blood components that may prime and activate neutrophils.

Activated white blood cells (WBCs) release several bioactive factors that may be important in the mechanism of TRALI. Nishimura and coworkers treated neutrophils with granulocyte-macrophage-colony-stimulating factor (GM-CSF) and found that the neutrophils produced tumor necrosis factor (TNF)- α , interferon- γ , and interleukin (IL)-18 after a challenge with anti-HLA Class II Ab.¹² Furthermore, treatment of neutrophils with immune complexes resulted in the acceleration of the production of TNF- α and Fas ligand.⁸ Similarly, Kopko and coworkers¹³ treated whole blood with serum from donors implicated in TRALI and found increased cytoplasmic expression of IL-1 β and TNF- α in monocytes. Although it is assumed that the harmful effects of these factors, alone or in combination, are critical in the development of TRALI, the precise mechanisms remain to be clarified.

Anaphylactic reactions are reported in recipients with immunoglobulin (Ig)A deficiency who developed IgA Abs and in whom the transfused blood component contained donor plasma with a normal amount of IgA.¹⁴⁻¹⁶ Similar reactions in C4 deficiency^{17,18} and haptoglobin deficiency¹⁹⁻²¹ have been reported. In those cases, the IgG class of Ab is usually observed, while in recipients with haptoglobin deficiency, the IgE class of Ab is frequently observed in addition to IgG Abs.²¹ In such cases, IgE-dependent Type I allergy may be the main pathway of anaphylactic reactions. The clinical presentation of allergic reactions such as rash, pruritus, and/or urticaria observed in patients with normal levels of plasma proteins suggests that the recipient was exposed to a foreign substance in the blood component to which the recipient was sensitized. Although causes of allergic reactions may include food (e.g., nuts and tomatoes) and medications (e.g., penicillin) that the donor ingested immediately before collection of the implicated blood component, a specific allergen is usually not identified. The only definite identified cause of a Type I allergy is the IgE class haptoglobin Ab in haptoglobin-deficient patients.²¹

PCs are the most common blood components that elicit NHTRs.^{22,23} Since washed PCs often effectively reduce NHTRs,²⁴⁻²⁶ it is likely bioactive substances present or accumulated in PCs play important roles in the onset of NHTRs. Early studies initially demonstrated that some NHTRs were caused by inflammatory cytokines released from WBCs in PCs during storage,²⁷⁻³¹ but allergic transfusion reactions to WBC-reduced PCs still occurred.^{26,32} More recently, platelet (PLT)-derived bioac-

tive substances have emerged as potential candidates responsible for NHTRs to PC transfusions. For instance, CD40L is expressed on the PLT surface and expelled after activation, and as such, a substantial amount of sCD40L is present in PCs.^{11,33} sCD40L has been shown to be associated with NHTRs, especially febrile reactions.^{33,34} However, the association with allergic reactions remains unclear.³⁴ RANTES, a proinflammatory CC chemokine stored in alpha granules of PLTs and which is released after stimulation,^{35,36} is another candidate because it is a chemoattractant for basophils^{37,38} and activates them to release histamine.³⁹ However, although the accumulated levels of RANTES in PCs are high enough for basophils to migrate, they are insufficient to elicit histamine release.⁴⁰ Thus, the precise mechanisms for the high incidence of NHTRs in PC transfusion remain largely unknown. Finally, febrile reactions are often associated with the HLA Class I Ab in patients.^{41,42} However, the association of HLA Class I Abs in patients to other NHTRs has not been established.

Recently, we developed several new laboratory assays that may help in the understanding of NHTRs, especially TRALI, hypotension, and allergic reactions. Below, we summarize these new techniques and other related tests.

DETECTION SYSTEMS FOR NON-HLA WBC ABS

Five-cell-lineage immunofluorescence test for Ab screening

HLA Class I;^{6,43,44} HLA Class II;^{12,46-48} and human neutrophil antigen (HNA)-1a,⁴⁹ -1b,⁵⁰ -2a,⁵¹ and -3a^{52,53} Abs have been associated with TRALI. Furthermore, monocyte Abs have been reported to be involved in a few cases of TRALI.⁴⁵ The recent introduction of new bead-based technologies such as Luminex has enabled easier detection of HLA Class I and Class II Abs. However, a similarly effective technology does not exist for detection of non-HLA WBC Abs. Although flow cytometric (FCM) analysis, such as the granulocyte immunofluorescence test (G-IFT), is a widely used approach for screening for non-HLA WBC Abs,⁵⁴ neutrophils exhibit high background reactivity, often making it difficult to examine the presence of Abs. Furthermore, to reduce the high background reactivity of monocytes, Fc gamma receptor Type I needs to be down regulated,^{44,55} which is a complicated procedure. In addition to WBC Abs, PLT Abs are implicated in NHTRs. It was shown that human PLT antigen (HPA)-1a Abs induced the release of RANTES from human PLTs in vitro.⁵⁶ Further, a case of life-threatening adverse effects and thrombocytopenia caused by passive transfused CD36 Ab (also known as Nak^a Ab),^{57,58} and a case of TRALI induced by transfused CD36 Ab,⁵⁹ were reported. CD36, also known as GPIV,⁶⁰ is a major glycoprotein of PLTs and is expressed by mono-

cytes. CD36-deficient individuals can be immunized to CD36 through blood transfusion or pregnancy and develop the CD36 Ab. Thus, it is important to develop the technology to examine PLT Abs.

To screen non-HLA WBC Abs, we recently developed a new FCM analysis system, termed the five-cell-lineage immunofluorescence test (IFT), in which four lineages of WBCs and PLTs were simultaneously tested (Fig. 1).⁶¹ To reduce background signals, whole blood was utilized as a cell source. A summary of the expression of representative WBC antigens, including HLA and HNA, of blood cells on their cell surfaces as determined by the five-cell-lineage IFT can be seen in Table 1. For example, both HNA-1 and HNA-2a are present only on neutrophils, HNA-4 is present on neutrophils and monocytes, HNA-5 is expressed by all the lineages except PLTs, and CD36 is present on PLTs and monocytes. These expression differences can aid in the identification of Ab specificity. It is worth noting that the expression of HNA-3a is controversial. In our preliminary experiments, HNA-3a was expressed on all the lineages of blood cells, which is in contrast to a previous report

showing only expression by neutrophils, lymphocytes, and potentially PLTs.⁶² The reason for these conflicting data is likely because the HNA-3a gene has not been identified, and its chemical properties are largely unknown.

At present, we have no data on the sensitivity and specificity of the five-cell-lineage IFT, since we have neither compared five-cell-lineage IFT with G-IFT, a gold standard assay, nor examined samples from patients without reactions. When we performed G-IFT, neutrophils exhibited high background reactivity; thus, no data were obtained. In the future, samples from patients without reactions are required to assess the specificity of this assay.

