Immunosuppressive Therapy of Factor VIII Inhibitors

By Mae B. Hultin, Sandor S. Shapiro, Herbert S. Bowman, Frances M. Gill, A. Thomas Andrews, Jose Martinez, M. Elaine Eyster, and William C. Sherwood

Immunosuppressive therapy was used in seven hemophiliac and three nonhemophiliac patients with factor VIII inhibitors. Permanent disappearance of the inhibitor occurred in three hemophiliac and two nonhemophiliac patients following treatment with cyclophosphamide and factor VIII. Critical factors influencing the response to therapy may include both the titer and duration of the inhibitor and the degree of intervening factor VIII exposure prior to immunosuppressive therapy. Two severe hemophiliacs with low titer inhibitors that disappeared without specific therapy are also reported.

During the past 10 years numerous studies have clearly demonstrated the antibody nature of acquired factor VIII inhibitors, which occur in 6%-10% of hemophiliacs and in some previously normal individuals. This finding suggested the possible benefit of immunosuppressive agents in the treatment of these patients. Since the initial report of the partial suppression of two factor VIII inhibitors with 6-mercaptopurine, immunosuppressive therapy has been attempted in many patients with varying success. Complete disappearance of the inhibitor has been reported following therapy in several nonhemophiliacs, but similar success has rarely been obtained in patients with hemophilia A. A recent report of the collective experience from several centers showed that regimens including factor VIII with cyclophosphamide may suppress the anamnestic response in hemophiliacs with inhibitors. Variables other than drug regimen could not be clearly analyzed because of the different methods used at the many centers contributing data.

The present analysis of seven hemophiliac and three nonhemophiliac patients treated at three centers using comparable assay techniques suggests that both the titer and duration of the inhibitor prior to therapy affect the outcome of immunosuppressive treatment. The degree of factor VIII exposure between inhibitor appearance and attempted immunosuppression may also be an important factor. In addition, interpretation of therapeutic outcome is complicated by the occasional spontaneous disappearance of the inhibitor. Since this phenomenon has not been well documented in severe hemophiliacs, we have included two such patients in this report.

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Table 1. Factor VIII Inhibitors in Hemophilia A

<table>
<thead>
<tr>
<th>Case</th>
<th>Clinical Severity</th>
<th>Previous Factor VIII Level (U/ml)</th>
<th>Age at Discovery (yr)</th>
<th>Maximum Inhibitor Titer</th>
<th>Known Duration of Inhibitor</th>
<th>Titer at Therapy</th>
<th>Therapy</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Severe</td>
<td>&lt;0.01</td>
<td>36</td>
<td>1:20</td>
<td>12 mo</td>
<td>Negative</td>
<td>Cp, VIII, Pred</td>
<td>Disappearance</td>
</tr>
<tr>
<td>2</td>
<td>Severe</td>
<td>0.019</td>
<td>7</td>
<td>1:5</td>
<td>1 wk</td>
<td>1.50</td>
<td>Aza</td>
<td>Persistence</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>20 mo</td>
<td>Exch T↓, Cp, VIII</td>
<td>Disappearance</td>
</tr>
<tr>
<td>3</td>
<td>Moderate</td>
<td>0.02-0.04</td>
<td>38</td>
<td>1.5</td>
<td>1 wk</td>
<td>1.5</td>
<td>Cp, VIII, Pred</td>
<td>Disappearance</td>
</tr>
<tr>
<td>4</td>
<td>Severe</td>
<td>0.02</td>
<td>29</td>
<td>1:1600</td>
<td>8 yr</td>
<td>1:2</td>
<td>CP, VIII, Pred</td>
<td>Persistence</td>
</tr>
<tr>
<td>5</td>
<td>Severe</td>
<td>0.02</td>
<td>21</td>
<td>1:300</td>
<td>2 wk</td>
<td>1:300</td>
<td>VIII, Pred, Exch T↓</td>
<td>Persistence</td>
</tr>
<tr>
<td>6</td>
<td>Severe</td>
<td>0.02</td>
<td>21</td>
<td>1:300</td>
<td>4 wk</td>
<td>1:300</td>
<td>Aza</td>
<td>Persistence</td>
</tr>
<tr>
<td>7</td>
<td>Severe</td>
<td>&lt;0.01</td>
<td>11</td>
<td>1:160</td>
<td>9 mo</td>
<td>1:160</td>
<td>Exch T↓, Aza</td>
<td>Persistence</td>
</tr>
<tr>
<td>8</td>
<td>Severe</td>
<td>0.02</td>
<td>13</td>
<td>1:2</td>
<td>—</td>
<td>—</td>
<td>Cp, VIII, Pred</td>
<td>Persistence</td>
</tr>
<tr>
<td>9</td>
<td>Severe</td>
<td>0.02</td>
<td>13</td>
<td>1:10</td>
<td>—</td>
<td>—</td>
<td>untreated</td>
<td>Disappearance</td>
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Cp, cyclophosphamide (i.v. unless otherwise stated); Pred, prednisone, p.o.; Aza, azathioprine p.o.; Exch T↓, exchange transfusion.

