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Fresh frozen plasma increases adhesion molecule expression on human pulmonary endothelial cells

Phillip A. Letourneau, MD^{1,2}, Shibani Pati, MD, PhD², Michael H. Gerber, BS², Fernando Jimenez, MS³, and John B. Holcomb, MD^{1,2}

¹Department of Surgery, University of Texas Medical School at Houston, Texas

²Center for Translational Injury Research, University of Texas Health Science Center at Houston, Texas

³Department of Pediatric Surgery, University of Texas Medical School at Houston, Texas

Abstract

Background—Current blood banking practices allow fresh frozen plasma (FFP) to be thawed and then stored for 5 days between 1-6°C. We hypothesized that aged plasma (Day 5 FFP) would be pro-inflammatory to the endothelium compared to fresh plasma (Day 0 FFP).

Materials and Methods—Human pulmonary endothelial cells (PECs) were treated with 1) media, 2) media + lipopolysaccharide (LPS), 3) lactated Ringer's (LR), 4)LR + LPS, 5) Day 0 FFP, 6)Day 0 FFP + LPS, 7) Day 5 FFP, and 8) Day 5 FFP + LPS. After a 24 hour incubation, the PECs were stained with antibodies for I-CAM, V-CAM, P-selectin, and E-selectin. The cells were subsequently analyzed by flow cytometry.

Results—In both PEC groups treated with FFP and stimulated with LPS, I-CAM, V-CAM, P-selectin and E-selectin were significantly up regulated compared to LR when stimulated by LPS.

Conclusion—FFP at both ages significantly increased expression of four different adhesion molecules compared to LR in PECs. This may represent a possible mechanism for increased leukocyte binding on the endothelium as a result of FFP transfusion.

Background

The advent of damage control resuscitation (DCR) has dramatically changed the way trauma patients are treated.^{1,2,3} One of the central tenets of DCR is early use of fresh frozen plasma (FFP) in high ratios to packed red blood cells (PRBCs).^{4,5} While increased survival is associated with implementation of DCR, studies have also demonstrated some potential risks, including multiple organ failure and acute respiratory distress syndrome (ARDS).^{6,7} However, these risks are not demonstrated in some of the larger studies.^{3,8} Inflammatory complications may be related to the amount of time blood products remain in storage prior to transfusion. Other investigators have demonstrated increased inflammatory gene expression and death associated with stored blood products, especially PRBCs.^{9, 10} Current blood banking practice allows for thawed plasma to be stored between 1-6°C for up to five days, facilitating its early

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use. This is based on storage guidelines for PRBCs and preservation of coagulation factors in plasma.^{11, 12}

Acute lung injury is mediated by leukocyte infiltration into alveoli and lung parenchyma. This infiltration is initiated at the endothelial level by interactions between endothelial cells and leukocytes. The first step in leukocyte migration is “rolling”, which occurs between endothelial adhesion molecules P-selectin and E-selectin and their ligands on leukocytes. Next is firm adherence to the endothelial cells, mediated by endothelial I-CAM-1 and V-CAM-1 and their respective ligands on leukocytes (LFA-1, and VLA-4). These adhesion molecules are increasingly expressed in response to various inflammatory stimuli, including tumor necrosis factor- α (TNF- α), interleukin-1 (IL-1), and lipopolysaccharide (LPS). Considering the association of FFP with transfusion related lung injury (TRALI) and the current practices ongoing in blood banks, we hypothesized that thawed plasma stored for 5 days at 4°C would demonstrate increased pro-inflammatory properties on primary endothelial cells compared to Day 0 plasma or lactated ringers (LR).

Materials and Methods

This *in vitro* experiment utilized human pulmonary endothelial cells (PECs, PromoCell, Stuttgart, Germany). Prior to the experiment the PECs were incubated in fully supplemented endothelial growth media-2 (Lonza, Basel, Switzerland). We defined plasma that is thawed the same day as transfusion as Day 0 FFP and plasma thawed 5 days before transfusion and stored at 4°C (as practiced by blood banks) as Day 5 FFP. FFP was purchased from Gulf Coast Regional Blood Bank Center in Houston, Texas, and the same donor was used for both Day 0 and Day 5 FFP. LR was obtained from Baxter (Deerfield, IL) and contained only the L-isomer. The groups were 1) unsupplemented media, 2) 30% LR, 3) 30% Day 0 FFP, and 4) 30% Day 5 FFP. LR and FFP were added to the same unsupplemented media as the media group. Ten units of Heparin per 1mL of media were also added to each group. These groups were further divided into subgroups that were either to be treated with LPS (Sigma-Aldrich, St. Louis) or not.

