

Allergic agonists in apheresis platelet products are associated with allergic transfusion reactions

William J. Savage, Jessica H. Savage, Aaron A.R. Tobian, Chris Thoburn, Robert G. Hamilton, John T. Schroeder, and Paul M. Ness

BACKGROUND: The mechanisms that underlie allergic transfusion reactions (ATRs) are not well characterized, but likely involve recipient, donor, and product factors. To assess product factors associated with ATRs, we investigated candidate mediators in apheresis platelet (PLT) products associated with ATRs and controls.

STUDY DESIGN AND METHODS: Using bead-based and standard enzyme-linked immunosorbent assays, we tested supernatants from 20 consecutive apheresis PLT transfusions associated with ATRs and 30 control products for concentrations of mediators in three categories: acute inflammatory mediators, direct agonists of basophils and mast cells, and growth and/or priming factors of basophils and mast cells.

RESULTS: Median concentrations of the direct allergic agonists C5a, brain-derived neurotrophic factor (BDNF), and CCL5 (RANTES) were 16.6, 41.8, and 13.9% higher, respectively, in the supernatant of apheresis PLT products that were most strongly associated with ATRs ($p < 0.05$ for each mediator). Other direct agonists (macrophage inflammatory protein-1 α , monocyte chemotactic protein-1, eotaxin-1, interleukin-8) were similar between groups. Concentrations of acute inflammatory mediators and basophil growth and/or priming factors were also similar between groups ($p > 0.2$ for all associations).

CONCLUSION: The allergic agonists C5a, BDNF, and CCL5 may be mediators of ATRs in apheresis PLT products. Acute inflammatory proteins and basophil and/or mast cell growth and priming factors do not appear to be associated with apheresis PLT products that cause ATRs.

Allergic transfusion reactions (ATRs) are the most common adverse reaction to transfused platelets (PLTs) and plasma and have an incidence of approximately 1% to 3%.¹⁻³ Although they are often self-limited reactions that involve erythematous rash, urticaria, and/or pruritus, more severe reactions with angioedema, bronchospasm, and anaphylaxis can occur. Furthermore, ATRs are problematic because prevention methods are limited.^{4,5} The mechanisms that underlie ATRs are not well characterized, with the exception of selective protein deficiencies and rare cases of passive transfer of immunoglobulin (Ig)E or allergen.⁶⁻¹⁰ These mechanisms have been associated with severe ATRs and likely do not account for the high frequency of common, mild ATRs.

Recipient, donor, and product characteristics are associated with ATRs. Previous studies have shown that recipient atopic predisposition is an important factor

ABBREVIATIONS: ATR(s) = allergic transfusion reaction(s); AUC = area under receiver-operator characteristic curve; BDNF = brain-derived neurotrophic factor; FNHTR = febrile nonhemolytic transfusion reaction(s); MCP-1 = monocyte chemotactic protein-1; MIP-1 α = macrophage inflammatory protein-1 α .

From the Department of Pathology, the Department of Medicine, and the Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University School of Medicine, Baltimore, Maryland.

Address reprint requests to: William Savage, MD, Department of Pathology, Johns Hopkins University School of Medicine (JHUSOM), Carnegie 667, 600 N. Wolfe Street, Baltimore, MD, 21287; e-mail: wsavage1@jhmi.edu.

This study was supported in part from internal funds provided by the Johns Hopkins DACI Reference Laboratory. WS acknowledges funding support from 5K12HL087169. JHS acknowledges support from T32AI007056-31.

Received for publication May 10, 2011; revision received June 30, 2011; and accepted July 5, 2011.

doi: 10.1111/j.1537-2995.2011.03310.x

TRANSFUSION **, **.* **.

associated with ATRs.^{2,11,12} At the same time, a plasma component is necessary to cause an ATR.^{5,13} Thus, ATRs are likely the result of a necessary plasma mediator that is introduced into a susceptible recipient, but the plasma factors are unclear. Specific agonists of basophils and mast cells, for example, CCL5 (regulated upon activation, normal T-cell expressed, and secreted [RANTES]) have been evaluated with inconclusive results.^{14,15}