Panel cell lines stably expressing HNA-1a, -1b, -1c, -2a, -4a, -4b, -5a, and -5b and a cell line expressing CD36 (termed HP-Nak cell) for determining Ab specificity

To unambiguously determine the specificity of non-HLA WBC Abs, we established panel cell lines expressing HNAs and CD36. To ensure stable expression of the antigens over

a long period of time, we applied a retrovirus vector for the transduction of HNA and CD36, by which the genes were integrated into the host DNA and long-lasting expression of the genes was achieved.^{63,64} Additionally, puromycin and/or neomycin selection is expected to rescue the cells from transgene silencing because of the cotranscription of both the drug-resistant gene and each HNA and CD36 gene as a bicistronic mRNA, mediated via the internal ribosome entry site in the vector.^{65,66} The whole schematic structure of the vector can be seen in Fig. 2. Next, to ensure a low background reactivity of the panel cell lines, we chose the human erythroleukemic cell line K562 as a parental cell line, because it does not express HLA, HNA, or HPA and exhibits very low background reactivity to normal human serum samples⁶⁷ (and unpublished

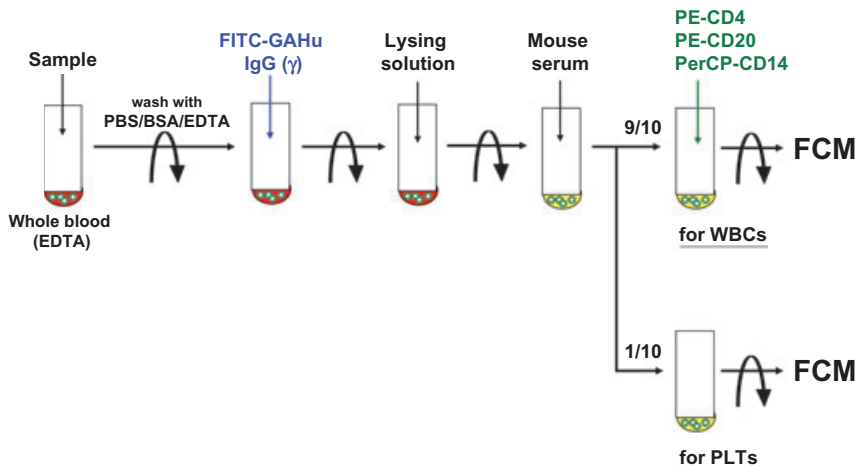


Fig. 1. Schematic presentation of staining procedure in five-cell-lineage IFT.

BSA = bovine serum albumin; EDTA = ethylenediaminetetraacetic acid; FITC-GAHu IgG (γ) = FITC-conjugated goat anti-human IgG (γ-chain-specific); PBS = phosphate-buffered saline.

TABLE 1. Expression of WBC antigens on five lineages of blood cells

WBC antigen	Allotype	Neutrophil	Monocyte	CD20+ B cell	CD4+ T cell	PLT
HNA-1	a,b,c	+				
HNA-2	a	+				
HNA-3*	a	+	+	+	+	+
HNA-4	a,b	+	+			
HNA-5	a,b	+	+	+	+	
HLA Class I	Numerous	+	+	+	+	+
HLA Class II	Numerous		+	+		
CD36 (Nak)			+			+

* The expression pattern of HNA-3 is controversial.

Step 1: Packaging



Step 2: Infection

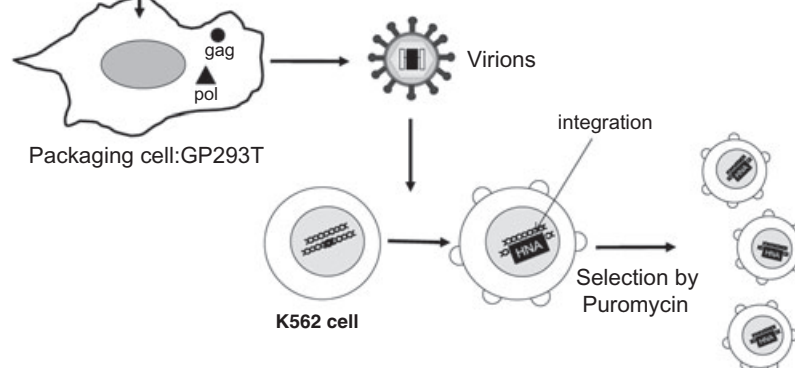


Fig. 2. Schematic presentation of retrovirus vector transfection.

data). Using these methods we established panel cell lines that stably expressed HNA-1a, -1b, -1c, -2a, -4a, -4b, -5a, and -5b antigens (termed KY-1a, -1b, -1c, -2a, -4a, -4b, -5a, and -5b cells, respectively);^{67,68} the cell line expressing HNA-3a has not been established because its gene has not been identified. In addition to the KY cells, we recently established a cell line expressing CD36 (termed HP-Nak cell).⁶⁹ HNA-1 and -2 and CD36 are single-chain glycoproteins, while HNA-4 and HNA-5 are integrins that consist of two transmembrane glycoprotein subunits, alpha and beta. Thus, a technique to establish cell lines expressing single-chain or heterodimer glycoproteins is now available and ready to be applied to establish cell lines expressing HPAs. However, although these panel cell lines are useful, a flow cytometer is required, which is only available in a limited number of laboratories. Therefore, bead-based technology, ELISA, or microarray technology needs to be introduced in future.

Non-HLA WBC Abs found in NHTRs

In our blood center, to screen for WBC Abs, we now detect HLA Abs by first using the microbead test (LABScreen, One Lambda, Inc., Canoga Park, CA). When HLA Abs are present, we then determine their specificity. Next, we screen for non-HLA Abs by five-cell-lineage IFT and finally determine their specificity using KY cells. Using this protocol, the only HNA Ab we are unable to detect with KY cells is that against HNA-3a. However, it is not difficult to identify HNA-3a Ab with five-cell-lineage IFT as the HNA-3a Ab reacts to neutrophils, lymphocytes, and potentially to all the rest of blood cells (as described above), while the frequency of a HNA-3a-negative person

is approximately 1 of 10 to 20, and we have already found HNA-3a-negative people in our blood center. We recently retrospectively examined 85 cases of severe NHTRs reported in our blood center during August 2003 to October 2007 with respect to the samples from patients and blood components implicated in those cases.⁷⁰ The cases included TRALI (n = 9), possible TRALI (n = 6), and other severe NHTRs (n = 70), including dyspnea only (n = 37), dyspnea and hypotension (n = 18), hypotension (n = 9), and anaphylactic reactions (n = 6). We found that 26% of the patients' sera and 6% of the blood components contained HLA Class I and/or Class II Abs, while unexpectedly, 36% of patients' sera and 18% blood components contained non-HLA WBC Abs. Most non-HLA Ab-positive samples with reactions reacted to neu-

trophils only or to neutrophils and other cell lineage(s). FCM analysis using five-cell-lineage IFT and KY cells revealed that specificity was determined in only one of the non-HLA Ab-positive samples, but that the rest of the samples were not the well-characterized HNA-1a, -1b, -1c, -2a, -3a -4a, -4b, -5a, or -5b. These data suggest that there may be additional neutrophil antigens that evoke immune responses and potentially cause NHTRs. Thus, it is very important to clarify these neutrophil antigens.

