HLA CLASS I ANTIBODIES IN THALASSEMIC PATIENTS

Oytip Nathalang[1], Kitti Torcharus[2], Ampaiwan Chuansumrit[3], Punpaka Punyaprasiddhi[1], Thip Sriphaisal[1] and Noriyuki Tatsumi[4]

[1] Department of Pathology, Pramongkutklao College of Medicine; [2]Department of Pediatrics, Pramongkutklao Hospital; [3]Department of Pediatrics, Faculty of Medicine Ramathibodi Hospital, Bangkok, Thailand; [4]Department of Clinical Hematology, Osaka City University, Osaka, Japan

Abstract. This study was undertaken to demonstrate the prevalence of HLA class I antibodies among 62 polytransfused patients. The diagnosis included β–thalassemia major, β–thalassemia/Hb E disease and severe Hb H disease. Their ages ranged from 1 year to 23 years with the mean age of 10.7 years. The number of packed red cell transfusions ranged from 3 to 235 with the mean of 60 episodes per patient. The standard microlymphocytotoxicity test was performed using 50 panels of lymphocytes which specifically identified the majority of HLA class I antibodies. 31/62 cases (50%) were positive for HLA class I antibodies. The detection of single or multiple antibodies depended upon the number of blood transfusions and the patients' ages. These antibodies were induced by the leukocytes present in the transfused packed red cells. Therefore, leukocyte-reduced packed red cells prepared by either additional inverted centrifrugation or leukocyte filter is suggested for the routine blood bank service.

INTRODUCTION

It is well documented that the human leukocyte antigen (HLA) system antigens and antibodies play important roles in transfusion-related events. These include alloimmunization and platelet refractoriness, febrile non -hemolytic (FNH) transfusion reactions, transfusion-related acute lung injury and post-transfusion graft versus host disease (GVHD). HLA antigens are highly immunogenic. In response to pregnancy or transfusion, immunologically normal recipients are more likely to form antibodies to HLA antigens than to any other antigen system. The prevalence of HLA alloimmunization and platelet refractoriness among patients receiving repeated transfusions of cellular components is 30-60%. HLA alloimmunization is provoked by residual leukocytes in blood components. Once provoked, the recipient's immune response is generally directed against class I antigens. The threshold level of leukocytes required to provoke an HLA alloimmune response is unclear and is likely to vary among different recipients. The HLA antibody response in the transfused individuals may be directed against private alloantigens present on donor cells or against public alloantigens (American Association of Blood Banks, 1993 ; Larison and Cook, 1994). The precise specificities are often difficult to determine since the polymorphisms of the HLA system require a large panel of cells for detecting antibodies against the majority of HLA antigens by conventional microlymphocytotoxicity test (LCT) (Decary *et al,* 1984).

In Thailand, the prevalence of thalassemia and hemoglobinopathy is high. Most patients of these diseases require repeated blood transfusions in order to maintain their normal activity (Wasi *et al,* 1980). This study was undertaken to demonstrate the prevalence of HLA class I antibodies among polytransfused patients.

MATERIALS AND METHODS

Subjects

Sixty-two sera samples were obtained from thalassemic patients in the Department of Pediatrics, Pramongkutklao Hospital and from the Department of Pediatrics, Faculty of Medicine, Ramathibodi Hospital, Bangkok, Thailand. The diagnosis included β–thalassemia major, 6 cases; β–thalassemia/Hb E disease, 55 cases; and severe Hb H disease, one case. Their ages ranged from 1 year to 23 years with the mean age of 10 years 8 months.

Correspondence: Col Oytip Nathalang, Department of Pathology, Pramongkutklao College of Medicine, Rajavithi Road , Bangkok 10400, Thailand.
Tel : (662) 247-1075; Fax : (662) 644-7369.

Methods

One microliter of serum obtained from thalassemic patients was dispensed under oil in a microtest plate and kept at -30°C until tested. The plates were thawed immediately before use. The lymphocytes obtained from 50 HLA-typed people were used as a panel which included the majority of specifically identified HLA class I antibodies. The lymphocytes were isolated by ficoll-hypaque method and a nylon wool column was used for separating T and B cells. T-lymphocytes were adjusted to a concentration of 1,000 per µl. Standard LCT was performd by 30 minute serum and cell incubation with an additional 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 (staining) beyond that of the background or negative control as recommended by the ASHI standard scoring system (Terasaki *et al,* 1978; Milken, 1987).

Statistical methods

The data was analysed by using the multivariate analysis of variance. A p-value of less than 0.05 was considered significant.

RESULTS

Sixty-two patients were included in this study. They received packed red cell transfusion when the hematocrit were less than 20% or whenever indicated. The interval of transfusion varied from one to three months. The packed red cells were prepared by conventional method. However, few patients received leukocyte-reduced red blood cells (LR-RBCs) prepared by inverted centrifugation with the residual leukocyte of $5x10^8$ cells. (American Association of Blood Banks, 1993). The number of packed red cell transfusions ranged from 3 to 235 with the mean of 60 episodes per patient. In addition, each patient took daily oral multivitamins and folic acid. Iron chelation was seldom given.

