PAS-C PLATELETS CONTAIN LOWER SUPERNATANT ISOHEMAGGLUTININ TITERS AND HLA ANTIBODY SPECIFICITIES AND INCREASED SOLUBLE CD40 LIGAND VERSUS PLASMA PLATELETS

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INTRODUCTION/ABSTRACT

Platelet Additive Solution 3 (PAS-3, Intersol, Fresenius Kabi USA, Lake Zurich, IL) – a balanced salt solution - may be used to replace approximately two thirds of human plasma for the storage of apheresis platelets collected on the Amicus separator (Fresenius Kabi USA, Lake Zurich, IL). Platelets stored in 65% PAS-3 / 35% plasma (PAS C platelets) maintain functional integrity for up to 5 days, and reduction in donor plasma may be beneficial for platelet recipients [1].

PAS C platelets are presumed to reduce recipient exposure to donor plasma components; however, the effects of PAS C on platelet supernatant composition are poorly defined.

OBJECTIVES

To better define the effects of PAS on platelet supernatant composition, we compared the following factors in supernatants of PAS-C platelets to plasma platelets.

- Total protein
- Isohemagglutinin titers
- HLA antibodies
- In-vitro neutrophil priming activity

MATERIALS AND METHODS

Sample collection. The study comprised 100 SDPs collected by apheresis from consecutive group O donors at the New York Blood Center with 50 collected into 100% plasma and 50 collected into 65% PAS C/35% plasma. After collection, a 5 mL sample was withdrawn from each unit, the platelets were pelleted and the supernatant was withdrawn and frozen for further testing.

Total protein measurement. Total protein was measured using a modified bichromatic assay on a Dimension Vista analyzer (Siemans, Munich, Germany).

Isohemagglutinin titers. Anti-A and anti-B isohemagglutinin titers were determined by serologic tube testing with straight and serial two-fold dilutions of platelet supernatant. In each reaction, 100 µL of straight or diluted SDP supernatant was added to 50 µL of type A1 or B reverse typing cells (Ortho Clinical Diagnostics, Raritan, NJ), and the samples were read for direct agglutination upon immediate spin. For each SDP supernatant sample, testing was performed with straight supernatant and increasing serial two-fold dilutions of SDP supernatant into 0.9% saline until agglutination was no longer detected. The titer was defined as the highest dilution that produced at least 1+ agglutination.

HLA antibody assessment. Screening for HLA antibodies was performed using the One Lambda (Canoga Park, CA) LabScreen mixed Luminex assay. Samples which exceeded the positive threshold were further tested with single antigen beads (LAB-Screen, One Lambda Inc.) to identify Class I and II antibody specificities.

Neutrophil isolation and priming. Neutrophils (PMNs) were isolated from heparinized whole blood after informed consent was obtained from healthy volunteer donors under an approved protocol by Colorado Multi-Institution Review Board at the University of Colorado Denver by standard technique using dextran sedimentation, ficoll-paque separation, and hypotonic lysis, as previously described [2]. PMNs (3.5 x10^5 cells) were incubated in Krebs Ringer Phosphate with Dextrose, pH 7.35 (KRPD) buffer along with supernatant or lipid samples (10%) for 5 min at 37°C then activated with 1µM formyl-Met-Leu-Phe (fMLF) and the superoxide dismutase inhibitable maximal rate of superoxide (O_2^-) was measured at 550 nm, as previously reported [2]. Priming activity was measured as the augmentation of the maximal rate of superoxide production by PMNs activated with fMLF.

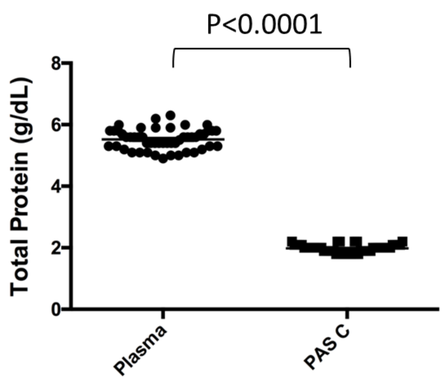
Lipid Extractions. Lipids were isolated from the plasma samples using a 1:1:1 chloroform:methanol:water:0.2% acetic acid extraction, as previously published.18 Lipids were then solubilized with 1.25% fatty acid free human serum albumin and used to assess in PMN priming activity, as previously reported [2, 3].

