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## HLA ALLOIMMUNIZATION OF SURGICAL PATIENTS BY TRANSFUSION WITH BEDSIDE LEUKOREduced BLOOD COMPONENTS

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**Abstract:** High efficiency leukocyte reducing filters can remove more than 99.9–99.99% of white cells from cellular blood components and are considered to be effective in decreasing HLA alloimmunization of patients with haematological malignancies.

A multi-institution study was performed to determine whether white cell filtration would also be effective in preventing alloimmunization in surgical transfusion recipients. Patients who were to receive red cell blood transfusions during and/ or within 48 hours after surgery were randomly assigned to receive red cells/ fresh frozen plasma that had been leukoreduced using a high efficiency filter at the bedside or buffycoat-depleted red cells transfused through an aggregate filter.

Of 87 patients with no alloantibodies at entry, 17% (8/47) of those in the leukoreduction group, who received a mean of  $0.3 \times 10^6$  leukocytes as a result of their transfusions, produced lymphocytotoxic antibodies at day 14 after transfusion, compared to 5% (2/40) in the buffycoat-depleted group, who had received a mean of  $1,234.2 \times 10^6$  leukocytes. This difference in the alloimmunization rate between the two arms was not statistically significant.

Reduction of leukocytes by bedside filtration does not appear to be effective in preventing HLA alloimmunization in surgical transfusion recipients. The alloimmunized cases suggest that an indirect allorecognition pathway may be involved in the formation of anti-HLA. Further measures are needed to reduce alloimmunization of immunocompetent patients.

**Keywords:** alloimmunization, surgical patient, leukoreduction, filtration, transfusion

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## INTRODUCTION

Formation of alloantibodies against a donor's human leukocyte antigens (HLA) class I can be initiated through direct allorecognition where T cell receptors of the recipient's T helper cells directly recognize the donor's HLA class I peptides present in the groove of HLA class II on antigen-presenting cells (APC)<sup>1)</sup>. A number of clinical studies, including the large multicenter Trial to Reduce Alloimmunization against Platelets (TRAP)<sup>2)</sup>, have confirmed that leukocyte reduction of allogeneic blood can decrease the incidence of HLA alloimmunization in patients with hematologic malignancies. Some studies have suggested that patients who receive allogeneic transfusions have an increased risk of postoperative infection<sup>3)4)5)</sup> and tumor recurrence<sup>6)7)</sup> compared to patients who do not receive transfusion. We previously observed that buffycoat removal (i.e. a 60–70% reduction in leukocytes) is not sufficient to reduce the risk of alloimmunization<sup>8)</sup> in surgical recipients. There have not yet been clinical trials published the efficiency of leukocyte-reduced blood components in preventing HLA alloimmunization in a population of immunocompetent patients who receive transfusions. In addition to cytokines and bioactive substances in blood and blood components for transfusion, HLA alloantibody formation could be a surrogate marker for transfusion-associated immunomodulation, but clinical evidence for any direct association between alloimmunization and either tumor recurrence or postoperative bacterial infections in transfusion recipients is lacking. Thus, it cannot be determined at this time whether alloimmunization results in clinically relevant postsurgical complications and in a generally poor outcome.

High efficiency leukocyte-reducing blood filters can remove more than 99.9–99.99% of white blood cells from whole blood, red cells, and platelets. Buffycoat-depleted red cells containing approximately  $10^9$  leukocytes is the standard component for red cell transfusion in Japan. That quantity of residual leukocytes is 10,000 times greater than the number in leukocyte-reduced red cells produced by high efficiency filters, which contain around  $10^5$  white cells.

We report a randomized trial of bedside leukoreduction for surgical patients requiring blood transfusions. HLA alloimmunization was monitored in patients receiving red cells that had leukocyte levels that were reduced at the bedside or by standard buffycoat removal methods.