IgE and complement have classically been associated with allergic mediator release from basophils and mast cells,¹⁶ but other chemokines and cytokines have known effects on these cells. These factors can generally be categorized as growth and/or priming factors or agonists that can directly induce release of mediators from basophils and mast cells, for example, histamine and leukotrienes. Factors that promote effector cell growth and survival include interleukin (IL)-4, IL-5, IL-13, and granulocyte-macrophage-colony-stimulating factor (GM-CSF).^{16,17} Factors that have been reported to directly induce effector cell mediator release include C5a, CCL2 (monocyte chemoattractant protein-1 [MCP-1]), CCL3 (macrophage inflammatory protein-1 α [MIP-1 α]), CCL5 (RANTES), CCL11 (eotaxin-1), and IL-8.^{18,19} Brain-derived neurotrophic factor (BDNF) is not yet known to be a direct agonist for basophils or mast cell histamine release, but it is thought to stimulate production of neuropeptides that cause pruritus and is therefore categorized as an agonist.^{20,21}

The goal of this study is to evaluate the association of ATRs with concentrations of three classes of plasma proteins in apheresis PLT products. Specifically, we investigated proteins that mediate acute inflammation, proteins that are agonists for acute mediator release from basophils and mast cells and proteins that are primarily priming and growth factors for basophils and mast cells. Many of these factors are stored and released by PLTs, making them plausible candidates for ATR mediators.²²

MATERIALS AND METHODS

Study samples

We evaluated products from 20 PLT transfusions that were associated with ATRs and returned to the blood bank between February 2010 and April 2010. In seven cases during the study period, the reporting of ATRs was not accompanied by a product returned to the blood bank; therefore, the ATRs studied were not sequential. According to hospital policy, ATRs were identified by reports of ATRs to the Division of Transfusion Medicine at Johns Hopkins Hospital by the primary clinical team and/or a team of oncology PLT coordinators who provide active surveillance of PLT transfusions. Transfusion reactions were adjudicated as allergic by the attending physician, according to Centers for Disease Control and Prevention hemovigilance criteria (http://www.cdc.gov/nhsn/wc_

[bio_hemo_overview.html](#)),²³ which include at least two signs and/or symptoms of urticaria, pruritus, morbilliform rash, angioedema, flushing, bronchospasm, chemosis, and hypotension. All allergic reactions were included, regardless of severity. All PLT products at this institution are leukoreduced, apheresis PLTs. The study was approved by the Johns Hopkins University School of Medicine Institutional Review Board.

Control PLT products were obtained from sequential apheresis products issued from the blood bank that were confirmed transfused and did not cause any transfusion reaction. To improve the specificity of our analysis, we matched controls to cases at a 1.5:1 ratio. Samples were centrifuged twice for 5 minutes at 15,000 $\times$ g at 4°C and supernatant was stored at -80°C until assayed. All laboratory testing was performed at the same time to reduce technical variation.

Quantitative immunoassays

Chemokines and cytokines were measured using an immunoassay platform (Bioplex 200, Bio-Rad, Hercules, CA). This multiplexed bead-based immunoassay utilized a 25-plex human cytokine kit (Invitrogen, Carlsbad, CA) and BDNF kit (Millipore, Billerica, MA), which were performed according to the manufacturer's protocols and supplied standards. The concentration was determined using a five-parameter log curve fit using the supplied software.

C5a concentrations were determined by measurement of C5a-desArg using an immunoassay according to the manufacturer's instructions (OptEIA, BD Biosciences, Franklin Lakes, NJ). C5a-desArg is a stable degradation product of C5a, which is rapidly cleaved by carboxypeptidases in plasma.²⁴ C5a-desArg testing was performed on a subset of 13 control and 18 ATR samples that had sufficient volume for this assay. Laboratory testing for total and allergen-specific IgE in pretransfusion patient samples and supernatants from apheresis PLT products was performed using the ImmunoCAP system (Phadia, Kalamazoo, MI), as described previously.¹¹