Statistics. The data is presented as the mean with± 95% confidence interval (CI) or the median with the range from 25th percentile to 75th percentile depending on the data analyzed. Statistical analysis and data visualization was performed using Prism software with unpaired t-tests employing the Mann-Whitney U test or Fisher's exact test depending upon the data analyzed (GraphPad, San Diego, CA).

RESULTS

Observational studies show decreased allergic and febrile non-hemolytic transfusion reactions in recipients of PAS C platelets compared to plasma platelets [1]. These effects are attributed to dilution of plasma protein antigens, and cytokines. The supernatant of PAS C platelets is expected to contain 35% of the total protein concentration of conventional plasma platelets. We measured the total protein in supernatant of plasma and PAS C SDPs.

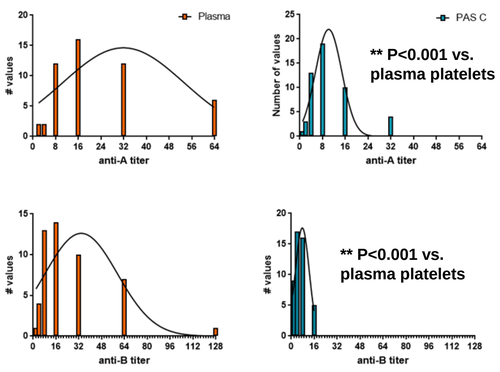
TOTAL PROTEIN LEVEL IN PAS C VERSUS PLASMA PLATELET SUPERNATANTS.



Dot plots of the total protein concentration in g/dL of spun supernatants are depicted as the function of treatment group: PAS C and plasma SDPs (n=50 for each group). The line indicates the mean and bars indicate the 95% confidence interval.

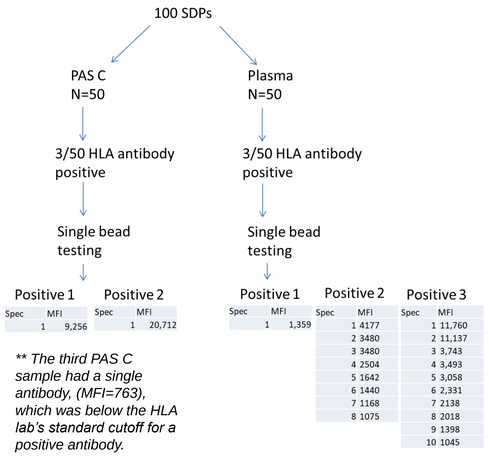
Plasma from group-O platelet donors may contain high levels of anti-A and anti-B isohemagglutinins, and replacing the donor plasma with 65% PAS C is expected to decrease anti-A and anti-B isohemagglutinin titers, which may prevent hemolysis after transfusion of ABO incompatible platelet units. To quantify the degree to which isohemagglutinin titers are reduced in PAS C compared to plasma platelets, titration for direct agglutination at immediate spin was performed on A1 and B red cells using supernatant from conventional plasma and PAS C SDPs from group O donors.

ANTI-A AND ANTI-B IGM TITERS IN PAS C VERSUS PLASMA PLATELET SUPERNATANTS.



Histograms of the anti-A (top) and anti-B (bottom) titers of spun supernatants from group O donors are shown as a function of treatment group: PAS C (right, blue) and plasma SDPs (left, orange) (n=50 per group). Each histogram is depicted with the best fit Gaussian curve.

HLA ANTIBODIES IN PAS C VERSUS PLASMA PLATELET SUPERNATANTS.



