The Characteristics of ABO Antibodies in Group O Thai Blood Donors

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This study aimed to characterize anti-A and anti-B hemolysins, IgM, and IgG titers in Thai blood donors. Altogether, 300 serum samples from group O donors at the National Blood Centre, Thai Red Cross Society, were screened for anti-A and anti-B hemolysins and treated with 0.01 M dithiothreitol to characterize IgM and IgG titers by standard tube technique. Antibody titers were compared with hemolysis grade. Male and female ratio = 1:1.3 and ages ranged from 17 to 60 years. The overall prevalence of anti-A and anti-B hemolysins was 69%. Anti-A and anti-B hemolysins comprised 18.3% and 16.7%, respectively and 34% had both antibodies. High titers of anti-A hemolysins were

associated with females ($P < 0.05$), and only anti-B IgM titers were associated with age ($P < 0.05$). Interestingly, the association of anti-A IgM titers, anti-A IgG titers, and hemolysin grade was demonstrated ($P < 0.05$). A significant association between hemolysin grade and anti-B IgM titers was found ($P < 0.05$). The prevalence of anti-A and anti-B hemolysins and high titers of IgM and IgG in Thais are high. Hemolysin grade showed significant associations with IgM titers; therefore, when providing ABO-incompatible platelet transfusion, especially for female plateletpheresis donors, IgM high titers of anti-A and anti-B screening is suggested. *J. Clin. Lab. Anal.* 26:223–226, 2012. © 2012 Wiley-Periodicals, Inc.

Key words: ABO antibodies; hemolysins; IgM and IgG titers; group O; Thais

INTRODUCTION

ABO compatibility and platelet transfusion is clinically important because platelet increments are decreased after transfusion of ABO incompatible platelets (1). Additionally, acute hemolytic transfusion reactions, venoocclusive disease, and increased morbidity in allogeneic transplants caused by minor-ABO incompatible platelet transfusions have been reported (2–7). Recent studies showed that approximately 10% to 40% of group O donors have high titer of IgG anti-A and anti-B (1, 8, 9), and not only IgM and IgG titers but also anti-A and anti-B hemolysins, which are IgG can cause hemolysis of red blood cells when group O blood/plasma is transfused to nongroup O patients and cause hemolytic disease of the fetus and newborn (10). A case of severe fetal anemia due to ABO incompatibility caused by high anti-B IgG titers of African mother was reported (11). The high frequency of hemolysins in group O blood donors was commonly found in Nigerian and Indian populations with the prevalence ranging from 23.2%

to 62.8% (12–15). A previous study of ABO antibody levels among Japanese, Lao, and Thai blood donors in 2006 showed that IgM anti-A and anti-B titers were low in Thais, similar to the Japanese antibody titers reported in 2001, but IgG anti-A and anti-B titers were high, similar to those reported in Japanese in 1986 (16). According to the requirements of the Standards for Blood Banks and Transfusion Services, it is recommended that the blood bank have a policy concerning transfusion of components containing significant amounts of ABO incompatible antibodies (17). Screening for donor anti-A and anti-B hemolysins, as well as high titers of IgM and IgG is

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suggested when using platelets containing ABO incompatible plasma (18). Since information on immunoglobulin types and concentrations of ABO hemolysins in the Thai population is still unclear, the purpose of this study was to characterize anti-A and anti-B hemolysins, IgM, and IgG titers in group O Thai blood donors.

MATERIALS AND METHODS

Subjects

Altogether, 300 blood samples obtained from group O Thai blood donors of the National Blood Centre, Thai Red Cross Society, Bangkok, Thailand were included in this study. There were 128 males and 172 females (M:F = 1:1.3) and their ages ranged from 17 to 60 years. Informed consent was obtained from each subject. This study was approved by the Committee on Human Rights Related to Research Involving Human Subjects, Thammasat University, Pathumtani, Thailand.

Methods

ABO blood group was determined by cell and serum grouping according to methods previously described (19). Anti-A and anti-B antiserum for cell grouping were obtained from the National Blood Centre, Thai Red Cross Society and pooled cells for serum grouping were prepared in-house. Test for hemolysins and scoring was performed by standard procedure (20). Hemolysin was graded as follows: 3+ (complete hemolysis), and 2+ (partial > 50% but not complete), 1+ (trace hemolysis), and negative (no visible sign of hemolysis). Serum samples having a score of 3+ and 2+ were considered strongly hemolytic. IgM anti-A and anti-B titers were determined using the saline method with incubation at room temperature for 5 min. The IgG antibody titers were determined by an indirect antiglobulin test (IAT) and an incubation of 30 min at 37°C. Before the IAT, the sera were treated with 0.01 M dithiothreitol for 15 min at 37°C using the method as described by Brecher (21), to destroy IgM antibodies in their sera. The results of hemolysins, titers for IgM, and IgG ABO antibody testing can be obtained within 20 min, 10 min, and 60 min, respectively.

