The Idiotypic Characteristics of Human Antibodies to Factor VIII

By Beatriz de la Fuente and Leon W. Hoyer

We have prepared an antiidiotype antibody that specifically reacts with and inactivates a human autoantibody to factor VIII (VIII:C). The antiidiotype specificity of this rabbit antibody was evident in solution, as specific inhibition of anti-VIII:C activity, and in solid-phase studies. The antiidiotype antibody inhibited both plasma anti-VIII:C and Fab' fragments prepared from that plasma's IgG. Although the antibody had a greater inhibitory effect on the anti-VIII:C used for immunization than it did for other anti-VIII:C, partial inhibition was identified with ten of 25 other human anti-VIII:C. The demonstration that antiidiotype antibodies can be prepared to human anti-VIII:C indicates the feasibility of this approach to the immunochemical characterization of human anti-VIII:C inhibitors. Moreover, antiidiotype reagents have a potential role in the treatment of inhibitor patients.

HUMAN ANTIBODIES that inactivate factor VIII procoagulant activity (VIII:C, antihemophilic factor) are detected in 5% to 15% of repeatedly transfused patients with severe classic hemophilia (hemophilia A) and, less commonly, as autoantibodies that develop in individuals with previously normal hemostasis.1 These antibodies, IgG in almost all cases, cause serious difficulty for the affected patients, as they inactivate most VIII:C produced by patients with autoantibodies and inactivate any transfused VIII:C. Thus, they prevent specific treatment of factor VIII deficiency by transfusion therapy.

These antibodies have been characterized in several ways: by their potency, eg, titer in Bethesda units,2 by their kinetics and the completeness of VIII:C inactivation,3,5 by their immunoglobulin subclass composition, and by the VIII:C epitopes with which they react.6 These studies have clarified the nature of individual antibodies, and they provide information that is at times quite useful in considering alternative therapies. They have not, however, provided information about the factors involved in the control of anti-VIII:C production, nor have they identified the characteristics that might predispose hemophilic patients to inhibitor formation. Studies of the idiotype properties of human anti-VIII:C have the potential capacity to address these issues.7,8 This article describes an approach to the preparation of antibodies that react with idiotypic determinants of human anti-VIII:C and summarizes the properties of such an antiidiotype antibody.

MATERIALS AND METHODS

Human Antibodies to Factor VIII

Antibody (Ab) plasmas were obtained from 15 patients with severe hemophilia who developed an inhibitor (Ab 7–9, 21–29, and 31–33) and from 11 individuals with no previous bleeding disorder whose anti-VIII:C were considered to be autoantibodies (Ab 1–4, 6, 16, 18, 30, and 34–36). All of these antibodies were type I in their kinetic pattern, except for Ab16, 18, and 36, which had type II properties according to the criteria of Biggs and co-workers.9,10 The properties of ten of these antibodies have been previously described in detail.11 They had titers between 25 and 9,000 Bethesda units/mL2 and had been kept as frozen samples for one to 16 years. These inhibitor plasmas were obtained through the helpful cooperation of Drs Liberto Pechet (Ab1), Yogendra Shah (Ab3), Dennis O'Leary (Ab4), Helen Glueck (Ab6), Jessica Lewis (Ab7), Richard Lutes (Ab8), Edward Tuddenham (Ab9), Harvey Weiss (Ab19), Alan Klatsky (Ab21), David Green (Ab22), Kim Ritchey (Ab23 and 29), Emily Czapek (Ab24), Sandor Shapiro (Ab28 and 32), Jack Levin (Ab30), Peter Levine (Ab31), Robert Breckenridge (Ab33 and 34), Lionel Clyne (Ab35), and Rene Lerner (Ab36). Ab2 was purchased from George King Biomedical, Inc (Overland Park, Kan).

Preparation of Fab' Fragments From Human Anti-VIII:C Plasmas

The IgG of seven human anti-VIII:C plasmas (Ab 1, 6–9, 16, 18) was separated from other proteins by caprylic acid precipitation, and the Fab' fragments were obtained by pepsin digestion as previously described.9 Fab' preparations had protein concentrations between 2.9 and 4.7 mg/mL. The Fab' had anti-VIII:C activity comparable to the IgG from which they had been prepared when the values were expressed as Bethesda units/mg.