Statistical analysis

Differences in concentrations between groups were compared using two-tailed Mann-Whitney rank-sum tests. Correlations were assessed using Spearman rho coefficients unless otherwise indicated. Area under receiver-operator characteristic curve (AUC) was calculated to summarize the discriminatory ability of candidate mediators to classify apheresis products as ATR or control in exploratory analyses. For analyses investigating allergen-specific IgE, a sum of quantitative results of food and aeroallergen-specific IgE screens (reported previously¹¹) was used. To assess the combined associations of C5a, BDNF, and CCL5 with ATRs, concentrations of these

mediators were converted to an ordinal scale according to order of increasing concentrations. The sum of the three ranked concentrations represents the combined levels of these mediators compared to the other products in the study for the subset of products that had C5a concentrations measured. A *p* value of less than 0.05 was considered significant, and no adjustments were made for multiple comparisons. For the five instances in which two apheresis products were associated with an ATR and returned to the blood bank, values for candidate mediators were averaged between the two products. Statistics were calculated using computer software (Stata v11.1, StataCorp, College Station, TX).

RESULTS

Allergic reactions to apheresis PLT products

Twenty apheresis PLT transfusions associated with ATRs (*n* = 25 apheresis PLT products) and 30 control apheresis products were evaluated. Five transfusions consisted of two apheresis units transfused consecutively before the ATR: three transfusions consisted of two apheresis units that were split products from the same apheresis collection, and two transfusions consisted of two apheresis units that were from different donors. None of the apheresis products were plasma concentrated or washed. Urticaria or pruritus was reported in all ATRs. Wheezing (1/20, 5%) and angioedema (1/20, 5%) were reported in addition to pruritus in two ATRs. No patient had anaphylaxis.

Relationship of candidate mediators to ATRs

Figure 1 shows the concentrations of the acute inflammatory proteins IL-1 β , IL-6, tumor necrosis factor (TNF)- α , and interferon (IFN)- γ in control apheresis products and apheresis products that caused ATRs. Levels of these proteins that induce acute inflammation were not significantly different between groups (*p* > 0.4 for all observations).

Figure 2 shows concentrations of the effector cell agonists BDNF, C5a, CCL5 (RANTES), MCP-1, MIP-1 α , IL-8, and CCL11 (eotaxin-1) in control and ATR-associated PLT product supernatants. Figure 3 shows concentrations of the priming and/or growth factors IL-4, IL-5, IL-13, and GM-CSF. The significant differences between control and ATR groups were increases in BDNF, CCL5, and C5a among apheresis products that caused ATRs. As BDNF is a

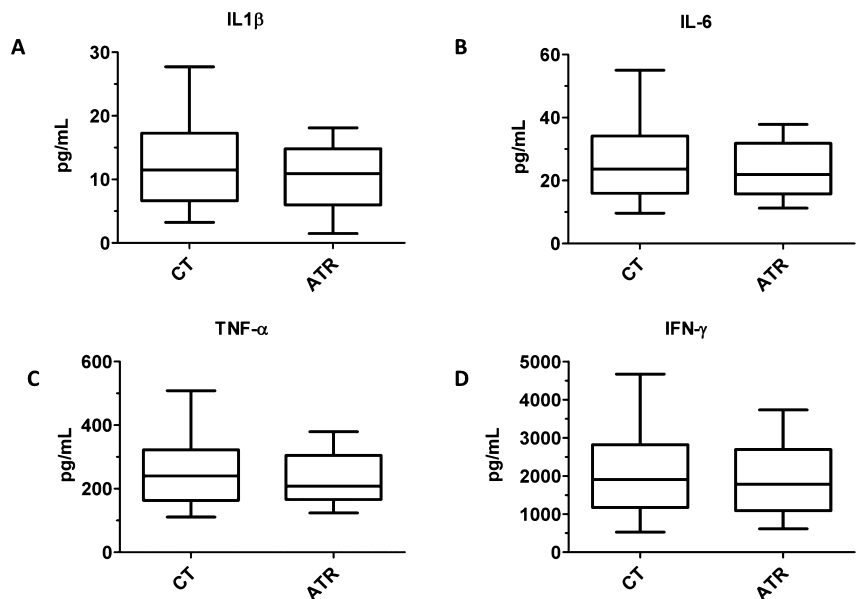


Fig. 1. Acute inflammatory mediators in apheresis PLT supernatants are not associated with ATRs. Concentrations of (A) IL-1 β , (B) IL-6, (C) TNF- α , and (D) IFN- γ are compared between control (CT) apheresis PLT products and products that were associated with ATRs. *p* > 0.4 for all comparisons.

candidate mediator of pruritus, the association of BDNF was stronger when an ATR is defined as the presence of pruritus (*p* = 0.01), compared to the presence of either urticaria or pruritus (*p* = 0.04).

