HLA ALLOIMMUNIZATION IN PATIENTS RECEIVING MULTITRANSFUSIONS OF RED BLOOD CELLS

Ampaiwan Chuansumrit[1], Oytip Nathalang[2], Somporn Wangruangsathit[3], Phongjan Hathirat[1], Pimol Chiewsilp[4] and Parttraporn Isarangkura[1]

Departments of [1]Pediatrics, [4]Pathology, Faculty of Medicine, Ramathibodi Hospital; [2]Department of Pathology, Phramongkutklao College of Medicine, Bangkok; [3]Pisanulok Provincial Hospital, Pisanulok, Thailand

Abstract. HLA antibodies were studied in 88 patients with chronic hemolytic anemia who received multitransfusions of red blood cells prepared by conventional (PRC-C), inverted centrifugation (LR-I) and leukocyte filter (LR-F) techniques. Their mean age was 8 years and 4 months with a duration of transfusion of 8 years. The patients were divided into five groups: group 1, receiving PRC-C (n=20); group 2, receiving LR-I (n=33); group 3, receiving LR-F (n=11); group 4, subsequently receiving LR-I and LR-F (n=10); and group 5, receiving PRC-C followed by LR-I and LR-F (n=14). The HLA class I antibodies were found in 30 out of 88 patients (34%). All were against HLA antigens commonly found in the Thai population. The patients receiving PRC-C exhibited HLA antibodies of 65%, which was significantly higher than those of patients receiving LR-I (24%) and LR-F (0%). Consequently, the transfusion reactions of fever, chill, rash and urticaria were also commonly found in patients receiving PRC-C (13.4%), which was significantly higher than patients receiving LR-I (0.4%) and LR-F (0%). The leukocyte filter technique has been shown to be effective in preventing HLA alloimmunization and transfusion reactions but the price is rather high. For the inverted centrifugation technique, only transfusion reactions were effectively prevented and the HLA alloimmunization continued to develop. A more effective and low-cost method for the removal of leukocytes should be investigated for these multitransfusion patients.

INTRODUCTION

Patients with chronic hemolytic anemia, such as thalassemia disease, require life-long red blood cell (RBC) transfusion. Conventionally prepared RBC is widely available in the hospital blood banks in developing countries with limited resources. However, HLA antigens from the contaminated leukocytes are highly immunogenic to induce an HLA antibody response in patients receiving multitransfusions of RBC. It plays an important role in transfusion-related events, such as platelet refractoriness, febrile non-hemolytic transfusion reaction and post-transfusion graft versus host disease. Since febrile transfusion reactions are common (Barton, 1981), the inverted centrifugation technique for the partial removal of leukocytes from RBC prior to transfusion has been conventionally recommended (American Association of Blood Banks, 1981). This technique has been routinely practiced in the University and Regional Hospital Blood Banks in Thailand since 1987. Recently, modern leukocyte reduction methods using various filters (Andreu *et al*, 1988; Sirchia *et al*, 1990; Koerner *et al*, 1991) are widely available. They are simple and practical at both the bedside and in the blood bank during the prestorage period (Popovsky, 1996). However, the main constraint is the high price, which patients and the health care system cannot afford, especially in developing countries.

Correspondence: Dr Ampaiwan Chuansumrit, Department of Pediatrics, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Rama IV Road, Bangkok 10400, Thailand.
Tel: (662) 2011748 to 9; Fax: (662) 2011850, 2462123; E-mail: raajs@mahidol.ac.th

This study presents the HLA alloimmunization and transfusion reactions among patients who received sequential RBC preparations from conventional, centrifugation and leukocyte filter techniques over the last two decades.

MATERIALS AND METHODS

Patients

Eighty-eight patients receiving multiple transfusions of RBC from 1979 to 1999 at the Department of Pediatrics, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok and the Pediatric Division, Pisanulok Provincial Hospital, Pisanulok were enrolled in the study. The diagnosis included β thalassemia major (n = 22), β thalassemia/Hb E disease (n = 61), β / δβ thalassemia disease (n = 1), severe hemoglobin H disease with Constant Spring (n = 1) and other chronic hemolytic anemias (n = 3). The male to female ratio was 1.3. Their ages ranged from one year and six months to 19 years, with a mean of eight years and four months. They received PRC transfusion when their hematocrits were less than 20% or whenever indicated. The interval of transfusion varied from one to three months. The duration of transfusions ranged from one to 18 years with a mean of eight years. The patients were divided into five groups according to the preparation of transfused PRC as shown in Table 1. Group 1, 20 patients, received conventional packed red cells (PRC-C); group 2, 33 patients, received leukocyte-reduced packed red cells prepared by inverted centrifugation (LR-I); group 3, 11 patients, received leukocyte-reduced packed red cells prepared by bedside leukocyte filter (LR-F); group 4, 10 patients, subsequently received LR-I and LR-F; and group 5, 14 patients, received PRC-C followed by LR-I and LR-F. The vital signs and body temperatures were determined before, during and after each transfusion. The number of transfusions, the volume of transfused RBC and transfusion reactions, such as fever, chill, rash, urticaria, as well as medications, were all precisely recorded.