NEUTROPHIL ACTIVATION TEST

Neutrophil activation test

Although the mechanisms by which TRALI is induced are poorly understood, a number of studies have demonstrated the importance of neutrophil activation.^{1-4,6,8-10} To examine the involvement of neutrophils we recently developed a neutrophil activation test (NeuAT), a simple in vitro whole blood cell culture assay. In NeuAT, heparinized whole blood was stimulated by test reagents or blood components, and the activation of neutrophils was monitored by a cell surface marker, Mac-1, which is a plasma membrane protein with a function as adhesion molecule and also an activation marker of neutrophils.^{71,72} The most important reservoir of Mac-1 that is incorporated into the plasma membrane during stimulation with inflammatory mediators is secretory vesicles.⁷³ Using this assay system, we demonstrated that HLA MoAb (Clone W6/32), HLA antisera, and immune complexes evoke rapid (within 30 min) neutrophil activation via Fcγ receptors, CD16, and CD32 expressed on neutrophils.⁷⁴ Thus, neutrophil degranulation can be assessed using this test, although it does not detect other types of

neutrophil activation, such as reactive oxygen species. In this assay system, 200 μ L of whole blood was mixed with 10 μ L of serum or plasma sample. This mixture mimics the clinical situation in which a patient having 5 L of blood receives a transfusion of 250 mL of FFP or PCs possessing WBC Abs.

Possible involvement of heparin-binding protein in NHTRs, particularly TRALI

Heparin-binding protein (HBP) is stored in azurophilic granules and secretory vesicles of neutrophils^{75,76} and has recently been shown to have an exclusive role in mediating the blood vessel wall permeability evoked by β 2-integrin-mediated neutrophil activation.^{77,78} β 2-Integrin is the key integrin that mediates chemotaxis of neutrophils to endothelial cells. The neutrophils adhere to endothelial cells, and Ab cross-linking of the common β 2-integrin subunit CD18 mimics adhesion-dependent receptor engagement.⁷⁷ Herwald and colleagues^{77,78} demonstrated that β 2-integrin-mediated neutrophil activation increases blood vessel wall permeability via release of HBP. More recently, they described a similar role for HBP in streptococcal toxic shock syndrome characterized by excessive plasma leakage and multiorgan failure.⁷⁹ These reports led us to examine whether HLA Ab or immune complex can evoke HBP release from neutrophils in NeuAT described above.⁷⁴ In parallel with Mac-1

up regulation, HBP was released from neutrophils within 30 minutes after stimulation of immune complexes and HLA MoAb and also patients' clinical serum and plasma containing HLA Class I Abs. These data indicate that, as for Mac-1, HBP can be used as an indicator in NeuAT and that NeuAT may be utilized to verify neutrophil activation. However, further confirmation is required from clinical studies. In addition, Mac-1 up regulation and HBP release are not the only events that occur upon neutrophil activation, and HBP is not the only neutrophil-derived biologic response modifier that could be implemented in TRALI. Other biologic response modifiers such as oxygen species¹¹ and leukotriene B₄⁸⁰ should be simultaneously examined to improve the reliability of NeuAT.

TESTS FOR MAST CELL/BASOPHIL-MEDIATED NHTRs

Allergen-dependent and -independent pathways of allergic transfusion reactions

The activation of mast cells and basophils and their subsequent histamine release are mediated via the allergen-dependent and -independent pathways (Fig. 3A). In the allergen-independent pathway, bioactive substances such as activated complement components, cytokines, and chemokines directly activate mast cells/basophils. Although allergens in blood components may

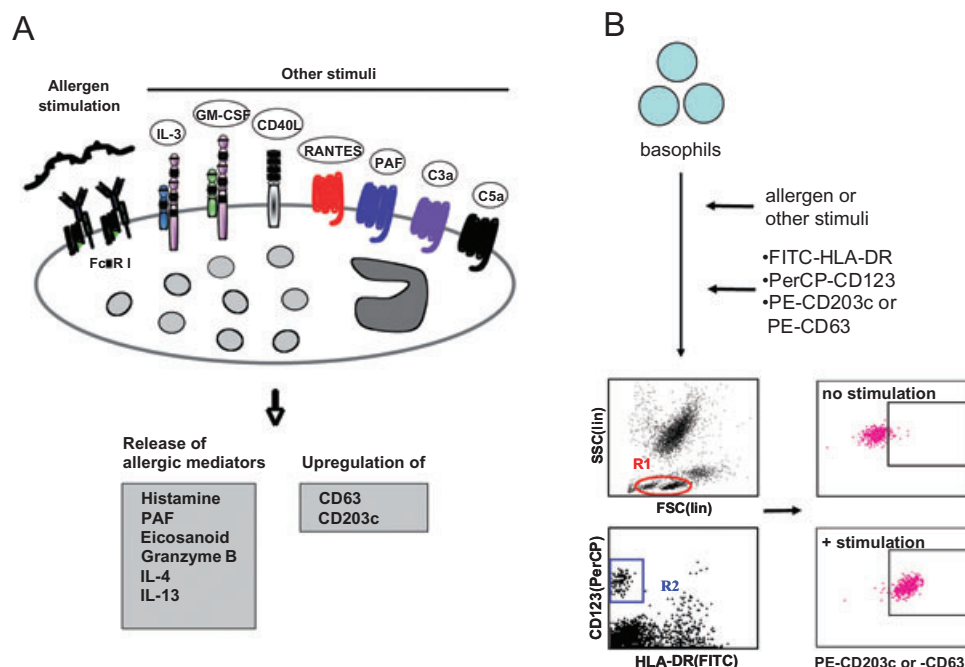


Fig. 3. Principle of the basophil activation and BAT. (A) Allergen and nonallergen extracellular stimuli, surface receptors, and events that occurred upon basophil activation are presented. (B) Basophils are identified on the basis of forward scatter versus side scatter location and the expression of HLA-DR and CD123. The increase in CD203c expression before and after the stimulation challenge reflects the basophil activation/degranulation in response to the challenge.

include food or medications ingested by the donor immediately before blood collection, such specific allergens have not been identified. The only allergens identified in blood components that can induce transfusion allergic reactions are plasma proteins including IgA, haptoglobin, and C4; transfusion to patients who are deficient in those plasma proteins reportedly causes serious allergic reactions. Considering that allergic transfusion reactions are the most common adverse effects associated with use of PCs, allergic reactions after PC transfusion are likely elicited mainly via the allergen-independent pathway, although the roles of bioactive substances in this pathway remain largely unknown.^{34,40,81}