MATERIALS AND METHODS

Laboratory Methods

Venous blood for coagulation studies was collected into a volume 3.8% citrate, either in evacuated glass tubes or in plastic syringes. Plasma was separated within 1 hr by centrifugation at 2800 g for 15 min at 4°C and kept on ice until assayed, or quick-frozen in acetone dry ice. Inhibitor assays were often done on frozen samples, while other assays were performed on fresh samples within 2 hr of plasma separation. Factor VIII procoagulant activity was measured either by the one-stage partial thromboplastin time (PTT) correction of a hemophilic substrate plasma (cases 1, 3 5, 8 10, 12) or by the two-stage thromboplastin generation test (cases 2, 6, 7, 11). Factor VIII inhibitor assays were performed at Cardeza Foundation Hemophilia Center (CFHC) of Thomas Jefferson University Hospital (TJUH) and Children’s Hospital of Philadelphia (CHOP) as previously described,10 by a method similar to the Bethesda assay.11 Dilutions of patient plasma in 0.025 M imidazole-buffered normal saline were incubated with normal plasma at 37°C for 2 hr and factor VIII assayed by the modified one-stage PTT method. In case 2, the inhibitor assay method of Strauss was used.12 The titer for those specimens has been converted to the approximate equivalent titer by the usual method, using a conversion factor (2:1) experimentally determined in our laboratory. On occasion, when an inhibitor was first detected, inhibitor assays were performed on older frozen plasma specimens which had been stored at −30°C to −70°C.

Patients

Nine patients were males with hemophilia A and three were females with spontaneously acquired factor VIII inhibitors. Summaries of the pertinent clinical data are given in Tables 1 and 2. Decisions concerning immunosuppressive therapy were made on an individual basis by the physician caring for each patient. In all patients, with the exception of case 12, immunosuppressive therapy was used during treatment of a bleeding episode. Therapy in a hemophiliac was considered successful if the inhibitor assay became negative shortly after treatment and if subsequent exposure to factor VIII alone did not result in reappearance of the inhibitor. This definition of clinical success does not imply total eradication of the inhibitor since patients whose inhibitor assays are negative may still demonstrate decreased in vivo yield or half-life of infused factor VIII. In nonhemophiliacs disappearance of the inhibitor directly following immunosuppressive therapy was the only criterion for success since recovery of their normal factor VIII levels made further exposure to factor VIII unnecessary. Cases 1 and 3 have been partially reported previously (ref. 9, cases 2 and 15).
Factor VIII Inhibitors

Table 2. Factor VIII Inhibitors in Nonhemophiliacs

<table>
<thead>
<tr>
<th>Case</th>
<th>Underlying Disease</th>
<th>Age at Inhibitor Discovery (yr)</th>
<th>Maximum Inhibitor Titer</th>
<th>Known Duration of Inhibitor</th>
<th>Therapy</th>
<th>Outcome</th>
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<tr>
<td>10</td>
<td>Scleroderma</td>
<td>55</td>
<td>1:20</td>
<td>3 wk</td>
<td>Cp, Pred, VIII</td>
<td>Disappearance</td>
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<td>6 wk</td>
<td>Cp p.o.</td>
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<tr>
<td>11</td>
<td>None</td>
<td>72</td>
<td>1:4</td>
<td>2 wk</td>
<td>Aza, Pred</td>
<td>Disappearance</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td>4 wk</td>
<td>Exch T, VIII, Cp</td>
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<tr>
<td>12</td>
<td>None</td>
<td>51</td>
<td>1:320</td>
<td>6 mo</td>
<td>Pred</td>
<td>Persistence</td>
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<td></td>
<td></td>
<td></td>
<td>14 mo</td>
<td>Cp, VIII, Pred</td>
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Cp, cyclophosphamide (i.v. unless otherwise stated); Pred, prednisone p.o.; Aza, azathioprine p.o.; Exch T, exchange transfusion.

Case Reports

Hemophiliacs

Treated Cases

Case 1 (Fig. 1). J.W. is a 39-yr-old black male who has been followed at TJUH since the age of 8 (in 1944). His factor VIII level has been consistently <0.01 U/ml. Many bleeding episodes in childhood had required plasma infusions. One of his four brothers also has severe hemophilia A and a factor VIII inhibitor.

J.W. was first noted to develop a factor VIII inhibitor in March 1973 during a prolonged hospitalization for trauma. Following discovery of the inhibitor, no further factor VIII therapy was given. The inhibitor reached a maximum titer of 1:20 1 mo later and by March 1974 was undetectable. At that time he suffered a severe hemarthrosis which did not respond to conservative management. Factor VIII concentrate, 3000 units every 12 hr, was given for 7 days. Following the second infusion, 800 mg of cyclophosphamide (11 mg/kg) were given intravenously. During this period postinfusion factor VIII levels ranged between 0.8 and 1.4 U/ml. No corticosteroids were administered. From day 8 to 18, 2000 units of factor VIII concentrate were given every 12 hr, with a continuing satisfactory factor VIII response. At no time was factor VIII inhibitory activity detected.