Previous studies have shown that BDNF²⁵ and CCL5²⁵⁻²⁷ concentrations increase during PLT storage. Increasing concentrations of these factors during storage were one reason to select them as candidate mediators. On the other hand, varying storage times could confound the relationship between ATRs and concentrations of BDNF and CCL5. To address this concern, we correlated BDNF and CCL5 concentrations with time to supernatant freezing among ATR products and found no significant correlations by either Pearson or Spearman methods. Although exploratory, candidate ATR mediators CCL5, BDNF, and C5a were modestly able to discriminate ATR versus control products, with C5a having a slightly higher discriminatory ability (AUC, 0.71; 95% confidence interval [CI], 0.53-0.90) than CCL5 (AUC, 0.69; 95% CI, 0.53-0.84) and BDNF (AUC, 0.68; 95% CI, 0.52-0.84).

Relationships among mediators

To evaluate the possibility of synergistic effects among allergic agonists in apheresis PLT products that cause ATRs, we analyzed correlations among the candidate mediators BDNF, C5a, and CCL5. Levels among these mediators were not significantly associated with levels of the other mediators (*p* = -0.04 to 0.22; *p* > 0.3 for all comparisons). However, when concentrations of these three

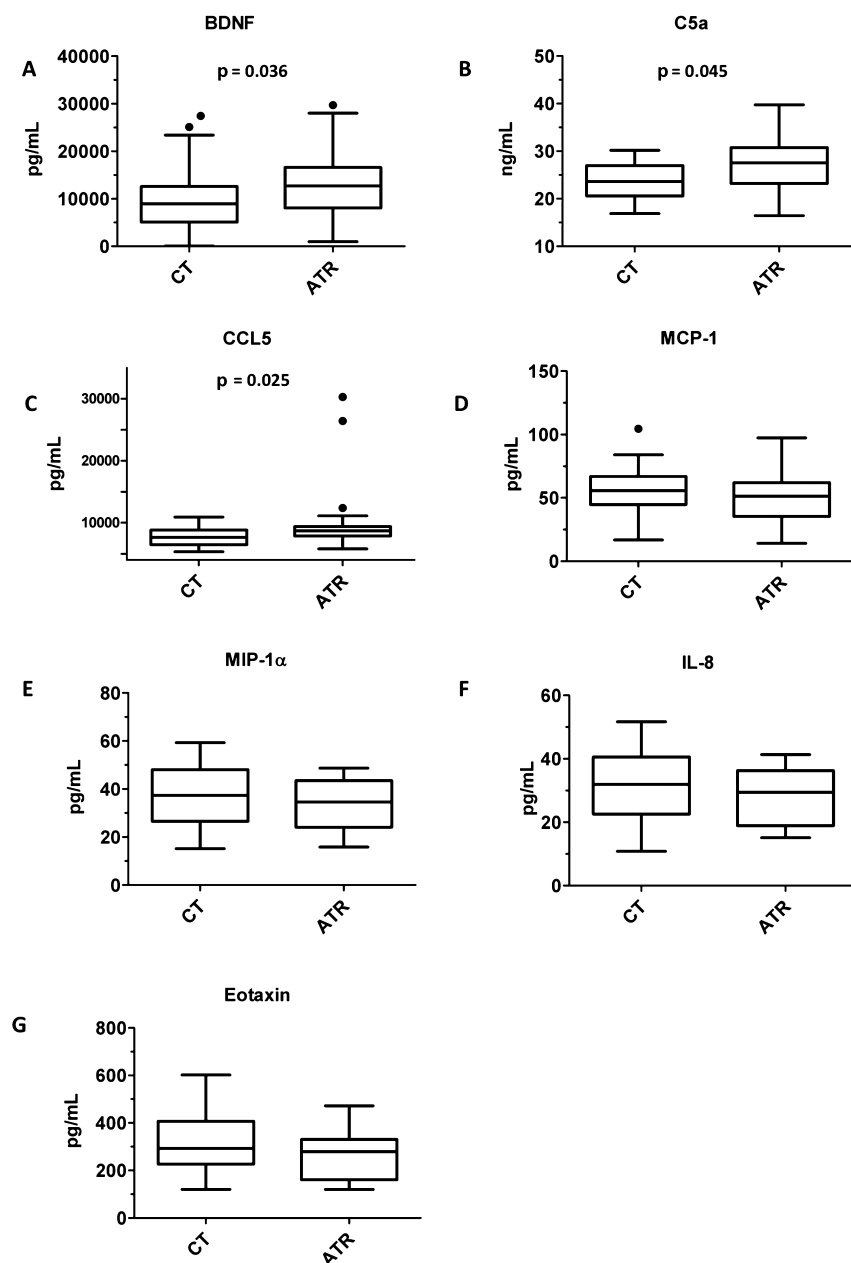


Fig. 2. Three agonists of allergic effector cells are associated with ATRs in apheresis PLT supernatants. Concentrations of (A) BDNF, (B) C5a, (C) and CCL5 (RANTES) were elevated in ATR products as compared to controls (CT). Concentrations of other agonists were not associated with ATRs ($p > 0.2$ for all comparisons): (D) MCP-1 (CCL2), (E) MIP-1 α (CCL3), (F) IL-8 (CXCL8), and (G) eotaxin-1 (CCL11).

mediators were combined into a single measurement, the sum of these mediators differentiated controls from ATR PLT products, which had higher total levels of these factors ($p = 0.003$, Fig. 4).

To investigate whether concentrations of these mediators were associated with allergic sensitization in donors, we further correlated BDNF, C5a, and CCL5 with total and allergen-specific IgE concentrations in