Factor VIII Measurements

Factor VIII procoagulant activity (VIII:C) was measured by a one-stage method using factor VIII-deficient human plasma as substrate.9 Pooled normal human plasma, prepared as previously described,11 served as standard (1 U/mL) for all factor VIII measurements.

Anti-VIII:C Measurements

Inhibition of VIII:C procoagulant activity was determined by incubating equal volumes of pooled normal human plasma and a dilution of anti-VIII:C at 37 °C for two hours. The residual VIII:C activity was then measured and the antibody titer expressed in

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Bethesda units. This value was obtained by determining the reciprocal of that dilution of test plasma or Fab' preparation that reacted with 0.5 U of VIII:C during the two-hour incubation.

Inhibition of anti-VIII:C activity was determined by incubating dilutions of heated and calcium phosphate absorbed rabbit antiserum (0.1 mL) (or control normal rabbit serum) with 0.01 mL of the human anti-VIII:C (or Fab' fragments prepared from these plasmas) diluted to contain 20 to 30 Bethesda units/mL. The residual anti-VIII:C activity was determined after this mixture had been held at 37 °C for one hour and at 4 °C overnight.

Similar incubations were carried out to determine the effect of rabbit IgG coupled to agarose. The residual human anti-VIII:C reactivity was measured after the same incubation schedule, and the beads were removed by centrifugation. After the beads were washed, bound anti-VIII:C was eluted from them using 0.05 mol/L glycine and 0.1 mol/L NaCl, pH 2.4.

Anti-IgG Measurements

Initial screening for anti-IgG in rabbit antisera was carried out by Ouchterlony analysis using 0.01 to 1.00 mg/mL human gamma globulin (Cohn fraction II, ca 99% IgG by electrophoresis, Sigma Chemical Company, St Louis). Similar testing was carried out to monitor the effectiveness of absorption of rabbit antisera with normal human IgG. Anti-IgG was also measured by the ELISA assay described by McCoy and co-workers. Flat-bottomed microtiter plates (Dynatech Laboratories, Inc, Alexandria, Va) were coated with IgG (10 μg/mL; 100 μL/well) in 0.05 mol/L sodium carbonate, pH 9.6. After incubation for three hours at 37 °C, the coated plates were washed five times with phosphate-buffered saline (PBS) containing 0.5% Tween 20 (PBS-T20). Bovine serum albumin (BSA) in PBS (1 mg/mL) was then added to each well, and the plates were incubated for an additional 30 minutes at 37 °C. After the plates were washed three times with PBS-T20, dilutions of rabbit antiserum (100 μL) were added for a one-hour incubation at 37 °C. The plates were then washed five times with PBS-T20 and to each well was added 100 μL of alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin (heavy and light chain) (Miles Laboratories, Elkart, Ind) in 0.1 mol/L Tris-HCl, pH 8.0, containing 1 mg/mL BSA. After one-hour incubation at 37 °C, the wells were washed five times with PBS-T20 and 100 μL of p-nitrophenyl phosphate (Sigma) (1 mg/mL in 0.05 mol/L carbonate, pH 9.6) were added. After a 15- to 20-minute incubation at room temperature, the optical density of the solution was measured at 405 nm, using a Titer-tek Multiskan spectrophotometer (Flow Laboratories, McLean, Va).

