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Leukoreduction and UV treatment reduce both the magnitude and duration of the anti-HLA antibody response

Rachael P. Jackman¹, Xutao Deng¹, Douglas Bolgiano², Garth H. Utter³, Cathy Schechterly⁴, Mila Lebedeva¹, Eva Operskalski⁵, Naomi L. Luban⁶, Harvey Alter⁴, Michael P. Busch^{1,7}, Sherrill J. Slichter^{2,8}, and Philip J. Norris^{1,7,9}

¹Blood Systems Research Institute, San Francisco, CA

²Puget Sound Blood Center, Seattle, WA

³University of California, Davis, CA

⁴National Institutes of Health, Bethesda, MD

⁵University of Southern California, Los Angeles, CA

⁶Children's National Medical Center, Washington, DC

⁷Departments of Laboratory Medicine University of California, San Francisco, CA

⁸University of Washington School of Medicine, Seattle, WA

⁹Departments of Medicine, University of California, San Francisco, CA

Abstract

BACKGROUND—Both leukoreduction and UV light treatment of blood products have been shown to reduce the incidence of HLA antibody development in recipients, but the impact of these treatments on the magnitude and persistence of the antibody response is less clear.

STUDY DESIGN AND METHODS—Longitudinal samples from 319 subjects taken from 4 different study cohorts were evaluated for HLA antibodies to determine the effects of leukoreduction and UV treatment on HLA antibody generation and persistence.

RESULTS—Subjects receiving leukoreduced or UV treated blood products were less likely to generate class I HLA antibodies, and those receiving leukoreduced blood were also less likely to generate class II HLA antibodies. Among those receiving non-leukoreduced blood, 55% developed class I HLA antibodies and 51% developed class II HLA antibodies compared with 28% (class I) and 15% (class II) for those receiving leukoreduced blood and 36% (class I) and 54% (class II) for those receiving UV treated blood. Among alloimmunized subjects, leukoreduction resulted in a significant 2-fold reduction in the magnitude of class I HLA antibodies, and UV treatment resulted in a significant 3-fold reduction in the magnitude of class II HLA antibodies. Both treatments resulted in shorter persistence of class I HLA antibodies.

CONCLUSIONS—These data demonstrate that leukoreduction and UV treatment of blood products results not only in a reduction in the incidence of HLA antibody production, but also in lower and more transient HLA antibody levels among sensitized transfusion recipients.

Corresponding Author (also contact author for reprint requests): Rachael P. Jackman 270 Masonic Ave., San Francisco, CA, 94118, USA Phone: (415) 749-6606 ext. 714 Fax: (415) 567-5899 rjackman@bloodsystems.org.

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Keywords

Alloimmunization; HLA Antibodies; Leukoreduction; UV treatment

INTRODUCTION

Blood transfusion is one of the most common sources of allogeneic exposure, and recipients of blood products are exposed to numerous alloantigens expressed on the surface of donor WBCs, RBCs, and platelets. Many blood recipients become alloimmunized to human leukocyte antigens (HLA) on WBC and platelets, which can limit subsequent transfusion of these cellular products.

Following transfusion of red cell products, patients can generate an alloresponse against RBC and/or WBC antigens. Anti-RBC responses can cause dangerous hemolytic transfusion reactions, so efforts are made to match major red cell antigens between donor and recipient to prevent their development. These efforts are effective and rates of RBC alloimmunization are fairly low, although some highly transfused patient populations (such as sickle cell and thalassemia patients) are at increased risk of developing RBC antibodies (Abs).¹⁻⁷ In contrast, alloimmunization against WBC antigens (predominantly HLA) is common following transfusion of red cells due to contaminating WBCs and because pre-transfusion HLA matching is not only impractical, but has been associated with graft versus host disease.⁸⁻¹¹

Following receipt of platelet transfusions, the alloimmune response is focused on antigens expressed on WBCs and platelets, including class I HLA and other platelet antigens. Antibody responses against these antigens can be highly problematic, resulting in refractoriness to subsequent platelet transfusions.^{12,13} Interestingly, the magnitude of the HLA Ab response appears to be important in determining if immune mediated refractoriness will occur, as low-to-moderate level Abs do not predict platelet transfusion failure.¹⁴