Tests used in the field of allergic diseases

In allergic diseases such as asthma and atopic dermatitis, mast cells and basophils are generally considered to be involved. Skin tests and detection of specific IgE are tests widely used to diagnose allergic diseases. The tryptase test is also useful because tryptase is the most abundant secretory granule-derived serine proteinase contained in mast cells, and elevated levels of tryptase in serum, plasma, and other biologic fluids are consistent with mast cell activation in systemic anaphylaxis and other immediate hypersensitivity allergic reactions.^{82,83} Recently, numerous studies have demonstrated that FCM is a reliable tool for monitoring basophil activation upon allergen challenge by detecting surface expression of the degranulation/activation markers, CD63 or CD203c, both of which are almost equally used in the basophil activation test (BAT)⁸⁴ (Fig. 3). In brief, small aliquots of heparinized whole blood are incubated with test samples. The cells are then stained with anti-HLA-DR, anti-CD123, and anti-CD63 or anti-CD203c. HLA-DR-negative CD123-positive cells in the blast cell window are identified as basophils, and the expression of CD63 or CD203c is determined. Some of these techniques might be applied to assess allergic transfusion reactions, as described below.

Application of tryptase in transfusion medicine

Serum tryptase levels in patients suffering from NHTRs are occasionally measured in Japan using the ImmunoCAP tryptase kit (Phadia, Uppsala Sweden; <http://www.phadia.se/>). When serum tryptase was elevated by more than 5 µg/L after transfusion when compared with before transfusion, the serum tryptase test was considered positive. The results of 1165 cases included positive tests in 11% (50/438) of urticaria cases, 13% (23/177) of anaphylactic reaction cases, 48% (137/286)

of anaphylactic shock cases, and 12% (8/69) of hypotension cases. As no serum samples from patients without reactions were available, those from nonallergic reaction cases were examined as negative controls. In those controls, tryptase elevation was scarcely observed, with positive results in only 0.6% (1/154) of febrile reaction cases and 2.4% (1/41) of TRALI cases (personal communication with Dr T. Mazda, of Department of Research and Development, Central Blood Institute, Japanese Red Cross Society, Tokyo, Japan), indicating the involvement of mast cells in a significant part of allergic reactions. However, it is often difficult to obtain patient serum samples for a tryptase test because the half-life time of tryptase, once released into the circulation, is only 2 hours.⁸⁵ Furthermore, only a small amount of tryptase is produced by basophils.^{86,87} Fortunately, BAT has no restriction on the timing of patient sample collection and assesses basophil activation. As such the tryptase test and BAT would be complementary to each other.

Application of BAT in transfusion medicine

Recently, we assessed whether the BAT could be applied to transfusion medicine using PC supernatants (PC-SNs) from nine cases with allergic transfusion and 12 cases without transfusion reaction as test samples (Table 2).⁸⁸ the PC-SNs were the same as those used in the previous study,⁴⁰ which were kept frozen. As basophil sources, we used whole blood samples from healthy people instead of those from the patients as no Abs against plasma proteins were detected in any of the patients by in-house ELISA and PHA systems,⁴⁰ and therefore, it is likely that the allergic reactions were elicited mainly via the allergen-independent pathway, as described above. Furthermore, if the reactions were caused via the allergen-independent pathway, it is likely that blood samples from healthy people can be used as a basophil source, because differences in the responsiveness of basophils between the specific patients and healthy persons would be minimal. Five microliters of PC-SNs was incubated with 100 µL of whole blood (sample : blood, 1:20). This mimics a clinical setting in which 250 mL of PC is transfused to a patient with 5 L of blood. Next, HLA-DR-negative CD123-positive blast cells were identified as basophils, and their CD203c expression was determined. To calculate CD203c-positive basophils,

TABLE 2. Summarized data of BAT performed on supernatants of PCs with and without allergic transfusion reactions*

Allergic transfusion reactions	Number of PC-SNs tested	Number of BAT-positive PC-SNs
+	9	3 (5/5, 3/5, 1/5)
–	12	1 (1/5)

* In parentheses, the number of BAT-positive panel blood per total of five panel blood tested is presented with respect to each of BAT-positive PC-SNs. Adapted from Matsuyama et al.⁸⁸

TABLE 3. Summary of the assays

Test	Purpose	Method	Sensitivity and specificity	Clinical correlation	
				Current status	In future
Five-cell-lineage IFT ⁶¹	To screen for non-HLA WBC Abs	FCM analysis, a modified G-IFT assay using whole blood as blood cell source	There are no data available. Data of samples from patients without reactions need to be obtained in a future study to define the specificity.	In a study examining 85 NHTR cases, 36% patients' sera (29/81) and 18% blood components (12/66) contained non-HLA Abs. In contrast, approximately only 2% to 3% of normal donor samples were non-HLA WBC Ab-positive. ⁷⁰	It needs to be clarified whether the presence of non-WBC Abs leads to neutrophil activation and initiate NHTRs. NeuAT would help to approach this issue.
FCM analysis using KY cells ^{67,68}	To determine the specificity of non-HLA WBC Abs	FCM using K562 cell lines transfected with genes encoding HNA-1a, -1b, -1c, -2a, -4a, -4b, -5a, and -5b.	The information is limited. Only two, one, one, and one antisera against HNA-1a, -1b, -2a, and -4a were available whose specificity had been determined in other laboratories. The presence of HNA Abs and their specificity were confirmed by KY cells. All the KY cells can detect MoAbs at the level of, at least, 1 µg/mL ⁶⁷ (and unpublished data).	Among the above 85 NHTR cases, 41 samples in total contained non-HLA WBC Abs, but only one sample contained HNA-1b Ab. None of the remaining samples had reactivity to HNA-1a, -1b, -1c, -2a, -4a, -4b, -5a, and -5b. ⁷⁰	Since it is likely that neutrophil antigens other than HNA-1 to -5 are supposed to be involved in NHTRs, those antigens need to be identified, and KY cells expressing those antigens are required to be established. More convenient detection systems, like bead-based technology, ELISA, or microarray technology, need to be introduced.
NeuAT ⁷⁴	To examine the involvement of neutrophil activation	Whole blood is stimulated by test reagents, and the activation of neutrophils was monitored by up regulation of Mac-1 expression and the release of HBP.	There are no data available.	There is no clinical evidence. In a preclinical study, HLA MoAb and immune complex activated neutrophils ⁷⁴	Experiments using patient blood and transfused blood components need to be performed to show the usefulness of this assay in clinical settings. Mac-1 up regulation and HBP release are not the only events that occur upon neutrophil activation, and therefore, other biologic response modifiers, such as reactive oxygen species, should be simultaneously examined to improve the reliability of NeuAT.
Tryptase level in patient serum	To examine whether the reaction is allergic in nature and to examine the involvement of mast cells	Measured using ImmunoCAP Tryptase (Phadia; http://www.phadia.se/). When elevation more than 5 µg/L was observed after transfusion compared to before transfusion, it was classified as positive.	See the Phadia Web site and "current status" column. The measuring range of the kit is 1-200 µg/L.	Serum tryptase was elevated in 11% (50/438) of urticaria, 13% (23/177) of anaphylactic reaction, 48% (137/286) of anaphylactic shock, and 12% (8/69) of hypotension cases. In contrast, elevation was scarcely observed in nonallergic cases, that is, in 0.6% (1/154) of febrile reaction and 2.4% (1/41) of TRALI cases.	
BAT ⁸⁸	To examine whether the reaction is allergic in nature and to examine the involvement of basophils	Whole blood was stimulated by blood components, and the activation of neutrophils was monitored flow cytometrically by up regulation of CD203c.	There are no data available.	There is no clinical evidence. The only supporting evidence is that three of nine SNs from PCs which caused allergic reactions activated basophils of healthy volunteers, while only 1 of 12 SNs from PCs which caused no transfusion reaction marginally activated basophils. ⁸⁸	A larger scale of similar study as well as a study using patient blood instead of a normal person's blood are necessary to assess the issues of the sensitivity and specificity and the clinical application.

