Immunologic Studies of Antihemophilic Factor (AHF, Factor VIII): Cross-Reacting Material in a Genetic Variant of Hemophilia A

By Leon W. Hoyer and Robert T. Breckenridge

Plasma antihemophilic factor (AHF, Factor VIII) activity is reduced in patients with hemophilia A (classical hemophilia) and von Willebrand's disease. In neither disease has it definitely been established whether a functionally defective AHF-like protein is synthesized or whether there is a simple deficiency of AHF. These alternatives are of special interest in von Willebrand's disease for there is apparent AHF synthesis after transfusion of normal plasma, hemophilic plasma or serum.

Anticoagulants which immunologically inactivate AHF make it possible to consider the presence of cross-reacting material (CRM) which shares antigentic characteristics with AHF though unable to function as AHF in coagulation assays. It has been reported that rabbit anti-human AHF is neutralized by hemophilic plasma, but anticoagulant specificity was not documented in these studies. Goudemand and co-workers found that hemophilia A plasmas appeared to inactivate an antibody to AHF which developed in a patient with hemophilia A after repeated transfusions. These data are presented only as a mean value for 13 hemophilic plasmas and it is not possible to evaluate variation between patients, effect of AHF level, or possible differences between families. Cross-reacting material was not found in other studies and data for 41 CRM-free patients with hemophilia A have been reported. In one series the plasmas from 20 additional patients with moderate hemophilia A (AHF activity of 1–12 units/100 ml.) were considered separately for they had variable capacity to inactivate anti-AHF. Mc Lester and Wagner found no evidence for CRM in plasma from dogs with a coagulation defect which in all respects mimics hemophilia A.

No CRM has been recognized in patients with von Willebrand’s disease. Only 18 patients have been reported, however, and it is not known how many different families have been tested.

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We have measured the capacities of AHF deficient plasmas to neutralize an anticoagulant to AHF which developed in a previously normal 33 year old Puerto Rican male following a penicillin reaction. Plasmas from 34 patients of 27 families with hemophilia A and six patients of four families with von Willebrand's disease were examined. Cross-reacting material was present in plasmas of six patients with hemophilia A and five of them were members of one family. Patients with this genetic variant of hemophilia A have normal amounts of a structurally altered AHF-like protein in their plasma but this material lacks procoagulant activity.

**MATERIALS AND METHODS**

*Citrate Plasmas.* Venous blood samples were drawn using plastic syringes and added to plastic tubes which contained one-ninth volume 3.8 per cent sodium citrate. Plasma was separated by centrifugation at 1200 g for fifteen minutes at 4 C. and used immediately or stored in plastic tubes at -20 C. until used.

*Hemophilic Plasma* was obtained from patients with hemophilia A who did not have circulating anticoagulants as determined by a previously published technic in which the plasma mixture incubates three hours prior to assay.

*Hemophilic Substrate Plasma used for assay* was made platelet deficient and stored in nonsiliconized plastic containers at -20 C. until used. It contained less than one unit of AHF per 100 ml. when compared to normal control plasma supplied by Dr. David Aronson of the National Division of Biological Standards. All AHF values are expressed in the units proposed by an ad hoc committee of the National Research Council in which one unit of AHF is the amount in one ml. of an average normal plasma.

*von Willebrand's plasma* was obtained from six individuals who had the three major characteristics of this disease: family history consistent with autosomal dominant inheritance, reduced AHF level and prolonged bleeding time.

*Antihemophilic Factor* was assayed by a one stage method. A single substrate was used in the studies reported, but two of the von Willebrand's plasmas and ten hemophilia A plasmas were reassayed with another substrate with similar results. Fresh plasma obtained from a single normal donor (L.W.H) was used as standard in these studies. This plasma contained 100 units of AHF per ml when compared with the lyophilized standard obtained from the Division of Biologic Standards. All clotting studies were performed in uncoated Pyrex tubes with an internal diameter of 8 mm.

*The circulating anticoagulant* used in these experiments was from a single plasma sample obtained from A.R. The characteristics and the specificity of this anticoagulant have been reported elsewhere.

Neutralization of anticoagulant activity by test plasmas was measured by incubating 0.05 ml. of a 1:25 dilution of the anticoagulant plasma, 0.5 ml. of the test plasma, and 0.1 ml. of normal plasma in an uncoated polystyrene tube (internal diameter of 11 mm.) at 37 C. for 90 minutes. At the end of this period the mixture was placed in a melting ice waterbath, a suitable dilution made with cold buffer, and the AHF activity determined. The residual AHF activity was compared with the AHF added in the 0.5 ml. test plasma and the 0.1 ml. normal plasma. Table 1 indicates the steps required in the neutralization assay and the calculations required to determine the percentage of AHF remaining after incubation.