Rates of antibody generation against HLA antigens following transfusion are high, ranging from 7-64%, depending on study, patient populations, and number and type of transfusions.^{12,15-23} Previously pregnant women and previously transfused patients have been shown to have a higher frequency of HLA antibody production when subsequently transfused.^{19,21,22,24} HLA Abs can usually be detected within two weeks after exposure and while sometimes only short-lived, can persist for long periods in some recipients.^{15,16,22,25-27} Historically, HLA Abs have been detected by lymphocytotoxicity assays (LCA), which detect high level, complement fixing antibodies.^{23,28,29} In comparison, newer and more sensitive multianalyte bead-based assays additionally detect low to moderate level antibodies, as well as non-complement fixing Abs and those directed against a potentially broader range of HLA antigens.³⁰⁻³³ The introduction of leukoreduction has been shown in most studies to reduce but not eliminate the frequency of alloimmunization,^{15,17-20,23,34} with a less clear impact on previously pregnant recipients.³⁵ Leukoreduction reduces the number of WBCs from approximately 5×10^8 cells/unit to less than 5×10^6 cells/unit, and generally less than 1×10^6 cells/unit with current methods. The observed reductions in rates of alloimmunization most likely reflect this decreased dose of antigen.

The use of UV treated products, while less extensively studied, has similarly been shown to reduce the rates of alloimmunization.²³ UV treatment is a component of some commercially available pathogen reduction methods used clinically in Europe in the treatment of platelets and plasma components. While alloimmunization appears to be less frequent when products

are exposed to UV light, interpretation of published studies has been complicated, as patients receiving UV treated platelets also often receive untreated red cells since there is no currently available UV treatment for red cell components. Studies in animal models have shown that in the absence of these additional untreated alloexposures, UV treatment of platelet rich plasma may be able to block the alloresponse.³⁶⁻³⁸ As this blockade seems to hold even when WBCs are enriched within the transfused products, the mechanisms most likely have little to do with the dose of antigen,³⁸ and more to do with functional changes in the treated WBCs.³⁹

In this study we used a sensitive, multi-analyte bead-based assay to examine the effects of leukoreduction and UV treatment of transfused blood products on the frequency, duration, and magnitude of HLA Ab responses in transfusion recipients. Samples from transfused patients enrolled in four different study cohorts employing different methods of product treatment were analyzed, including patients in the TRAP Study (Trial to Reduce Alloimmunization to Platelets),²³ Microchimerism Study (trauma patients),⁴⁰ Transfusion-Transmitted Viruses Study (TTVS),⁴¹⁻⁴⁴ and the Transfusion-Related Infections Prospective Study (TRIPS).⁴⁵

MATERIALS AND METHODS

Subjects and samples

One hundred and ninety subjects were selected from the TRAP study (out of 530), including 170 LCA⁻ patients, of whom 70 were clinically refractory (CR⁺) and 100 were clinically non-refractory (CR⁻), along with 20 LCA⁺ patients, of whom 10 were CR⁺ and 10 were CR⁻.²³ CR⁺ patients were those with a one-hour post-transfusion corrected count increment of less than 5000 after 2 sequential transfusions of ABO-compatible platelets as described in the original TRAP analysis.²³ LCA⁺ samples were included as positive controls for detection of HLA Abs, and as many LCA⁻ samples were included as were available. Subjects with missing data were excluded, including 27 subjects missing 1 corrected count increment data point, 3 subjects who were refractory to only their last transfusion, and 7 who received at least one non-ABO compatible transfusion to which they were refractory. An additional 129 subjects were selected from 3 other studies (Table 1). Seventy-two trauma patients were selected from a larger study of microchimerism (out of 451 total subjects).⁴⁰ Thirty-seven subjects were included from the Transfusion-Transmitted Viruses Study (TTVS) (out of 1513 subjects).⁴¹⁻⁴⁴ Twenty subjects were included from the Transfusion-Related Infections Prospective Study (TRIPS) (out of 607 adult subjects).⁴⁵ These samples were selected from the larger cohorts based on the availability of serial samples, and for the TRIPS study were also excluded if they had been previously transplanted or had been put on high levels of immunosuppressive medications. Together, these three studies provided 20 non-transfused controls and an additional 75 leukoreduced and 34 non-leukoreduced transfusion recipients. Serum samples were collected for the TRAP and TTV studies while plasma samples were collected for the microchimerism and TRIP studies. Longitudinal panels including pre- and post-transfusion time points were tested in a blinded fashion. Samples were collected under IRB approved protocols that included written informed consent.

