Normal Pregnancy in a Patient With a Prior Postpartum Factor VIII Inhibitor: With Observations on Pathogenesis and Prognosis

By Barry S. Coller, Mae B. Hultin, Leon W. Hoyer, Frederick Miller, Joan V. Dobbs, Michael H. Dosik, and E. Roy Berger

A 22-yr-old primigravada developed a hemorrhagic diathesis 6 days after delivering a normal female infant and was found to have an immunoglobulin inhibitor of factor VIII (15 Bethesda units). The patient was treated with prednisone and the bleeding stopped soon thereafter. Her inhibitor titer decreased over the next 8 mo, at which time no inhibitor was detectable. Nine months later she became pregnant again and proceeded to have an uneventful pregnancy and delivery of a normal female infant without evidence of a recurrence of the inhibitor. Studies of H-thymidine incorporation into the patient’s lymphocytes in the presence of her own plasma or plasmas from her husband, normals, a von Willebrand patient, and a hemophilic patient yielded equivocal results. Analysis of VIII:CAg using 2 different antisera failed to discern immunologic differences between the VIII:CAg of the patient, her husband, or her first child. A review of the literature revealed that there were no recurrences with second pregnancies in any of the 8 patients with postpartum factor VIII inhibitors whose inhibitors had completely disappeared prior to delivery. While the pathogenesis of this disorder remains uncertain, the apparently favorable prognosis for such patients should be considered in counseling with regard to future pregnancies.

While the development of immunoglobulin inhibitors of factor VIII has been well documented to occur in the postpartum state, there is scant information on the mechanism of such antibody production and the prognosis of these patients with regard to subsequent pregnancies. The occurrence of such an inhibitor in a young woman after her first pregnancy stimulated us to investigate these issues further.

Materials and Methods

Coagulation and Platelet Aggregation Studies

Blood was collected into polypropylene tubes containing 0.01 volume 40% sodium citrate. It was centrifuged either at 1000 g at 4°C for 3.5 min for platelet-rich plasma (for aggregation studies) or 3500 g at 4°C for 30 min for platelet-poor plasma (for all other assays). Prothrombin times (PT) employed a rabbit-brain source of tissue factor (Simplastin, General Diagnostics, Morris Plains, N.J.) and activated partial thromboplastin times (PTT) and specific factor assays employed celite as the activator (Platelein-Plus, General Diagnostics). Factor VIII coagulant activity (VIII:C) and factor IX coagulant activity were measured by a modified one-stage clotting assay using known hemophilic plasmas as substrates. Assay for factor VIII inhibitor titer was performed by the method of Kasper et al. and expressed as Bethesda units. Immune-complex assays were performed as previously reported using the 40% saturated ammonium sulfate fractions of rabbit antisera to human IgG, IgA, IgM, kappa, and lambda light chains (Behring-Calbiochem, Somerville, N.J.). Von Willebrand factor (ristocetin cofactor) was assayed with a timed macroscopic clumping assay using formaldehyde-fixed platelets. Factor VIII/von Willebrand factor antigen was measured by electroimmunoassay according to a modification of the method of Laurell in 1% agarose utilizing a water-cooled electrophoresis apparatus. Factor VIII coagulant antigen (VIII:CAg) was measured by an immunoradiometric assay employing radiolabeled and affinity-purified Fab' prepared from two different high-titer human factor VIII antisera. The VIII:CAg studies were all performed on a single occasion on samples that had been frozen at −70°C for between 3 and 9 mo.

Platelet Retention

Ten milliliters of blood were drawn without bubbling into a syringe containing 40 μl of 1000 U/ml heparin (Panheprin, Abbott Labs, North Chicago, Ill.). The syringe was gently twirled and the blood pumped at 5.7 ml/min through a column of polyvinyl tubing (Becton-Dickinson, Rutherford, N.J., no. 6238, internal diameter 0.118 inch) containing 2.6 g of glass beads of 0.5 mm diameter (Potter Industries, Carlstadt, N.J.). The blood was collected in 1 ml amounts into plastic tubes containing 10 μl of 10% Na₂EDTA. Platelet retention was calculated by subtracting the mean platelet count of the 5th and 6th milliliters from the platelet count of the heparinized blood and expressing it as the percentage retained.