200,000 to 400,000 cells (between passages 4-7) were seeded with their respective media and incubated for 2 hours in 6 well plates. At that point the LPS groups were treated with 1 μ g/mL of LPS. After a 24 hour incubation the PECs were collected and stained with fluorescent antibodies (BD Biosciences, San Jose, CA) for flow cytometry. Pulmonary cells were analyzed for I-CAM-1, V-CAM-1, p-selectin, and e-selectin. Flow cytometry was run on a BD LSR II (BD Biosciences, San Jose, CA). Data analysis was performed using one-way ANOVA with post hoc Bonferroni contrast on Analyse-it software (Leeds, UK). All cell culture experiments were performed in replicates of five and duplicated twice. P-values of ≤ 0.05 were considered significant.

Results

PECs that were incubated with FFP and stimulated with LPS demonstrated increased expression of all adhesion molecules examined when compared to LR and media. Numerical data is presented in Table 1. Expression of I-CAM-1 was increased 10.5 fold ($p < 0.0001$) in the Day 0 FFP + LPS group compared to the LR + LPS group. There was no statistical difference between Day 0 FFP and Day 5 FFP when examining I-CAM-1 (Figure 1). V-CAM-1 was also found to be increased in PECs treated with plasma and LPS. There was a 4.3 fold increase in V-CAM-1 expression when comparing Day 0 FFP to media, and a 4 fold increase compared to LR ($p < 0.0001$) (Figure 2). Again, no differences between the plasma groups were noted.

When examining P-selectin, the observed increase in expression of the LPS group was 3.4 fold greater in the Day 0 FFP group compared to media, and 4.5 times when compared to LR ($p < 0.0001$). P-selectin was also increased by plasma (both Day 0 and Day 5) without LPS ($p < 0.0001$). The fold increase in P-selectin expression after treatment with Day 0 FFP to LR and media was 2.5. No differences were observed between Day 0 and Day 5 FFP (Figure 3). Similarly, E-selectin was increased in PECs treated with FFP and stimulated with LPS. Expression of E-selectin was increased by 7.4 times when Day 0 FFP is compared to media, and by 3.15 times when compared to LR ($p < 0.0001$). No differences between Day 0 and Day 5 FFP were observed when examining E-selectin (Figure 4). Though there was a trend of increased expression of E-selectin when contrasting plasma to LR and media without LPS, the differences did not reach statistical significance after ANOVA.

Discussion

The increased and earlier use of FFP in resuscitation of seriously injured and massively transfused trauma patients has been associated with improved survival when used aggressively with PRBCs. However, some studies have demonstrated an association between multiple organ failure and ARDS and the use of FFP. While these observations have been made, the cellular mechanisms are not well understood.

Previous studies have demonstrated increased inflammation and infectious processes associated with blood product use.^{13, 14} In both human data and cell culture experiments, PRBCs have been implicated in inflammatory gene expression and increased infection rates.^{9,13,21} It has been shown that administration of only one unit of PRBCs can have a detrimental effect on surgical patients.¹⁵ On the other hand, there are no randomized control trials in trauma patients. While investigations in our laboratory demonstrate endothelial repair activity with Day 0 FFP, these effects diminish over time.¹⁶

This study looks to identify one possible mechanism for the observed increased incidence of inflammation amongst patients who receive high ratios of FFP. Our results show that treatment with plasma at both Day 0 and Day 5 time points increases adhesion molecule expression on PECs when compared to treatment with LR or media. Nevertheless, there is no significant difference between the two plasma groups. This suggests that endothelial cells are primed for adhesion of leukocytes and migration with subsequent inflammation irrespective of the age of the plasma transfused.

