Maternal Platelet Antibody Levels in Neonatal Isoimmune Thrombocytopenia

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ABSTRACT

A case of neonatal isoimmune thrombocytopenia (NIT) is described in which maternal platelet antibody levels decreased during the course of pregnancy. It is suggested that if this disorder is clinically suspected, maternal serum should be assessed for anti-platelet specificity using immunoblot analysis.

Introduction

Neonatal isoimmune thrombocytopenia occurs in approximately one of every 2,000 births and is due to maternal IgG antibody directed against antigens present on the platelet of the child but absent on maternal platelets. Sensitization may occur relatively early in gestation since it has been shown at PL A1 and Lek antigenic groups appear as early as 16 to 19 weeks of gestation.

It has been suggested that changes in the level of maternal antibody during the course of pregnancy may be useful in predicting the occurrence of isoimmune neonatal thrombocytopenia; however, no definitive studies are yet available. Our experience is presented with the use of a sensitive platelet antibody assay system and the suggestion that monitoring maternal levels of platelet antibody is not ideal.

Case Report

In November 1987, a 27 yr G1 PO delivered a child with severe thrombocytopenia. Maternal platelet count was normal. A post partum maternal serum sample was positive for the presence of anti-platelet antibodies up to a dilution of 1:20 of her serum, but no studies were performed to determine the platelet antigen specificity. In August 1989, at approximately 32 weeks gestation with her subsequent pregnancy, a serum sample was again analyzed for anti-platelet antibody reactivity. Although antibody could still be detected, the titer had decreased, now being positive in the neat sample only. Several anti-human leukocyte antigen (HLA) antibodies were detected in her serum including anti-B5, B35, B53 and B17. Approximately eight weeks later she delivered a child with marked thrombocytopenia, but her platelet count was again normal. A repeat platelet antibody assay this time showed a marked increase in her titer, being positive at a 1:40 dilution of her serum and in strength of reactivity. On the assumption that the cli-
nician was dealing with a case of isoimmune neonatal thrombocytopenia, the child received a transfusion of washed maternal platelets and had a successful outcome. There was no evidence of intracranial hemorrhage and the child continues to do well.

Methods

An indirect immunofluorescence platelet antibody assay* was performed as previously described. In brief, a 0.1 ml aliquot of thawed HLA type platelet pool at a final concentration of $250 \times 10^9$ per ml after washing was incubated with 0.01 ml of either normal control serum or patient serum or dilutions of each for one hour at room temperature. After centrifugation and repeated washings, the platelet pellet was incubated with 0.1 ml fluorescein-conjugated goat (Fab')3 antihuman IgG, A, M antiserum for 15 minutes at room temperature. After repeated centrifugation and washing, the platelet pellet was resuspended in phosphate-buffered saline to a final volume of two ml. The amount of fluorescein conjugate bound in each platelet suspension was then measured using flow cytometric analysis.

For this assay, region A on the spectrum III was defined as consisting of all 255 fluorescence intensity channels. The intensity of fluorescence, expressed as the mean value or region A, is proportional to the amount of serum IgG, A, M bound to the platelet membrane. The mean channel A reading for the test serum plus platelet mixture was then compared to that of the control serum plus platelet mixture, and a ratio of relative fluorescence was determined. A ratio of $>1.3$ of test serum/control serum is compatible with the presence of platelet-directed antibody in the test serum.

Patient serum samples were obtained on three occasions: immediately post-partum in 1987, at approximately 32 weeks gestation in 1989, and immediately post-partum at approximately 40 weeks gestation in 1989. The serum samples were screened for the presence of anti-HLA antibodies using a panel of lymphocytes from 40 HLA-type normal donors.

PLATELET PREPARATION/ ELECTROPHORESIS/IMMUNOBLOTTING

Platelet rich plasma was prepared from blood collected in ethylenediamine tetraacetic acid (EDTA) tubes by centrifugation at 150 g for 20 minutes. The supernatant was then centrifuged at 1300 g for 10 minutes. The platelet button was then resuspended in Ringer's-EDTA containing 10 mM benzamidine HCL and 100 jg per ml of soybean trypsin inhibitor. After several washings, sufficient volume was added to achieve a platelet concentration of $1 \times 10^9$ per ml. To this final suspension was then added 1:40 volume of 40 percent Triton X-100. The platelet suspension was then incubated at 37°C for one hour and refrigerated at 4°C overnight.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis was carried out as described by Laemmli using a five percent to 20 percent gradient gel as the separating gel and five percent polyacrylamide as the stacking gel. Twenty-five jg of platelet suspension ($1 \times 10^9$ platelets) were mixed with 25 jg of a solution containing 0.002 percent bromphenol blue, four percent sodium dodecyl sulfate and 0.125M Tris, and 20 percent glycerol, pH 6.0 and incubated for five minutes at 100°C prior to loading. Electrophoresis was carried out for 16 hours at 20 volts. The voltage was increased to 80V for two additional hours.