Statistical Analysis

Chi-square test was used to determine the association between grade of hemolysins and IgM and IgG antibody titers. A level of $P < 0.05$ was accepted as significant.

TABLE 1. Distribution of Age and Sex in 300 Blood Donors

Age (year)	Sex		Total (%)
	Male (%)	Female (%)	
≤ 30	36 (28.1)	72 (41.9)	108 (36.0)
31–40	36 (28.1)	49 (28.5)	85 (28.3)
41–50	39 (30.5)	38 (22.1)	77 (25.7)
> 51	17 (13.3)	13 (7.6)	30 (10.0)
Total	128 (100.0)	172 (100.0)	300 (100.0)

Results

The distribution of male and female donors among different age groups (≤30, 31–40, 41–50, and >50 years) showed significant association ($P < 0.05$). The highest numbers of female donors (41.9%) was found in the younger than 30 age group, while that of male blood donors was 30.5% in the 41 to 50 year group, as shown in Table 1.

Hemolysins

The overall prevalence of anti-A and anti-B hemolysins in serum samples from group O Thai blood donors was 69%. Prevalence of anti-A and anti-B hemolysins was 18.3% and 16.7%, respectively, while that of donors having both anti-A and anti-B hemolysins in their sera was 34%. Anti-A and anti-B hemolysins were not associated with age, while high titers of anti-A hemolysins were associated with females ($P < 0.05$).

IgM Titers

In this study, IgM anti-A and anti-B titers in group O blood donors ranged from 8 to 1,024 (IgM anti-A titer ≥64 was 75.7% and IgM anti-B titer ≥64 was 80.0%), and no association was noted between the sexes. Only a decrease of anti-B IgM titers was associated with an increase of donor age in both males and females ($P < 0.05$).

IgG Titers

Regarding IgG anti-A and anti-B titers, the distribution was between 16 and 4,096 (IgG anti-A titer ≥64 was 93.0% and IgG anti-B titer ≥64 was 95.3%) and no association was found between sex and age.

Association of Hemolysins, IgM Titers, and IgG Titers

Interestingly, the association among anti-A IgM titers, anti-A IgG titers, and anti-A hemolysin grade were

TABLE 2. Comparison of Hemolysin Grades and Immunoglobulin Titers

Hemolysin grade/titer	Anti-A titer				Anti-B titer			
	IgM*		IgG		IgM*		IgG	
	<64	≥64	<64	≥64	<64	≥64	<64	≥64
0 and 1	44.8%	55.2%	9.0%	91.0%	33.8%	66.2%	6.8%	93.2%
2 & 3	5.2%	94.8%	5.2%	94.8%	6.6%	93.4%	2.6%	97.4%

* P value < 0.05.

demonstrated ($P < 0.05$), whereas significant association was found ($P < 0.05$) between anti-B hemolysin grade and anti-B IgM titer, as shown in Table 2.

DISCUSSION

It is suggested that environmental factors, enteric bacteria, and other parasites may have affected the production of anti-A and anti-B titers (22–24). Moreover, a high frequency of strongly hemolytic anti-A and anti-B hemolysins has been reported in Asian and Black populations compared with Caucasians (12,23,25), which may be due to mosquito bites and intestinal parasitic infections (15). In addition, high titers of ABO antibodies in group O individuals can also be the outcome of vaccination or other antigen exposures (26).

The data on the Ig types and concentration of ABO antibodies in the Thai population are obscure. The high frequency of anti-A and anti-B hemolysins in group O blood donors in this study is higher than other previous studies in Nigerian and Indian populations because of the increasing numbers of female donors. High titers of anti-A hemolysins were also associated with female blood donors, which may be caused by pregnancies.

A previous study in Japanese group O blood donors showed that IgM anti-A, and anti-B titers were low, and no differences were noted between sexes (16), while in this study IgM anti-A and anti-B titers in group O Thai blood donors were high. Only IgM anti-B titers were significantly decreased according to an increase of donor age.

Additionally, IgG titers of anti-A and anti-B in Japanese donors showed differences between sexes and increase in donor age, especially in female blood donors (16). In contrast, no association was found between sex and age with IgM and IgG anti-A and anti-B titers in group O Thai blood donors.

Correlation between IgM and IgG levels has been demonstrated in Zimbabweans (25). We also found significant association among anti-A IgM titers, anti-A IgG titers, and anti-A hemolysin grade, while only significant association between anti-B hemolysin grade and anti-B IgM titer was demonstrated.

In cases of emergency, it is common practice to transfuse ABO-incompatible platelets to patients because of limited supply and short shelf life of platelets. Therefore, it is important to avoid transfusion of immune anti-A and anti-B antibodies to nongroup O patients. This study reports a high frequency of immune ABO antibodies in group O Thai blood donors. In addition to screening for hemolysins and high IgG titers, which is labor-intensive and time-consuming, screening for high IgM titers is beneficial because of its simplicity. Therefore, it can be used as an alternative to screen female group O apheresis donors.

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