Conversely, the effects of FFP are pleiotropic. The observed endothelial permeability caused by hypoxia and thrombin after HS may be initially beneficial, leading to the movement of interstitial fluid into the vascular space. However, the ongoing inducement of permeability with iatrogenic resuscitation fluids may contribute to vascular instability, edema, and other detrimental effects of injury and resuscitation. Since we have demonstrated in previous experiments that fresh (Day 0) plasma stabilizes the endothelium, the overall effect may be decreased permeability and an overall benefit, despite increased leukocyte-endothelial binding. The mechanisms and true incidence of potential complications remain obscured.

These observations are also supported by clinical data. Multiple retrospective studies have demonstrated increased survival associated with increased plasma to PRBC ratios. Holcomb et al demonstrated improved survival in 466 massively transfused patients (59.6% vs. 40.1%) for those patients that received high ratios of FFP to PRBCs, and no increased incidence of multiple organ failure or ARDS.³ In another large retrospective study Teixeira and colleagues demonstrated increased mortality (49%) amongst patients who received lower ratios of FFP to PRBCs compared to high ratios (25%).¹⁷ High ratios of FFP to PRBCs also were found to

benefit patients in New Orleans, with the high ratio group at 87.5% survival and the low ratio group survival at 26%.¹⁸

Fewer articles demonstrate no benefit or risk. Scalea et al, demonstrate no survival benefit in their high FFP to PRBC ratio group, although their massive transfusion patients numbered only 81.¹⁹ Another paper, from Denver, demonstrated increased risk of multiple organ failure. Using Cox proportional hazard regression, the researchers found a 2.1% increased risk of MOF with every unit of FFP transfused. The risk of ARDS increased 2.5% with each unit of FFP. However, the group also reported that each unit of FFP decreased the risk of mortality by 2.9%.²⁰

This study is limited by its dependence on *in vitro* experiments, without an *in vivo* model. Still, the use of LPS (a bacterial product) to induce stress is better suited to a septic model, rather than a HS or injury model. In spite of this, previous studies have used LPS in a two hit model of transfusion related lung injury.²¹ These *in vitro* studies are not representative of the complex milieu that exists after injury and hemorrhagic shock. Further cell culture, such as using PRBCs, and animal studies examining the balance between permeability and adhesion will help define this relationship. Other methods of examining variable expression would also increase the validity of these results. In addition, we recognize the rapidly changing activity involved in trauma response. Additional time point collections could elucidate appropriate pathways and associated genes.

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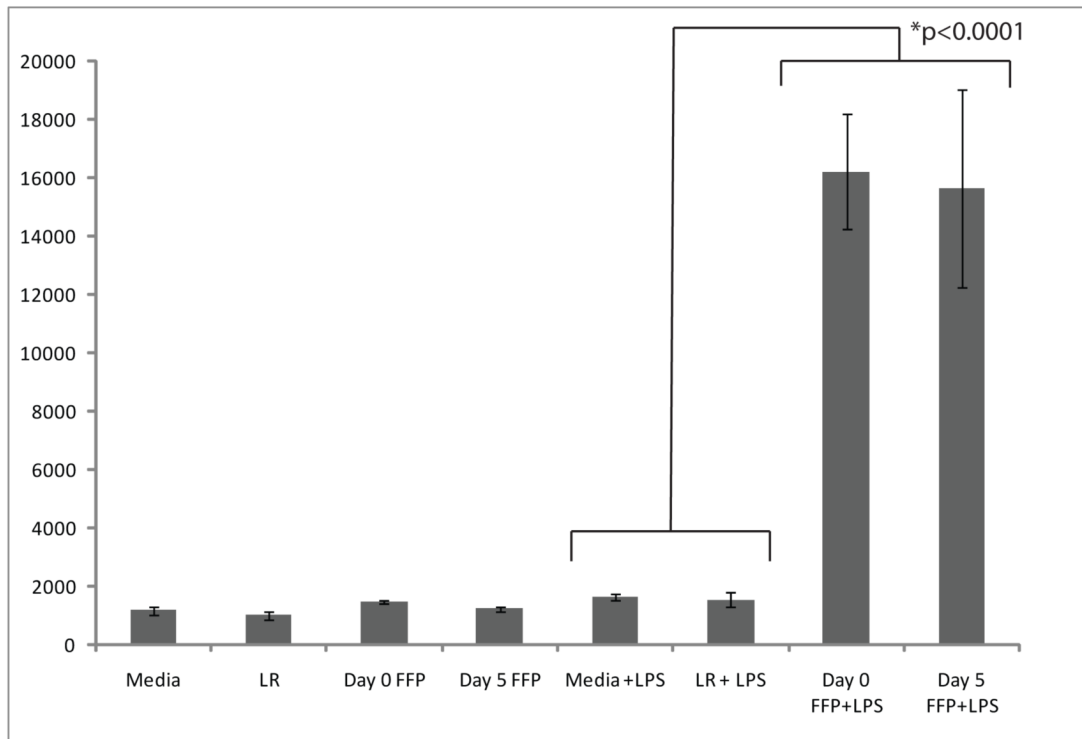