Purification of Human Anti-VIII:C

The method of anti-VIII:C purification was modified slightly from that developed for separation of 125I-labeled human anti-VIII:C. Immune complexes were obtained by incubating antihemophilic factor concentrate (Armour Laboratories, Phoenix, Ariz) with 0.5 mL human anti-VIII:C plasma for four hours at 37 °C. Complexed anti-VIII:C was recovered in void-volume fractions obtained by agarose gel filtration using a 1.6 × 84 cm column of Sepharose 6B (Pharmacia Fine Chemicals, Piscataway, NJ) in borate-buffered saline. Anti-VIII:C was dissociated from the antigen by acidification at pH 3.5 for 30 minutes (a procedure that destroys VIII:C antigenic determinants), and the solution was then adjusted to pH 7.0. After the volume was reduced to 5 to 8 mL using Aquacide IA (Calbiochem, Inc, La Jolla, Calif), IgG fractions were separated by Sephadex G-200 (Pharmacia) gel filtration in borate-buffered saline using a 1.6 × 87 cm column. The second (IgG) peak from this fractionation was absorbed to protein A-Sepharose (Pharmacia) for two hours at 37 °C using an IgG/PAS ratio (10 mg/mL packed vol) well below the PAS capacity. The PAS beads were then washed three times with PBS containing 1 mg/mL rabbit albumin, and the absorbed IgG was eluted with 0.05 mol/L glycine, 0.1 mol/L NaCl, pH 2.4. The eluate was neutralized by adding 1/10 vol borate buffer, pH 8.4, and dialyzed against borate-buffered saline prior to anti-VIII:C measurement and IgG quantification by ELISA assay. The IgG standard was a human serum of known IgG content obtained from Calbiochem (SHS, lot 1006D).

Preparation of Antiidiotype Antibodies

A modification of the method described by Zanetti and Bigazzi was used in these studies. The immunizing antigen (human anti-VIII:C 15 to 40 μg IgG containing 40 to 90 Bethesda units inhibitor activity) was mixed with an equal volume (1 mL) of complete Freund's adjuvant prior to intradermal injection in multiple sites, including both foot pads. Subsequent injections were carried out at two-week and four-week intervals, using similar amounts of antigen in incomplete Freund's adjuvant. The rabbits were bled ten to 15 days after each injection; an average of ten serum samples was obtained from each of three rabbits.

Sera were heated to 56 °C for 30 minutes, clarified by centrifugation at 2,000 g for 15 minutes at 4 °C, and absorbed with 10 mg/mL rabbit anti-human IgG (J.D. Baker, Phillipsburg, NJ) for ten minutes at 25 °C. They were then centrifuged at 2,000 g for 15 minutes at 4 °C. The heat-inactivated and calcium phosphate-absorbed sera were stored at −70 °C until used.

Reactivity with normal human IgG was removed by both fluid-phase and solid-phase absorptions. The former were carried out by adding human IgG prepared from pooled normal serum (Sigma) at a ratio of 10 mg/mL rabbit antiserum and incubating the mixture for one hour at 37 °C and 16 hours at 4 °C. A small amount of precipitate was formed, and this was removed by centrifugation (2,000 g for 30 minutes at 4 °C). Some samples were absorbed a second time, but with only an additional 5 mg/mL rabbit serum.

Solid-phase absorptions were carried out using human IgG coupled to agarose (Sepharose 2B-CL, Pharmacia) by the cyanogen bromide method. The coupling ratio was 10 mg IgG/mL activated Sepharose, and over 98% of the protein was coupled. The washed beads were stored in borate-buffered saline at 4 °C. Absorptions were carried out in 16 × 100 cm polystyrene tubes containing 10 (or 5) mg normal human IgG-agarose/mL rabbit serum. After incubation on a rocking platform for one hour at 37 °C and 16 hours at 4 °C, the beads were separated by centrifugation (2,800 g) for 15 minutes at 4 °C.

Some of the rabbit antibodies were also coupled to agarose for use in solid-phase studies. IgG was separated from other serum proteins by caprylic acid precipitation, as previously described, and coupled to cyanogen bromide-activated agarose at a ratio of 10 mg/mL beads. IgG-agarose suspensions were prepared using IgG derived from normal rabbit serum, potent antisera that reacted with human anti-VIII:C, and those potent antisera after absorption with normal human IgG.

The completeness of (human) IgG removal by these beads was determined by ELISA assay using microtiter plates coated with goat anti-human IgG (Cappel Laboratories, West Chester, Pa) and peroxidase-goat anti-human IgG (Cappel).

