Identification of Bak\textsuperscript{b}, a New Platelet-Specific Antigen Associated With Posttransfusion Purpura

By T.S. Kickler, J.H. Herman, K. Furihata, T.J. Kunicki, and R.H. Aster

\textbf{Bak} is a platelet alloantigen whose putative allele, Bak\textsuperscript{b}, has not been identified previously. By using a serum, "Har," obtained from a patient with posttransfusion purpura, we describe the platelet alloantigen Bak\textsuperscript{a}. The Har serum reacted with an NP-40-extractable platelet membrane protein of 142 kd with mobility similar to platelet glycoprotein IIbα. We found that the antigen recognized by the Har serum is inherited in an autosomal dominant mode with an apparent gene frequency of .39. Chi-square analysis of observed and expected phenotype frequencies indicated that serum Har recognizes Bak\textsuperscript{b}, the anticipated allele of Bak\textsuperscript{a}. Our findings provide new evidence for polymorphism of glycoprotein IIb and for the association of posttransfusion purpura with alloimmunization to determinants on this glycoprotein.

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300,000 cpm/mL. After an incubation of one hour at room temperature, the blot was repeatedly washed with Tris-saline buffer. Finally, the nitrocellulose paper was dried and autoradiography carried out by using Kodak Xomatic XRP-6 film for 24 hours as previously described.\(^{13}\)

Two-dimensional electrophoretic analysis. Platelet membrane extracts were analyzed by the method of O’Farrell under nonreduced conditions.\(^{14}\) Fifty micrograms platelet protein was electrophoresed. This was followed by electrophoresis into the second dimension by using SDS-polyacrylamide gel electrophoresis (PAGE) under reduced conditions as described by Phillips and Poh.\(^{15}\) The lane at the left margin of the slab gel was used to electrophorese the platelet lysate in only one dimension under the same conditions as the lysate electrophoresed two-dimensionally. This one-dimensional lane serves as a reference for interpreting the two-dimensional blot. Immunoblots of these two-dimensional gels were then prepared as described in the immunoblotting section.

Solid-phase enzyme-linked immunosorbent assay. To perform the population and family studies, a solid-phase immunobilized antigen assay was used.\(^{16}\) This permitted using the Har serum that contained not only the Bak\(^{b}\) antibody but also multispecific HLA antibody. The principle of the assay is that AP2 (monoclonal antibody specific to IIb-IIIa) is bound to a microtiter well under alkaline conditions. Platelet membrane antigens solubilized in Triton X-100 are then added to the wells, which allows GPIIb-IIIa complex to bind to the AP-2 monoclonal. Approximately 5 \(\mu\)g of total platelet protein is added per well. After washing, antiserum is added to each well, and antibody binding is determined by using a biotin-conjugated murine monoclonal antibody (diluted 1:750 in PBS/gelatin; HB-43, American Type Culture Collection, Rockville, MD) specific for the Fc portion of IgG. HB-43 binds to all IgG subclasses in a 1:1 molar ratio.\(^{17,18}\) HLA-specific antibodies are not detected in the assay.\(^{16}\) The Har serum and anti-Bak\(^{b}\) were diluted 1:25. All antibody incubations were for 60 minutes at room temperature. Serologically typed Bak\(^{a}\), Bak\(^{b}\), P1\(^{A}\), and P1\(^{B}\) donors were used as controls with each experiment.

RESULTS

Serological Studies

The patient’s platelets phenotyped as P1\(^{A}\), P1\(^{A}\), Bak\(^{a}\), P1\(^{B}\), Pen\(^{a}\)-positive, which suggests that the patient’s serum contained an alloantibody to an antigen other than to these known platelet-specific antigens.

On lymphocytotoxic testing of the patient’s serum, it reacted with all 20 of a lymphocyte panel. Testing of the serum against platelets HLA identical to the patient (HLA-A1, -25, -B8, -18) showed reactivity with six of eight panels. The serum did not react with the patient’s autologous platelets or with Glanzmann’s (Type I) thrombasthenic platelets. These results suggested that the serum contained an antibody to a platelet-specific antigen associated with glycoprotein IIb (GPIIb) or GPIIIa and was not an autoantibody.

Western Blotting

To define the antigen against which this antibody was directed, we used SDS-PAGE under nonreduced conditions followed by Western blotting (Fig 1). The Har serum was partially purified by absorption and elution from platelets.\(^{3}\) We found that the Har antibody reacted strongly at 142 kd with a protein that was not detected on Glanzmann’s plate-
autologous ELISA using GPIIb/IIIa immobilized with antigen-capture.

Frequency and estimated gene frequency of the antigen recognized by Har and to determine whether the gene

Family studies. When the solid-phase ELISA assay was used, a pattern consistent with codominant inheritance of Bak and the antigen recognized by Har serum was seen.

Population study. To determine the phenotypic frequency and estimated gene frequency of the antigen recognized by serum Har and to determine whether the gene determining this antigen is in genetic equilibrium with the one determining Bak, platelets from 91 randomly selected, unrelated blood donors were tested with anti-Bak and serum Har in the solid-phase ELISA assay.

Two-Dimensional Immunoblots

Further evidence that the antigen of interest is on GPIIb was obtained by blotting the patient's antibody against an NP-40 membrane extract of platelets after two-dimensional gel electrophoresis under nonreduced and then reduced conditions (Fig 2). In this procedure, any protein that does not have a disulfide bond will be on the diagonal line because its mobility will be the same in both directions. Any shift above or below the line indicates that the protein contains disulfide bonds. As described by Phillips and Poh Agin,13 GPIIbα characteristically falls below the diagonal line and GPIIIa above. In the blot shown in Fig 2, reactivity below the diagonal line at approximately 125 kd is seen showing that the antigen is on IIbα rather than IIIa.