## MATERIALS AND METHODS

*Patients*

Adult patients admitted to six hospitals in the Fukushima area of Japan for elective surgery between October 1998 and January 2000 were included in the study. Patients were not admitted to the study if they were less than 18 years old, had

emergency surgery, liver cirrhosis, renal failure, disseminated intravascular coagulation (DIC), or sepsis, or were taking immunoglobulins or immunosuppressive drugs (including steroids). Patients who had received anticancer chemotherapy and/or radiation therapy either during or after surgery, were not excluded. The patients were transfused according to a randomized procedure whenever transfusion was indicated.

The study protocol was approved by the institutional grants review board and written informed consents including blood transfusion were obtained from all participants before surgery.

#### *Preparation of Blood Components for Transfusion*

When transfusions were indicated, patients were randomly allocated to receive either buffycoat-depleted blood prepared at the regional blood center from which aggregates were removed at the bedside, or leukocyte-reduced red cells. Buffycoat-depleted red cells were prepared according to a standard protocol that removes a constant amount of plasma and buffycoat from centrifuged packed red cells suspended in 46 ml (for a 200 ml donation) or 92 ml (for a 400 ml donation) additive solution (saline, glucose, citrate, mannitol, adenine, and phosphate; MAP). The final component contains a mean of  $1.3 \times 10^9$  leukocytes per 400 ml (40% of the original leukocyte number)<sup>9</sup>, or  $0.71 \times 10^9$  leukocytes per 200 ml (50% of the original). An aggregate-removing filter (SQ40, Pall, NY) was used at the bedside for every standard transfusion of buffycoat-depleted red cells. Such microaggregate filtration removes a mean of 57% of the leukocytes present in a unit<sup>10</sup>.

Leukocyte-reduced cells were prepared using a leukocyte filter (Sepacell RZ-200, Asahi Medical, Tokyo), which reduced the leukocyte content of the red cells to a mean of  $2.1 \times 10^5$  ( $n=6$ ; range  $0.7-3.4 \times 10^5$ ) per 400 ml (i.e., a 4-log reduction) in our pilot study. The participants in the study had been instructed in the appropriate use of the filter at the bedside.

Fresh frozen plasma (FFP), when needed, was transfused using a red cell leukocyte-reduction filter (RZ-200) for the patients in the leukoreduction group, whereas filters were not used for the patients in the buffycoat-depleted group. Filtered platelet concentrate obtained from a random donor by apheresis using a platelet leukoreduction filter (Sepacell PLX, Asahi Medical) was transfused to one patient in the leukocyte reduction group, but none of the patients in the buffycoat-depleted group received platelets.

The numbers of leukocytes in blood components were estimated using the data from the Japanese Red Cross Blood Centers<sup>9</sup>.

To prevent post-transfusion graft-versus-host disease<sup>11</sup>, all cellular components were gamma-irradiated at 15 Gy within 24 hours of donation.

#### *Antibody Testing*

Serum specimens were obtained by routine sampling before transfusion (day 0)

and on days 1, 7, and 14 after surgery. Alloantibodies were monitored using a standard NIH microlymphocytotoxicity test (LCT) against HLA class I antigens with a panel of 20 HLA-typed frozen cells, which covers >95% of the phenotypes of the Japanese population. Serum samples were considered antibody positive when they caused at least 60% cytotoxicity in one or more cells or at least 40% cytotoxicity in two or more cells in the panel<sup>2)</sup>.

### *Statistical Analysis*

Statistical analyses were done with chi-square (with or without Yate's correction) or Fisher's exact tests for frequency data, and with two-tailed Student's *t* test or Mann-Whitney test for quantitative data. A finding of  $p < 0.05$  was considered to be statistically significant.

## RESULTS

### *Patients*

One hundred patients from six hospitals were initially enrolled in the study, and were randomized to receive either standard buffycoat-depleted blood with SQ40 microaggregate-depleting filtration or leukocyte-reduced blood components. Of these, 8 patients were found to have HLA antibodies at entry, 2 patients erroneously received red cells that had not been categorized, post-transfusion samples from 2 patients were not obtained correctly and 1 patient had two surgical procedures within 14 days. These 13 patients were excluded from the study, leaving 87 patients (47 in the filtered group and 40 in standard group) eligible for analysis; their characteristics are shown in Table 1. The trial arms were evenly balanced with respect to age, sex, disease, previous alloantigen exposure, and anticancer chemotherapy. Although more female recipients were assigned to the leukocyte-reduced transfusion group than to the standard transfusion group and more patients with a previous transfusion were assigned to the standard group than to the leukocyte-reduced transfusion group, the overall history of alloantigen exposure was similar in the two arms. Two patients in the standard group had been treated with both chemotherapy and radiation, but no other patients had received radiation therapy.