Preparation of leukocyte-reduced packed red cells

LR-I was prepared by the inverted centrifugation of conventional packed red cells at 3,000 rpm for ten minutes and transferred to another transfusion bag. LR-F was prepared by Pall RCXL bedside filters.

Residual leukocyte determination

The residual leukocytes were detemined in the PRC prepared by different techniques, 30 units for each. Using an automated Coulter, the residual leukocytes of the PRC-C and LR-I were determined with a mean number of 1.87×10^9 cells/unit (Buranakijsin *et al*,1997) and 4×10^8 cells/unit (Kaewkamol *et al*,1993), respectively. Additionally, the residual leukocytes in the LR-F were determined using a manual Nageotte counting chamber (Brandwein and Dickstein, 1991) with a mean number of 0.75×10^6 cells/unit (Buranakijsin *et al*,1997).

HLA antibody determination

One microliter of serum obtained from each patient was dispensed under oil in a microtest plate and kept in a -30ºC refrigerator. The plates were thawed immediately before testing. The lymphocytes, prepared from 50 HLA-typed Thai adults, were used as a panel, which included the majority of specifically identified HLA class I antigens. The lymphocytes were isolated by ficoll-hypaque method and a nylon wool column was used for the separation of T and B cells. The T lymphocytes were adjusted to a final concentration of 1,000 cells per μl. The standard microlymphocytotoxicity test was performed by a 30-minute incubation of serum and lymphocytes and followed by a one-hour incubation with rabbit complement. Then, ten microliters of Stain-Fix™ (One Lambda Inc, USA) were added to each well. All reactions were examined under an inverted phase contrast microscope and scored by estimating the percentage of cell deaths beyond that of the back-

ground or negative control as recommended by the ASHI standard scoring system (Terasaki *et al*, 1978; Milken *et al*, 1987).

Statistics

The occurrence of HLA antibodies induced by the contaminated leukocytes from different methods of RBC preparations was calculated by the proportion (Z) test. The association of HLA antibodies and parameters related to RBC transfusion was calculated by chi-square test. The risk of HLA antibodies was expressed by the odds ratio. A p value of less than 0.05 was considered significant.

RESULTS

The patients' descriptive data is shown in Table 1. The HLA class I antibodies were found in 30 out of 88 patients (34%) as shown in Table 2. Twelve patients had one type of antibody, 11 patients had two types, four patients had three types and three patients had multispecific antibodies. All of them were against HLA antigens commonly found among the Thai population, which included A1, A2, A11, A19, A24, A28, A31, A33, A203, B5, Bw4, Bw6, B7, B8, B13, B16, B18, B22, B35, B40, B46, B51, B52, B54, B55 and B60.

The occurrence of HLA antibodies in patients receiving PRC-C in group 1 (65%) was significantly higher than those of patients receiving LR-I in group 2 (24%) with a p-value of < 0.001. Their descriptive data were not significantly different ($p > 0.05$). Additionally, patients subsequently receiving LR-I and LR-F in group 4 had 20% of HLA antibodies, which was similar to patients in group 2. Also, patients sequentially receiving PRC-C, LR-I and LR-F in group 5 had 50% of HLA antibodies, similar to patients in group 1. Importantly, patients receiving only LR-F in group 3 did not possess any HLA antibodies.

The occurrence of HLA antibodies was also based on the volume and number of transfusions. Patients in group 2 receiving LR-I of more than 8,000 ml and 35 transfusion episodes had a higher chance of developing HLA antibodies with the p-values of 0.004 and 0.01, respectively. They had 16-fold and 15-fold increased risks of HLA antibodies with the odds ratio of 2.3-108 and 1.6-142 (95% confidential interval), respectively. However, there was no association of HLA antibodies and the transfused RBC in group 1 patients receiving PRC-C since the alloimmunization was rather high.