the plasma of apheresis products. No significant correlation was found ($\rho = -0.21$ to 0.19 ; $p > 0.1$ for all comparisons). In five ATR events, two apheresis products were transfused sequentially before an ATR. Our primary analysis used a mean of the mediators in the two products, but it remained a possibility that an ATR could be due to a high mediator level in only one of two products transfused. Among the two product transfusions, concentrations of all mediators in each unit were in the ranges of concentrations presented in Figs. 1 to 3. To summarize the variability of mediator concentrations in two apheresis unit transfusions, we calculated the coefficient of variations (CVs) between the two apheresis products for each of the 15 mediators. For transfusions of split products derived from the same apheresis collection, the mean of the 15 CVs for all mediators was 18.0%. For transfusions of two products collected from two donors, the mean of the 15 CVs was 45.6%.

DISCUSSION

We evaluated the associations of acute inflammatory proteins, allergic effector cell agonists, and allergic cell priming and/or growth factors in the supernatants of apheresis PLT products that did or did not cause an ATR. Increased concentrations of C5a, BDNF, and CCL5 in apheresis PLT products are associated with ATRs. There appears to be a stronger association of these mediators with ATRs when the sum of their concentrations is analyzed in aggregate.

If multiple plasma factors can contribute to ATRs to varying degrees, then the inconsistent association of CCL5 with ATRs^{14,15} may be explained by varying contributions of CCL5 to the ATRs studied. It will be helpful in future

studies of ATRs to measure multiple mediators simultaneously so that the relative contribution of each can be determined.

Given that ATRs occur during or within 2 hours of transfusion, we hypothesized that allergic effector cell agonists would be lead candidates for mediators of ATRs. In contrast, we demonstrate that acute inflammatory proteins and effector cell priming and growth factors that do