Thirty-one out of 62 sera (50%) showed HLA class I antibodies. Twelve sera demonstrated monospecific antibodies and 19 sera had multispecific antibodies, (Tables 1, 2). The specificities of monospecific antibodies found in 12 serum samples were A2, A11, A24, B8, B17, B22, B38, B40, B44 and B52, (Table 3) which were immunized by the HLA class I antigens common among the Thai population. (Chandanayingyong, 1997). Moreover, it was found that the detection of single or multiple antibodies depended upon the number of blood transfusions as well as the patients' ages ($p < 0.05$).

DISCUSSION

Transfusion therapy for thalassemia is defined as the prevention of discomfort and the maintenance of as high a criteria of health as possible.

Table 1

HLA class I antibodies in polytransfused patients of various ages.

Age (years)	Total	No. of patients: HLA class I antibodies (%)	Type of antibody: One type	Two types	Multispecific
1-5	14	2 (14 .3)	1	1	0
6-10	16	5 (31.3)	3	1	1
11-15	19	15 (79.0)	3	8	4
16-20	11	8 (72.7)	5	2	1
21-25	2	1 (50.0)	0	0	1
Total	62	31 (50.0)	12	12	7

Table 2
Correlation between HLA class I antibodies and number of blood transfusions.

No. of blood transfusions	No. of patients				
	Total	HLA class I antibodies (%)	Type of antibody		
			One type	Two types	Multispecific
3-10	8	1 (12.5)	1	0	0
11-20	8	1 (12.5)	1	0	0
21-30	3	1 (33.3)	0	1	0
31-40	2	2 (100)	2	0	0
41-50	7	4 (57.1)	1	3	0
51-70	16	5 (31.3)	2	2	1
> 70	18	17 (94.4)	5	6	6
Total	62	31 (50.0)	12	12	7

Table 3

HLA specificities of monospecific antibodies found in 12 serum samples.

Antibody specificity	No.
A2	1
A11*	2
A24*	2
B8	1
B17	1
B22*	1
B38	1
B40	1
B44	1
B52	1

* The r values of these sera > 0.8

Group and type-specific red cells that are compatible should be used in order to prevent isoimmunization in all patients facing long-term transfusion therapy. However, the incidence of red cell alloantibodies and autocontrol positive responses in these patients was found to be 9.5% and 20.6%, respectively (Srijinda *et al,* 1996). Furthermore, alloimmunization by prior blood transfusion is also a causative stimulus for antibody formation. These antibodies are predominantly HLA or lymphocytotoxic antibodies (Sujirachato *et al,* 1989; Larison *et al,* 1994). In this study, the prevalence of HLA class I antibodies among thalassemic patients was 50%. The antibody formation was significantly increased by the number of blood transfusions and the patients' ages. These antibodies were induced by the contaminated leukocytes in the transfused packed red cells. Several different methods have become available for the preparation of leukocyte-reduced red blood cells (LR-RBCs). Leukocyte-adsorption filters are highly efficient at achieving significant leukocyte reduction of blood components ($<5x10^6$ cells). The drawback to these filters is that they are expensive. LR-RBCs prepared by the inverted centrifugation method is shown to reduce the leukocyte number to $< 5x10^8$. However, there is no single definite standard for any leukocyte-reduced blood component since the required degree of leukocyte removal depends on the therapeutic goal (American Association of Blood Banks, 1993). In addition, 5 out of 12 sera that had monospecific antibodies, showed strong lymphocyte killing activity (>70%) with an r value more than 0.8 (Schicbl, 1987). These sera can be used as the self-screening antisera for routine use which is beneficial to the HLA laboratory with a limited budget.

ACKNOWLEDGEMENTS

This study is supported by the Association of Military Surgeons of Thailand.

REFERENCES

American Association of Blood Banks. Technical manual. 11th ed. Bethesda: American Association of Blood Banks, 1993 ; 287-307.

Chandanayingyong D, Stephens AF, Klaythong R, *et al.* HLA-A, -B, -DRB1, -DQA1 and -DQB1 polymorphism in Thais. *Hum Immunol* 1997; 53 : 174-82.

Decary F, Ferner P, Giavedoni L, *et al.* An investigation of non-hemolytic transfusion reactions. *Vox Sang* 1984 ; 46 : 277-85.

Larison PJ, Cook LO. Adverse effect of blood transfusion. In : Harmening DM, ed. Modern blood banking and transfusion practices. 3rd ed. Philadelphia: FA Davis, 1994 ; 351-74.

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

Schicbl B. HLA-typing techniques. HLA typing problems and solutions. Biotest AG. special ed no.7, 1987 ; 15-23.

Srijinda S, Na Thalang O, Suwanasit T, *et al* : Red cell alloantibodies in thalassemic patients by the gel test. *Thai J Hematol Transfus Med* 1996 ; 6: 32-8.

Sujirachato K, Chiewsilp P, Isarangkura P. Leukocyte antibodies in thalassemic patients. *Birth Defects* 1988 ; 23 : 283-5.

Terasaki PI, Bernoco D, Park MS, *et al.* Microdroplet testing for HLA-A,-B,-C and -D antigens. *Am J Clin Pathol* 1978 ; 69 : 109-20.

Wasi P, Pootrakul S, Pootrakul P, *et al.* Thalassemia in Thailand. *Ann NY Acad Sci* 1980 ; 344 : 352-60.