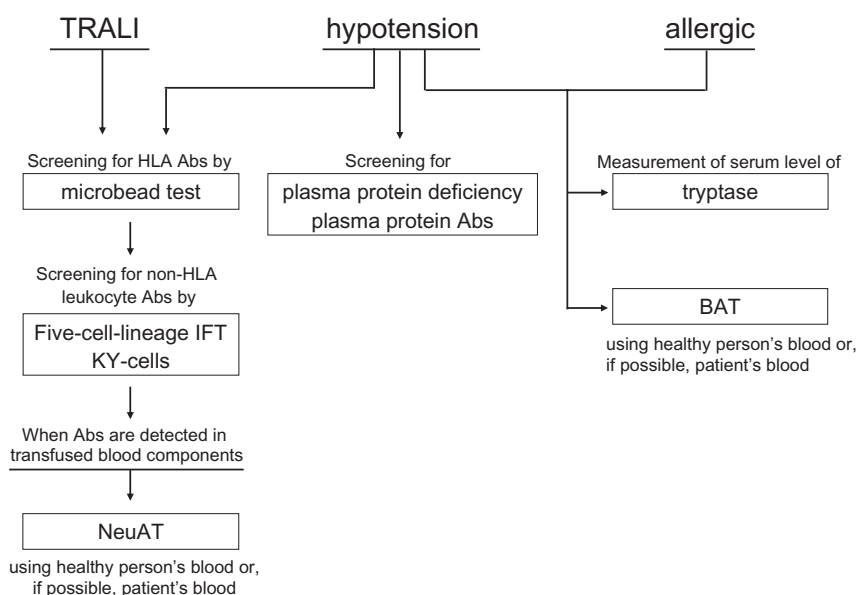


Fig. 4. Flow chart indicating the order in which assays should be performed upon clinical suspicion of TRALI, hypotension, and allergic reaction.

the cursor was set so that 5% of basophils were CD203c-positive after incubation without a stimulus. When the percentage of CD203c-positive basophils after stimulation by each PC-SN exceeded the mean + 3 SDs of 12 PC-SNs without reactions, the PC-SN was categorized as BAT-positive.

Three of the nine PC-SNs with allergic reactions activated basophils in at least one of five blood samples. One PC-SN activated basophils in all the blood samples, while the other two activated basophils in three or one of the five blood samples, respectively. Although the PC-SN positive to all the blood samples elicited the largest up regulation of CD203c among the three BAT-positive PC-SNs, while the PC-SN positive to only one blood sample exhibited only a slight up regulation of CD203c, it is unclear as to why the two PC-SNs were BAT-positive only to a limited number of blood samples. It is also unknown why six of the nine PC-SNs with allergic reaction were BAT-negative. In contrast to these data, one of the 12 PC-SNs without allergic reaction unexpectedly elicited basophil activation. However, because that PC-SN was BAT-positive only to one of the five blood samples, and because the extent of CD203c up regulation was very small and close to the cutoff point, this is likely a false BAT-positive result.

Taken together, these data suggest that BAT may be a useful research tool for examining allergic transfusion reactions, although many issues such as the sensitivity and specificity and the significance remained to be clarified. To resolve these issues, a similar, but larger-scale study is required. Simultaneously, a study using patient blood needs to be performed to examine whether

BAT can be applied to diagnose and predict allergic transfusion reaction.

CONCLUSION

We described several recently developed assay systems useful for developing a greater understanding of the etiology of NHTRs. However, there are several potential limitations, and, thus, at present the assay systems should only be used as research tools in reference laboratories. First, although the tryptase test is an established test with detailed information regarding its sensitivity/specificity and clinical results, the other assay systems are all handmade, and the fundamental information including the sensitivity/specificity and clinical data are lacking. Furthermore, the other assay systems cannot be performed routinely because of the requirement for panel cell preparation, cell line maintenance,

a flow cytometer, and an in-house ELISA system for HBP. Key information about each assay is summarized in Table 3. A flow chart (Fig. 4) is also presented in which we recommend which assays should be performed in which order on clinical suspicion of TRALI, hypotension, and allergic reaction. When TRALI is suspected, HLA and non-HLA WBC Abs should be screened for. When Abs are detected in transfused blood components, NeuAT should be performed using the transfused blood components and the patient blood to assess the involvement of neutrophil activation. If the patient blood is unavailable, blood from a healthy person can be used. When allergic reactions are suspected, patient serum tryptase level should be measured and compared between before and after the transfusion. Simultaneously, BAT should be conducted using the transfused blood components and the patient blood. Again, if the patient blood is unavailable, blood from a healthy person can be used. When hypotension is observed, all the assays for TRALI and allergic reactions should be performed. In addition, patients should be examined for plasma protein level and plasma protein Abs. Further studies are required for a better understanding of the etiology of NHTRs, which will help to develop novel strategies aimed at lowering the risk for developing NHTRs among transfusion patients.

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CONFLICT OF INTEREST

There are no conflicts.