Figure 1. Median I-CAM-1 expression on PECs as quantified by mean fluorescence intensity

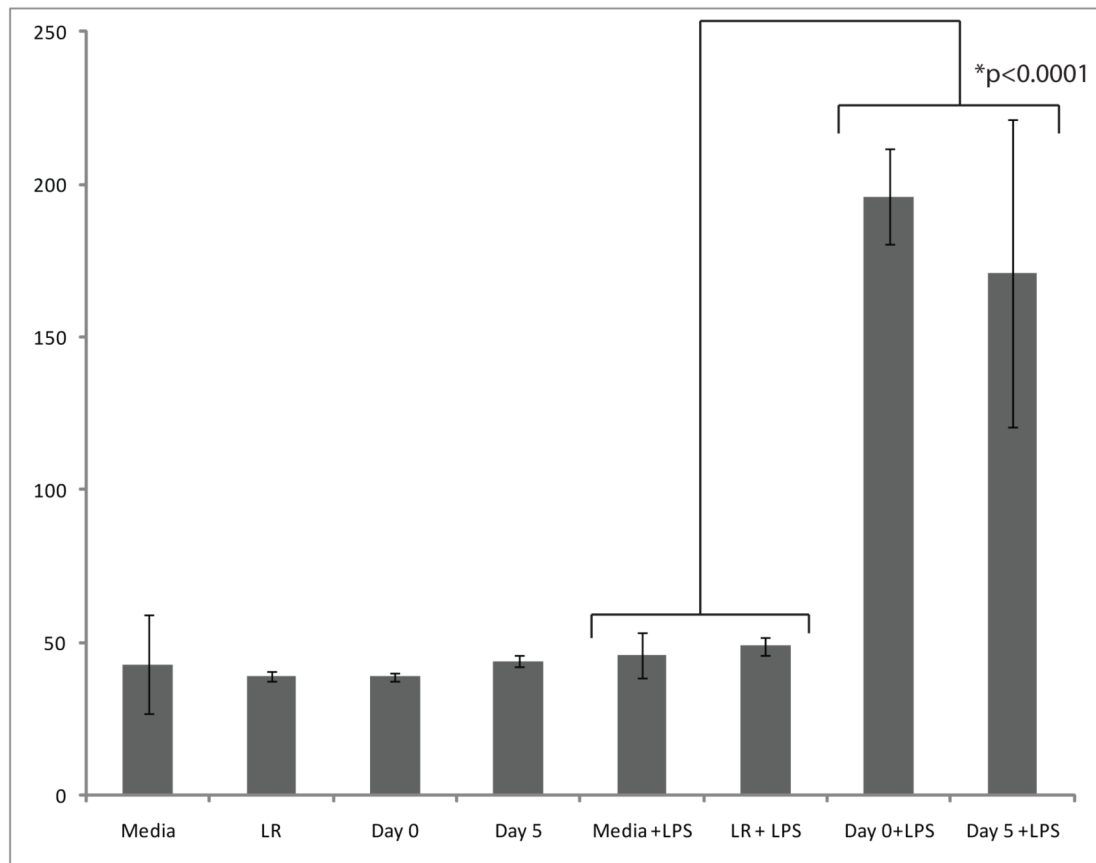


Figure 2.
Median expression of V-CAM-1 on PECs as quantified by mean fluorescence intensity