Transfusion reactions of fever, chill, rash and urticaria were found in 141 out of 1,052 episodes (13.4%) in patients receiving only PRC-C in group 1. However, the reactions were found in 6 out of 1,457 episodes (0.4%) in patients receiving LR-I in group 2, which was significantly lower than those in group 1 ($p<0.0001$). Additionally, patients in group 4 exhibited a similar reaction rate of 0.9% while subsequently receiving LR-I and LR-F. Patients in group 5 sequentially receiving PRC-C, LR-I and LR-F, had a decreased reaction rate from 5.8% to 0.8% as shown in Table 2. Interestingly, patients in group 3 receiving solely LR-F did not exhibit any reactions.

The association of HLA antibodies and transfusion reactions was evaluated. The patients in group 1 exhibiting reactions had HLA antibodies of 75% (9/12), which was slightly higher than those of patients not having reactions (4/8 = 50%). However, the patients in group 2 exhibiting reactions had HLA antibodies of 80% (4/5), which was significantly higher than those of patients not having reactions (4/28 = 14.3%) with the p-value of 0.0008.

DISCUSSION

RBC transfusion is essential for patients with chronic hemolytic anemia in order to maintain their activities. Group and type specific RBC are required for preventing isoimmunization to the transfused RBC. However, the contaminated leukocytes will induce HLA

Table 1
The mean descriptive data of 88 patients receiving red blood cells sequentially prepared by the conventional (C), inverted centrifugation (I) and leukocyte filter (F) techniques.

Group	Red blood cells	Patients (n)	Studied age	Age at initial transfusion	Transfused red blood cells			
					Preparation	Duration	Number	Volume
1	Conventional PRC	20	7 yr, 8m	2 yr	PRC-C	5yr, 8m	52 (4-80)	8,586 (400-17,540)
2	LR-inverted centrifugation	33	8yr, 1m	2yr, 9m	LR-I	5yr, 6m	42 (4-238)	7,916 (600-53,640)
3	LR-leukocyte filter	11	3yr, 7m	1yr, 11m	LR-F	1yr, 9m	13 (4-49)	2,348 (410-9,810)
4	LR-I → LR-F	10	8yr, 6m	1yr, 11m	LR-I	3yr, 8m	32 (3-81)	5,819 (370-14,930)
					LR-F	2yr, 9m	21 (5-54)	5,106 (353-14,870)
5	PRC-C → LR-I → LR-F	14	13yr, 7m	1yr, 6m	PRC-C	3yr, 6m	24 (3-80)	4,822 (400-17,540)
					LR-I	6yr, 8m	56 (2-102)	15,435 (300-30,770)
					LR-F	1yr, 8m	16 (4-48)	6,874 (950-21,870)

PRC = packed red cells, LR = leukocyte - reduced; The figures in parentheses are the range.

Table 2
HLA antibodies and transfusion reactions in 88 patients receiving red blood cells sequentially prepared by the conventional(C), inverted centrifugation (I) and leukocyte filter (F), techniques.

Group	Patients (n)	HLA antibodies n (%)	Transfusion reactions		
			Preparation	Episodes / Total	%
1	20	13 (65)	PRC-C	141 / 1,052	13.4
2	33	8 (24)	LR-I	6 / 1,457	0.4
3	11	0	LR-F	0 / 147	0
4	10	2 (20)	LR-I	3 / 324	0.9
			LR-F	2 / 211	0.9
5	14	7 (50)	PRC-C	20 / 342	5.8
			LR-I	11 / 791	1.4
			LR-F	2 / 231	0.8
Total	88	30 (34)			

PRC = packed red cells, LR = leukocyte - reduced.

antibodies or lymphocytotoxic antibodies (Murphy *et al*, 1986). In this study, the occurrence of HLA antibodies among patients receiving conventional RBC preparation was 65%, which was similar to other studies (Sniecinski *et al*, 1988; Sirchia *et al*, 1986) and was significantly higher than those of patients receiving LR-I (24%) and LR-F (0%). Although the amount of transfused RBC in patients receiving solely LR-F are rather small, the absence of HLA antibodies did not differ from other study (Sirchia *et al*, 1986). The HLA antibodies are not only based upon the method of RBC preparation but also the number and volume of transfused RBC. They directly reflect the number of exposed donor HLA antigens. The higher volume and number of transfused RBC component create an increased risk of developing HLA antibodies in patients receiving LR-I. Therefore, the prominent etiologies inducing HLA antibodies among multitrasfusion patients include larger amounts and higher numbers of transfusion episodes as well as a high number of remaining leukocytes in the RBC component, especially when prepared by conventional method.