Anti-HLA antibody detection

Antibodies against class I and class II HLA antigens were measured in serum or plasma samples using the One Lambda (Canoga Park, CA) LabScreen mixed Luminex assay, run according to manufacturer's instructions. The results are reported as normalized background (NBG) ratios for each of eight multi-antigen beads, and the highest value for each sample was used. A NBG ratio greater than 10.8 for class I HLA antibodies and 6.9 for class II HLA

antibodies was used as the cutoff for a positive result based on previous work in non-alloexposed populations.⁴⁶

Leukoreduction

For the TRAP study platelets were leukoreduced with PL-100 filters and the red cells were leukoreduced using either RC-100 or BPF-4 filters (Pall, East Hills, NY). Of the 100 TRAP subjects that received leukoreduced platelets, 56 received pooled platelet concentrates, and 44 received single donor apheresis platelets. For the microchimerism study red cells were leukoreduced using either the Fenwal whole blood in-line filter system or the Fenwal Flex-Excel spin first system (Fenwal, Lake Zurich, IL). For the TRIPS study, red cells were leukoreduced using the Pall Leukotrap RC collection system (Pall, East Hills, NY) within 8 hours of collection. In addition, all blood products used for the TRIPS study were gamma irradiated at a minimum dose of 25 Gy.

UV treatment

UV treated platelets were irradiated in a Stericell bag (Dupont, Wilmington, DE) with a maximum volume of 420 ml using an ultraviolet B irradiator (Haemonetics, Braintree, MA) delivering a total dose of 1480 mJ per square centimeter.²³

Statistical analysis

NBG ratios <1 were assigned a value of 1. For comparisons of NBG ratios, one-way ANOVA was used with Tukey's multiple comparisons post-test using GraphPad Prism (GraphPad Software, Inc, La Jolla, CA). Differences in the frequency of new antibody development between two groups were evaluated using the χ^2 test in GraphPad Prism. To evaluate persistence of Ab responses, start times were normalized to the first time-point with an NBG ratio above the threshold of 10.8 for class I and 6.9 for class II, and time to loss below these thresholds was evaluated by Kaplan-Meier survival analysis using R. Differences in persistence of Abs between groups was examined by the log-rank test using R.

RESULTS

Frequency of HLA alloimmunization

HLA Abs were measured in longitudinal samples from 4 studies of transfusion recipients that included recipients of leukoreduced and non-leukoreduced RBCs and platelets, UV treated platelets, and non-transfused patient controls. The frequency of new class I and class II HLA Abs detected was significantly reduced in the leukoreduced group compared to the non-leukoreduced group, with 55% of the non-leukoreduced group and 28% of the leukoreduced group developing new class I HLA Abs ($p<0.001$), and 51% and 15% developing new class II HLA Abs ($p<0.001$), respectively (Table 2). The frequency of new class I, but not class II, HLA Abs was significantly reduced with UV treatment compared to non-leukoreduced ($p<0.05$), 36% and 55% of recipients of UV-treated products developed new class I and class II HLA Abs, respectively (Table 2). No new HLA Abs were seen in the non-transfused group.

Magnitude of the HLA Ab response

Peak NBG ratios for class I and class II HLA Abs (Figure 1A-B) were compared between recipients of non-leukoreduced, leukoreduced, and UV-treated transfusions, as well as non-transfused controls, excluding those positive for HLA Abs pre-transfusion (or at study onset for non-transfused controls). Significantly higher mean NBG ratios were found in the non-leukoreduced group compared to all the other groups for both class I and class II HLA Abs.