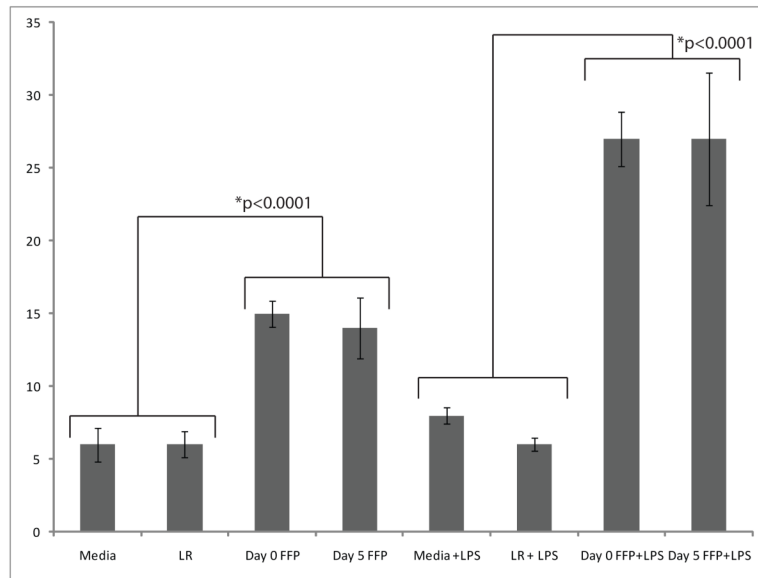


Figure 3.
Median expression of P-selectin on PECs as quantified by mean fluorescence intensity

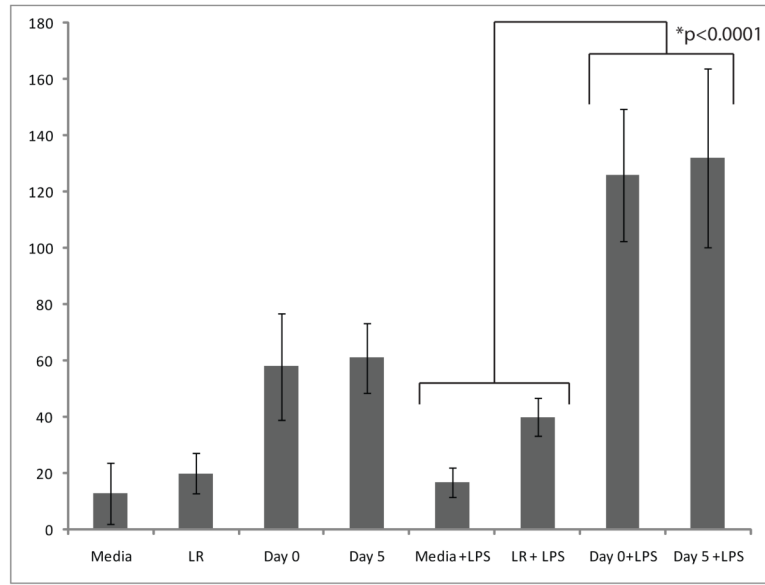


Figure 4. Median E-selectin expression on PECs as quantified by mean fluorescence intensity.

Table 1

Median expression of adhesion molecules on PECs

	Media Median (range)	LR Median (range)	Day 0 FFP Median (range)	Day 5 FFP Median (range)	Media + LPS Median (range)	LR + LPS Median (range)	Day 0 FFP +LPS Median (range)	Day 5 FFP + LPS Median (range)
I-CAM (n=5)	1163 (946-1315)	998 (874-1245)	1466 (1374-1524)	1222 (1036-1265)	1613 (1570-1825)	1545 (1148-1806)	1624 (13488-18641)	15619 (12711-20020)
V-CAM (n=5)	43 (41-79)	39 (38-42)	39 (38-41)	44 (41-46)	46 (43-59)	49 (44-52)	196 (177-219)	171 (83-218)
P-selectin (n=5)	6 (4-7)	6 (4-6)	15 (15-17)	14 (12-17)	8 (7-8)	6 (5-6)	27 (26-30)	27 (26-32)
E-selectin (n=5)	13 (12-68)	20 (14-50)	58 (34-142)	61 (29-89)	17 (14-43)	40 (13-41)	126 (106-236)	132 (91-273)