### *Results of Transfusion*

The number of red cells and FFP transfusions did not represent a significant difference between the two groups (Table 2). Three of the red cell transfusions (two in the leukoreduced and one in the regular transfusion group) were associated with adverse reactions including allergic skin reaction and fever, but there were no differences in the frequencies of reactions between the two groups.

### *HLA alloimmunization*

During the 14 days of the study period after transfusion, lymphocytotoxic

Table 1. Patient Characteristics and Prior Exposure to Alloantigens

	Leukoreduced	Buffycoat-Depleted	<i>p</i>
Enrolled	55	45	
Excluded	8	5	
Alloimmunized at entry	5	3	
Miscellaneous	3	2	
Patients studied	47	40	
Age (years)			
Median	72	73	NS
Range	28-84	46-90	
Female	25 (53%)	16 (40%)	NS
Previous pregnancy	23	16	
Previous transfusion	3 (6%)	8 (20%)	NS
Previous pregnancy and/or transfusion			
	25 (53%)	21 (53%)	NS
Anti-cancer chemotherapy	21 (45%)	17 (43%)	NS
Surgery for			
Gastric cancer	16	17	
Rectal cancer	10	8	
Colon cancer	4	1	
Esophageal cancer	2	2	
Pancreatic cancer	2	2	
Bile duct/ gall bladder cancer	2	2	
Cancer metastasis in liver	2	1	
Other malignant disease	2	3	
Ileus	3	1	
Trauma/ bone fracture	3	1	
Other benign disease	1	2	

NS; not significant

antibodies developed in 17% (95% confidence interval [CI] ; 8-31%) of the patients in the leukocyte-reduced group, as compared with 5% (95% CI ; 1-17%) in the buffycoat depleted group (Table 2). HLA-alloantibodies formed mainly in patients with malignant disease in both groups (Table 3).

Among the patients who had a history of pregnancy and/or transfusion, lymphocytotoxic antibodies developed more frequently (24%) in the leukoreduction group than in the buffycoat depleted group (10%), but the difference was not significant. Although also not significant statistically, a similar tendency was observed in patients with no previous donor exposure (9% in the leukoreduced and 0% in the buffycoat-depleted group). As shown in Table 3, lymphocytotoxic antibodies did not appear to develop sooner in recipients who had at least once been pregnant and/ or transfused; five (63%) of eight women with previous alloantigen exposure (pregnancy) and one of two alloimmunized male patients with no history of

Table 2. Results of Transfusion and Alloimmunization

Variable		Leukoreduced ( <i>n</i> = 47)	Buffycot-depleted ( <i>n</i> = 40)	<i>p</i>
Transfusions				
Red cells	Units per patient (mean)	2.7	2.5	NS
	Standard deviation	1.8	1.5	
	Median	2	2	
	Range	1-10	1-7	
FFP #	Units per patient (mean)	5.0	5.7	NS
	Standard deviation	4.2	6.6	
Platelets	No. of patients	1	0	
Estimated white cells transfused per patient ( $\times 10^6$ ) <sup>\$</sup>				
	Mean	0.30	1234.2	<0.001
	Standard deviation	0.21	2356.4	
	Median	0.26	1134.2	
	Range	0.07-1.2	305.3-3913.0	
Patient receiving $\geq 5 \times 10^6$ leukocytes		0 (0%)	40 (100%)	<0.001
Alloimmunization				
Overall				
	At day 1	0/47	0/40	NS
	At day 7	5/47 (11%)	1/40 (3%)	NS
	At day 14	8/47 (17%)	2/40 (5%)	NS
	[95% CI]	[8-31%]	[1-17%]	
Recipients with prior alloantigen exposure				
	At day 1	0/25	0/21	NS
	At day 7	4/25 (20%)	1/21 (5%)	NS
	At day 14	6/25 (24%)	2/21 (10%)	NS
	% [95% CI]	[9-45%]	[1-30%]	
Recipients with no prior alloantigen exposure				
	At day 1	0/22	0/19	NS
	At day 7	1/22 (5%)	0/19	NS
	At day 14	2/22 (9%)	0/19	NS
	% [95% CI]	[1-29%]	[0-18%]	