Positive 1 / Positive 2

Spec	MFI
1	9,256

Spec	MFI
1	20,712

Positive 1

Spec	MFI
1	1,359

Positive 2

Spec	MFI
1	4177
2	3480
3	3480
4	2504
5	1642
6	1440
7	1168
8	1075

Positive 3

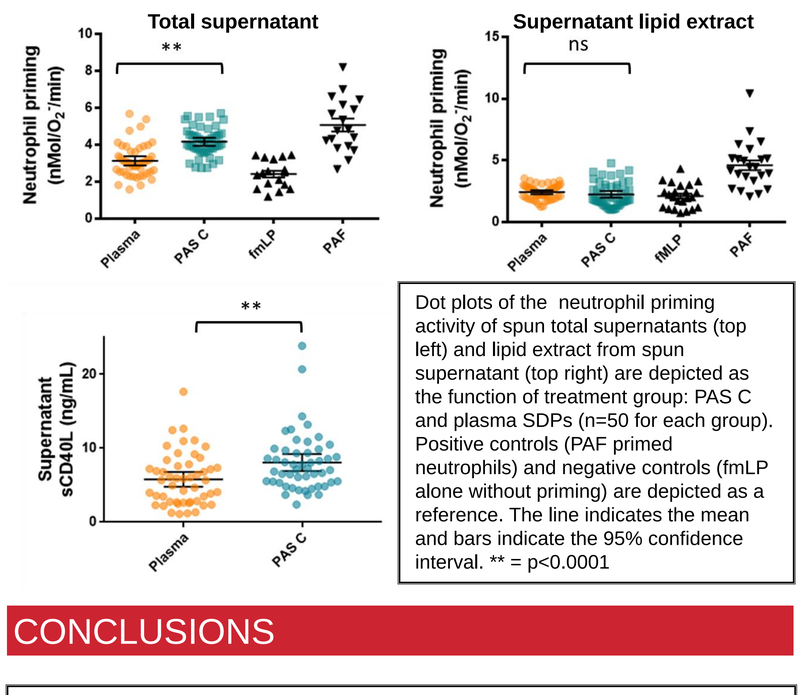
Spec	MFI
1	11,760
2	11,137
3	3,743
4	3,493
5	3,058
6	2,331
7	2138
8	2018
9	1398
10	1045

*** The third PAS C sample had a single antibody, (MFI=763), which was below the HLA lab's standard cutoff for a positive antibody.*

Transfusion related acute lung injury (TRALI) is one of the top causes of transfusion-related death in the United States. Anti-HLA antibodies are central to TRALI pathogenesis. The use of PAS C platelets has been proposed as a TRALI mitigation strategy. To determine if PAS C platelets had fewer HLA antibodies than plasma platelets, the HLA antibodies were initially measured using a Luminex-based screening assay [4]. Positive samples were further tested using a single bead assay to determine the specificity and strength of the HLA antibodies.

Neutrophil priming by bioactive lipids in donor plasma has been causally linked to TRALI [5]. To determine if neutrophil priming by bioactive lipids is reduced in PAS C compared to plasma platelets we assayed neutrophil priming activity in both the total supernatant and the lipid extractable fractions. Lipid extractable neutrophil priming activity was equivalent in PAS C vs plasma platelets, but priming activity in the total supernatant was increased in PAS C platelets. Because sCD40L is a water soluble cytokine that primes neutrophils and may be released from the platelet surface after collection, we quantified sCD40L levels in supernatant of PAS C and plasma platelets.

NEUTROPHIL PRIMING ACTIVITY AND SCD40L LEVELS IN PAS C VERSUS PLASMA PLATELET SUPERNATANTS



Dot plots of the neutrophil priming activity of spun total supernatants (top left) and lipid extract from spun supernatant (top right) are depicted as the function of treatment group: PAS C and plasma SDPs (n=50 for each group). Positive controls (PAF primed neutrophils) and negative controls (fmLP alone without priming) are depicted as a reference. The line indicates the mean and bars indicate the 95% confidence interval. ** = p<0.0001

CONCLUSIONS

Decreased plasma proteins likely underlie lower rates of allergic and febrile non-hemolytic transfusion reactions seen with use of PAS-C platelets. Decreased anti-A and anti-B titers may prevent hemolysis from minor ABO mismatch. Lower HLA-antibody specificities may mitigate transfusion related acute lung injury (TRALI). Increased PMN priming by PAS-C platelets is likely due to platelet membrane release of sCD40L and not bioactive lipids. Although sCD40L has been associated with TRALI, only PMN priming with lipid - not cytokine - agents has been causally linked with TRALI. The mechanism and clinical impact of increased sCD40L in PAS-C platelets remain to be elucidated.

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