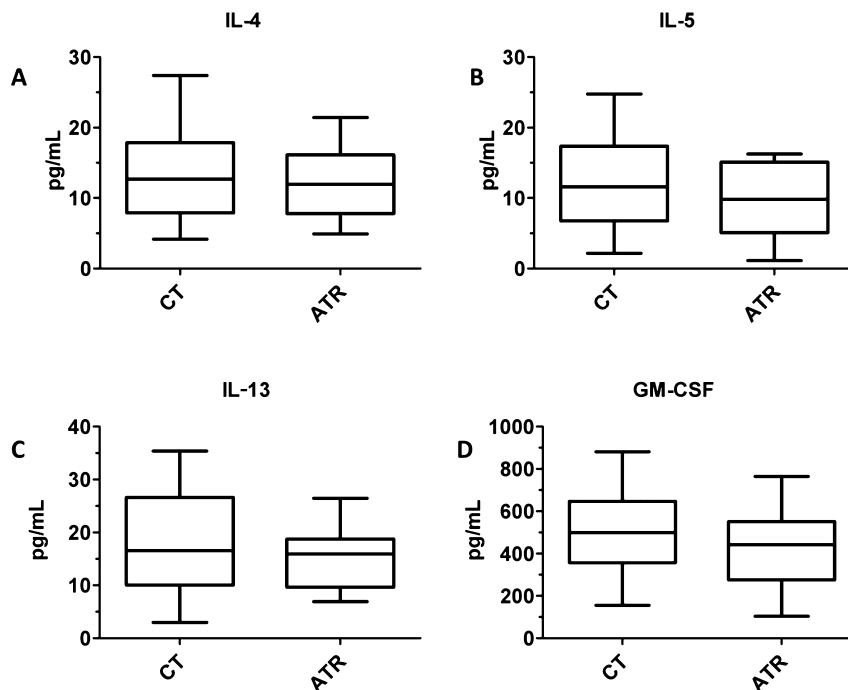


Fig. 3. Mast cell and basophil growth and priming factors are not associated with ATRs. Concentrations of (A) IL-4, (B) IL-5, (C) IL-13, and (D) GM-CSF are compared between control (CT) apheresis PLT products and products that were associated with ATRs. $p > 0.2$ for all comparisons.

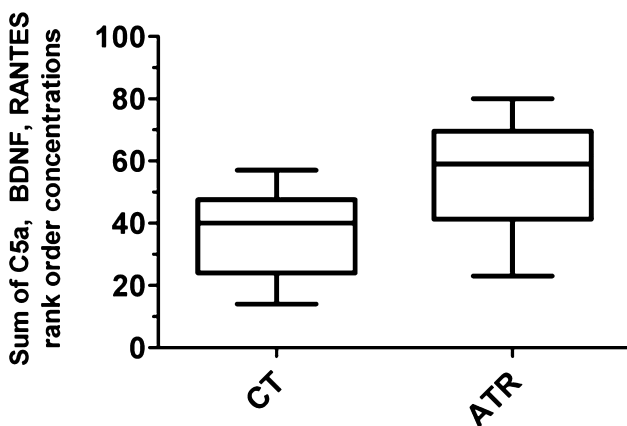


Fig. 4. The sum of C5a, BDNF, and CCL5 concentrations more strongly distinguish ATR and control (CT) PLT products than concentrations of individual mediators. To combine concentrations of C5a, BDNF, and CCL5 into a single metric, concentrations were converted to an ordinal scale according order of increasing concentrations. The sum of the three ranked concentrations is presented on the y-axis and compared between ATR and control PLT products. $p = 0.003$.

not typically mediate clinical signs and symptoms within minutes would not be elevated in apheresis PLT products associated with ATRs. Nevertheless, cumulative exposure to acute inflammatory and growth and/or priming factors

from prior transfusions may have a role in increasing recipient susceptibility to ATRs on subsequent transfusions. PLT recipients had a median of 22 apheresis PLT exposures before the ATR captured in this study, and studies that evaluate cumulative exposure of allergic mediators are needed to address the role of allergic effector modulation in transfusion recipients.

Acute inflammatory mediators have previously been reported to be associated with febrile nonhemolytic transfusion reactions (FNHTRs), as leukoreduction decreases the concentrations of these mediators and the incidence of FNHTRs.²⁸ The increasing prevalence of leukoreduction has led to a decrease in FNHTR, but not ATRs.^{29,30} Thus, we hypothesized that these cytokines and/or chemokines would not be associated with ATRs, and our data confirm prior observations.

The observation that BDNF, CCL5, and C5a are associated with ATRs does not necessarily indicate that they are part of the causal mechanism for ATRs.