In addition, the transfusion reactions among patients receiving different preparations of RBC were evaluated. Similarly, the highest occurrence was found among patients receiving PRC-C. The reactions were directly proportional to the occurrence of HLA antibodies. Moreover, the reactions were also based upon the volume and number of transfused RBC. Both group 1 and 5 patients received PRC-C. The patients in group 1, who received a larger volume and a higher number of PRC-C transfusions, experienced more frequent reactions (13.4%) than those of patients in group 5 (5.8%). However, the reactions were lower in patients receiving LR-I and LR-F. Since the residual leukocytes in LR-I were as low as 10^8 cells/unit of RBC, febrile non-hemolytic reaction was reduced (American Association of Blood Bank, 1996). However, the HLA alloimmunization continued to develop in patients receiving LR-I. Although the patients possessed many specific HLA antibodies against antigens commonly found among the Thai population, they seldom exhibited reactions while receiving LR-I and LR-F. This is most likely due to the low number of leukocytes remaining in the transfused RBC prepared by either inverted centrifugation or leukocyte filter method.

As a result, the contaminated leukocytes in the transfused RBC consequently induce HLA alloimmunization and transfusion reac-

tions. The most effective prevention method is using leukocyte filter technique but the high price is a constraint in developing countries with limited resources. The preparation of RBC by inverted centrifugation should be routinely performed in every blood bank. In addition, a more effective and low-cost method should be investigated for these multitransfusion patients.

ACKNOWLEDGEMENTS

The authors would like to express their gratitude to the staff, nurses and paramedical personnel at the Blood Bank and the Department of Pediatrics, Faculty of Medicine, Ramathibodi Hospital and Pisanulok Hospital who provided packed red cells and excellent care for the patients.

REFERENCES

American Association of Blood Banks: Technical Manual, 8th ed. Philadelphia: American Association of Blood Banks, 1981: 41-2.

American Association of Blood Bank: Technical Manual, 12th ed. Bethesda: American Association of Blood Banks, 1996: 42.

Andreu G, Dewailly J, Leberre C, *et al.* Prevention of HLA alloimmunization with leukocyte-poor packed red cell and platelet concentrates obtained by filtration. *Blood* 1988; 72: 964-9.

Barton JC. Non-hemolytic, non-infectious transfusion reactions. *Semin Hematol* 1981; 18: 95-121.

Brandwein H, Dickstein R. Leucocyte filtration : Understanding counting method and their implication. Pall Corporation, 1991: 1-23.

Buranakijsin S, Chuansumrit A, Tardtong P, Apibal S, Hathirat P. Evaluation of two units of leukocyte-depleted packed red cell prepared by a single leukocyte filter. *Thai J Hematol Transf Med* 1997; 7: 105-12.

Kaewkamol K, Bejrachandra S, Mahakittikun B, Chongkolwatana V, Rungpitarangsi B. Evalution of three procedures for the preparation of leukocyte poor blood. *Thai J Hematol Transf Med* 1993; 3: 105-12.

Koerner K, Sahlmen P, Zimmermann B, Kubanek B. Preparation of leukocyte-poor red cell concentrate : comparison of five different filters. *Vox Sang* 1991; 60: 61-2.

Popovsky MA. Quality of blood components filtered before storage and at the bedside: implications for transfusion practice. *Transfusion* 1996; 34: 470-4.

Milken SL. Reagent antisera for HLA typing. Tissue typing reference manual. South-Eastern Organ Procurement Foundation, 2nd ed. 1987: 1-11.

Murphy MF, Metcalfe P, Thomas H, *et al*. Use of leukocyte-poor blood components and HLA-matched-platelet donors to prevent HLA alloimmunization. *Br J Haematol* 1986; 62: 529-34.

Sirchia G, Rubella P, Mascaretti L, *et al*. The clinical importance of leukocyte depletion in regular erythrocyte transfusions. *Vox Sang* 1986; 51: 2-8.

Sirchia G, Wenz B, Rebulla P, Parravicini A, Carnelli V, Bertolini F. Removal of white cells from red cells by transfusion through a new filter. *Transfusion* 1990; 30: 30-3.

Sniecinski I, O'Donnell MR, Nowicki B, Hill LR. Prevention of refractoriness and HLA alloimmunization using filtered blood products. *Blood* 1988; 71: 1402-7.

Terasaki PI , Bernoco D, Park MS, Ozturk G, Iwaki Y. Microdroplet testing for HLA-A, -B, -C and -D antigen. *Am J Clin Pathol* 1978; 69: 109-20.