# FFP; fresh frozen plasma. \$; calculated with the data obtained by our pilot study. NS; not significant

previous exposure became alloimmunized on day 7. In all six, the antibodies that developed at day 7 were still present on day 14.

Anticancer chemotherapy for surgical patients did not suppress the alloimmunization incidence; anti-HLA developed in 4 (19%) of 21 patients treated with chemotherapy, compared with 4 (15%) of 26 patients with no chemotherapy in the leukoreduced group. Similarly, the rate of 1 (6%) of 17 patients with chemotherapy was favorable with that of 1 (4%) of 23 with no chemotherapy in the standard arm.

Table 3. Alloimmunized Patients after Blood Transfusion

Unique Patient Number	Age (y)	Sex	Filter	Disease	Anti-cancer				WBC Tx ( $\times 10^6$ )	Number* Alloantibody at day			
					History of chemo- Preg./Tx.	therapy	Transfusion RBC	FFP		0	1	7	14
109	76	F	RZ	Rectal cancer	Y/ N	Y	3	12	0.27	-	-	-	+
203	76	F	RZ	Metastatic cancer in liver	Y/ N	N	4	14	0.46	-	-	-	+
205	80	F	RZ	Gastric cancer	Y/ N	Y	1	0	0.13	-	-	+	+
301	58	F	RZ	Ovarian cancer	Y/ N	Y	2	20	0.26	-	-	+	+
403	79	F	RZ	Rectal cancer	Y/N	Y	1	0	0.13	-	-	+	+
501	83	M	RZ	Rectal cancer	-/N	N	2	0	0.20	-	-	-	+
502	82	F	RZ	Gastric cancer	Y/N	N	2	0	0.20	-	-	+	+
507	66	M	RZ	Trauma in urinary bladder	-/N	N	3	10	0.21	-	-	+	+
139	73	F	SQ	Gastric cancer	Y/N	N	2	8	1127.6	-	-	-	+
412	46	F	SQ	Gastric cancer	Y/N	Y	1	0	559.0	-	-	+	+

y; years, F; female, M; male, Preg.; pregnancy, Tx.; transfusion, Y; yes, N; no, RBC; red cells, FFP; fresh frozen plasma, Number WBC\*; Numbers of leukocytes transfused were calculated with the data obtained by our pilot study.

Antibody positive was defined by at least 60% cytotoxicity in one or more cells, or at least 40% cytotoxicity in two or more cells.

Cytotoxicity at day 14 in seroconverted individuals: unique patient number (UPN) 109; >80% in one cell, UPN 203; 61-80% in one cell and 41-60% in two cells, UPN 205; >80% in three cells, 61-80% in six cells and 41-60% in one cell, UPN 301; >80% in two cells and 61-80% in three cells, UPN 403; >80% in nine cells, 61-80% in 10 cells and 41-60% in one cell, UPN 501; >80% in one cell and 41-60% in one cell, UPN 502; 61-80% in one cell and 41-60% in four cells, UPN 507; 61-80% in four cells and 41-60% in two cell, UPN 139; >80% in 13 cells and 61-80% in two cells, and UPN 412; >80% in three cells and 61-80% in five cells.

## DISCUSSION

In this randomized study, we attempted to verify the clinical efficacy of bedside filtration for surgical patients. However, we did not demonstrate that leukocyte reduction at the bedside reduced the incidence of HLA alloimmunization.

