Circulating Factor IX Antigen-Inhibitor Complexes in Hemophilia B Following Infusion of a Factor IX Concentrate

By Scott H. Goodnight, Jr., Catherine W. Britell, Kirk D. Wuepper, and Bjarne Østerud

A persistent low-titer factor IX inhibitor was discovered in a patient with severe hemophilia B. The inhibitor was very likely an immunoglobulin, since it was present in serum, was not dialyzable, retained its potency after heating to 56°C, and was bound by staphylococcal protein A (SPA). When the hemophilia B patient with the inhibitor was given therapeutic infusions of factor IX concentrate, the survival of factor IX antigen ([IX]₉₉₉) was markedly prolonged (t½ approximately 60 hr) compared to the survival of [IX]₉₉₉ in infused hemophilia B patients lacking an inhibitor (t½ 5 hr). The prolonged survival of [IX]₉₉₉ suggested the possibility of circulating immune complexes composed of [IX]₉₉₉ and factor IX inhibitor ([IX]ᵢ₉₉₉). Since immunoglobulins bind to SPA via the Fc portion of the molecule, the Fab region is free to bind to antigen. Immune complexes were formed in vivo after a factor IX concentrate infusion, [IX]₉₉₉ would be retained by SPA-sepharose due to the linking of [IX]₉₉₉ with the inhibitor molecule. As expected, postinfusion plasma from the inhibitor patient (but not from other hemophilia B patients) showed binding of [IX]₉₉₉ to the SPA-sepharose. Further evidence for circulating complexes was provided by crossed immunoelectrophoresis using an antibody to purified factor IX. Preparations containing factor IX alone showed a single fast-moving peak in the gel, whereas postinfusion plasma from the inhibitor patient as well as mixtures in vitro of [IX]₉₉₉ and [IX]ᵢ₉₉₉ showed an additional slow-moving peak. These studies document the presence of circulating factor IX antigen-inhibitor complexes in a patient with hemophilia B and a persistent low-titer factor IX inhibitor. To date, clinical evidence of immune complex disease has not been observed.

The management of 5%–15% of patients with hemophilia A and a lesser percentage of patients with hemophilia B may be complicated by the development of circulating plasma inhibitors to factor VIII or factor IX. Their etiology is unknown, although exposure to clotting factor antigens from therapeutic infusions of plasma or plasma concentrates occurs regularly. Most factor IX inhibitors were shown to be immunoglobulins of IgG subclasses and, when examined in detail, were found to be of monoclonal origin in one instance but polyclonal in others. The concentration of the antibody may rise dramatically following clotting factor administration or may persist at low levels for extended periods of time. In those patients with lower titers of inhibitor, the treatment of bleeding episodes often

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consists of the infusion of large quantities of concentrated clotting factor to exhaust the inhibitor and provide hemostasis.

Whether or not infusions under these circumstances would result in circulating immune complexes composed of clotting factor antigen and circulating inhibitor is not known. If so, vascular or organ damage due to immune complexes might develop after repeated therapeutic infusions.

We studied a boy with hemophilia B who had a persistent low-titer inhibitor to factor IX and who regularly received factor IX concentrates (Konyne) for treatment of bleeding episodes. This report defines some of the characteristics of the inhibitor and provides evidence for circulating factor IX antigen-inhibitor complexes following Konyne infusions in this patient.

**MATERIALS AND METHODS**

**Plasma collection and storage.** Blood was obtained by atraumatic venipuncture using plastic syringes and was mixed 9:1 with Ware's anticoagulant (6 parts 0.1 M sodium citrate plus 4 parts 0.1 M citric acid). After centrifugation for 10 min at 11,400 g at 4°C, the plasma was removed, flash-frozen in polyethylene tubes using dry ice and acetone, and subsequently stored at −30°C.

