Frequency and Characterization of Platelet-Specific Antibodies in Patients who Received Multiple Platelet Transfusions

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Multiple platelet transfusions can induce alloimmunization. Alloimmunization involving platelet membrane antigens has been characterized by failure to achieve the expected post-transfusion platelet levels; this is a clinical status frequently referred to as refractoriness to platelet transfusions [1]. Platelet alloantibodies are divided into two types: antibodies to human leukocyte antigen (HLA) class I determinants, and antibodies to platelet-specific glycoprotein antigens, so-called platelet-specific antibodies [1, 2]. HLA antibodies have been found in 30% to 70% of patients who received multiple blood transfusions and are considered the major immunologic cause of platelet refractoriness [3, 4]. The incidence of platelet-specific antibodies is less well known and was previously considered rare [3]. With the development of more effective detection techniques, platelet-specific antibodies have been reported more frequently and have also been implicated as causes of platelet refractoriness.

The distribution patterns of platelet-specific antigens are different among Taiwanese and Caucasian populations [5]. The frequencies and patterns of platelet-specific antibodies may also be different, although there have been few studies of the frequency and specificity of platelet-specific antibodies in Taiwan [6, 7]. Previous studies in Taiwan utilized non-specific
methods (chloroquine-stripping method) to estimate the incidence of platelet-specific antibodies, which did not allow determination of the antigen targets. These studies did not detect any clinically significant amounts of platelet-specific antibodies in patients who had received multiple transfusions. The aim of this study was to characterize platelet-specific antibodies in Taiwanese patients who had received multiple platelet transfusions using the phase-III platelet detection method.

**Materials and Methods**

From June through December 1998, blood samples from 103 patients who had received multiple platelet transfusions were submitted to our laboratory for examination of platelet alloantibodies. All patients had more than 10 platelet donor exposures and suffered from clinical refractoriness to platelet transfusions. In our laboratory, platelet antibodies are routinely tested using a non-discriminative screening method and a solid phase red cell adherence method (Capture-P, Immucor Inc, Norcross, GA, USA). This method has been reported to be sensitive for the detection of platelet antibodies, and has been adapted for platelet cross-matching [8, 9]. We performed the tests as previously described [9]. Briefly, solid phase platelet monolayers were formed by adding 100 µL of platelet-rich plasma to polystyrene U-bottom microplate wells that had been pretreated with rabbit anti-human platelet antibodies. One drop of test serum and three drops of low ionic strength solution were added. After incubation and washing, platelet antibodies were detected by the addition of one drop of anti-immunoglobulin (Ig) G coated indicator red cells. This method cannot distinguish platelet-specific antibodies from HLA antibodies. Forty patients (39%) had positive results for platelet-specific antibodies using this screening method. These 40 patients were considered alloimmunized and recruited for the platelet-specific antibodies study. The median age of this patient population was 41 years (range, 1–81 yr). All patients had received multiple platelet and/or red cell transfusions. Thirteen adult female patients had also been exposed to possible previous immunization due to pregnancy. Blood samples were stored at –70°C until further assay.

**Detection of platelet-specific antibodies**

We used a commercially available ELISA test (GTI PakPlus; Brookfield, WI, USA) to detect the platelet-specific antibodies. A recent report showed that this test permits ready differentiation between alloantibodies directed to HLA and those directed to platelet-specific glycoproteins [10]. This test is a phase-III platelet antibody detection method; that is, it can be used to identify the targets of antibodies at the glycoprotein level [11]. The technique used microwells coated with platelet glycoproteins (platelet glycoproteins IIb/IIIa, Ia/IIa, Ib/IX and IV) or HLA class I antigens incubated with test sera. After incubation, any antibody bound to the microwell was detected using an alkaline-phosphatase-conjugated anti-human globulin reagent (anti-IgG/A/M). After adding the appropriate substrate, the optical density (OD) of the resulting mixture was measured at 405 or 410 nm by a spectrophotometric reader. The OD ratio was defined as the mean OD of the tested sera over that of negative controls. The tests were performed according to the manufacturer’s instructions.

**Statistical analysis**

Nominal data were evaluated using contingency tables and chi-square tests and, if necessary, substituted using the two-sided Fisher’s exact test. Student’s t-test was used to correlate transfusions with alloimmunization. A p value of less than 0.05 was considered significant.