Lymphocyte Transformation Studies

Lymphocytes were separated from heparinized blood by centrifugation on a Ficoll-Hypeaque gradient and then washed 3 times in culture medium (RPMI 1640, Gibco, Grand Island, N.Y.) containing 100 U/ml penicillin and 0.1 mg/ml streptomycin. The cells were then suspended in the above culture medium to which was added the plasma to be tested (10% v/v) and heparin (10 U/ml). The cells were then aliquoted into microculture plates so as to contain 10³ cells/well (0.2 ml) and incubated at 37°C for 6 hr in a 5% CO₂ environment. At that point, 10 μl of methyl-1H-thymidine (New England Nuclear, Boston, Mass.; 6 Ci/mM) was added and the cells incubated for an additional 18 hr. The cells were then collected and processed on a multiple-sample automatic harvester (Otto Miller Co., Madison, Wis.) and processed as described previously. Samples were counted in a scintillation counter and expressed as dpm using external standards ratios. Each value represents the mean ± SEM of quintuplicate or sextuplicate cultures. The signifi-
CANCE OF DIFFERENCES BETWEEN SAMPLES WAS DETERMINED BY STUDENT'S T TEST.

CASE REPORT

The patient was a 22-yr-old primigravida, estimated date of confinement 8/27/77, who had the onset of labor with spontaneous rupture of membranes on 8/25. The labor initially progressed slowly, requiring the use of oxytocin, but then progressed well with delivery of a normal 6 lb 14 oz female infant (Apgar 10) on 8/26 by low forces over a median episiotomy. The patient suffered a third degree laceration of the perineum, but the repair and her immediate postpartum course were considered uneventful and she was discharged on 8/29. On 9/2 the patient had severe vaginal bleeding and was readmitted. She was treated with 2 U of blood, methergine, and intravenous oxytocin and then underwent a curettage during which a small amount of decidual tissue and 500 ml of blood and blood clots were removed from the vagina and uterus. Postoperatively, the patient became febrile and was treated with antibiotics. On 9/7 vaginal bleeding was again noted, which persisted despite oxytocin and methergine treatment. Three PTTs were performed over the course of that day with values of 37.5, 31.0, and 42.5 sec (normal < 35 sec). Other data obtained at that time were: PT 11.0 sec/control 11.0 sec, fibrinogen 300 mg/dl, platelet count 300 x 10^9/liter, fibrin(ogen) degradation products > 10 but < 40 μg/ml, clot lysis normal, bleeding time 5.5 min. A second curettage was performed on 9/8. A small amount of bleeding was noted from the episiotomy site; despite suturing this continued to ooze. In addition, there was some bleeding from the cervix at the tenacula sites but this was said to respond well to suturing. The patient remained stable until 9/11, but then began having additional vaginal bleeding and was treated with oxytocin and 1 U of fresh plasma. The bleeding persisted and fresh frozen plasma was given every 6 hr. On 9/13 a preparation of conjugated estrogens was added to the regimen. Coagulation studies on 9/14 revealed a PTT of 44.0 sec and a PTT of a 1:1 mixture of patient and normal plasma tested immediately after mixing of 29.1 sec. The PTT remained elevated at 44-47 sec over the next 4 days. A sample of the patient's plasma was sent to the State University of New York at Stony Brook on 9/18, where the results shown in Table 1 were obtained. The patient was diagnosed as having an inhibitor to factor VIII, and prednisone (60 mg/day) was started and plasma infusions discontinued. On 9/20 the bleeding became minimal and the patient was discharged. As shown in Fig. 1, the patient's inhibitor titer declined over the next several months and her factor VIII activity returned to the normal range. The steroid therapy was tapered and then stopped over the same period. The patient subsequently became pregnant again in March 1979. The pregnancy was uneventful and she went into labor on 12/10/79. The fetus was found to be a double footling breech and when progression of labor stopped after approximately 7 cm of cervical dilatation, a primary cesarian section was performed with the uneventful delivery of a normal female infant. The postpartum course was unremarkable. Factor VIII determinations on the first child's and the husband's plasmas in September 1980, 3 yr after the first delivery, were within normal limits (158 and 122%).

RESULTS

Immunoneutralization assays performed on plasma obtained three weeks after the delivery in 1977 showed partial neutralization of the inhibitor by anti-IgG (70%), anti-IgM (40%), anti-kappa (40%), and anti-lambda (50%). No neutralization was seen with anti-IgA (the presence of excess anti-IgA in the assay being proven by Ouchterlony analysis). Thus the inhibitor was polyclonal, with both IgG and IgM being detectable as well as both light chain types.