The higher NBG ratios seen in the non-leukoreduced group could be due to the increased frequency of new antibodies within this group alone or could be further enhanced by larger responses within this group. To determine if the magnitude of the HLA Ab responses were greater in the recipients of non-leukoreduced blood, peak NBG ratios were compared between the recipients of non-leukoreduced, leukoreduced, and UV-treated blood among those positive for new antibody responses. Leukoreduction and UV treatment led to a 2-fold reduction in the magnitude of the class I HLA Ab response compared with the non-leukoreduced group, though the shift was only significant with leukoreduction ($p < 0.05$), (Figure 2A). In contrast, leukoreduction had no significant effect on the magnitude of the class II HLA Ab response, though UV treatment led to a significant 3-fold reduction compared with the non-leukoreduced group ($p < 0.05$), (Figure 2B).

Persistence of the HLA Ab response

To assess the impact of leukoreduction and UV treatment on the persistence of the HLA Ab response, class I and class II HLA Ab NBG ratios were evaluated over time for recipients of non-leukoreduced blood, leukoreduced blood, or UV-treated blood, excluding those positive for HLA Abs pre-transfusion. Kaplan-Meier survival analysis was performed to assess the persistence of HLA Abs above the NBG threshold of 10.8 for class I and 6.9 for class II. Significant differences were seen in the persistence of class I Abs, with a less prolonged Ab response in recipients of leukoreduced blood ($p = 0.0411$) or UV-treated blood ($p = 0.0175$) compared with non-leukoreduced blood (Figure 3A). No significant differences were observed in the persistence of class II Abs (Figure 3B).

DISCUSSION

Transfusion of allogeneic blood products can result in the generation of antibodies against HLA, leaving recipients vulnerable to platelet transfusion failure as well as problems related to eligibility and matching for hematopoietic stem cell or solid organ transplant. Here we have shown that leukoreduction of blood products, or UV-treatment of platelet products can reduce not only the frequency of this HLA Ab response, but also the magnitude and duration of the response among those who do generate HLA Abs.

The frequency of new class I and class II HLA Abs was significantly reduced among recipients of leukoreduced as compared with non-leukoreduced blood. This is consistent with previous studies including the original analysis of the TRAP subjects using LCA to screen for HLA Abs.^{15,17-20,23,34} The reduction in frequency observed with leukoreduction was accompanied by a significant decrease in both the magnitude and persistence of class I HLA Abs, although no significant decreases were seen in the magnitude or persistence of class II Abs. One potential bias that could lead to the observed differential effect on class I and class II Abs was the inclusion of samples selected for refractoriness, where class I antibodies are more likely to play a role. To assess this possibility, we compared the levels of antibodies between recipients of leukoreduced and non-leukoreduced blood, excluding the TRAP samples from subjects who were clinically refractory to platelet transfusions. The same 2-fold difference in class I HLA NBG ratios was seen between recipients of leukoreduced and non-leukoreduced products ($p = 0.0109$), and no significant difference was seen in class II HLA NBG ratios amongst those with new HLA Abs (data not shown). Another explanation for the different observed effects of leukoreduction on new class I vs. class II HLA Abs is that there is a differential effect of leukoreduction on cells expressing class I versus class II HLA. While all nucleated cells express class I HLA, only a subset of these cells express class II. It is possible that leukoreduction is less successful in removing these antigen-presenting cells from the blood than other white blood cells or that the leukoreduction process activates professional antigen presenting cells and up-regulates HLA-DR expression.⁴⁷ It is also possible that because of this more limited expression

profile, the dose of class II HLA antigen is already below a threshold needed for stronger antibody production. The TRAP subjects receiving leukoreduced platelets received either leukoreduced pooled random donor or leukoreduced apheresis single donor platelets. We saw no significant difference in the HLA Ab responses between these two groups (data not shown), consistent with the original TRAP analysis.²³

UV treatment had similar, though not identical, effects to leukoreduction. The decreased frequency of new class I HLA Abs was accompanied by a reduction in persistence, but not a significant reduction in magnitude. The trend in magnitude appeared to be the same as that seen with leukoreduction, and the failure to show statistical significance is most likely the result of smaller sample size. Despite failure to reduce the frequency of new class II HLA Ab with UV-treated blood in this study, there was a significant reduction in the magnitude of the class II HLA Ab response, suggesting that even though these recipients were generating Abs against class II HLA antigens, the responses were weakened. The significant decrease in the persistence of class I HLA Abs we observed in this study with either leukoreduction or UV treatment was not observed in the original analysis of the TRAP study which employed the LCA to measure antibodies, although they did see the same trend.²⁷ In addition, our analysis used only a subset of the original TRAP subjects and added additional subjects for the non-leukoreduced and leukoreduced groups, and this may account for the differences observed. Further, the isolation of class I results from class II results may have unmasked differences that were obscured in the original analysis using the LCA, which does not distinguish between class I and II Abs.