RESULTS

Purification of Human Anti-VIII:C

Specific anti-VIII:C was purified from two plasmas (Ab1 and Ab6), which have been characterized in detail in a previous publication. The technique, similar to that used to purify 125I-labeled anti-VIII:C for...
use in immunoradiometric assays, includes two gel filtration steps and can be carried out during a 48-hour period. The data for 11 Ab1 purifications are summarized in Table 1. The overall recovery of anti-VIII:C activity was 8.1%, and the overall purification was 121-fold. The anti-VIII:C/IgG ratio was increased nearly 25-fold during the purification.

Properties of Rabbit Anti-Ab1

Two rabbits were immunized with purified Ab1, and a third rabbit was immunized with purified Ab6. While all three rabbits formed anti-human IgG, there was no detectable antiidiotype reactivity when the anti-Ab6 and one of the anti-Ab1 sera were absorbed with normal human IgG. These two antisera were not useful for further studies. In contrast, the third rabbit formed antibodies that were specific for antigenic determinants present on purified Ab1 but not present on normal human IgG. As is summarized in Table 2, the anti-Abl activity was detected four weeks after initial immunization, and this serum inhibited Ab1 activity. While the anti-human IgG titer was stable in subsequent samples, anti-Abl activity increased until weeks 10 and 13 and subsequently fell. Anti-IgG precipitates could be detected by Ouchterlony assays at dilutions of rabbit serum as great as 1:30.

It was recognized that the anti-human IgG reactivity of the rabbit serum was not specific for Ab1 determinants. Evidence of antiidiotype activity was obtained by absorbing the anti-Abl serum with normal human IgG until there was no detectable anti-IgG activity. The adequacy of the absorption was subsequently verified by the more sensitive ELISA technique. Addition of 10 mg normal human IgG/mL antiserum regularly removed more than 99.9% of the anti-IgG activity (Fig 1), and subsequent absorption with an additional 5 mg human IgG/mL removed 90% of the residual activity. Absorption of the rabbit serum with human IgG coupled to agarose was equally effective in depleting the serum of anti-IgG activity, and the absorbed serum had similar antiidiotype activity.

As apparent in Table 2, the absorbed rabbit antisera had very little residual anti-normal human IgG, but they retained much of the anti-Ab1 activity. This is seen more clearly in Fig 1, an analysis of the serum obtained after ten weeks of immunization. The upper panel illustrates the anti-IgG reactivity before and after absorption, and the bottom panel indicates the

![Graph](image1)

**Figure 1.** The effect of absorption of anti-Ab1 with normal human IgG. The upper panel indicates the anti-IgG activity of Ab1 before absorption (0--0), after absorption with 10 mg normal human IgG/mL serum (O--O), and after a second absorption with an additional 5 mg normal human IgG/mL serum (O--O). The lower panel indicates the residual Ab1 anti-VIII:C activity after incubation with dilutions of anti-Ab1 serum. The symbols identify the same samples as those in the upper panel. Comparable results were obtained when the anti-Ab1 was absorbed with normal human IgG coupled to agarose.

<p>| Table 2. Anti-Human IgG and Anti-Ab1 Properties of Sera Obtained After Immunization With Purified Ab1 |
|-------------------------------------------------|------------------------|------------------------|</p>
<table>
<thead>
<tr>
<th>Weeks After Initial Immunization</th>
<th>Anti-IgG Content*</th>
<th>Anti-Ab1†</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>28,000 &lt;10</td>
<td>0 &lt;0</td>
</tr>
<tr>
<td>4</td>
<td>29,000 &lt;10</td>
<td>6 &lt;0</td>
</tr>
<tr>
<td>6</td>
<td>30,000 &lt;10</td>
<td>21 &lt;0</td>
</tr>
<tr>
<td>8</td>
<td>30,000 &lt;10</td>
<td>30 &lt;10</td>
</tr>
<tr>
<td>10</td>
<td>25,000 &lt;10</td>
<td>66 &lt;25</td>
</tr>
<tr>
<td>13</td>
<td>15 &lt;3</td>
<td>58 &lt;14</td>
</tr>
<tr>
<td>15</td>
<td>25,000 &lt;10</td>
<td>38 &lt;3</td>
</tr>
<tr>
<td>21</td>
<td>9,000 &lt;10 &lt;10</td>
<td>18 &lt;3</td>
</tr>
</tbody>
</table>

* Dilution of rabbit serum that caused an absorbance of 0.8 (405 nm) in the anti-IgG ELISA.
† Dilution of rabbit serum that reduced the Ab1 anti-VIII:C activity by 50%. These determinations were done with mixtures that contained 2 to 3 Bethesda units/mL.
‡ The unabsorbed sera were tested after heat inactivation and incubation with calcium phosphate. The absorbed sera had been subsequently incubated with 10 mg normal human IgG/mL serum.