**Coagulation tests.** Factor IX coagulant activity (IXC) was assayed using a one-stage kaolin-activated method with added adsorbed bovine plasma. Factor IX antigen (IXAg) was measured using Laurell electroimmunoassay as previously described. A highly specific antiserum was prepared by immunization of rabbits with a homogeneous factor IX antigen prepared by adsorption and elution from BaSO4 and DE-11 Whatman cellulose, preparative polyacrylamide gel electrophoresis, and heparinagarose affinity chromatography. The lower limit of detection for IXAg was 0.05–0.10 U/ml.

If a hemophilia B plasma contained reduced but equivalent amounts of IXC and IXAg, it was designated B-. A hemophilic plasma with a normal ox brain thromboplastin time and containing IXAg in excess of IXC was termed B+. A hemophilic plasma with a normal ox brain thromboplastin time and containing IXAg in excess of IXC was termed B+.

Factor IX inhibitor (IXih) was assayed using a modification of the Bethesda method for factor VIII inhibitors. A dilution of test plasma (0.2 ml) was incubated for 5 min with an equal volume of pooled normal plasma obtained from 25 normal males. Previous studies showed that this incubation period allowed complete neutralization of IXC by the inhibitor. An aliquot was removed and diluted 1:5 in imidazole buffer, and 0.1 ml of the dilution was added to a mixture of factor IX-deficient plasma, adsorbed bovine plasma, kaolin, and cephalin that had been previously incubated for 8 min at 37°C. Immediately thereafter, 0.1 ml 55 mM CaCl2 was added and the clotting time determined. By definition, one inhibitor unit will inactivate 50% of IXC in an equal volume of normal pooled plasma.

**Preparation of adsorbed plasma and serum.** For some experiments plasma was adsorbed by adding 1 part 50% Al(OH)3 (Cutter Laboratories, Berkeley, Calif.) to 9 parts citrated plasma, incubating for 3 min at 37°C, and centrifuging at 11,400 g for 10 min. The precipitate was discarded and the adsorption repeated.

Serum was prepared by allowing 10 ml blood to clot in a glass tube for 2 hr at 37°C. The serum was removed, mixed 9:1 with Ware's anticoagulant, heated to 56°C for 30 min, and adsorbed twice with Al(OH)3 as described above.

**Other tests.** Blood chemistries, urinalyses, and hematologic tests were performed using standard methods. Serum complement components (C3, C4), quantitative immunoglobulins, and anti-native DNA antibodies were assayed in the Immunology Research Laboratory. Immunoelectrophoresis and immunodiffusion were performed using standard methods.

**Staphylococcal protein A (SPA) chromatography.** SPA, covalently bonded to crosslinked Sepharose 4B, was obtained from Pharmacia Fine Chemicals (Piscataway, N.J.), and 15 ml of the gel prepared according to the manufacturer's instructions was packed in a 1.5 × 15 cm column. After equilibration at room temperature with 0.05 M Tris buffer in 0.15 M NaCl, 4.0 ml of test plasma was applied to the column and chromatographed at a flow rate of approximately 1 ml/min at room temperature. After elution of the initial protein peak (280 nm) with the Tris buffer, 3 M NaSCN was substituted to elute the immunoglobulins. Fractions (2 ml) were collected and dialyzed for 12 hr against 3.5% citrated saline at 4°C, concentrated five- to tenfold using Minicon B-15 (Amicon, Lexington, Mass.) filter concentrators, and assayed immediately for IXC, IXAg, and IXih.
**Factor IX infusion studies.** When therapeutically indicated for joint hemorrhages, standard therapeutic doses (e.g., 15-30 U/kg) of a factor IX concentrate (Konyne, Cutter Laboratories), each containing similar $X_{PI} / x_{AI}$ ratios, were administered intravenously to four patients with severe hemophilia B at flow rates of about 2.5 ml/min. Aliquots of the infused concentrate and blood samples drawn from the patients prior to and at intervals following the infusions were assayed for $X_{PI}$, $X_{AI}$, and $X_{SH}$.