The use of four different study cohorts had several advantages. The inclusion of these different study subjects allowed for greater numbers, and some dilution of selection biases of individual studies. In addition, our mixed cohort had greater diversity in terms of underlying health, which may further influence alloimmunization. Pooling of cohorts also allowed for the inclusion of patients receiving blood products that were collected and processed in different places and time periods, allowing, at least in the case of the leukoreduction data, some variation in the number and quality of residual leukocytes. However, there were also some disadvantages to the use of a mixed cohort. Sample processing was not the same for all 4 studies, with serum used for screening the TRAP and TTVS subjects, and plasma used for the microchimerism and TRIPS subjects. This may have introduced some bias in the different groups, as a previous study has shown that serum gives a NBG ratio about 1.3 times higher than paired plasma samples,⁴⁸ and the cohorts screened using serum samples were the sole source of recipients of non-leukoreduced blood. However, the TRAP study was also a major source of recipients of leukoreduced blood and the sole source of recipients of UV treated blood, which may have partially countered this bias. Furthermore, the mean NBG ratio for recipients of non-leukoreduced blood was 3.5 times higher than seen in recipients of leukoreduced blood, so sample type is unlikely to account for more than a small portion of that difference. Different patient populations might also differ in the number of transfusions received, resulting in different degrees of alloexposure. The bulk of the patients were from the TRAP and MC studies, both of which are made up of subjects typically given multiple blood units, though this exposure would be in a shorter time-frame among trauma or surgical patients than in cancer patients. The timing of sample collections differed somewhat between individuals and between study cohorts used. Any biases in timing may have limited our analysis of the kinetics of the response. For example, our Kaplan-Meier analysis was done using the number of days from appearance of antibodies above threshold to loss below threshold, but these start and stop times are approximate, as we do not have daily readings, or equal frequency of readings between all patients. It is also possible that individual peak responses were missed or underrepresented due to the timing of sample collection, though we would not expect this to differ between groups.

In summary, while it has been shown previously that leukoreduction results in a decrease in the rates of alloimmunization, we have extended this finding to show that there are additional benefits of leukoreduction. Our analysis, using different patient populations, has found not only a decrease in the frequency of HLA Abs, but also in the magnitude and persistence of this antibody response. Given the association of higher levels of HLA Abs with refractoriness to platelet therapy, the reduction in both the magnitude and persistence of the Ab response in those receiving leukoreduced blood may lower the clinical impact of alloimmunization, even if not completely abrogating the HLA-directed immune response.

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Figure 1. Mean anti-HLA antibody NBG ratios are higher in recipients of non-leukoreduced blood

Peak class I (A) and class II (B) HLA antibody NBG ratios were compared among recipients of non-leukoreduced (Non-LR), leukoreduced (LR), and UV-treated (UV) blood products, as well as with non-transfused surgical and trauma patients (No Tx). Mean values were compared using a one-way ANOVA with Tukey's multiple comparison post-test, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Dashed line used to mark the NBG cutoff used to distinguish positive and negative results. Error bars display mean and standard error.

Figure 2. Mean HLA antibody NBG ratios are higher among responders in recipients of non-leukoreduced blood

Peak class I (A) and class II (B) HLA antibody NBG ratios were compared among recipients who developed new antibodies following transfusions with non-leukoreduced (Non-LR), leukoreduced (LR), or UV-treated (UV) blood. Mean values were compared using a one-way ANOVA with Tukey's multiple comparison post-test, * $p < 0.05$. Dashed line used to mark the cutoff used to distinguish positive and negative results. Error bars display mean and standard error.