![Table 1](image2)

**Table 1. Purification of Human Anti-VIII:C**

<table>
<thead>
<tr>
<th>Material*</th>
<th>Volume (ml)</th>
<th>Anti-VIII:C (Bethesda Units)</th>
<th>IgG (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>0.5</td>
<td>691 ± 71</td>
<td>8,170 ± 723</td>
</tr>
<tr>
<td>Void-volume fractions after agarose gel filtration</td>
<td>36.0</td>
<td>314 ± 56</td>
<td>197 ± 26</td>
</tr>
<tr>
<td>Second peak after Sephadex G-200 gel filtration</td>
<td>17.0</td>
<td>109 ± 9</td>
<td>56 ± 8</td>
</tr>
<tr>
<td>Eluate from protein A-Sepharose</td>
<td>1.1</td>
<td>56 ± 8</td>
<td>27 ± 4</td>
</tr>
</tbody>
</table>

*The mean values (±SE) are indicated for data obtained during II Ab1 purifications.
anti-VIII:C plasma or Fab' with 0.1 mL of

The mixture had 2 to 3 Bethesda units/mL when normal rabbit plasma absorbed with normal human IgG coupled to agarose. The anti-Abi 675 IDIOTYPIC PROPERTIES OF ANTI-VIII:C

These properties demonstrate the antiidiotype specific-

anti-VIII:C activity. Nearly 50% of the anti-Abi reactivity remained after absorption of more than 2.3, 2.2, 2.4 Bethesda units/mL, and 2.4, 2.2, 2.2 Bethesda units/mL, respectively.

Fig 2. The effect of absorbed anti-Abi on the anti-VIII:C properties Abi plasma (O--O) and anti-VIII:C IgG purified from Abi plasma (■—■). In the control mixtures (data not shown), Abi plasma and the purified Abi, incubated with dilutions of normal rabbit serum (1:3, 1:10, and 1:30), had comparable values: 2.3, 2.2, 2.4 Bethesda units/mL, and 2.4, 2.2, 2.2 Bethesda units/mL, respectively.

Table 3. Inhibition of Plasma and Fab' Anti-VIII:C Activity by Absorbed Anti-Abi

<table>
<thead>
<tr>
<th>Anti-VIII:C Plasm</th>
<th>Dilution Tested</th>
<th>Fab' (mg/mL)</th>
<th>Residual Anti-VIII:C* (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ab1</td>
<td>1:50</td>
<td>0.19</td>
<td>57.5 ± 6.2</td>
</tr>
<tr>
<td>Ab1</td>
<td>1:50</td>
<td>0.19</td>
<td>73.0 ± 2.0</td>
</tr>
<tr>
<td>Ab6</td>
<td>1:20</td>
<td>0.20</td>
<td>9.8 ± 5.7</td>
</tr>
<tr>
<td>Ab7</td>
<td>1:12</td>
<td>0.39</td>
<td>6.2 ± 16.9</td>
</tr>
<tr>
<td>Ab7</td>
<td>1:300</td>
<td>0.014</td>
<td>9.6 ± 5.0</td>
</tr>
<tr>
<td>Ab8</td>
<td>1:150</td>
<td>0.15</td>
<td>12.3 ± 6.3</td>
</tr>
<tr>
<td>Ab8</td>
<td>1:300</td>
<td>0.15</td>
<td>21.5 ± 12.3</td>
</tr>
<tr>
<td>Ab9</td>
<td>1:166</td>
<td>0.19</td>
<td>26.1 ± 12.3</td>
</tr>
<tr>
<td>Ab16</td>
<td>1:20</td>
<td>0.53</td>
<td>15.3 ± 15.8</td>
</tr>
<tr>
<td>Ab1</td>
<td>1:50</td>
<td>0.19</td>
<td>6.2 ± 16.9</td>
</tr>
<tr>
<td>Ab1</td>
<td>1:10</td>
<td>0.19</td>
<td>10.4 ± 3.2</td>
</tr>
<tr>
<td>Ab1</td>
<td>1:30</td>
<td>0.19</td>
<td>12.3 ± 6.3</td>
</tr>
<tr>
<td>Ab1</td>
<td>1:50</td>
<td>0.19</td>
<td>15.3 ± 15.8</td>
</tr>
<tr>
<td>Ab1</td>
<td>1:10</td>
<td>0.19</td>
<td>12.3 ± 6.3</td>
</tr>
<tr>
<td>Ab1</td>
<td>1:50</td>
<td>0.19</td>
<td>15.3 ± 15.8</td>
</tr>
</tbody>
</table>