**Two-dimensional immunoelectrophoresis.** Crossed immunoelectrophoresis was carried out in 0.9% agarose (Industrie Biologique Francoise). Barbital buffer pH 8.6 was prepared by adding 3.6 g barbituric acid and 20.52 g sodium barbital to 2 liters distilled water. Disodium EDTA (0.5 g) and 2.25 g agarose were added to 250 ml barbital buffer. The solution was heated with constant stirring to 100°C and allowed to cool to 56°C before use. Cleaned 75 × 50 mm disposable slides (Corning) were covered with 9 ml agarose; after cooling, a well was punched in one corner of the gel.

Test material (40 μl) was added to the well and electrophoresis was carried out with cooling at 15 V/cm on the long axis of the slide at 4°C for 4–5 hr. The gel was sliced 0.25 cm from the well parallel to the direction of electrophoresis, and the larger portion was discarded. Fresh agarose containing 2.65%–3.5% antiserum to factor IX was added to the plate and electrophoresis carried out in the second direction (90° counterclockwise) at 5 V/cm for 16 hr. The gels were then washed, stained with Coomassie Brilliant Blue, dried, and photographed.

**Disc electrophoresis.** Normal pooled plasma (50 μl) or plasma from patient A (50 μl) was incubated with 50 μl 125I-factor IX (0.4 U/ml)36 for 30 min at room temperature. A 6 μl aliquot of each incubation mixture was added to 50 μl electrophoresis buffer (0.0125 M Tris, 0.19 M glycine, pH 8.3) and one drop of glycerol and applied to SDS polyacrylamide gels (Eastman, Rochester, N.Y.) for 2 hr using alkaline gels (pH 8.9) and a constant 180 V. Following electrophoresis, the gels were removed, divided into 1.3 mm slices, and counted in an automatic gamma counter (Searle, model 1197).

**CASE REPORTS**

Four patients with clinically severe hemophilia B who had repeated episodes of spontaneous bleeding were studied.

**Patient A (hemophilia B−, inhibitor).** An 11-yr-old boy with hemophilia B− and a low-titer inhibitor ($X_{PI} < 0.01$ U/ml, $X_{SH}$ nondetectable, normal ox brain thromboplastin time, and $X_{SH}$ 1.0–2.1 U/ml) had had recurrent spontaneous hemorrhages and soft-tissue hemorrhages since infancy. He was treated with plasma from age 6 mo to 8 yr. Since then, factor IX concentrates had been used. Infusion of 24 U/kg of factor IX reliably resulted in cessation of bleeding. He was otherwise well and without clinical or laboratory evidence of immunologic disease (normal serum immunoglobulins, anti–native DNA antibodies, C3 and C4 levels, urinalyses, and serum creatinine). Following infusion there was no evidence of hemolysis or fall in serum C3 concentration. Although there was no family history of hemophilia, the patient’s mother had $X$ of 0.40 U/ml and $X_{AI}$ of 0.40 U/ml. Patient A was followed prospectively for 18 mo; in spite of frequent infusions of 500–1000 units factor IX for hemorrhages and chronic synovitis, $X_{SH}$ never exceeded 2.1 U/ml when measured 2–3 wk following infusion.

**Patients B and C (hemophilia B−, no inhibitor).** A 54-yr-old man with hemophilia B− and no evidence of an inhibitor ($X_{PI} < 0.01$ U/ml, $X_{SH}$ and $X_{SH}$ undetectable) was effectively treated with multiple infusions of plasma and factor IX concentrates over a 20-yr period. One complication of his hemophilia was moderately severe arthropathy. A second, nonrelated adult patient, age 32 yr, with severe hemophilia B− who required frequent infusions for joint disease was also studied ($X_{PI} < 0.01$ U/ml, $X_{SH}$ and $X_{SH}$ undetectable).

**Patient D (hemophilia B−, no inhibitor).** A 13-yr-old boy with hemophilia B−, normal thrombotest, and no evidence of an inhibitor ($X_{PI} < 0.01$ U/ml, $X_{SH}$ 0.40 U/ml, $X_{SH}$ undetectable) was effectively treated with plasma or factor IX concentrates over an 11-yr period, principally for hemorrhages. He had no family history of hemophilia; however, his mother had a $X_{PI}$ of 0.18 U/ml and a $X_{SH}$ of 0.90 U/ml.