REFERENCES

- Kleinman S, Caulfield T, Chan P, Davenport R, McFarland J, McPhedran S, Meade M, Morrison D, Pinsent T, Robillard P, Slinger P. Toward an understanding of transfusion-related acute lung injury: statement of a consensus panel. *Transfusion* 2004;43:177-89.
- Webert KE, Blajchman MA. Transfusion-related acute lung injury. *Transfus Med Rev* 2003;17:252-62.
- Bux J. Transfusion-related acute lung injury (TRALI): a serious adverse event of blood transfusion. *Vox Sang* 2005;89:1-10.
- Bux J, Sachs UJ. The pathogenesis of transfusion-related acute lung injury (TRALI). *Br J Haematol* 2007;136:788-99.
- Win N, Chapman CE, Bowles KM, Green A, Bradley S, Edmondson D, Wallis JP. How much residual plasma may cause TRALI? *Transfus Med* 2008;18:273-5.
- Popovsky MA, Moore SB. Diagnostic and pathogenetic considerations in transfusion-related acute lung injury. *Transfusion* 1985;25:573-7.
- Holness L, Knippen MA, Simmons L, Lachenbruch PA. Fatalities caused by TRALI. *Transfus Med Rev* 2004;18:184-8.
- Nishimura M, Ishikawa Y, Satake M. Activation of polymorphonuclear neutrophils by immune complex: possible involvement in development of transfusion-related acute lung injury. *Transfus Med* 2004;14:359-67.
- Silliman CC, Paterson AJ, Dickey WO, Stroneck DF, Popovsky MA, Caldwell SA, Ambruso DR. The association of biologically active lipids with the development of transfusion-related acute lung injury: a retrospective study. *Transfusion* 1997;37:719-26.
- Silliman CC, Voelkel NF, Allard JD, Elzi DJ, Tuder RM, Johnson JL, Ambruso DR. Plasma and lipids from stored packed red blood cells cause acute lung injury in an animal model. *J Clin Invest* 1998;101:1458-67.
- Khan SY, Kelher MR, Heal JM, Blumberg N, Boshkov LK, Phipps R, Gettings KF, McLaughlin NJ, Silliman CC. Soluble CD40 ligand accumulates in stored blood components, primes neutrophils through CD40, and is a potential cofactor in the development of transfusion-related acute lung injury. *Blood* 2006;108:2455-62.
- Nishimura M, Mitsunaga S, Ishikawa Y, Satake M. Possible mechanisms underlying development of transfusion-related acute lung injury: roles of anti-major histocompatibility complex Class II DR antibody. *Transfus Med* 2003;13:141-7.
- Kopko PM, Paglieroni TG, Popovsky MA, Muto KN, MacKenzie MR, Holland PV. TRALI: correlation of antigen-antibody and monocyte activation in donor-recipient pairs. *Transfusion* 2003;43:177-84.
- Vyas GN, Perkins HA, Fudenberg HH. Anaphylactoid transfusion reactions associated with anti-IgA. *Lancet* 1968;2:312-5.
- Schmidt AP, Taswell HF, Gleich GJ. Anaphylactic transfusion reactions associated with anti-IgA antibody. *N Engl J Med* 1969;280:188-93.
- Sandler SG, Mallory D, Malamut D, Eckrich R. IgA anaphylactic transfusion reactions. *Transfus Med Rev* 1995;9:1-8.
- Lambin P, Le Pennec PY, Hauptmann G, Desaint O, Habibi B, Salmon C. Adverse transfusion reactions associated with a precipitating anti-C4 antibody of anti-Rodgers specificity. *Vox Sang* 1984;47:242-9.
- Westhoff CM, Sipherd BD, Wylie DE, Toalson LD. Severe anaphylactic reactions following transfusions of platelets to a patient with anti-Ch. *Transfusion* 1992;32:576-9.
- Morishita K, Shimada E, Watanabe Y, Kimura H. Anaphylactic transfusion reactions associated with anti-haptoglobin in patient with ahaptoglobinemia [letter]. *Transfusion* 2000;40:120-1.
- Koda Y, Watanabe Y, Soejima M, Shimada E, Nishimura M, Morishita K, Moriya S, Mitsunaga S, Tadokoro K, Kimura H. Simple PCR detection of haptoglobin gene deletion in anahaptoglobinemic patients with antihaptoglobin antibody that causes anaphylactic transfusion reactions. *Blood* 2000;95:1138-43.
- Shimada E, Tadokoro K, Watanabe Y, Ikeda K, Niihara H, Maeda I, Isa K, Moriya S, Ashida T, Mitsunaga S, Nakajima N, Juji T. Anaphylactic transfusion reactions in haptoglobin-deficient patients with IgE and IgG haptoglobin antibodies. *Transfusion* 2002;42:766-73.
- Chambers LA, Kruskall MS, Pacini DG, Donovan LM. Febrile reaction after platelet transfusion: the effect of single versus multiple donors. *Transfusion* 1990;30:219-21.
- Heddle NM, Klama LN, Griffith L, Roberts R, Shukla G, Kelton JG. A prospective study to identify the risk factors associated with acute reactions to platelet and red cell transfusion. *Transfusion* 1993;33:794-7.
- Vo TD, Cowles J, Heal JM, Blumberg N. Platelet washing to prevent recurrent febrile reactions to leukocyte-reduced transfusions. *Transfus Med* 2001;45-7.
- Buck SA, Kickler TS, McGuire M, Braine HG, Ness PM. The utility of platelet washing using an automated procedure for severe platelet allergic reactions. *Transfusion* 1987;27:391-3.
- Azuma H, Hirayama J, Akino M, Miura R, Kiyama Y, Imai K, Kasai M, Koizumi K, Kakinoki Y, Makiguchi Y, Kubo K, Atsuta Y, Fujihara M, Homma C, Yamamoto S, Kato T, Ikeda H. Reduction in adverse reactions to platelets by the removal of plasma supernatant and resuspension in a new additive solution (M-sol). *Transfusion* 2009;49:214-8.
- Muyllle L, Wouters E, De Bock R, Peetermans ME. Reactions to platelet transfusion: the effect of the storage time of the concentrate. *Transfus Med* 1992;2:289-93.
- Muyllle L, Joos M, Wooters E, De Bock R, Peetermans ME. Increased tumor necrosis factor alpha (TNF-alpha),