The bleeding resolved, and the patient was discharged. However, 2 wk later he rebled in the same area and was rehospitalized. At that time his inhibitor was undetectable. He was retreated with factor VIII concentrate, 3000 units every 12 hr, a single dose of cyclophosphamide, 800 mg intravenously, and prednisone, 40 mg orally (p.o.) daily for 1 wk. Although the factor VIII response to this therapy was consistently poorer than during the prior admission, no factor VIII inhibitor was detected during this hospitalization or 1 mo following discharge. Since then, he has been placed on a home therapy program with factor VIII replacement three times weekly and has experienced no further significant bleeding. Factor VIII inhibitor assays have been consistently negative, but in vivo factor VIII yields have been less than predicted.

Case 2. M.K. is an 11-yr-old white male with severe hemophilia A who has been followed at Harrisburg Hospital since the age of 7 (in 1971). In early childhood he had repeated hemarthroses which responded to fresh plasma or cryoprecipitate infusions. Sometime before age 7 a factor VIII level of 0.02 U/ml was determined at another medical center. The patient's two brothers are unaffected, and there is no other family history of hemophilia.

In May 1971 large amounts of factor VIII were given for a fracture of the left femur. The hemostatic response was adequate, but no factor VIII or inhibitor levels were obtained. When seen initially at Harrisburg Hospital in September 1971, he had a severe acute hemarthrosis, as well as extensive chronic joint deformities. Infusion with 1400 units of cryoprecipitate did not produce a measurable factor VIII level, and an inhibitor was detected at a concentration of 100 U/ml (titer of approximately 1:50 by the CFHC assay).

No further factor VIII therapy was given, and azathioprine, 2 mg/kg p.o. daily, was begun. Over the following 20 mo, despite continued chemotherapy, the factor VIII inhibitor assay re-
Fig. 1. Case 1. Disappearance of inhibitor associated with immunosuppressive therapy in a severe hemophiliac.
Case 1. C.S. is a 39-yr-old white male with severe hemophilia A who has had numerous hemarthroses throughout his life, leaving him severely deformed. A factor VIII inhibitor was detected in 1965, when he was first seen at the CFHC, at age 29. The patient has no siblings, and there is no family history of hemophilia.

In 1969, an episode of severe gastrointestinal bleeding was treated with an initial infusion of 5000 units of cryoprecipitate, which neutralized the inhibitor and produced a factor VIII level of 0.40 U/ml. Despite continued factor VIII therapy, the inhibitor reappeared on the third day and reached a titer of 1:1600 in 2 wk. The inhibitor titer gradually fell to a level of 1:5 in October 1972 and 1:2 in May 1973, when he was readmitted for gastrointestinal and retroperitoneal hemorrhage. Therapy was begun with packed red blood cells, 3200 units of cryoprecipitate, a single 300-mg infusion of cyclophosphamide (6 mg/kg), and 60 mg prednisone orally. Immediately following the infusion, the factor VIII level was 1.09 U/ml, a negative inhibitor assay, and rapid resolution of the hematuria. At the same time, he was given a single 850 mg intravenous dose of cyclophosphamide (7 mg/kg), and started on prednisone, 90 mg p.o. daily. Factor VIII therapy was continued at 2000-3000 units of concentrate every 12 hr for 4 days, and prednisone was continued with 850 mg cyclophosphamide intravenously, and 20,000 units of factor VIII concentrate. Immediately following the infusion, the factor VIII level was 0.66 U/ml, and no inhibitor was detectable. Five days later, the inhibitor assay was negative. Since that time the patient has required factor VIII infusions on at least ten occasions, with satisfactory postinfusion yields and no recurrence of inhibitor.

Case 2 (Fig. 2). C.S. is a 41-yr-old black male with hemophilia A who has been followed at the Cardeza Foundation since the age of 24 (in 1958). Two brothers had hemophilia A and died of bleeding. His bleeding history was not severe prior to 1973, and his factor VIII levels were approximately 0.03 U/ml. In 1967 and 1971 good in vivo factor VIII responses were documented following factor VIII infusions given for trauma.

In February 1973 he developed hematuria, for which he was treated with factor VIII on at least three occasions at another hospital without improvement. Three weeks later, when first seen at the TJUH Emergency Ward, he was treated twice in 3 days with a total of 2400 units of cryoprecipitate. Because of a poor clinical response, he was hospitalized and treated initially with 1600 units of cryoprecipitate, followed by 1200 units every 12 hr. On the fifth hospital day, despite continued therapy, the factor VIII level was <0.01 U/ml, and a factor VIII inhibitor was detected at a titer of 1:5. Study of prior frozen plasma samples revealed the presence of a factor VIII inhibitor at a titer of 1:5 on the day he was first seen in the Emergency Ward, 3 days prior to admission. On the sixth hospital day, he was given 28,000 units of factor VIII concentrate, resulting in a factor VIII level of 1.09 U/ml, a negative inhibitor assay, and rapid resolution of the hematuria. At the same time, he was given a single 850 mg intravenous dose of cyclophosphamide (25 mg/kg). The hemarthrosis rapidly resolved, and no further factor VIII was given. Azathioprine was continued through this episode, until discharge 1 wk later. Two months later, when serial casting was performed, the inhibitor assay was negative, the factor VIII level was 0.01 U/ml, and postinfusion factor VIII levels were adequate. Since July 1973, the inhibitor assay has remained negative. In 1974 a home infusion program was begun, and the clinical response has been consistently satisfactory.