*Percent of control measured after incubation of 0.01 mL dilute anti-VIII:C plasma or Fab' with 0.1 mL of 1:3 and 1:10 dilutions of anti-Abi absorbed with normal human IgG coupled to agarose. The mixture had 2 to 3 Bethesda units/mL when normal rabbit plasma replaced the anti-Abi.

effect of the absorbed and unabsorbed serum on Ab1 anti-VIII:C activity. Nearly 50% of the anti-Abi reactivity remained after absorption of more than 99.99% of the anti-(normal human) IgG reactivity. These properties demonstrate the antiidiotype specificity of the absorbed rabbit serum.

The specificity of the absorbed anti-Abi was verified in an additional experiment in which it was tested with Fab' prepared from Abi. The removal of the Fc fragment antigenic determinants from Abi had no effect on the inhibitor properties of absorbed anti-Abi (Table 3). While the incomplete inactivation of Abi anti-VIII:C activity (Fig 1) might have been due to a relatively low antibody content in the absorbed anti-

Ab1, it is also possible that the absorbed antiserum did not include antibodies that react with some of the anti-VIII:C in Ab1 plasma. Immunochemical heterogeneity has been recognized in several previous studies, and the "resistant population" may have had different antigenic determinants. This was tested by comparing the effects of absorbed anti-Abi when incubated with Abi plasma and with purified Abi anti-VIII:C. In contrast to the limited inactivation of plasma anti-VIII:C activity (50% to 60% in most cases, as noted in Fig 1 and Tables 1 through 3), there was complete (>95%) inactivation of purified anti-Abi (Fig 2). When dilutions of the absorbed anti-Abi were tested with comparable amounts of anti-VIII:C, there was nearly a 100-fold increase in the amount of antiidiotype antibody needed to inactivate the anti-VIII:C activity. This suggests that the purification method recovers only a subpopulation of the anti-VIII:C, and the antiidiotype antibody prepared by immunization with that subpopulation is very effective in inactivating its anti-VIII:C reactivity. The increased reactivity was not simply due to the lower IgG content of the purified anti-VIII:C, as addition of comparable amounts of IgG (cross-reacting material-negative hemophilic plasma free of an inhibitor) to purified Abi had no effect on its inactivation by absorbed anti-Abi.

Reactivity of Absorbed Anti-Abi With Other Human Anti-VIII:C

In order to determine whether the absorbed anti-Abi would react with all human anti-VIII:C 25 other human antibodies were tested in the same way as Abi (Fig 3). Appropriate dilutions of each anti-VIII:C plasma were incubated with 1:3 and 1:10 dilutions of normal rabbit serum, anti-Abi (containing antinormal human IgG), and absorbed anti-Abi specific for Abi idiotype determinants. The experimental variation inherent in these measurements was estimated from the differences between the residual anti-VIII:C activities for the control incubations. The average value for the 1:3 and 1:10 dilutions of the normal rabbit serum incubation mixtures was defined as 100%. The standard deviation of these measurements was 8.5%, and a reduction of twice this value (17%) was taken as a conservative estimate of the possible loss of anti-VIII:C activity that was not due to specific inactivation by anti-Abi. In every case, the unab-

sorbed anti-Abi removed >75% of the anti-VIII:C activity, establishing that these inhibitors were, in fact, IgG antibodies. When absorbed anti-Abi was incubated with 26 anti-VIII:C plasmas, the maximal inhibition was obtained, as expected, with Abi (Fig 3).
Solid-Phase Properties of Anti-Ab1