Proteins were transferred from the polyacrylamide gel to nitrocellulose membrane as described by Towbin et al.10 The portion of the paper carrying the molecular weights standards was

* Ortho Spectrum III flow cytometer.
stained with 0.125 percent Coomassie blue, and the remainder was cut into strips corresponding to the electrophoresis lanes and placed in blocking buffer containing five percent non-fat dry milk in 0.01 M Tris, 0.17 M sodium chloride, pH 7.5.

After overnight incubation at room temperature, the strips were extensively washed and then incubated with appropriate antibody-containing preparations including 1:10 dilution of each of the patient’s serum samples as indicated or normal control serum. Mouse monoclonal anti-human GP IIa was diluted in 1:500 in Tris buffered saline. The strips were incubated at room temperature overnight on a shaker bath. After washing, a solution of peroxidase-conjugated goat antibody to either mouse IgG or human IgG was added to appropriate strips and the strips incubated for one hour at room temperature. The strips were then developed using 4-chloral-1-naphthol as an indicator reagent.

Results

The anti-platelet antibody activity of the patient’s serum samples is shown on table I. The November 1987 sample showed a significant antibody titer; however, since the assay utilized a HLA-type platelet pool as target cells, no distinction between anti-HLA or anti-platelet specific antibody could be made. It is of interest to note that on the patient’s second sample (August 1989) there was actually a decrease in her antibody titer at a time when she was approximately 32 weeks gestation with her second pregnancy. Four specific anti-HLA antibodies were detectable in her serum at this point. Eight weeks later, at the time of her delivery, the patient’s serum antibody had again increased in titer, now being strongly positive at a 1:40 serum dilution, and showed an increase intensity of reactivity with a MCH A ratio of 2:35.

Because of the unexpected thrombocytopenia in the newborn and the sudden change in maternal serum anti-platelet reactivity, all three serum samples were analyzed to determine antibody specificity to normal platelet glycoproteins. These results are illustrated in figure 1. Although not present on the November 1987 sample, there is a distinct band of reactivity with a 98 Kd glycoprotein noted in the August 1989 sample. The intensity of this band is increased in the October 1989 sample. The control lane using mouse anti-human glycoprotein IIIa shows these bands to be the same. Unfortunately, platelet antigen typing could not be performed on the mother to confirm her PLA<sup>1</sup> status.

Discussion

The diagnosis of neonatal isoimmune thrombocytopenia in this case would seem certain: the newborn was severely thrombocytopenic while maternal platelet count was normal at all times. There was no indication of sepsis. Maternal antibodies were present in increasing titer which reacted with a 98 Kd platelet glycoprotein, i.e., GPIIIa implying that she lacked an epitope on this protein which resulted in sensitization during pregnancy. Statistically, this is probably
an anti-PLA\textsuperscript{1} antibody; however, it is also known that the Yuk(Pen) antibody can also be associated with neonatal isoimmune thrombocytopenia (NIT).\textsuperscript{5} It is also theoretically possible that the antibody developed to an as yet undefined platelet antigen group. Since platelets were not available for antigen typing from either the parents or the child, no definitive conclusion can be reached regarding the platelet antigen lacking on the maternal platelets.

This study, although only a single case, thus suggests that monitoring maternal anti-platelet antibody titers, especially in our assay system which detects not only anti-platelet glycoprotein but also anti-HLA antibodies, can be misleading. The authors were aware that this patient had several anti-HLA antibodies, including anti-B5, B35, B53, and B17. The HLA sensitization during pregnancy is relatively common; however, such antibodies have only rarely been reported to cause NIT.\textsuperscript{9} The relatively low antibody titer on the August '89 serum sample, compared to that of November '87, was interpreted as indicating that maternal sensitization was minimal at best.

It is known that platelet specific antigens are present early in gestation and that if sensitization had occurred evidence of such sensitization, i.e., increasing antibody titers, would have been evident by approximately 32 weeks gestation. Our findings of decreasing titer is similar to report by Kaplan et al\textsuperscript{4} in a study of seven patients with NIT secondary to anti-PLA\textsuperscript{1} antibody. It was shown that in two patients the titer had actually decreased during the course of pregnancy, and, in a third, no serum antibody was evident at any time. The basis for a decrease in titer during a period of active sensitization is unclear. It is evident, however, from the immunoblot analysis in our case that antibody activity against GPIIIa was evident in the August '89 sample despite the low serum antibody activity determined by flow cytometric analysis. Whether or not increased sensitivity could be achieved by performing immunoblot assays on suspected cases needs to be further studied.

References