Case 3. W.R. is a 41-yr-old black male with hemophilia A who has been followed at the Cardeza Foundation since the age of 24 (in 1958). Two brothers had hemophilia A and died of bleeding. His bleeding history was not severe prior to 1973, and his factor VIII levels were approximately 0.03 U/ml. In 1967 and 1971 good in vivo factor VIII responses were documented following factor VIII infusions given for trauma.

In February 1973 he developed hematuria, for which he was treated with factor VIII on at least three occasions at another hospital without improvement. Three weeks later, when first seen at the TJUH Emergency Ward, he was treated twice in 3 days with a total of 2400 units of cyclophosphamide (25 mg/kg). The hemarthrosis rapidly resolved, and no further factor VIII was given. Azathioprine was continued through this episode, until discharge 1 wk later. Two months later, when serial casting was performed, the inhibitor assay was negative, the factor VIII level was 0.01 U/ml, and postinfusion factor VIII levels were adequate. Since July 1973, the inhibitor assay has remained negative. In 1974 a home infusion program was begun, and the clinical response has been consistently satisfactory.

Case 4 (Fig. 2). C.S. is a 39-yr-old white male with severe hemophilia A who has had numerous hemarthroses throughout his life, leaving him severely deformed. A factor VIII inhibitor was detected in 1965, when he was first seen at the CFHC, at age 29. The patient has no siblings, and there is no family history of hemophilia.

In 1969, an episode of severe gastrointestinal bleeding was treated with an initial infusion of 5000 units of cryoprecipitate, which neutralized the inhibitor and produced a factor VIII level of 0.40 U/ml. Despite continued factor VIII therapy, the inhibitor reappeared on the third day and reached a titer of 1:1600 in 2 wk. The inhibitor titer gradually fell to a level of 1:5 in October 1972 and 1:2 in May 1973, when he was readmitted for gastrointestinal and retroperitoneal hemorrhage. Therapy was begun with packed red blood cells, 3200 units of cryoprecipitate, a single 300-mg infusion of cyclophosphamide (6 mg/kg), and 60 mg prednisone p.o. daily. Subsequently, 1600 units of cryoprecipitate were given every 12 hr for 5 days. Immediately following the infusion, the factor VIII level was 0.21 U/ml. Immediately following the infusion, the factor VIII level was 0.24 U/ml and the inhibitor was not measurable. During the first week, factor VIII levels continued to be satisfactory and the inhibitor assay remained negative. However, on day 7 the inhibitor titer was 1:2, and by day 8, when cryoprecipitate and prednisone therapy had been discontinued, the factor VIII inhibitor titer was 1:10. The patient was clinically well and was discharged on day 13. The inhibitor titer reached a maximum of 1:640 on the 23rd day, subsequently falling to 1:20 by November 1973, and to 1:2 by August 1974. At that time he was hospitalized with a large hematoma of the left arm, associated with a severe allergic dermatitis. An infusion of 10,000 units of factor VIII concentrate neutralized the inhibitor and achieved a factor VIII level of 0.21 U/ml. Immediately following this infusion, 550 mg of cyclophosphamide (11 mg/kg), were given intravenously, together with 60 mg of prednisone orally. Factor VIII concentrate, 2000 units every 8 hr, and prednisone, 60 mg daily, were continued for the next 5 days.
Fig. 2. Case 4. Persistence of inhibitor following immunosuppressive therapy in a severe hemophiliac.
FACTOR VIII INHIBITORS

The hematoma resolved and no further bleeding occurred. The inhibitor titer rose to a maximum of 1:320 on day 19, falling to 1:20 by day 77.

Case 5. R.J. is a 25-yr-old black male with hemophilia A who has been followed at the CFHC since the age of 10 (in 1960). As a child he suffered frequent severe spontaneous hemorrhages. At the age of 12 he developed a seizure disorder which has been controlled with diphenylhydantoin and phenobarbital. Weak factor VIII inhibitory activity was first detected in his plasma in 1965 at the age of 15. In 1968 he was treated with cryoprecipitate on two occasions with a poor clinical response. In December 1970 he received cryoprecipitate prior to a tooth extraction, and his postinfusion factor VIII level was only half of the expected value. He experienced mild oozing from the socket for 6 days postoperatively. Between 1970 and 1973 he required factor VIII therapy on only one occasion. His only brother is also known to have hemophilia A, but no inhibitor has been detected despite frequent treatment.