Immunogenetic Studies

Fig 3. PI^A/PI^A^2 phenotypes of 35 normal donors determined by antigen-capture ELISA using GPIIb/IIIa immobilized with monoclonal antibody AP-2. Antibodies used were KRO (anti-PI^A^) and McF (anti-PI^A^2) at a dilution of 1:50. Values on the ordinate are the ratios of optical density (OD) obtained with antibody to OD obtained with autologous serum used at the same dilution. Actual ODs for positive reactions ranged from 0.360 to 1.20. Donors are ranked according to phenotyping results. Donors 1 to 24 tested negative with anti-PI^A^ and strongly positive with anti-PI^A^2. Donors 25 to 34 gave intermediate reactions with both antisera. Donor 35 reacted strongly with anti-PI^A^ but negative with anti-PI^A^2. This individual tested PI^A^-negative in the platelet suspension immunofluorescence test.

Fig 4. Reactions of platelets from 34 randomly selected normal donors with serum KEE containing anti-Bak antibody and serum HAR at a dilution of 1:25 in antigen-capture ELISA using GPIIb/IIIa immobilized with monoclonal antibody AP-2. Values on the ordinate are the ratios of OD obtained with antibody to OD obtained with autologous serum used at the same dilution. Donors are ranked according to presumptive Bak phenotype. Donors 1 to 11 reacted strongly with anti-Bak and were negative with serum HAR. Donors 12 to 30 gave intermediate reactions with both sera. Donors 31 to 34 reacted strongly with serum HAR but were negative with anti-Bak.

Donors 12 to 30 were negative with serum Har and to determine whether the gene
Table 1. Bak Phenotypes of 91 Unrelated White Donors

<table>
<thead>
<tr>
<th>Bak&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Bak&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Bak&lt;sup&gt;ab&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observed</td>
<td>38</td>
<td>42</td>
</tr>
<tr>
<td>Expected</td>
<td>33.67</td>
<td>43.68</td>
</tr>
</tbody>
</table>

\[ \chi^2 = 1.14; P = .30 \] \to \chi^2 = 1.074 \to 1.642. \] \( \chi^2 = 3.841 \) at \( P = .05 \).

Har recognizes the allele of Bak<sup>a</sup>. These findings together with the fact that no "null" platelets were observed for Bak<sup>a</sup> and the putative Bak<sup>b</sup> antigen indicate that serum Har recognizes Bak<sup>b</sup>, the predicted allele of Bak<sup>a</sup>. The calculated gene frequencies of Bak<sup>a</sup> and the putative Bak<sup>b</sup> gene are .61 and .39, respectively. Phenotype frequencies are Bak<sup>a</sup> homozygous, .37; Bak<sup>b</sup> homozygous, .15; and heterozygous, .48.

**DISCUSSION**

Patients transfused with blood may make anti-HLA antibodies and/or antibodies to platelet-specific antigens. The simultaneous development of both types of antibodies complicates the identification of platelet-specific antibodies. To date, laborious serological procedures involving absorption, elution, or serial dilution of antibodies have been used for this purpose. Recently, selective destruction of platelets, these approaches are not always reliable and generally do not permit quantitative analysis of antigen strength, which is helpful in determining homozygosity or heterozygosity. The solid-phase, antigen-capture ELISA assay permits accurate phenotyping of platelets and allows immunogenetic studies to be performed even when sera are contaminated with HLA-specific antibodies.

To date, at least five well-characterized platelet-specific antigen systems have been described. These include PI<sup>4</sup> (Zw), PI<sup>5</sup>, Bak (Lek), Ko, and Pen (Yuk).<sup>1</sup> These antigens are also those beginning to be characterized biochemically. The PI<sup>4</sup> antigen has been localized to an epitope on GPIIIa.<sup>21</sup> PI<sup>5</sup> on GPIIbα,22 Bak<sup>a</sup> on IIb,<sup>23</sup> and Pen on GPIIIa.<sup>16</sup> Our studies demonstrate that the new antigen we describe, Bak<sup>b</sup>, is on GPIIbα as would be expected of an allele of Bak<sup>a</sup>

These studies also demonstrate the genetic polymorphism of platelet GPIIIb. In addition to this report, three other previously uncharacterized antigens have been described within the last 2 years. These antigens include Pen,<sup>5</sup> Yuk,<sup>4</sup> which may be identical to Pen,<sup>16</sup> and Yuk.<sup>4</sup> All antigen systems allow for at least four genetically determined variants of GPIIIa and two variants of GPIIb.

Since the original description of posttransfusion purpura and the implication of the PI<sup>4</sup> immunogen in the pathogenesis of this disorder by Shulman et al,<sup>1</sup> other alloantigens have been shown to be capable of inciting posttransfusion purpura. Ziegler and coworkers first suggested that posttransfusion purpura may be caused by platelet-specific antigens other than PI<sup>4</sup>. Others subsequently implicated Bak<sup>a</sup>,<sup>7</sup> PI<sup>4</sup>,<sup>25</sup> and Pen.<sup>6</sup> Our report shows that posttransfusion purpura can occur in association with alloimmunization against Bak<sup>a</sup>. The cause of posttransfusion purpura is not completely understood. Investigations of its pathogenesis should take into consideration that this condition can apparently be induced by numerous platelet-specific alloantigens.

**REFERENCES**


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