**RESULTS**

**Characterization of the inhibitor.** The inhibitor of patient A was found in serum or plasma, was not adsorbed by aluminum hydroxide, neutralized factor IX...
Table 1. Properties of the Inhibitor From Patient A

<table>
<thead>
<tr>
<th>Test Material</th>
<th>IX&lt;sub&gt;inh&lt;/sub&gt; Assay</th>
<th>Clotting Time&lt;sup&gt;*&lt;/sup&gt; (sec)</th>
<th>Inhibitor Concentration (units/ml)</th>
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<tbody>
<tr>
<td>Buffer</td>
<td></td>
<td>58</td>
<td>0</td>
</tr>
<tr>
<td>Normal plasma</td>
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<td>53</td>
<td>0</td>
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<tr>
<td>Inhibitor plasma</td>
<td></td>
<td>88</td>
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</tr>
<tr>
<td>Normal serum†</td>
<td></td>
<td>58</td>
<td>0</td>
</tr>
<tr>
<td>Inhibitor serum†</td>
<td></td>
<td>73</td>
<td>1.9</td>
</tr>
<tr>
<td>Adsorption with Al(OH)&lt;sub&gt;3&lt;/sub&gt;</td>
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<td></td>
<td></td>
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<tr>
<td>Normal plasma</td>
<td></td>
<td>58</td>
<td>0</td>
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<tr>
<td>Inhibitor plasma</td>
<td></td>
<td>83</td>
<td>2.1</td>
</tr>
<tr>
<td>Onset of Activity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 sec incubation</td>
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<td>83</td>
<td>2.1</td>
</tr>
<tr>
<td>1 min incubation</td>
<td></td>
<td>85</td>
<td>2.1</td>
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<tr>
<td>5 min incubation</td>
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<td>88</td>
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<tr>
<td>60 min incubation</td>
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<tr>
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<td>1.1</td>
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<td>56° × 1 hr</td>
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<td>73</td>
<td>1.1</td>
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<tr>
<td>66° × 1 hr</td>
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<tr>
<td>76° × 1 hr</td>
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<td>59</td>
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</tr>
</tbody>
</table>

* 1:1 Mixture of test material and normal pooled plasma.
† Heated and adsorbed with Al(OH)<sub>3</sub>

almost immediately, and was heat stable for 1 hr at 56°C (Table 1). No loss of inhibitor occurred after dialysis for 15 hr at 4°C.

Plasma of patient A neutralized purified factor IX as well as factor IX contained in normal plasma. When 0.2 ml purified factor IX (0.2 U/ml)<sup>4</sup> with a blank time of 100 sec was mixed with 0.1 ml plasma from patient A, the clotting time in a factor IX assay after 5 min incubation was 90 sec. The clotting time of a control mixture of factor IX and hemophilia B<sup>+</sup> plasma without an inhibitor was 65 sec.

Chromatography of inhibitor plasma on SPA coupled to Sepharose 4B provided purification of the inhibitor as well as evidence that it was an immunoglobulin (Table 2). When a total of 4 units (4 ml) of factor IX contained in normal plasma was chromatographed, IX<sub>Ag</sub> (2.0 U) and IX<sub>PTC</sub> (2.9 U) did not bind to the SPA and were found only in the first protein peak. In contrast, when plasma from patient A containing 5.6 Bethesda units of inhibitor (IX<sub>PTC</sub> and IX<sub>Ag</sub> undetectable) was chromatographed, 4.2 units of inhibitor were retained by the SPA and subsequently measured after elution with NaSCN and dialysis.

Fractions collected following SPA chromatography and NaSCN elution of normal or inhibitor plasma showed precipitin lines against monospecific antisera to IgG, IgA, and IgM using double diffusion in agar. Immunoelectrophoresis of these fractions against anti-whole human serum showed only IgG (strong) and IgA and IgM (weak) globulins. The presence of other serum proteins was not detected.