As inactivation assays are subject to possible VIII:C measurement artifacts caused by residual antibody or antigen, several of the studies were repeated using anti-Ab1 coupled to agarose. In this case, anti-Ab1 should remove Ab1 IgG from solution instead of forming an inactive complex or precipitate.

Similar results were obtained when human anti-VIII:C were tested with anti-Ab1 IgG coupled to agarose. Eighty percent of Ab1 anti-VIII:C activity was removed when it was incubated with coupled (unabsorbed) anti-Ab1 at a ratio of 15 mg rabbit IgG/mL Ab1 plasma, and 59% was removed when the anti-Ab1 serum had been absorbed with normal human IgG prior to the isolation of rabbit IgG and its coupling to agarose. Control experiments with Ab6 and Ab9 demonstrated absorption of anti-VIII:C activity by the unabsorbed anti-Ab1 (69% and 82%, respectively, at the same ratios of IgG to plasma), but there was <15% reduction in antibody activity when they were incubated with agarose beads to which absorbed anti-Ab1 had been coupled.

Although extensive experiments were not carried out, anti-VIII:C could be recovered from the immunoabsorbents that had been washed with borate-buffered saline and eluted with a pH 2.4 buffer. Anti-VIII:C activity was obtained from anti-Ab1 agarose that had been incubated with Ab1, Ab2, Ab6, and Ab9. In contrast, anti-VIII:C activity was recovered from adsorbed anti-Ab1-agarose incubated with Ab1, but no anti-VIII:C activity could be detected in similar studies carried out with Ab6 or Ab9.

**DISCUSSION**

The development of antibodies to factor VIII (VIII:C, antihemophilic factor) is a serious complication of transfusion therapy in 5% to 15% of patients with severe classic hemophilia.1 Similar antibodies have been detected in individuals with no previous bleeding disorder, and these autoantibodies can also have a profound clinical effect.18 Although a number of different therapeutic approaches have been attempted, none have the consistent hemostatic effect that is noted when factor VIII is used to treat uncomplicated hemophilia. Our studies of anti-VIII:C formation have been carried out in order to develop better ways to manage this important clinical problem.

Although several laboratories have characterized the immunochemical properties of human anti-VIII:C,1,5,6,17-20 there is, to our knowledge, no previous information about their idiotypic properties. The present article demonstrates the feasibility of preparing antiidiotype antibodies to human anti-VIII:C, and it identifies heterogeneity in a small group of potent anti-VIII:C.

The recognition of unique antigenic determinants of myeloma proteins21 and on specific antibodies22,23 has led to our present understanding of idiotype properties.
In essence, this designation identifies immunoglobulin antigenic determinants that are not isotypic or allotypic. The “individual antigenic specificities” have subsequently been shown to reflect heavy and/or light chain variable (V) region determinants. Although these determinants are a part of the antigen binding region of the immunoglobulin, antigen inhibits idiotype reactivity in only some instances.24 There is variable cross-reactivity when antiidiotype reagents are tested with antibodies to the same antigen that have been obtained from different individuals.25-27 Common cross-reactive idiotype determinants have been recognized in several studies, eg, some human anti-hepatitis B surface antigen antibodies25 and rabbit antiidiotype reagents that react with human IgM rheumatoid factors.26 In other instances, each antiidiotype antibody appears to have an individual idiotype specificity.27,28

In the present study, a rabbit antiidiotype antibody has been obtained after immunization with a purified human autoantibody to VIII:C. As expected, the immunized rabbit also formed a variety of antibodies that reacted with common IgG determinants. When these were removed by exhaustive absorption, the residual serum had no detectable activity with normal human IgG, but it continued to inactivate Abl anti-VIII:C (Fig 1, Table 2). When 25 other human anti-VIII:C were tested, ten appeared to have partial cross-reactivity, but 15 other inhibitors did not interact with the anti-Ab1 to a detectable level (Fig 3). This pattern is different from human autoantibodies to IgG (rheumatoid factors), which have been found to share common idiotypic determinants in many instances.26,29 Further studies are in progress to determine the idiotype heterogeneity of human anti-VIII:C.