The HLA alloimmunization rate in the buffycoat-depleted arm of this study was 5%, which is lower than our previous retrospective report<sup>8</sup>); in which it was 20-23% of the overall surgical patients and 30 to 39% of those with prior pregnancy and/or transfusion. The alloimmunization rate in the bedside leukocyte reduction group in this study was 9% (2/22) in those without a history of risk and 24% (6/25) in patients with prior alloantigen exposure. In patients who were transfused using a high efficiency leukoreduction filter at the bedside, the incidence of lymphocytotoxic antibodies was not lower than in those transfused with buffycoat-depleted blood, whether or not they had ever been exposed to alloantigen. The results show that bedside leukoreduction to a level of less than  $5 \times 10^6$  cells does not always prevent primary HLA alloantibody formation in immunocompetent patients. In those with a history of prior contact with allogeneic antigens, a memory immune response is



apparently boosted by either a smaller number of blood cells bearing HLA class I or its soluble form. This is in agreement with previous studies<sup>12,13</sup> which demonstrated that leukocyte-reduced platelet transfusions do not prevent a secondary response although they could prevent primary alloimmunization.

Patients with aplastic anemia, who are considered to be immunocompetent, are reported to frequently develop HLA antibodies (around 50%) after receiving transfusions with regular blood components<sup>14</sup>. In the present study, bedside-filtered red cells, platelets, and FFP were used. Although no studies are available comparing HLA alloimmunization following transfusion with prestorage-filtered versus bedside-filtered blood components in immunocompetent patients who were not sensitized, our results agree with those of Novotny *et al.*<sup>15</sup> who found a higher rate of alloimmunization in presensitized patients (31%) with aplastic anemia than in non-presensitized patients (3%) when prestorage filtered red cells and platelets were administered.

Our observation is concordant with a report<sup>16</sup> that 10% of multitransfused preterm infants alloimmunized to HLA class I after transfusion of prestorage WBC-reduced red cells.

The TRAP study<sup>2</sup> confirmed that leukoreduction significantly reduced alloimmunization, however, a percentage of transfusion recipients still became alloimmunized. The mechanism of alloimmunization of patients receiving leukoreduced blood remains poorly understood, but these cases suggest that an indirect allorecognition pathway, via which donor's HLA antigens are processed in recipient APC, may stimulate HLA alloimmunization. Rodent model studies<sup>17,18,19</sup> show that allogeneic platelets and/ or major histocompatibility complex (MHC) class II depleted leukocytes, independently of donor APC, are potent immunogens with respect to MHC class I antigens. Our results would be concordant with these studies if the indirect pathway is involved in alloimmunization to donor HLA class I antigens in humans, because a residual of around 1-0.1% of the original numbers of platelets, leukocyte fragments and soluble HLA are transfused even when leukoreduction filters are used for red cell component transfusion.

The critical threshold of leukocyte load necessary to cause sensitization in individuals with no prior alloantigen exposure is generally accepted as  $5 \times 10^6$  cells per transfusion<sup>15</sup>. However, there is little experimental evidence to support whether this level of leukoreduction is optimal for completely preventing alloimmunization. A leukocyte level as low as  $1/\mu\text{L}$  is reported to stimulate an antidonor alloantibody response<sup>20</sup>. In humans, we have found that transfusion of regular FFP that contains donor leukocyte fragments and/ or soluble HLA results in the onset of primary HLA alloimmunization<sup>21</sup>. In spite of the fact that our patients received stored blood components (including FFP) after using a high efficiency bedside filter, the possibility that residual platelets, fragmented leukocytes and soluble HLA may have had a role in the formation of anti-HLA antibodies cannot be excluded.

We did not perform the HLA typing of donors or recipients, however, whether

primary or secondary alloimmunization was involved in the recipients with previous alloantigen exposure might have been revealed by studying the combination of HLA between the recipients and the donors.

In conclusion, primary HLA-alloimmunization in hematological malignancies can be prevented when the number of leukocytes is reduced below the threshold for alloimmunization<sup>22)</sup>, however, such numbers of leukocytes may still cause primary as well as secondary responses in immunocompetent surgical patients. Hence, future studies dealing with the prevention of transfusion-induced HLA-alloimmunization should focus on the threshold of allosensitization and new strategies for its prevention.

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