Survival studies following the infusion of a factor IX concentrate. Infusion of factor IX concentrate into two patients with hemophilia B<sup>+</sup> without an inhibitor showed recovery of nearly identical levels of IX<sub>Ag</sub> and IX<sub>PTC</sub> with time (Fig. 1). The
Table 2. Assays of Factor IX Coagulant Activity (IX<sub>PTC</sub>), Antigen (IX<sub>Ag</sub>), and Inhibitor (IX<sub>Inh</sub>) Before and After Staphylococcal Protein A Chromatography (Total Units*)

<table>
<thead>
<tr>
<th></th>
<th>Prior to Chromatography</th>
<th>Fraction 1</th>
<th>Fraction 2</th>
<th>Fraction 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal plasma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IX&lt;sub&gt;PTC&lt;/sub&gt;</td>
<td>4.0</td>
<td>2.9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IX&lt;sub&gt;Ag&lt;/sub&gt;</td>
<td>4.0</td>
<td>2.0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IX&lt;sub&gt;Inh&lt;/sub&gt;</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Inhibitor plasma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IX&lt;sub&gt;PTC&lt;/sub&gt;</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IX&lt;sub&gt;Ag&lt;/sub&gt;</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IX&lt;sub&gt;Inh&lt;/sub&gt;</td>
<td>5.6</td>
<td>0</td>
<td>0</td>
<td>4.2</td>
</tr>
</tbody>
</table>

*Total units of IX<sub>PTC</sub>, IX<sub>Ag</sub>, and IX<sub>Inh</sub> applied to or recovered from the column.
†Fraction 1 includes the nonadsorbed plasma protein peak, fraction 2 the area between the peaks, and fraction 3 the Ig peak obtained following elution with NaSCN and subsequent dialysis.

Fig. 1. Survival of IX<sub>PTC</sub> and IX<sub>Ag</sub> following infusion with a factor IX concentrate. Survival curves of patient C (not shown) were similar to those of patient B. Values for patient B are averages for two infusions. (A) Patient B; (B) patient D.
slopes of the curves indicated an initial survival \(t_{1/2}\) of approximately 5 hr followed by a second component with a \(t_{1/2}\) of about 12 hr. Findings were quite similar when a patient with hemophilia B* was studied, with the exception that IX\(_{Ag}\) was 0.4 U/ml both prior to and 48 hr following infusion (Fig. 1).

Contrasting results were obtained on two occasions following administration of Konyne to patient A (Fig. 2). IX\(_{Ag}\) rose to a level almost three times that of IX\(_{PTC}\) with a survival \(t_{1/2}\) in excess of 60 hr. Survival of IX\(_{PTC}\) was similar to the survival of IX\(_{PTC}\) in the patient with hemophilia B lacking an inhibitor. Factor IX\(_{inh}\) was not detectable during the first 10 hr following the infusion.

**Evidence for circulating antigen-inhibitor complexes.** Circulating IX\(_{Ag}\)-IX\(_{inh}\) complexes were sought using SPA-Sepharose chromatography. Since SPA has been shown to bind to the Fc region of immunoglobins, the Fab region may remain complexed with antigen.

When SPA chromatography was performed on plasma obtained from a hemophilia B patient lacking an inhibitor during the 12 hr following Konyne infusion, IX\(_{PTC}\) and IX\(_{Ag}\) were recovered only in the nonadsorbed fractions (Table 3). In contrast, chromatography of plasma from the patient with the factor IX inhibitor during the 12 hr following Konyne infusion not only showed in IX\(_{Ag}\) and IX\(_{PTC}\) in the nonadsorbed fraction but also showed IX\(_{Ag}\) and a trace of IX\(_{PTC}\) in the Ig fractions. IX\(_{inh}\) was not detected.

