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PLATELET TRANSFUSION IN A PATIENT AFFECTED BY GLANZMANN'S THROMBASTHENIA WITH ANTIBODIES AGAINST GPIIb-IIIa

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ABSTRACT

Patients affected by Glanzmann's thrombasthenia often require blood and platelet transfusions due to bleeding. They may develop antibodies against platelet antigens and become refractory to platelet transfusions.

In this study we present our approach to platelet transfusion in a thrombasthenic patient with platelet antibodies directed against gpIIb/IIIa. We found that platelets from two HPA1b/b donors were weakly positive when matched with the patient's serum. The patient was submitted to dental surgery and platelets were transfused before and after surgery to prevent bleeding. The patient did not experience transfusion reaction and good platelet recovery was observed.

Key words: CD41, cross-match, fibrinogen receptor, Glanzmann's thrombasthenia, platelet transfusion

Glanzmann's thrombasthenia is one of the most frequent inherited disorders of platelet function. It is characterized by a marked reduction in or absence of the platelet fibrinogen receptor (gp IIb/IIIa), which leads to a clinical picture that includes mucocutaneous bleeding, normal or slightly decreased platelet count and normal platelet morphology.[1-3]

These patients are often submitted to platelet and/or blood transfusions due to frequent bleeding and they may develop antibodies against the fibrinogen receptor gpIIb/IIIa glycoprotein. These sera do not usually react with thrombasthenic platelets. Thus it is often difficult to find a suitable donor for bleeding therapy and/or prevention in thrombasthenic patients.

In this study we describe our approach to platelet transfusion in a thrombasthenic patient undergoing dental surgery. The program was designed to prevent possible bleeding due to several dental extractions.

Patient and Methods

The patient was a 52-year-old female affected by Glanzmann's asthenia; her condition fulfilled all the diagnostic criteria mentioned above. The patient had received several blood and platelet transfusions during her life due to gastrointestinal bleeding. In the last two years had experienced some dental problems which required extraction of several teeth as final therapy. Since during her last transfusions she had developed severe nonhemolytic febrile reactions that were refractory to corticorticosteroid pretreatment, she was referred to our Service in the hope of finding a suitable donor.

The patient's platelets and serum, obtained as previously described,[4] were matched with a panel of mAbs (Table 1) and a series of HPA1-typed platelets from donors. A series of mAbs were used to evaluate the phenotype of the patient's platelets, namely CD41a, CD29 (from Coulter Immunology, Hialeah, FL), CD42a, CD61, CD31 (from Becton Dickinson, San José CA), CD36 (from ORTHO, Raritan, NJ), CD41b (from Pharmingen, San Diego, CA), and CD49b, CD42b (from CLB, Amsterdam, the Netherlands); positivity was assessed by standard immunofluorescence and flow cytometry.

An indirect test was used to check the reactivity of the patient's serum with HPA-1a-positive and -negative (HPA1 b/b) platelets, and the analysis was performed by flow cytometry, as described.[4] A cross-match was performed before each transfusion and evaluated by both immunofluorescence and solid phase assay (Capture P, Immucor, Norcross).[5]

Anti-lymphocyte antibodies were screened by standard lymphocytoxicity assay and immunofluorescence.

The patient underwent dental surgery three times over a 4-month interval. For each procedure a suitable donor was submitted to platelet apheresis after giving informed consent and a cross-match was also performed immediately before the transfusion with a new sample of the patient's serum; this last test was designed to check whether different degrees of positivity were found.

The platelet apheresis was divided into two aliquots, one of which was administered before the dental extraction and the other immediately after the procedure. The patient received prednisone (20 mg i.v.) before each transfusion. Platelet counts and the presence of CD41a-positive platelets were determined before and after surgery (1 hour after the end of the platelet transfusion).

Results

As expected, the phenotype of the patient's platelets revealed that gpIIb/IIIa was missing, as demonstrated by negativity for the CD41a, CD41b and CD61 mAbs, while all the other antigens tested were positive.