In January 1973 he was admitted to TJUH for extraction of an abscessed tooth. He was treated with 1700 units of cryoprecipitate just prior to surgery and 1700 units 10 hr later. Despite this therapy, he bled excessively during the first postoperative day. The factor VIII level was 0.04 U/ml 7 hr following the second cryoprecipitate infusion, and a factor VIII inhibitor was found at a titer of 1:2. Because of the development of a large facial and cervical hematoma he was given 50,000 units of cryoprecipitate and commercial concentrate, followed immediately by 400 mg of cyclophosphamide (7.5 mg/kg) intravenously. Further therapy consisted of factor VIII concentrate, 5000 units every 12 hr for 4 days, decreasing to 2000 units every 12 hr for 3 days, and cyclophosphamide 100 mg orally daily on days 3-5. No corticosteroid therapy was given. During this time postinfusion factor VIII levels were 0.14-0.70 U/ml and inhibitor was undetectable. The facial hematoma resolved completely in 1 wk. The factor VIII inhibitor reappeared on day 8 at a titer of 1:10 and rose to 1:20 on day 9. No recurrence of bleeding was noted, and he was discharged on the 12th hospital day. His inhibitor titer rose to 1:400 by the third week following therapy, but had fallen to 1:10, 9 mo later.

Case 6. M.R. is a 27-yr-old black male with severe hemophilia A who has been followed at Harrisburg Hospital since 1965 (age 17). The diagnosis was made at age 2, and he has experienced many spontaneous bleeding episodes requiring repeated transfusions. There was no family history of hemophilia.

In May 1967 he received large quantities of cryoprecipitate and factor VIII concentrate because of a soft-tissue hemorrhage. His preinfusion factor VIII level was 0.01 U/ml, and in vivo factor VIII yields were consistent with the amounts infused. In August 1967, following trauma, he developed rapidly expanding hematomata of the face, left arm, and left leg. A factor VIII assay was <0.01 U/ml, and an inhibitor was detected at a titer of 1:160. After an 18-unit exchange transfusion with fresh CPD blood and 2500 units of factor VIII concentrate, the inhibitor titer fell to 1:5. He was begun on 150 mg azathioprine p.o. daily (2 mg/kg), which was later decreased to 100 mg daily. During almost 2 yr on this regimen, the inhibitor titer gradually de-
increased and was not measurable by May 1972. No factor VIII therapy was given between February 1970 and April 1974, when intracranial bleeding required therapy. He was treated with 3000 units of factor VIII concentrate intravenously (i.v.) every 12 hr, along with prednisone orally and one dose of 350 mg of cyclophosphamide i.v. (6 mg/kg). Factor VIII therapy produced the expected in vivo response for the first 5 days, but by the seventh day, postinfusion factor VIII levels were <0.01 U/ml, and a factor VIII inhibitor was again detected. The inhibitor reached a maximum titer of 1:75 3 wk later, and had fallen to 1:5 by August 1974.

Untreated Cases

Case 8 (Fig. 3). J.B. is a 16-yr-old white male who has been followed at Children's Hospital of Philadelphia since 1960, when the diagnosis of severe factor VIII deficiency was made at the age of 6 mo. He has been treated on multiple occasions for hemarthroses, dental bleeding, and gastrointestinal hemorrhage. He has several maternal uncles and nephews with hemophilia A. On a routine evaluation in May 1973 his factor VIII level was <0.01 U/ml and no inhibitor was found. In June 1973 he received cryoprecipitate for a soft-tissue hemorrhage, and for the first time showed little clinical response. A postinfusion factor VIII level was <0.01 U/ml and an inhibitor was detected at a titer of 1:2. A test dose of 1500 units of factor VIII concentrate produced a factor VIII level of only 0.07 U/ml 2 hr later. Further factor VIII treatment was withheld. No inhibitor was detected on repeat studies 5 wk and 15 wk later.

In October 1973, the patient developed a left iliopsoas hemorrhage with femoral nerve compression. No inhibitor was detectable. Factor VIII concentrate was given with a good clinical response and satisfactory postinfusion factor VIII levels. Shortly thereafter he sustained a minor left fibular fracture, which was treated successfully with posterior splinting and factor VIII concentrate. Over several months a pseudotumor developed at the site and required surgical intervention. No inhibitor was measurable, and a test dose of factor VIII concentrate produced the expected factor VIII postinfusion level. However, the half-life of infused factor VIII was less than 4 hr. Under cover of factor VIII, the pseudotumor was removed successfully with no excessive bleeding and normal healing. The inhibitor was intermittently measurable at a maximum titer of 1:2 throughout the 18-day period of therapy but not subsequently. He has been placed on a home infusion program and has been treated successfully for multiple hemarthroses and for one subdural hematoma, despite the fact that the in vivo factor VIII yield following infusion is less than expected.