**Table 3. Assays of IX\(_{PTC}\), IX\(_{Ag}\), and IX\(_{inh}\) on Plasma and Staphylococcal Protein A Chromatographic Fractions (Total Units*) From Patients A and B 12 hr Following Konyne Infusion**

<table>
<thead>
<tr>
<th></th>
<th>SPA Chromatography</th>
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<tbody>
<tr>
<td></td>
<td>Plasma</td>
</tr>
<tr>
<td><em><em>Patient A (B</em>: inhibitor)</em>*</td>
<td></td>
</tr>
<tr>
<td>IX(_{PTC})</td>
<td>1.2</td>
</tr>
<tr>
<td>IX(_{Ag})</td>
<td>2.8</td>
</tr>
<tr>
<td>IX(_{inh})</td>
<td>0</td>
</tr>
<tr>
<td><em><em>Patient B (B</em>: no inhibitor)</em>*</td>
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</tr>
<tr>
<td>IX(_{PTC})</td>
<td>1.4</td>
</tr>
<tr>
<td>IX(_{Ag})</td>
<td>1.3</td>
</tr>
<tr>
<td>IX(_{inh})</td>
<td>0</td>
</tr>
</tbody>
</table>

*Total units of IX\(_{PTC}\) or IX\(_{Ag}\) applied to or recovered from the column.
Complexes in vivo were also sought using two-dimensional (crossed) immunoelectrophoresis. When normal plasma was studied, a single fast-moving peak of factor IX was identified (Fig. 3A). Similar findings were observed when a purified preparation of factor IX was used (Fig. 3B). Such peaks were entirely absent when plasma from patients with hemophilia B or from the patient with the factor IX inhibitor was tested.

When plasma containing the factor IX inhibitor was mixed with an equal volume of normal plasma, a second, slower-moving peak was also seen. A comparable pattern was found when inhibitor plasma was mixed with purified factor IX (Figs. 3C, 3D).

The Ig fraction from SPA chromatography of IXinh plasma was dialyzed, concentrated, and mixed with purified factor IX. Two peaks were again evident (Fig. 3E).

When plasma obtained from patient A (with the inhibitor) 45 min following an infusion of Konyne was studied, both a fast-moving peak corresponding to factor IX and a second, slower-moving peak were seen (Fig. 3F). Plasma from patient B (who lacked an inhibitor) following Konyne infusion showed only a single fast-moving peak (not shown).

A third experiment designed to demonstrate inhibitor-factor IX complex formation utilized 125I-labeled purified factor IX and disc electrophoresis on SDS.
Fig. 4. Disc gel electrophoresis of (A) a mixture of \textsuperscript{125}I-factor IX (0.4 U/ml) and normal plasma or (B) a mixture of \textsuperscript{125}I-factor IX and plasma from patient A.

Polyacrylamide gel. Electrophoresis of a mixture containing equal volumes of \textsuperscript{125}I-factor IX and normal plasma yielded a single sharp zone of radioactivity (Fig. 4A). When labeled purified factor IX was added to plasma from patient A (inhibitor titer 2.1 units), a second, slower peak was found (Fig. 4B).

DISCUSSION

The inhibitor discovered in this 11-yr-old boy with hemophilia B\textsuperscript{−} had persisted at this writing for at least 2 yr but remained low in titer despite repeated therapeutic infusions of a factor IX concentrate. Because other hemophilic inhibitors have been shown to be immunoglobulins (Ig) and since this patient’s inhibitor was present in serum, was heat stable, and was nondialyzable (Table 1), it seemed likely that it, too, would prove to be an antibody. However, initial attempts to neutralize its inhibitory activity using heterologous antisera to purified Ig failed, most likely because of the low titer of the inhibitor.

A second attempt to characterize the inhibitor used staphylococcal protein A, a protein isolated from cultures of \textit{Staphylococcus aureus}, which was reported to bind the Fc fragments of immunoglobulins of IgG subclasses 1, 2, and/or 4.\textsuperscript{11} When inhibitor plasma from patient A was chromatographed on a column containing SPA bound to Sepharose 4B, the inhibitor was retained and could subsequently be recovered following elution with NaSCN. Our studies and those of others\textsuperscript{12} showed that small amounts of IgA and IgM as well as IgG bind to SPA. Other nonimmunoglobulin proteins were not detected by standard immunoelectrophoresis of the eluate, although this technique is relatively insensitive. Because of the
physicochemical characteristics of the inhibitor and the fact that it binds to SPA, it is likely, although not proven, that it is an immunoglobulin, probably an IgG of subclass 1, 2, and/or 4. This would be consistent with reports in the literature where one high-titer inhibitor in hemophilia B was shown to be IgG₄.²