- interleukin 1, and interleukin 6 (IL-6) levels in the plasma of stored platelet concentrates: relationship between TNF-alpha and IL-6 levels and febrile transfusion reactions. *Transfusion* 1993;33:195-9.
29. Heddle NM, Klama L, Singer J, Richards C, Fedak P, Walker I, Kelton JG. The role of plasma from platelet concentrates in transfusion reactions. *N Engl J Med* 1994;331:625-8.
 30. Aye MT, Palmer DS, Giulivi A, Hashemi S. Effect of filtration of platelet concentrates on the accumulation of cytokines and platelet release factors during storage. *Transfusion* 1995;35:117-24.
 31. Stack G, Snyder EL. Cytokine generation in stored platelet concentrates. *Transfusion* 1994;34:20-5.
 32. Wilhelm D, Klouche M, Fiebelkorn A, Gorg S, Kluter H, Kirchner H. Non-haemolytic transfusion reactions after platelet substitution. *Lancet* 1993;342:364.
 33. Phipps R, Kaufman J, Blumberg N. Platelet derived CD154 (CD40 ligand) and febrile responses to transfusion. *Lancet* 2001;357:2023-4.
 34. Blumberg N, Gettings KF, Turner C, Heal JH, Phipps RP. An association of soluble CD40 ligand (CD154) with adverse reactions to platelet transfusions. *Transfusion* 2006;46:1813-21.
 35. Klinger MH. Platelets and inflammation. *Anat Embryol* 1997;196:1-11.
 36. Bagglioni M, Dahinden CA. CC chemokines in allergic inflammation. *Immunol and Today* 1994;15:127-33.
 37. Bischoff SC, Krieger M, Brunner T, Rot A, von Tscharnner V, Baggiolini M, Dahinden CA. RANTES and related chemokines activate human basophil granulocytes through different G protein-coupled receptors. *Eur J Immunol* 1993;23:761-7.
 38. Hartmann K, Beiglbock F, Czarnetzki BM, Zuberbier T. Effect of CC chemokines on mediator release from human skin mast cells and basophils. *Int Arch Allergy Immunol* 1995;108:224-30.
 39. Kuna P, Reddigari SR, Schall TJ, Rucinski D, Viksman MY, Kaplan AP. RANTES, a monocyte and T lymphocyte chemotactic cytokine releases histamine from human basophils. *J Immunol* 1992;149:636-42.
 40. Wakamoto S, Fujihara M, Kuzuma K, Sato S, Kato T, Naohara T, Kasai M, Sawada K, Kobayashi R, Kudoh T, Ikebuchi K, Azuma H, Ikeda H. Biologic activity of RANTES in apheresis PLT concentrates and its involvement in nonhemolytic transfusion reactions. *Transfusion*. 2003;43:1038-46.
 41. de Rie MA, van der Plas-van Dalen CM, Engelfriet CP, von dem Borne AE. The serology of febrile transfusion reactions. *Vox Sang* 1985;49:126-314.
 42. Petrányi GG, Réti M, Harsányi V, Szabó J. Immunologic consequences of blood transfusion and their clinical manifestations. *Int Arch Allergy Immunol* 1997;114:303-15.
 43. Popovsky MA, Haley NR. Further characterization of transfusion-related acute lung injury: demographics, clinical and laboratory features, and morbidity. *Immunohematology* 2000;16:157-9.
 44. Popovsky MA, Abel MD, Moore SB. Transfusion-related acute lung injury associated with passive transfer of antileukocyte antibodies. *Am Rev Respir Dis* 1983;128:185-9.
 45. Kopko PM, Paglieroni TG, Popovsky MA, Muto KN, MacKenzie MR, Holland PV. TRALI: correlation of antigen-antibody and monocyte activation in donor-recipient pairs. *Transfusion* 2003;43:177-84.
 46. Kopko PM, Popovsky M., MacKenzie MR, Paglieroni TG, Muto KN, Holland PV. HLA Class II antibodies in transfusion-related acute lung injury. *Transfusion* 2001;41:1244-8.
 47. Win N, Brown C, Navarrete C. TRALI associated with HLA Class II antibodies. *Transfusion* 2003;43:545-6.
 48. Flesch BK, Neppert J. Transfusion-related acute lung-injury caused by human leukocyte antigen Class II antibody. *Br J Haematol* 2002;116:673-7.
 49. Leach M, Vora AJ, Jones DA, Lucas G. Transfusion-related acute lung injury (TRALI) following autologous stem cell transplant for relapsed acute myeloid leukemia: a case report and review of the literature. *Transfus Med* 1998;8:333-7.
 50. Yomtovian R, Kline W, Press C, Clay M, Engman H, Hammerschmidt D, McCullough J. Severe pulmonary hypersensitivity associated with passive transfusion of neutrophil-specific antibody. *Lancet* 1984;1:244-6.
 51. Bux J, Becker F, Seeger W, Kilpatrick D, Chapman J, Waters A. Transfusion-related acute lung injury due to HLA-A2-specific antibodies in recipient and NB1-specific antibodies in donor blood. *Br J Haematol* 1996;93:707-13.
 52. Nordhagen R, Conradi M, Dromtorp SM. Pulmonary reaction associated with transfusion of plasma containing anti-5b. *Vox Sang* 1986;51:102-7.
 53. Davoren A, Curtis BR, Shulman IA, Mohrbacher AF, Bux J, Kwiatkowska BJ, McFarland JG, Aster RH. TRALI due to granulocyte-agglutinating human neutrophil antigen-3a (5b) antibodies in donor plasma: a report of 2 fatalities. *Transfusion* 2003;43:641-5.
 54. Verheugt FW, von dem Borne AE, Decary F, Engelfriet CP. The detection of granulocyte alloantibodies with an indirect immunofluorescence test. *Br J Haematol* 1977;36:533-44.
 55. Kuijpers RW, Dooren MC, von dem Borne AE, Ouwehand WH. Detection of human monocyte-reactive alloantibodies by flow cytometry after selective downmodulation of the Fc receptor I. *Blood* 1991;78:50-6.
 56. Dettke M, Dreer M, Hocker P, Panzer S. Human platelet antigen-1 antibodies induce the release of chemokine RANTES from human platelets. *Vox Sang* 2001;81:199-203.
 57. Morishita K, Wakamoto S, Miyazaki T, Sato S, Fujihara M, Kaneko S, Yasuda H, Yamamoto S, Azuma H, Kato T, Ikeda H. Life-threatening adverse reaction followed by thrombocytopenia after passive transfusion of frozen plasma