Freshly prepared hemophilic plasmas had the same properties in the neutralization assay as plasmas from the same individual which had been stored at -20 C. for as long as 19 months. As repeated assays (4-8 separate experiment) of three CRM-negative plasmas (C.G., D. Cu. and R.Wa.) gave constant results, most hemophilic plasmas were assayed in a single experiment. Each of the hemophilia A plasmas found to have CRM was retested at least three times. All von Willebrand's plasmas were assayed using fresh plasma and a repeat assay done with plasma stored at -20 C. for less than three weeks.
Table 1.—Neutralization of Anticoagulant by Test Plasma

<table>
<thead>
<tr>
<th>Test plasma</th>
<th>Normal</th>
<th>Saline</th>
<th>D.Cu. 1 (CRM-negative Hemophilia A)</th>
<th>Z.L. 2 (CRM-positive Hemophilia A)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AHF activity of test plasma (units/100 ml.)</td>
<td>100</td>
<td>0</td>
<td>1.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Units AHF added:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5 ml. test plasma</td>
<td>.5</td>
<td>0</td>
<td>.0075</td>
<td>.0125</td>
</tr>
<tr>
<td>0.1 ml. normal plasma</td>
<td>.1</td>
<td>.1</td>
<td>.1</td>
<td>.1</td>
</tr>
<tr>
<td>Total</td>
<td>.6</td>
<td>.1</td>
<td>1.075</td>
<td>1.125</td>
</tr>
</tbody>
</table>

Five tenths ml. of test plasma, 0.05 ml. of a 1:25 dilution of anticoagulant plasma, and 0.1 ml. of normal plasma inoculated for 90 minutes at 37°C. The mixture was placed in a melting ice waterbath, a 1:5 dilution made with cold buffer, and the AHF activity determined.

<table>
<thead>
<tr>
<th>Clotting time (seconds)</th>
<th>91</th>
<th>133</th>
<th>135</th>
<th>110</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residual AHF activity (units/100 ml.)*</td>
<td>23</td>
<td>1.1</td>
<td>1.1</td>
<td>4.9</td>
</tr>
<tr>
<td>(units/0.65 ml.)</td>
<td>.150</td>
<td>.0074</td>
<td>.0074</td>
<td>.032</td>
</tr>
</tbody>
</table>

| % AHF activity remaining after incubation† | 25 | 7.4 | 6.9 | 28.4 |

*The AHF activity was determined using normal plasma incubated for 90 minutes at 37°C as standard.†Residual AHF activity (units/0.65 ml.) divided by the total units AHF added.

The barbital-saline buffer, pH 7.4–7.5, contained 7.3 Gm. NaCl, 2.76 Gm. barbital, 2.06 Gm. sodium barbital and distilled water to make the volume one liter. All dilutions were made using this buffer.

RESULTS

The decrease in free antibody associated with inactivation of AHF by immune anticoagulants makes it possible to detect anticoagulant neutralization by cross-reacting material (CRM). Barbital-saline buffer is the CRM-free standard to which test plasmas are compared, and reduced AHF inactivation is evidence for anticoagulant neutralization by CRM. The immune interaction is measured, therefore, in a manner like that of radioimmunoassay: the "labeled" functional AHF is displaced from the antibody by "unlabeled" biologically inactive CRM. Illustrative data for normal plasma, barbital-saline buffer, and hemophilic plasmas with (Z.L.) and without (D.Cu.) CRM are given in Table 1.

Plasmas from severe hemophiliacs can be compared with saline for they have insignificant AHF activity. Test plasmas with over 10 units AHF/100 ml. reduce the anticoagulant to AHF ratio, and, therefore, the percentage of AHF inactivated in the absence of nonfunctional CRM. This effect was measured for a series of mixtures of normal (L.W.H.) and hemophilic CRM-free (C.G.) plasma (Fig. 1). The percentage AHF remaining after 90 minutes incubation was directly related to the AHF activity of the test plasma.
Fig. 1.—Inactivation of AHF mixtures with different initial AHF activities. Five-tenths ml. of a mixture of normal and hemophilic plasma was added to a series of tubes containing 0.05 ml. of anticoagulant (1:25 dilution) and 0.1 ml. of normal plasma. The residual AHF activity was measured after incubation at 37 C. for 90 minutes and the per cent AHF retained was calculated. Each point represents a single determination.