The fate of infused factor IX antigen and factor IX procoagulant activity was studied after Konyne infusion in patient A. Of interest, the factor IX procoagulant activity showed an initial survival that was similar to recent reports (t½ 5 hr), whereas factor IX antigen showed a markedly prolonged survival with a half-life estimated to be greater than 60 hr. This finding was in striking contrast to the data obtained after infusion of patients with hemophilia B - and B' who lacked an inhibitor. In these patients the initial survival (t½) of IXAg was the expected 5 hr.

One explanation for these findings in patient A would be the formation in vivo of factor IXAg-factor IXinh complexes that resulted in a prolonged survival of IXAg. Therefore a search for factor IX-inhibitor complexes was undertaken using three methods: SPA chromatography, two-dimensional (crossed) immunoelectrophoresis, and disc gel electrophoresis.

If an immunoglobulin binds to SPA by its Fc fragment, then the Fab portion of the molecule would be free to bind an antigen. Therefore if plasma obtained after Konyne infusion in patient A contained IXAg·IXinh complexes, factor IXAg would be expected to be retained by the SPA and could subsequently be eluted by NaSCN. As was shown, factor IXAg was recovered in the Ig peak (Table 3). Factor IXinh was not observed in this fraction, possibly owing to the reassociation of antigen-antibody complexes after dialysis.

Two-dimensional immunoelectrophoresis carried out in vitro on mixtures of factor IX and inhibitor from patient A or of post-Konyne infusion plasma from this patient showed a second, slower-moving peak when compared to crossed immunoelectrophoresis of factor IX alone (Fig. 3). These findings would indicate that under these electrophoretic conditions complexes formed either in vitro or in vivo migrate at a slower rate in the gel.

A third method for detecting the presence of inhibitor–factor IX complexes utilized electrophoresis of radiolabeled factor IX incubated with plasma from patient A in disc gel electrophoresis (Fig. 4). The presence of two peaks of radioactivity in this test system and the presence of only one peak when normal plasma was added to the labeled factor IX provides additional evidence for formation of complexes in vitro.

The size, kinetics, and effects of these complexes forming in vivo following infusion of factor IX concentrates are not known. If the complexes were very large, e.g., 19S, the reticuloendothelial system should rapidly clear them from the circulation.² The prolonged survival of factor IXAg observed in our patient would suggest that the complexes must be smaller than 19S in size. Complexes of smaller size could possibly result in vascular or organ damage.²² Fortunately, as of this writing no evidence of immune complex disease had been found in patient A, although it is possible that such disease could occur in the future.

Symptomatic immune complex disease has not been previously described in hemophilic patients with inhibitors. Patients developing high inhibitor titers are not usually given large amounts of antigen for therapeutic purposes and may therefore
be spared. "Low responders," however, may constitute a group of patients at risk. The lack of reported immune complex disease in these individuals may be due to a low titer of circulating complexes or due to the fact that some of the inhibitors are IgG4, which fixes complement poorly. Moreover, it is possible that brief episodes of immune complex disease may be clinically inapparent and therefore overlooked.

The lack of an anamnestic immune response to the factor IX contained in the therapeutic infusions in this patient could be due to genetic factors, variation in lymphocyte function, or the size of the immune complexes. It is also conceivable that almost immediately following infusion the inhibitor obscures antigenic determinants on the factor IX molecule that are needed for additional recognition by the immune system.

In light of the recent surge of interest in circulating immune complexes and their relationship to disease, hemophiliacs with inhibitors offer a unique opportunity for study of these phenomena. Furthermore, physicians caring for patients with inhibitors who are receiving infusions of factor IX concentrates should be alert for the development of immune complex disease in these patients.

ACKNOWLEDGMENT

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REFERENCES


Circulating factor IX antigen-inhibitor complexes in hemophilia B- following infusion of a factor IX concentrate

SH Jr Goodnight, CW Britell, KD Wuepper and B Osterud

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