- containing anti-CD36 (Naka) isoantibody. *Transfusion* 2005;45:803-6.
58. Wakamoto S, Fujihara M, Urushibara N, Morishita K, Kaneko S, Yasuda H, Takayama H, Yamamoto S, Azuma H, Ikeda H. Heterogeneity of platelet responsiveness to anti-CD36 in plasma associated with adverse transfusion reactions. *Vox Sang.* 2005;88:41-51.
59. Nakajima F, Nishimura M, Hashimoto S, Okazaki H, Tadokoro K. Role of anti-Nak(a) antibody, monocytes and platelets in the development of transfusion-related acute lung injury. *Vox Sang.* 2008;95:318-23.
60. Tomiyama Y, Take H, Ikeda H, Mitani T, Furubayashi T, Mizutani H, Yamamoto N, Tandon NN, Sekiguchi S, Jamieson GA, Kurata Y, Yonezawa T, Tarui S. Identification of the platelet-specific alloantigen, Naka, on platelet membrane glycoprotein IV. *Blood* 1990;75:684-7.
61. Matsuyama N, Kojima Y, Hirayama F, Yasui K, Taniue A, Fukumori Y, Yoshimura K, Tabata N, Sakata N, Tani Y, Shibata H. Simultaneous five cell-lineage flow cytometric analysis system for detection of leukocyte antibodies. *Transfus Med* 2006;16:111-8.
62. Stroncek D. Neutrophil alloantigens. *Transfusion* 2002;16: 67-75.
63. Yasui K, Furuta RA, Matsumoto K, Tani Y, Fujisawa J. HIV-1-derived self-inactivating lentivirus vector induces megakaryocyte lineage-specific gene expression. *Microbes Infect* 2005;7:240-7.
64. Sabatino DE, Seidel NE, Cline AP, Anderson SM, Gallagher PG, Bodine DM. Development of a stable retrovirus vector capable of long-term expression of gamma-globin mRNA in mouse erythrocytes. *Ann N Y Acad Sci* 2001;938:246-61.
65. Adam MA, Ramesh N, Miller AD, Osborne WR. Internal initiation of translation in retroviral carrying picornavirus 5' nontranslated regions. *J Virol* 1991;65:4985-90.
66. Ghattas IR, Sanes JR, Majors JE. The encephalomyocarditis virus internal ribosome entry site allows efficient coexpression of two genes from a recombinant provirus in cultured cells and in embryos. *Mol Cell Biol* 1991;11:5848-59.
67. Yasui K, Miyazaki T, Matsuyama N, Kojima Y, Furuta RA, Fujisawa JI, Tani Y, Shibata H, Sato S, Kato T, Ikeda H, Hirayama F. Establishment of cell lines stably expressing HNA-1a, -1b, and -2a antigen with low background reactivity in flow cytometric analysis. *Transfusion* 2007;47: 478-85.
68. Yasui K, Hirayama F, Matsuyama N, Furuta RA, Kimura T, Odagiri M, Watanabe Y. New cell lines selectively expressing HNA-1c, -4a, -4b, -5a, and -5b antigen established for the detection of HNA antibodies. *Transfusion* 2008;48: 1037-9.
69. J Hayashi T, Yasui K, Matsuyama N, Furuta RA, Hori Y, Tanaka S, Hirayama F, Tani Y, Shibata H, Inoue M. Establishment of a novel method for detecting Naka antibodies by using a panel cell line. *Transfusion* 2009;49:390-2.
70. Matsuyama N, Hirayama F, Kojima Y, Furuta RA, Kimura T, Taniue A, Fukumori Y, Tani Y, Shibata H. Non-HLA white cell antibodies in nonhemolytic transfusion reactions. *Transfusion* 2008;48:1526-8.
71. Klugewitz K, Ley K, Schuppan D, Nuck R, Gaetgens P, Walzog B. Activation of the $\beta 2$ integrin Mac-1 (CD11b/CD18) by an endogenous lipid mediator of human neutrophils and HL-60 cells. *J Cell Sci* 1997;110:985-90.
72. Diez-Fraile A, Meyer E, Paape MJ, Burvenich C. Analysis of selective mobilization of 1-selectin and Mac-1 reservoirs in bovine neutrophils and eosinophils. *Vet Res* 2003;34:57-70.
73. Jones DH, Anderson DC, Burr BL, Rudloff HE, Smith CW, Krater SS, Schmalstieg FC. Quantitation of intercellular Mac-1 (CD11b/CD18) pools in human neutrophils. *J Leukoc Biol* 1988;44:535-44.
74. Yasui K, Furuta RA, Matsuyama N, Fukumori Y, Kimura T, Tani Y, Shibata H, Hirayama F. Possible involvement of heparin-binding protein in transfusion-related acute lung injury. *Transfusion* 2008;48:978-87.
75. Tapper H, Karlsson A, Morgelin M, Flodgaard H, Herwald H. Secretion of heparin-binding protein from human neutrophils is determined by its localization in azurophilic granules and secretory vesicles. *Blood* 2002;99:1785-93.
76. Watorek W. Azurocidin-inactive serine proteinase homolog acting as a multifunctional inflammatory mediator. *Acta Biochim Pol* 2003;50:743-52.
77. Gautam N, Herwald H, Hedqvist P, Lindbom L. Signaling via $\beta 2$ integrins triggers neutrophil-dependent alteration in endothelial barrier function. *J Exp Med* 2000;191:1829-39.
78. Gautam N, Olofsson AM, Herwald H, Iversen LF, Lundgren-Akerlund E, Hedqvist P, Arfors KE, Flodgaard H, Lindbom L. Heparin-binding protein (HBP/CAP37): a missing link in neutrophil-evoked alteration of vascular permeability. *Nat Med* 2001;7:1123-7.
79. Herwald H, Cramer H, Morgelin M, Russell W, Sollenberg U, Norrby-Teglund A, Flodgaard H, Lindbom L, Bjorck L. M protein, a classical bacterial virulence determinant, forms complexes with fibrinogen that induce vascular leakage. *Cell* 2004;116:367-79.
80. Nishimura M, Hashimoto S, Satake M, Okazaki H, Tadokoro K. Interference with TRALI-causing anti-HLA DR alloantibody induction of human pulmonary microvascular endothelial cell injury by purified soluble HLA DR. *Vox Sang* 2007;93:78-82.
81. Cognasse F, Boussoulade F, Chavarin P, Acquart S, Fabrigli P, Lamy B, Garraud O. Release of potential immunomodulatory factors during platelet storage. *Transfusion* 2006;46: 1184-9.
82. Schwartz LB, Metcalfe DD, Miller JS, Earl H, Sullivan T. Tryptase levels as an indicator of mast-cell activation in systemic anaphylaxis and mastocytosis. *N Engl J Med* 1987; 316:1622-6.
83. Schwartz LB, Sakai K, Bradford TR, Ren SL, Zweiman B, Worobec AS, Metcalfe DD. The alfa form of human tryptase is the predominant type present in blood at baseline in normal subjects and is elevated in those with systemic mastocytosis. *J Clin Invest* 1995;96:2702-10.

84. Boumiza R, Debard AL, Monneret G. The basophil activation test by flow cytometry: recent developments in clinical studies, standardization and emerging perspectives. *Clin Mol Allergy* 2005;3:9-16.
85. Schwartz LB. Tryptase from human mast cells: biochemistry, biology and clinical utility. *Monogr Allergy* 1990;27:90-113.
86. Castells MC, Irani AM, Schwartz LB. Evaluation of human peripheral blood leukocytes for mast cell tryptase. *J Immunol* 1987;138:2184-9.
87. Foster B, Schwartz LB, Devouassoux G, Metcalfe DD, Prussin C. Characterization of mast-cell tryptase-expressing peripheral blood cells as basophils. *J Allergy Clin Immunol* 2002;109:287-93.
88. Matsuyama N, Hirayama F, Wakamoto S, Yasui K, Furuta RA, Kimura T, Taniue A, Fukumori Y, Fujihara M, Azuma H, Ikeda H, Tani Y, Shibata H. Application of the basophil activation test in the analysis of allergic transfusion reactions. *Transfus Med* in press 2009. 