Lymphocyte transformation studies were performed at 3, 6, and 23 mo postpartum, the last being performed when the patient was 4 mo into her second pregnancy (Table 2). At 3 mo the incorporation of 3H-thymidine into the patient's lymphocytes was significantly greater in the presence of her husband's plasma than her own plasma (p < 0.05), hemophilic plasma (p < 0.01), or von Willebrand plasma (p < 0.01). The response in the presence of a pool of 8 normal plasmas was intermediate; it did not differ significantly from the response in the presence of her husband's plasma (0.4 > p > 0.3), but when compared to the response in the presence of her own plasma, the increase was only of borderline significance (0.10 > p > 0.05). Also of note was the dramatic increase in thymidine incorporation of the patient's cells in the presence of normal serum (11,158 ± 353 dpm) when compared to our experience with 40 normals (895 ± 102 dpm). This can be taken to indicate an increase in

---

Table 1. Initial Laboratory Data

<table>
<thead>
<tr>
<th>Test</th>
<th>Result (Patient/Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prothrombin time</td>
<td>12.6/13.0 sec</td>
</tr>
<tr>
<td>Activated partial thromboplastin time</td>
<td>74/45 sec</td>
</tr>
<tr>
<td>Factor VIII</td>
<td>8%</td>
</tr>
<tr>
<td>Factor IX</td>
<td>90%</td>
</tr>
<tr>
<td>Factor VIII inhibitor</td>
<td>15 Bethesda units</td>
</tr>
<tr>
<td>Immunoneutralization</td>
<td>IgG and IgM (no IgA)</td>
</tr>
<tr>
<td>von Willebrand's factor (ristocetin cofactor)</td>
<td>kappa and lambda light chains</td>
</tr>
<tr>
<td>Factor-VIII-related antigen (von Willebrand's factor antigen)</td>
<td>90%</td>
</tr>
<tr>
<td>Platelet aggregation</td>
<td>184%</td>
</tr>
<tr>
<td>ADP</td>
<td>2 waves at 2.2 μM (normal)</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>2 waves at 2.7 μM (normal)</td>
</tr>
<tr>
<td>Ristocetin</td>
<td>Normal initial slope at 1.8 and 1.2 mg/ml</td>
</tr>
<tr>
<td>Collagen</td>
<td>Normal single wave</td>
</tr>
<tr>
<td>Platelet retention</td>
<td>&gt;94% (normal &gt;85%)</td>
</tr>
</tbody>
</table>
basal activity of the cells. At 6 mo, the response in the presence of the husband’s plasma and the normal pool were both significantly greater ($p < 0.01$) than the response in the presence of the patient’s own plasma, with the response to the husband’s plasma again being strongest. Control studies showed that there was no significant difference in the response of control cells incubated in the presence of the patient’s or her husband’s plasma. Technical problems unfortunately invalidated the results with hemophilic and von Willebrand plasmas. At 23 mo (during her second pregnancy), the patient’s lymphocytes again incorporated more $^3$H-thymidine into DNA in the presence of her husband’s plasma ($p < 0.01$) and normal pool plasma ($p < 0.01$) than her own plasma, but these studies were complicated by the finding that control cells also responded less well in the presence of the patient’s plasma than in the presence of the husband’s, pooled normal, hemophilic, or von Willebrand plasmas ($717 \pm 108$ dpm versus $1125 \pm 99$, $1868 \pm 197$, $2554 \pm 278$, and $1307 \pm 223$ dpm, respectively; $p < 0.05$ for comparison of patient to each of the other plasmas), raising the possibility that the patient’s plasma had an inhibitor of blast transformation. The presence of an inhibitor of blast transformation in the plasma of women during pregnancy has previously been observed by others.$^{12,13}$ The response of the patient’s cells to hemophilic or von Willebrand plasma was not significantly less than that to the patient’s own plasma.

Factor VIII coagulant antigen studies using the 2 antisera revealed 15% and 17% of normal VIII:CAg in the patient sample obtained at the time of diagnosis (9/18/77); that same sample contained 8% VIII:C. In contrast, a sample obtained 9 mo after the second pregnancy contained 112% and 108% VIII:CAg with the 2 antisera and 129% VIII:C. The husband’s and first child’s plasmas were found to contain 130% and 123% VIII:CAg, respectively (averages of the results with the two antisera; VIII:C values were 122% and 158%). Multiple dilutions of the patient’s plasma and the plasmas of her family members gave parallel lines.