First, there is large overlap in concentrations of these mediators between ATR and control products. Second, an alternative explanation could be that these markers are surrogates for allergic status of the donor and that other unidentified factors are causing ATRs. However, previous studies^{11,12,31} did not find an association of donor food or environmental sensitization with ATRs, and we did not find an association of donor IgE sensitization and BDNF, CCL5, or C5a concentrations in this study.

It is still possible that qualitative differences among donors or a storage effect may contribute to the plasma mediator milieu that causes ATRs. For example, PLT-derived mediators (e.g., CCL5) may be released during storage or in vivo to varying degrees because of qualitative PLT differences³² and not storage time.^{33,34} Correlating the allergic histories of donors with mediator levels in future studies may help more precisely define any qualitative associations. In vitro generation of pro-allergic mediators may depend on qualitative differences in how PLTs and plasma proteins react to the forces, temperatures, and material exposures in apheresis PLT processing.

In summary, apheresis PLT levels of acute inflammatory mediators and growth and/or chemotactic factors of basophils and mast cells do not appear to be associated with ATRs. BDNF, C5a, and CCL5 are candidate mediators of ATRs that may serve as necessary plasma factors in a

two-prong model of ATRs that requires both recipient susceptibility and plasma factors in the donor product.

CONFLICT OF INTEREST

None.

REFERENCES

- Robillard P, Nawej KI, Jochem K. The Quebec hemovigilance system: description and results from the first two years. *Transfus Apher Sci* 2004;31:111-22.
- Savage WJ, Tobian AA, Fuller AK, Wood RA, King KE, Ness PM. Allergic transfusion reactions to platelets are associated more with recipient and donor factors than with product attributes. *Transfusion* 2011. Epub Jan 7, 2011.
- Roback J. Technical manual. 16th ed. Bethesda (MD): American Association of Blood Banks Press; 2008.
- Tobian AA, King KE, Ness PM. Transfusion premedications: a growing practice not based on evidence. *Transfusion* 2007;47:1089-96.
- Tobian AA, Savage WJ, Tisch DJ, Thoman S, King KE, Ness PM. Prevention of allergic transfusion reactions to platelets and red blood cells through plasma reduction. *Transfusion* 2011. Epub Jan 7, 2011.
- Wibaut B, Mannessier L, Horbez C, Coupez B, Courbon B, Mizon P, Goudemand J. Anaphylactic reactions associated with anti-Chido Antibody following platelet transfusions. *Vox Sang* 1995;69:150-1.
- Shimada E, Tadokoro K, Watanabe Y, Ikeda K, Niihara H, Maeda I, Isa K, Moriya S, Ashida T, Mitsunaga S, Nakajima K, Juji T. Anaphylactic transfusion reactions in haptoglobin-deficient patients with IgE and IgG haptoglobin antibodies. *Transfusion* 2002;42:766-73.
- Vassallo RR. Review: IgA anaphylactic transfusion reactions. Part I. Laboratory diagnosis, incidence, and supply of IgA-deficient products. *Immunohematology* 2004;20:226-33.
- Arnold DM, Blajchman MA, Ditomasso J, Kulczycki M, Keith PK. Passive transfer of peanut hypersensitivity by fresh frozen plasma. *Arch Intern Med* 2007;167:853-4.
- Jacobs JF, Baumert JL, Brons PP, Joosten I, Koppelman SJ, van Pampus EC. Anaphylaxis from passive transfer of peanut allergen in a blood product. *N Engl J Med* 2011;364:1981-2.
- Savage WJ, Tobian AA, Savage JH, Hamilton RG, Ness PM. Atopic predisposition of recipients in allergic transfusion reactions to apheresis platelets. *Transfusion* 2011; May 13. doi: 10.1111/j.1537-2995.2011.03160.x. [Epub ahead of print].
- Wilhelm D, Kluter H, Klouche M, Kirchner H. Impact of allergy screening for blood donors: relationship to non-hemolytic transfusion reactions. *Vox Sang* 1995;69:217-21.
- 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.
- 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 non-hemolytic transfusion reactions. *Transfusion* 2003;43:1038-46.
- Kluter H, Bubel S, Kirchner H, Wilhelm D. Febrile and allergic transfusion reactions after the transfusion of white cell-poor platelet preparations. *Transfusion* 1999;39:1179-84.
- Schroeder JT. Basophils beyond effector cells of allergic inflammation. *Adv Immunol* 2009;101:123-61.
- Sin AZ, Roche EM, Togias A, Lichtenstein LM, Schroeder JT. Nerve growth factor or IL-3 induces more IL-13 production from basophils of allergic subjects than from basophils of nonallergic subjects. *J Allergy Clin Immunol* 2001;108:387-93.
- Petersen LJ, Brasso K, Pryds M, Skov PS. Histamine release in intact human skin by monocyte chemoattractant factor-1, RANTES, macrophage inflammatory protein-1 alpha, stem cell factor, anti-IgE, and codeine as determined by an ex vivo skin microdialysis technique. *J Allergy Clin Immunol* 1996;98:790-6.
- Schroeder JT, MacGlashan DW Jr., Lichtenstein LM. Human basophils: mediator release and cytokine production. *Adv Immunol* 2001;77:93-122.
- Nockher WA, Renz H. Neurotrophins in allergic diseases: from neuronal growth factors to intercellular signaling molecules. *J Allergy Clin Immunol* 2006;117:583-9.
- Stander S, Raap U, Weissshaar E, Schmelz M, Mettang T, Handwerker H, Luger TA. Pathogenesis of pruritus. *J Dtsch Dermatol Ges* 2011;9:456-63.
- Boehlen F, Clemetson KJ. Platelet chemokines and their receptors: what is their relevance to platelet storage and transfusion practice? *Transfus Med* 2001;11:403-17.
- Kuehn BM. CDC launches surveillance system to improve blood transfusion safety. *JAMA* 2010;303:1467.
- Bokisch VA, Muller-Eberhard HJ. Anaphylatoxin inactivator of human plasma: its isolation and characterization as a carboxypeptidase. *J Clin Invest* 1970;49:2427-36.
- Glenister KM, Payne KA, Sparrow RL. Proteomic analysis of supernatant from pooled buffy-coat platelet concentrates throughout 7-day storage. *Transfusion* 2008;48:99-107.
- Cardigan R, Sutherland J, Wadhwa M, Dilger P, Thorpe R. The influence of platelet additive solutions on cytokine levels and complement activation in platelet concentrates during storage. *Vox Sang* 2003;84:28-35.
- Fujihara M, Ikebuchi K, Wakamoto S, Sekiguchi S. Effects of filtration and gamma radiation on the accumulation of RANTES and transforming growth factor-beta1 in apheresis platelet concentrates during storage. *Transfusion* 1999;39:498-505.