Case 9. H.M. is a 15-yr-old white male who has been followed at the Children's Hospital of Philadelphia since 1965 (age 5). The diagnosis of factor VIII deficiency was made elsewhere at
Fig. 4. Case 10. Disappearance of inhibitor associated with immunosuppressive therapy in a patient with scleroderma.

age 2. Prior to 1973 he had been treated for multiple spontaneous hemarthroses and for two episodes of hematuria with good responses to transfusion. His two brothers are unaffected, and there is no other family history of hemophilia. Although no specific factor VIII assay is available from the period prior to inhibitor development, his clinical history is typical for severe hemophilia.

In January 1973 he was treated for a left elbow hemarthrosis with cryoprecipitate, and for the first time did not respond with a detectable rise in factor VIII. A factor VIII inhibitor was found at a titer of 1:10 and further replacement therapy was withheld. Seventeen days later the inhibitor titer was 1:20, but by 10 wk the titer had fallen to 1:2. Nine months later, with no intervening therapy, the inhibitor was no longer detectable.

In November 1973, he developed bleeding in the left wrist with median nerve compression. He had no detectable inhibitor and was treated with commercial factor VIII. Although a clinically satisfactory response was achieved, both the in vivo yield and the half-life of infused factor VIII were less than predicted. In January 1974 he was placed on a home infusion program, with continued good clinical response. The inhibitor assay has remained negative, although the in vivo yield of factor VIII is only 30% of the expected rise.

Nonhemophiliacs

Case 10 (Fig. 4). F.L. was a 55-yr-old black female with a 13-yr history of scleroderma, including fingertip ulcerations, arthritis, and Raynaud's phenomenon. She was referred to TJUH in May 1974 because of a 6-mo history of visual blurring and occipital headaches. There was no family history of hemorrhagic disease. She had been taking prednisone, 20 mg every other day, for at least 5 yr.

Physical examination revealed characteristic findings of scleroderma. Diagnostic procedures, including bilateral cerebral arteriography, led to a diagnosis of cerebral vasculitis. One day post arteriography, oozing appeared at the right femoral venipuncture site and swelling of the left thigh was noted at the site of an intramuscular injection. Coagulation studies showed a prolonged partial thromboplastin time, a factor VIII level of 0.12 U/ml and a factor VIII inhibitor with a
titer of 1:2. Therapy with prednisone, which had been discontinued for a few days, was reinstituted at 20 mg orally every other day, with improvement in vision and no further bleeding.

Three weeks later the patient developed a rapidly enlarging hematoma of the left thigh, requiring rehospitalization. The factor VIII inhibitor titer was 1:10 and the factor VIII level was 0.10 U/ml. On May 31 a single 1-g intravenous dose of cyclophosphamide (16 mg/kg) was given and prednisone was increased to 80 mg daily. Two days later a single infusion of 5000 units of factor VIII concentrate was given to control gastrointestinal bleeding, raising her factor VIII level from 0.06 to 0.22 U/ml. Her inhibitor titer rose to 1:20 over the next 2 wk. Because of continued bleeding and the lack of response of the factor VIII inhibitor, cyclophosphamide 100 mg p.o. was given daily for 3 wk starting on June 18. Within 1 wk there was a precipitous drop in inhibitor titer, and by July 17 (1 wk following cessation of immunosuppressive therapy) the inhibitor was not measurable and the factor VIII level was 0.32 U/ml. On August 4 her factor VIII level was 1.88 U/ml. Six months later, she was admitted to another hospital with extensive bilateral pneumonitis and rapidly expired. Postmortem examination revealed widespread evidence of scleroderma but no signs of hemorrhage.

Case II. H.F. was a 72-yr-old white female who had been in good health until November 1971, when she was hospitalized for hematuria and generalized ecchymoses. There was no past history or family history of any bleeding tendency. Two weeks previously she had an upper respiratory infection which was treated with aspirin and an antihistamine. She was first seen at Harrisburg Hospital 1 wk after the onset of symptoms. Physical examination revealed pallor, a warm, swollen right ankle, and large ecchymotic areas on both upper arms. The factor VIII assay was 0.02 U/ml and a factor VIII inhibitor was demonstrated. The bleeding persisted, and on the fourth hospital day azathioprine, 100 mg p.o. daily, and prednisone, 80 mg p.o. daily, were begun. On the 18th hospital day, because of continued bleeding, a 20-unit exchange transfusion was performed. Prior to the exchange her inhibitor titer was 1:4 (performed at CFHC). Immediately following the exchange her factor VIII level was 0.16 U/ml and her inhibitor titer was negative. After the procedure she received 4000 units of cryoprecipitate and 1.5 g of cyclophosphamide (20 mg/kg) intravenously. Azathioprine was increased to 150 mg daily orally.

The factor VIII level increased progressively, reaching 1.0 U/ml 2 wk later. Azathioprine and prednisone were discontinued 1 wk and 4 wk, respectively, after the procedure. The patient was discharged asymptomatic 5 wk after the exchange with a factor VIII level of 1.0 U/ml.