The patient's serum was tested with 52 different donors and no fully-matched one was found. Fifty samples showed a high fluorescence intensity [mean channel value (MCV) was 38±11] when

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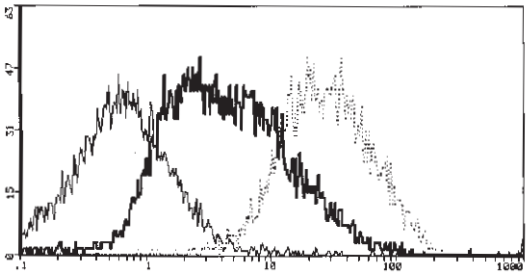

Figure 1. Three different examples of platelet cross-matching evaluated by immunofluorescence.
Negative control (platelet + normal AB serum): —
Weak positive: ▬ . Strong positive: - - -

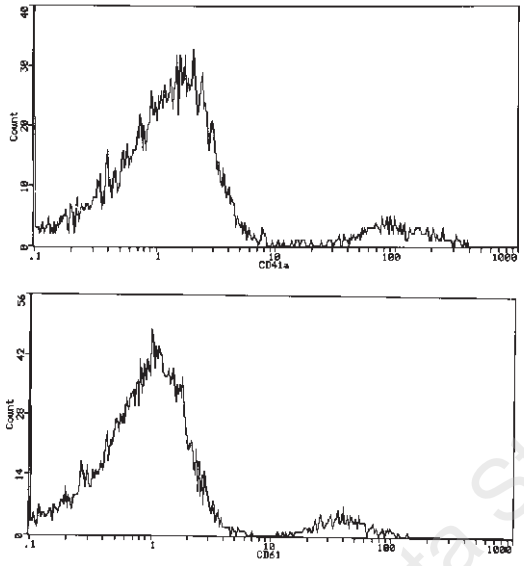

Figure 2. Evaluation of CD41-(upper histogram) and CD61-(lower histogram) positive platelets after transfusion with units obtained from the selected HPA1b/b donors. Histograms obtained after the first dental surgery procedure. The first peak (CD41/CD61-negative platelets) shows the thromboasthenic platelets, while the second peak (CD41/CD61-positive platelets) demonstrates the presence of donor platelets in the patient's blood.
X-axis: fluorescence intensity; Y-axis: number of events

matched with the patient's serum, but a weak positivity was observed with two donors who were HPA1b/b (scored both by immunofluorescence; MCV 3.5±1.5, and by solid phase assay). It should be noted that this latter positivity was termed *weak* in comparison to the kind of reactivity (MCV) obtained with platelets from HPA1a-positive donors (Figure 1). Statistical analysis with the χ-square test showed $p<0.05$. Concerning the solid phase assay, positivity was assessed against the *weak* positive control included in the kit. No anti-lymphocyte antibodies were found. For these reasons these last two donors were selected.

The patient underwent dental surgery three times during a 4-month interval and she experienced no transfusion reactions. The number of platelets transfused was 3.5×10^{11}, 4.2×10^{11}, 3.7×10^{11},and platelets counts were 175, 165, and 201×10^{9}/L before surgery and 196, 188, 224×10^{9}/L one hour after the end of platelet transfusions, respectively. The absolute number of CD41-positive platelets in her blood, as evaluated by flow cytometry (Figure 2), was 18, 21, 19x10^{9}/L, respectively, demonstrating that CD41-positive platelets from the donor were present, although the cross-match showed weak positivity. No bleeding was observed.

Comment

In our study a patient affected by Glanzmann's thrombasthenia, who had received many red blood cells and platelet transfusions during her life, developed anti-gpIIb/IIIa antibodies. To avoid non-hemolytic febrile reactions we used platelet cross-matching to find platelet units that were as compatible as possible. Two weakly reactive donors (HPA1b/b) were found and our patient did not experience any reaction during the transfusions; moreover, clinical benefit (i.e. no post-surgery bleeding) was obtained. We think that the weaker reactivity of these units might have been due to a lower titer of the anti-HPA1b component of the anti-gpIIb/IIIa antibodies with respect to the anti-HPA1a fraction.

Analysis of the absolute number of platelets and results of CD41 antigen analysis[6] after platelet transfusion (Figure 2) documented that the donor platelets survived long enough to prevent bleeding.

In conclusion, cross-matching procedures usually utilized in the presence of anti-HLA antibodies (lymphocytotoxicity)[7] and/or of anti-HPA-defined antibodies (immunofluorescence) may also be very useful in the presence of anti-gpIIb/IIIa antibodies, since weakly reactive HPA-typed donors may be found and clinical benefits may be obtained.

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