28. Heddle NM, Klama L, Meyer R, Walker I, Boshkov L, Roberts R, Chambers S, Podlosky L, O'Hoski P, Levine M. A randomized controlled trial comparing plasma removal with white cell reduction to prevent reactions to platelets. *Transfusion* 1999;39:231-8.
29. Paglino JC, Pomper GJ, Fisch GS, Champion MH, Snyder EL. Reduction of febrile but not allergic reactions to RBCs and platelets after conversion to universal prestorage leukoreduction. *Transfusion* 2004;44:16-24.
30. King KE, Shirey RS, Thoman SK, Bensen-Kennedy D, Tanz WS, Ness PM. Universal leukoreduction decreases the incidence of febrile nonhemolytic transfusion reactions to RBCs. *Transfusion* 2004;44:25-9.
31. Stern A, van Hage-Hamsten M, Sondell K, Johansson SG. Is allergy screening of blood donors necessary? A comparison between questionnaire answers and the presence of circulating IgE antibodies. *Vox Sang* 1995;69:114-9.
32. Hasegawa S, Tashiro N, Matsubara T, Furukawa S, Ra C. A comparison of FcepsilonRI-mediated RANTES release from human platelets between allergic patients and healthy individuals. *Int Arch Allergy Immunol* 2001;125 Suppl 1: 42-7.
33. Sarkodee-Adoo CB, Kendall JM, Sridhara R, Lee EJ, Schiffer CA. The relationship between the duration of platelet storage and the development of transfusion reactions. *Transfusion* 1998;38:229-35.
34. 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. ■