Five weeks after discharge, her factor VIII level was 0.84 U/ml. About 8 mo after discharge she was admitted to the original referring hospital with a myocardial infarction. Physical examination revealed no evidence of bleeding, and a normal partial thromboplastin time was found. She expired, and no autopsy was performed.

Case III. M.M. is a 56-yr-old white female who was in good health until May 1970, when she underwent a hysterectomy for leiomyoma at age 51. She had previously undergone several surgical procedures without difficulty. There was no family history of a bleeding disorder. Her postoperative course was complicated by poor wound healing, pelvic abscess and sepsisemia, disseminated intravascular coagulation, and pulmonary emboli. She received antibiotics (no penicillin), 30 units of whole blood and packed cells, and a course of heparin therapy.

In August 1970 she was first seen at the Carдеza Foundation because of hematuria and was found to have a factor VIII level of <0.01 U/ml, with a factor VIII inhibitor at a titer of 1:40. There was no evidence of any underlying disorder. In October 1970, because of persistent hematuria, she was admitted to another hospital where she received a single infusion of 1000 units of cryoprecipitate, with cessation of the hematuria. She was discharged on 50 mg of prednisone p.o. daily. Within a month the inhibitor titer rose to a maximum of 1:320, falling within 3 mo to the 1:10–1:20 range. She was treated with varying doses of prednisone after that, and the inhibitor titer was partially responsive to this drug. In 1971 she received cyclophosphamide, 1500 mg intravenously, and 3000 units of factor VIII at another hospital. This attempt at immunosuppression failed. It was not determined if a circulating factor VIII level was achieved. Over the ensuing years, she developed severe osteoporosis and several serious fractures. In an attempt to eliminate the need for continued prednisone, she received another course of immunosuppressive therapy in July 1974. Her initial factor VIII level was 0.03 U/ml and the inhibitor titer was 1:5. On July 2 she was treated with 7000 units of factor VIII concentrate, raising her factor VIII level to 0.41 U/ml, followed immediately by 600 mg of cyclophosphamide i.v. (10 mg/kg) and predni-
sone 80 mg p.o. Further therapy consisted of prednisone, 80 mg daily, and factor VIII concentrate, 2000 units every 12 hr for 1 wk. By July 10 the inhibitor was undetectable. Eleven days later, with no further factor VIII infusions, her factor VIII level was only 0.05 U/ml. In spite of continued prednisone therapy, the inhibitor reappeared, reaching a titer of 1:20 by September 1974, and subsiding to 1:5 by December 1974. She continues on prednisone, 20 mg daily, and is presently clinically asymptomatic, with an inhibitor titer of 1:5.

DISCUSSION

Inhibitors in Hemophilia A

Complete eradication of the inhibitor has occurred in only six patients with hemophilia A who have subsequently received factor VIII without reappearance of the inhibitor (refs. 6 and 7 and cases 1–3). An additional two patients, whose inhibitors disappeared following therapy, have not yet been reexposed to factor VIII. In a larger number of patients the anamnestic response has been blunted or even prevented. Our experience suggests that several clinical factors may contribute to the ultimate outcome of therapy.

The development of factor VIII inhibitors in hemophiliacs is clearly related to factor VIII infusion. If further factor VIII therapy is withheld, inhibitor titers generally fall, and may even reach undetectable levels, as in case 1 (Fig. 1). Reexposure to factor VIII typically causes an anamnestic rise in inhibitor titer, as illustrated in case 4 (Fig. 2). A few low-titer inhibitors in severe hemophiliacs, such as cases 8 and 9, may not show an anamnestic rise or may even disappear despite subsequent factor VIII exposure. Thus, the disappearance of a low-titer inhibitor following therapy cannot be ascribed with certainty to the therapeutic regimen.

The interpretation of therapeutic results is also complicated in less severe hemophiliacs, with factor VIII levels greater than 0.01 unit/ml. Inhibitors in this group are generally of low titer, may disappear without specific therapy, and may not recur despite reexposure to factor VIII (ref. 1, Table 3). It is interesting that five of the six successfully treated hemophiliacs were patients whose factor VIII levels prior to inhibitor development were >0.01 U/ml (refs. 6, 7, and 9, cases 2 and 3). The only example of eradication of an inhibitor in a hemophiliac whose prior factor VIII level was <0.01 U/ml is case 1 in this series. The clinical course in case 2 suggests that cyclophosphamide therapy abolished the inhibitor, but a similar conclusion is less certain in case 3 and in the other reported cases.

Early treatment of inhibitors may be important for successful outcome. Data from animal studies suggest that immunosuppressive drugs are more likely to be effective during primary immune responses or with the first reexposure to antigen than after multiple exposures to antigen. Cases 1–3 were all begun on some form of immunosuppressive therapy very early after the appearance of the inhibitor, with little or no intervening exposure to factor VIII, while three of our four failures (cases, 4, 5, and 7) had long-standing inhibitors with multiple reexposures to factor VIII prior to immunosuppressive therapy.