**Results**

**Characterization of patients according to immunization status**

Table 1 shows the characterization and transfusion history of patients according to their immunization status. The number of platelet transfusions was slightly higher in alloimmunized patients; however, the differences were not statistically significant. There were no differences in gender or age between the two groups.

**Frequency of platelet-specific antibodies in alloimmunized patients**

Sera from 40 alloimmunized patients were tested for the presence of platelet-specific antibodies. The results are shown in Table 2. HLA antibodies were detected in 34 patients (84% of alloimmunized patients) and were the most frequently detected platelet-reactive antibodies. Platelet-specific antibodies were found in 27 patients (68% of alloimmunized patients), often in the presence of HLA antibodies. Only five patients developed platelet-specific antibodies in the absence of HLA antibodies.

**Characterization of platelet-specific antibodies**

The platelet-specific antibodies were characterized ac-
According to their glycoprotein targets (Table 2). The frequencies of antibodies to platelet glycoproteins included: IIb/IIIa 48%, Ia/IIa 56%, Ib/IX 23%, and IV 23%. Glycoprotein Ib/IX antibodies occurred more frequently in patients who did not produce HLA antibodies (60% vs 12%, \( p < 0.001 \)). We further defined the reactivity of each glycoprotein antibody group according to their OD ratio (Fig.); the OD ratios for each group of platelet-reactive antibodies were as follows (median, range): glycoprotein IIb/IIIa 3.27, 2–7.9; Ia/IIa 3.2, 2–45; Ib/IX 2.1, 2–3; IV 3.15, 2.4–10.8; and HLA 6.63, 2.9–43. Antibodies against HLA and glycoprotein Ia/IIa had the highest reactivities among platelet-reactive antibodies and were the most prevalent platelet antibodies.

**Discussion**

We found that about 39% of blood samples from patients with clinical conditions suggestive of refractoriness contained platelet-specific antibodies and HLA antibodies, which were the most frequently found platelet-reactive antibodies. Platelet-specific antibodies were detected in 27 patients (26% of all screened patients, 68% of alloimmunized patients). The two platelet antigens to which the antibodies were most frequently reactive were glycoproteins IIb/IIIa and Ib/IX. These results are in contrast to previous reports in which no significant platelet-specific antibodies were found in a similar patient population [6, 7]. This discrepancy may have been due to the use of a less specific method in previous studies or to the assumption that all (or most) patients in previous studies who had platelet-reactive antibodies had HLA antibodies. Our results clearly show that platelet-specific antibodies were often detected in the presence of HLA antibodies; nevertheless, some patients developed platelet-specific antibodies only.

The natural history of HLA alloimmunization in patients receiving platelet transfusions was studied by Howard and Perkins in 1978 [12]. Sixty percent of patients developed lymphocytotoxic antibodies as early...
as 10 days after the primary exposure. However, the number of transfusions was not related to the likelihood of alloimmunization. A similar observation was made by Dutcher et al [13]. Lee and Schiffer found that HLA alloimmunization occurred sooner in patients with acute myelogenic leukemia than in those with acute lymphocytic leukemia, and it was more likely to occur during the induction of chemotherapy [14]. In the present study, we investigated alloimmunization retrospectively. Several potentially confounding factors were not controlled, including the use of leukocyte reduction filters, the timing of platelet antibody tests, and the disease status of patients. The marginal trend of a great number of platelet transfusions in allo-immunized patients should be interpreted carefully. A controlled cohort study is needed to address the effects of platelet dosage or disease status on HLA antibody production.

Platelet-specific antibodies have been implicated in several clinical situations including neonatal alloimmunization thrombocytopenia, post-transfusion purpura, and platelet refractoriness [1, 2]. The latter has been more commonly related to HLA antibodies. HLA antibody-related refractoriness can be successfully managed using HLA-matched platelet components [1, 2]. Management of platelet-specific antibodies related to refractoriness has been through either cross-matching techniques or provision of platelet alloantigen-deficient platelet components, which are not available in Taiwan. Our study highlights the need for the use of a platelet-specific antibody identification method such as MAIPA (monoclonal antibody-specific immobilization of platelet antigens) [15], and the genotyping of platelet antigens in platelet donors.

In conclusion, HLA antibodies were the most frequently found platelet-reactive antibodies, and platelet-specific antibodies were also common in Taiwanese patients in this series who had received multiple platelet transfusions.

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References