### Table 2. $^3$H-Thymidine Incorporation Studies on Patient’s Lymphocytes at 3, 6, and 23 mo Postpartum

<table>
<thead>
<tr>
<th>Plasma Source</th>
<th>3 mo</th>
<th>6 mo</th>
<th>23 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>13,751 ± 2,258</td>
<td>3,547 ± 231</td>
<td>1,201 ± 189</td>
</tr>
<tr>
<td>Husband</td>
<td>22,510 ± 2,637</td>
<td>6,937 ± 578†</td>
<td>2,925 ± 475†</td>
</tr>
<tr>
<td>Normal plasma pool</td>
<td>19,155 ± 1,745†</td>
<td>5,393 ± 172†</td>
<td>2,982 ± 305†</td>
</tr>
<tr>
<td>Hemophilic</td>
<td>15,601 ± 1,150§</td>
<td>3,889 ± 170†</td>
<td>2,460 ± 320†</td>
</tr>
<tr>
<td>von Willebrand</td>
<td>12,607 ± 940§</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* $^3$H-thymidine was added 18 hr before harvesting and determining the amount of $^3$H-thymidine incorporated into DNA.

† $p < 0.10$ compared to patient plasma.

§ Not significantly different compared to patient plasma.
in the immunoradiometric assay. Thus, the two anti-
sera used did not recognize significant antigenic
differences between the VIII:CAg from the patient
and her family members.

**DISCUSSION**

The pathogenesis of postpartum factor VIII anti-
bodies remains obscure. One hypothesis is that allo-
types of factor VIII exist and that the mother becomes
sensitized to the paternal allotypic determinants inher-
ited by the fetus and that the antibodies produced then
cross-react with the patient’s own factor VIII. In fact,
circumstantial evidence has been advanced to support
the idea that there are allotypes of factor VIII coagu-
ulant activity, and this would be quite reasonable
given the data with other proteins such as hemoglobin.
Our studies of blast transformation lend some addi-
tional support to this hypothesis, since the patient’s
lymphocytes incorporated more 3H-thymidine into
DNA when incubated in the presence of her husband’s
plasma than her own, hemophilic, or von Willebrand
plasma. These studies must be interpreted with great
cautions, however, since: (1) while the differences were
statistically significant, they were not dramatically
different, (2) whole plasmas rather than purified
factor VIII was employed, (3) a single concentration
of plasma was used, (4) the patient was taking steroids
at the time of the first assays, (5) the responses in the
presence of von Willebrand and hemophilic plasma at
23 mo did not differ from those employing the
husband’s or pooled normal plasma. This last objec-
tion may not be of great consequence since the patient
was many months away from the original pregnancy
and was now pregnant again. If the allotypic hypothe-
sis were correct, one would expect that inhibitors
would only occur during pregnancies with female
fetuses, since factor VIII is coded for on the X chro-
mosome and thus paternal transmission of an X chro-
mosome would result in the fetus being female. While
this criterion was met in our own case, in fact a review
of the literature revealed that of the 33 reported
cases, the sex of the child was reported in 12 and of
these 7 were males. Another observation that appears
to be at variance with the allotypic hypothesis is that
anamnestic responses to replacement therapy have not
been a problem in this disorder; there was no evidence
of such a response in our patient either. One might
anticipate that infusion of factor VIII from many
donors would elicit the same response as the husband’s
factor VIII. One would therefore have to postulate
that the pregnant state induces a change in the
immune system to permit it to recognize these allo-

---

**Table 3. Results of Second Pregnancies in Patients With Prior Postpartum Factor VIII Inhibitors**

<table>
<thead>
<tr>
<th>Year of Report</th>
<th>Author(s)</th>
<th>Age at Onset</th>
<th>Prior Obstetrical History</th>
<th>Implicated Pregnancy (Sex of Child)</th>
<th>Time to Symptoms Postpartum (Days)</th>
<th>Age at Second Pregnancy</th>
<th>In Remission at Delivery</th>
<th>Resulting Delivery (Sex of Child)</th>
<th>Recurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1946</td>
<td>Chargriff and West*</td>
<td>30</td>
<td>1 Spont ab</td>
<td>Normal (7)</td>
<td>0</td>
<td>32</td>
<td>No</td>
<td>Normal</td>
<td>1</td>
</tr>
<tr>
<td>1949</td>
<td>Hewlett and Haden</td>
<td>39</td>
<td>1 Spont ab</td>
<td>Normal (7)</td>
<td>90</td>
<td>41</td>
<td>Yes</td>
<td>Normal</td>
<td>0</td>
</tr>
<tr>
<td>1953</td>
<td>Frick</td>
<td>24</td>
<td>None</td>
<td>Normal (male)</td>
<td>90</td>
<td>25</td>
<td>No</td>
<td>Normal (male)</td>
<td>0</td>
</tr>
<tr>
<td>1957</td>
<td>Zbinden and Leopold</td>
<td>20</td>
<td>None</td>
<td>Normal (? )</td>
<td>270</td>
<td>20</td>
<td>Yes</td>
<td>Normal (? )</td>
<td>1</td>
</tr>
<tr>
<td>1961</td>
<td>Margolius et al.</td>
<td>27</td>
<td>6 Ni deliv</td>
<td>Normal (7)</td>
<td>90</td>
<td>29</td>
<td>No</td>
<td>Therapeutic ab-3rd month</td>
<td>1</td>
</tr>
<tr>
<td>1961</td>
<td>Margolius et al.</td>
<td>24</td>
<td>None</td>
<td>Normal (? )</td>
<td>60</td>
<td>26</td>
<td>Yes</td>
<td>Normal (Female)</td>
<td>10</td>
</tr>
<tr>
<td>1964</td>
<td>Walpot</td>
<td>25</td>
<td>?</td>
<td>Normal (? )</td>
<td>2</td>
<td>28</td>
<td>Yes</td>
<td>Normal Premature (? )</td>
<td>0</td>
</tr>
<tr>
<td>1977</td>
<td>Voke and Letsky</td>
<td>33</td>
<td>None</td>
<td>Normal (? )</td>
<td>20</td>
<td>33</td>
<td>Yes</td>
<td>Normal (? )</td>
<td>10</td>
</tr>
</tbody>
</table>