Figure 3. Class I HLA antibodies persist slightly longer in recipients of non-leukoreduced blood
 Longitudinal class I and class II HLA antibody NBG ratios were evaluated over time for recipients of non-leukoreduced (Non-LR), leukoreduced (LR), and UV-treated blood products. Kaplan-Meier survival analysis was used to evaluate the persistence of new class I (A) and class II (B) antibodies, with recipients of leukoreduced and UV-treated blood products compared with recipients of non-leukoreduced blood products using the log-rank test with associated p-values reported for each group compared with recipients of non-leukoreduced blood.

Table 1

Subject characteristics

Study	TRAP * (n=190)	MC * (n=72)	TTVS * (n=37)	TRIPS * (n=20)	Combined (n=319)
Focus of study	Platelet Alloimmunization	Microchimerism	Transfusion-transmission of HCV	Transfusion-transmitted infectious disease	
Subject population	Acute myeloid leukemia patients	Trauma patients	Routine surgery patients [†]	Mixed [‡]	
Subject enrolment	1991-1995	2006-2010	1974-1980	2002-2003	
Transfused [§] , n (%)	190 (100)	55 (76)	34 (92)	20 (100)	299 (94)
LR, n (%) ^{//}	100 (53)	55 (100)	0 (0)	20 (100)	175 (59)
UV, n (%) [¶]	47 (25)	0 (0)	0 (0)	0 (0)	47 (16)
Untreated, n (%) ^{**}	43 (23)	0 (0)	34 (100)	0 (0)	77 (26)
# of samples/subject ^{††}	6 (6,7)	3.5 (3,4)	9 (6,18)	5 (5,5)	6 (4,7)
Length of follow-up Days ^{††}	301 (161,364)	138 (26,237)	233 (157,301)	178 (175,189)	203 (154, 364)

* TRAP (Trial to Reduce Alloimmunization to Platelets), MC (Microchimerism), TTVS (Transfusion-Transmitted Viruses Study), TRIPS (Transfusion-Related Infections Prospective Study).

[†] Includes (n): Acute HCV infection from HCV⁺ transfusion (11), Chronic HCV infection from HCV⁺ transfusion (11), HCV- transfusion (12), non-transfused (3)

[‡] Includes (n): Von-Hippel Lindau (5), Melanoma (4), Renal Cell Carcinoma (2), Mucinous Cyst (1), Renal Cancer (1), Thalassemia (1), Mesothelioma (1), Adenocarcinoma (1), Systemic Lupus Erythematosus (1), Pheochromocytoma (1), Diabetes (1), Human Immunodeficiency Virus (1).

[§] For TRAP study, treatment refers to platelet products, with all patients receiving leukoreduced red cells. For remaining studies, treatment refers to red cell products.

^{//} Given leukoreduced blood products, % indicates the percentage of transfused patients.

[¶] Given UVB treated platelets, % indicates the percentage of transfused patients.

^{**} Given untreated blood products, % indicates the percentage of transfused patients.

^{††} Median (interquartile range).

Table 2

Frequency of new antibody production

	No Tx [*] (n=20)	Non-LR [*] (n=77)	LR [*] (n=175)	UV [*] (n=47)
Class I Abs				
Undetermined [†] , n	0	4	4	0
No pre-existing [‡] , n	18	58	152	44
Pre-existing [§] , n (%)	2 (10)	15 (21)	19 (11)	3 (6)
New, n (%)	0 (0)	32 (55)	43 (28) //	16 (36) ¶
Class II Abs				
Undetermined [†] , n	0	3	6	0
No pre-existing [‡] , n	18	63	152	38
Pre-existing [§] , n (%)	2 (10)	11 (15)	17 (10)	9 (19)
New, n (%)	0 (0)	32 (51)	23 (15) //	21 (55)

* No Tx (non-transfused controls), Non-LR (recipients of non-leukoreduced blood products), LR (recipients of leukoreduced blood products), UV (recipients of UV irradiated blood products)

[†] Positive for Abs at first time-point, but no available pre-tx sample, so could be either preexisting or the result of the tx. These were excluded from the analysis.

[‡] Used as denominator for new Ab frequency.

[§] % Patients positive for Abs prior to transfusion or at study onset for non-tx controls.

// p<0.001 for new Abs compared with Non-LR by χ^2 test.

¶ p<0.05 for new Abs compared with Non-LR by χ^2 test.