Inhibitor titer at the time of immunosuppression appears to influence the outcome, with low titer inhibitors more apt to respond. Compared to
the therapeutic failures, cases 1–3 had lower titers both at the time of therapy and in the past. Patients who have had high-titer anamnestic responses, but have a low titer at the time of therapy, probably should be considered "high-titer" patients. For example, patient 4 with a documented prior high titer, was a therapeutic failure despite a titer of 1:2 at the time of therapy. It is possible that patients whose inhibitors fail to demonstrate a high-titer response to antigenic stimulus represent a subgroup of "poor responders" who may be more susceptible to immunosuppressive therapy. In addition, it may be important to achieve in vivo factor VIII levels greater than 0.4–0.5 U/ml at the time of immunosuppressive therapy, a hypothesis that is supported by data in some cases, including our cases 1, 3, 4, and 5, but not in every case (ref. 8, case 3).

However, the achievement of a circulating factor VIII level may simply relate to the initial low inhibitor level, which itself is the significant variable.

Various immunosuppressive drug regimens have been used for hemophiliac inhibitors. Corticosteroid therapy alone has been ineffective. Despite animal studies documenting the effectiveness of both azathioprine and cyclophosphamide as immunosuppressive agents, in hemophiliacs therapeutic benefit has occurred most frequently with cyclophosphamide. In our cases 2, 6, and 7, prolonged trials of oral azathioprine (2 mg/kg daily) were unsuccessful. Cyclophosphamide, on the other hand, was associated both with disappearance (cases 1–3) and persistence (cases 4, 5, 7) of inhibitor when used as a single intravenous dose (6–25 mg/kg) combined with factor VIII. This experience is in agreement with previously reported cases. Cases 1–3 each received more than one course of immunosuppressive therapy. It is possible that further doses of immunosuppressive therapy following the initial dose may improve the chance of success.

**Inhibitors in Nonhemophiliacs**

The natural history of factor VIII inhibitors in nonhemophiliacs is extremely variable, and spontaneous remissions have been reported, especially in postpartum women and patients with autoimmune disorders. Thus one cannot be certain that the disappearance of the inhibitor in case 10, with scleroderma, occurred in response to immunosuppressive therapy. In patients with no underlying disease, such as cases 11 and 12, inhibitors respond to therapy less frequently, and spontaneous remission is uncommon. Therefore the disappearance of the inhibitor in case 11 following cyclophosphamide is more likely to have been the result of treatment.

Previous reports of immunosuppressive therapy in nonhemophiliacs do not show a clear correlation between therapeutic outcome and the several factors analyzed in the hemophiliac cases, although the duration and maximum titer of the inhibitor prior to therapy might be as relevant in these patients as in hemophiliacs. In cases 10 and 11 inhibitor titers were low, and treatment was begun early in the course of the inhibitor's presence. Intervening exposure to factor VIII may not be pertinent, since the development of inhibitors in nonhemophiliacs is probably not related to transfusion with factor VIII. However, an anamnestic-like rise in inhibitor titer following factor VIII infusion has been documented in two cases (our case 12 and Nilsson's case 513), which were both
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therapeutic failures by our definition. Factor VIII infusion may not be necessary in addition to immunosuppressive drugs, since regimens without factor VIII have been associated with disappearance of inhibitor in some nonhemophiliacs, \(^4^, \(^5^, \(^8^\) as in our case 10. The importance of achieving a circulating factor VIII level cannot be analyzed adequately on the basis of the data in prior reports or in our three cases.

The choice of drug regimen is even more difficult to evaluate in these patients than in hemophiliacs. Azathioprine and prednisone, alone or in combination, may have been effective in several patients. \(^4^, \(^5^\) Cyclophosphamide therapy was associated with the disappearance of inhibitor in our cases 10 and 11, as well as in several previous reports, \(^3^, \(^8^, \(^25^\) including some patients who had failed to respond to prednisone or azathioprine.

Thus, the treatment of factor VIII inhibitors with immunosuppressive therapy may be beneficial in some hemophiliac and nonhemophiliac patients, although the overall experience with these agents has so far been disappointing. The known and potential long-term toxicities of immunosuppressive drugs \(^2^6\) make the decision to use them a difficult one, especially in children and young adults. As an alternate or supplemental therapy for bleeding episodes, factor IX concentrates have been found to promote hemostasis in some factor VIII inhibitor patients. \(^2^7^, \(^2^9\) Unfortunately, thrombogenic complications have been reported in some patients with the use of these concentrates \(^3^0, \(^3^1\) and it may be premature to use this material in situations other than acute bleeding episodes. \(^3^2\)

In order to assess the effectiveness of immunosuppressive therapy, a better understanding of the variations in the natural history of factor VIII inhibitors is needed. A national cooperative study of factor VIII inhibitors in hemophiliacs, recently initiated by the National Institutes of Health, should provide this basis for designing a controlled clinical trial of therapeutic regimens in patients with factor VIII inhibitors.

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