The inhibition of human anti-VIII:C by the antiidiotype serum was verified in solid-phase studies. This was necessary for two reasons. A major concern was the use of a coagulation assay for the final measurement, as artifact and/or uninterpretable results are possible when a variety of different plasmas of different species are mixed together. Even though control mixtures are evaluated, it is difficult to avoid unwanted procoagulants in this kind of experiment. A second and more subtle reason for solid-phase studies was a concern about the possible role of “internal images” in the evaluation of antiidiotype antibodies.7,28-31 This concept, first articulated by Jerne in 1974,2 suggests that the original antigen and the antiidiotype antibodies may share structural features. The “insulin-like” mimetic effect of anti-(bovine antiinsulin) has been considered to be an example of such a relationship.31 If this were the case, the presence of an anti-anti-VIII:C could have had intrinsic VIII:C activity in coagulation assays that would mimic anti-VIII:C inhibition. By removing anti-VIII:C in a solid-phase system, this potential artifact was prevented. Moreover, the antiidiotype preparations did not have any intrinsic VIII:C activity when tested in standard VIII:C assays.

The inactivation of Fab' fragments prepared from the anti-VIII:C plasmas further demonstrated that the effect of absorbed anti-Ab1 was not due to reactivity with isotypic determinants, eg, those present on the Fc fragment. Moreover, the partial cross-reactivity with anti-VIII:C other than the immunizing antigen was comparable when whole plasmas (IgG) or Fab' were examined (Table 3).

The incomplete inactivation of Ab1 anti-VIII:C by relatively large amounts of absorbed rabbit anti-Ab1 raised a question about possible heterogeneity in the population of anti-VIII:C that was present in unfractionated Ab1 plasma. Evidence for heterogeneity was then obtained by demonstrating complete inactivation when purified Ab1 anti-VIII:C was tested instead of Ab1 plasma. This difference was not due to an effect of other IgG molecules, as addition of (noninhibitor) hemophilic plasma had no effect of the phenomenon and other purified anti-VIII:C were not inactivated. Thus, it would appear that Ab1 plasma contains at least two different antibody populations and that the method used for anti-VIII:C purification selectively isolates one of them. This will, of course, be difficult to verify unless it is possible to prepare monoclonal antibodies that react with the different anti-VIII:C in Ab1 plasma. At the present time, there is no alternative purification method, and these other determinants will have to be identified with reagents prepared by immunizing mice with the IgG fraction from Ab1 plasma.

Although the present study simply demonstrates that antiidiotype antibodies can be prepared using human anti-VIII:C as antigen, this type of reagent may have therapeutic potential. The inhibiting effect of antiidiotype antibodies on immune reactivity has been a topic of considerable interest, but it has been recognized that antibody formation can be either augmented or inhibited by such an infusion.8 Careful preliminary studies will be essential if antiidiotype antibodies are to be considered for the treatment of patients with anti-VIII:C. A more immediate application of antiidiotype antibodies would be the in vitro removal of specific antigen. As an immunoabsorbent, solid-phase antiidiotype antibodies could be used for extracorporeal perfusion of patient blood so that only specific antibodies would be removed. This approach may be more satisfactory than hemoperfusion using protein A adsorbents to remove IgG32 or intensive plasmapheresis.33 While the preparation of sufficient antiidiotype antibody for this kind of therapy would not be possible using rabbits, it is certainly feasible.
using monoclonal antibody methods. It remains to be determined, of course, if several specific antiidiotype antibodies will have to be developed for each individual patient’s population of anti-VIII:C, as our data would suggest, or if cross-reacting idiotypes can be identified when other human inhibitors are studied.

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The idiotypic characteristics of human antibodies to factor VIII

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