*Also reported by Conley et al. and Margolius et al.*
†Bleeding symptoms were present before second pregnancy and reappeared within several weeks after delivery, but apparently not during the pregnancy or delivery.
‡The patient had a partial clinical remission prior to the second pregnancy. The early pregnancy was uneventful, but gross hematuria and subcutaneous hemorrhages occurred 2 wk before term. The delivery was uneventful but diffuse hemorrhage occurred on the second postpartum day and persisted for 41 days.
§The inhibitor was present at the onset of the second pregnancy and diminished and finally disappeared before delivery.
¶There was no excessive bleeding associated with the abortion, but persistent menometrorrhagia began soon thereafter.
**The inhibitor was present at the onset of the second pregnancy, diminished in titer during the pregnancy and disappeared completely approximately 4 wk before delivery.
tropic changes or that there is something unique to the transplacental as compared to the intravenous route of sensitization. Our VIII:CAg results are also at variance with what would be expected from the allotypic hypothesis. Although a difference in the slope of the VIII: CAg assay has only been identified in a single hemophilic patient,29 a marked difference in antigenic structure might possibly lead to the recognition of a change in the assay slope when plasmas of the patient, her husband, or child are compared. No such differences were observed.

A review of the literature resulted in the identification of eight patients in addition to our own who became pregnant again following the occurrence of a postpartum factor VIII inhibitor (Table 3). Of these, five were in remission at the time of the second delivery and none of these patients had a recurrence of her inhibitor. Interestingly, two of these patients actually had the inhibitors present at the onset of their second pregnancies but their antifactor VIII titers continued to decrease and finally disappeared before the second delivery. In addition to these eight reported cases, we have been able to learn of three additional cases by personal communications with other investigators15-17 wherein patients with postpartum factor VIII inhibitors subsequently became pregnant again and had uneventful pregnancies. With our case, that means that subsequent pregnancies were uneventful in all nine cases of women with postpartum factor VIII inhibitors who were free of the inhibitor at the time of delivery. We do not know of any cases in this category in which relapse did occur. This might also be taken as evidence against the allotypic hypothesis since one would expect subsequent sensitization by approximately half of the second daughters. However, it is interesting to note that after pregnancy women appear to respond less well to their husband's lymphocytes in mixed lymphocyte culture.18,19 Since this effect is accentuated with increasing parity, it suggests that some active form of immunologic tolerance is involved. In conclusion, while the pathogenesis remains uncertain, it would appear that the prognosis for subsequent pregnancies in patients with postpartum factor VIII inhibitors is likely to be favorable in those achieving complete remissions.

ACKNOWLEDGMENT

We would like to thank Dorene Turi, Penny Hausser, James Hartnett, and Carl Carta for outstanding technical assistance and Jennifer Floyd and Vesta Carucci for outstanding secretarial assistance.

REFERENCES

7. Eyster ME, Jones MB, Moore T, Laurent D-B: Carrier detection in classic hemophilia by combined measurement of immunologic (VIII AGN) and procoagulant (VIII AHF) activities. Am J Clin Pathol 65:975, 1976
15. Nilsson IM: Personal communication
16. Biggs R, Rizza CR: Personal communication
17. Lurie A: Personal communication
Normal pregnancy in a patient with a prior postpartum factor VIII inhibitor: with observations on pathogenesis and prognosis

BS Coller, MB Hultin, LW Hoyer, F Miller, JV Dobbs, MH Dosik and ER Berger