AHF ACTIVITY OF TEST PLASMA IN UNITS/100 ml

Assay reproducibility was measured in four ways: 1) A single normal plasma (L.W.H.) was assayed 16 times in eight separate experiments over a two month period. The average percentage AHF retained was 20.0 with a standard deviation of 3.8 and a range of 15.0 to 28.0. 2) Plasmas from six normal individuals and from two patients with Christmas disease (Factor IX deficiency) who had normal AHF levels had an average percentage AHF retained of 21.2 (standard deviation of 3.0 and total range of 18.0 to 25.0). 3) Barbital-saline buffer in place of test plasma was assayed 14 times in eight separate experiments and had an average percentage AHF retained of 6.2 (standard deviation of 1.3 with range of 4.1 to 9.0). 4) Plasma from one CRM-negative hemophiliac (C.G.) was assayed in eight separate experiments and had an average percentage AHF retained of 7.2 (standard deviation of 1.5 with range of 5.4 to 9.6).

von Willebrand’s plasmas were tested for CRM in parallel with control mixtures of normal (L.W.H.) and CRM-free hemophilic (C.G.) plasma. Plasmas from six patients of four different families were studied (Table 2). These von Willebrand’s plasmas did not effect higher residual AHF activity after incubation with the anticoagulant than did the control mixtures adjusted to the same initial AHF activity. There was, therefore, no evidence for cross-reacting AHF-like material in von Willebrand’s disease.

Hemophilia A plasmas had less than one to 10 units AHF per 100 ml and were, therefore, compared with buffer in the neutralization assay (Table 3). Twenty-eight of the patients (25 families) had no demonstrable CRM and the residual AHF activity was no greater than control assays with buffer in place of test plasma. Six of the patients (W.Ka., Z.L., G.C., K.E., D.E. and S.E.) had CRM, however, and the neutralization assay indicated that their plasmas contained as much material which reacted immunologically with the anticoagulant as did normal plasmas. One patient (W.Ka., a 50 year old man with severe hemophilia A) died of renal disease shortly after this study was begun and had no living relatives with coagulation abnormalities. The other patients with CRM belong to a single family which includes three brothers (K.E., D.E. and S.E.), their maternal grandfather (G.C.) and a male cousin.
Table 2.—Neutralization of Anticoagulant to AHF by von Willebrand’s Plasmas

| Family | Patient | Sex | AHF units per 100 ml.* | Clotting Time (Seconds) | % AHF activity retained
|--------|---------|-----|------------------------|-------------------------|------------------------
| I.     | D.C.    | F   | 28                     | 129                     | 11%                    |
| I.     | E.C.    | F   | 60                     | 112                     | 10%                    |
| II.    | N.S.    | F   | 10                     | 155                     | 14%                    |
| III.   | G.V.E.  | M   | 28                     | 130                     | 10%                    |
| III.   | M.V.E.  | F   | 33                     | 161                     | 10%                    |
| IV.    | E.R.    | M   | 7.5                    | 145                     | 9%                     |

*AHF activity of fresh plasma.\textsuperscript{17}

\textsuperscript{1}Five one-hundredths ml. of a 1:25 dilution of the anticoagulant plasma was incubated with 0.5 ml. of the test plasma and 0.1 ml. of normal plasma for 90 minutes at 37 C. At the end of this period the mixture was placed in a melting ice waterbath, a suitable dilution made with cold buffer, and the residual AHF activity determined.

\textsuperscript{2}The AHF activity was determined using incubated normal plasma as standard. The percentage AHF present after incubation was then calculated comparing the units AHF present with the total AHF activity added in the 0.5 ml. test plasma and 0.1 ml. normal plasma.

§A mixture of normal and hemophilic plasmas adjusted to give the same AHF activity as the von Willebrand’s plasma.

(Z.L.). No other members of this family have known coagulation abnormalities. Additional family studies and an investigation of the nature of the CRM are in progress.

Repeated assays with fresh or frozen plasma gave similar results. The neutralization assay separated, without overlap, normals and hemophiliacs with CRM (over 17 percent of AHF retained) from buffer controls and hemophiliacs without CRM (less than 10 percent of AHF retained). Cross-reacting material was also recognized in plasmas from family XXVII when they were assayed by the slightly different inhibitor-neutralization assay of Abildgaard and co-workers.\textsuperscript{12}

**DISCUSSION**

A majority of patients with hemophilia A do not synthesize any AHF, or, alternatively, synthesize a defective molecule lacking those antigens recognized by this circulating anticoagulant to AHF. Two families have been recognized, however, in which nonfunctional but cross-reacting material (CRM) is present. The pattern of inheritance in CRM-positive family XXVII is that of a sex-linked recessive characteristic typical of hemophilia A.

Our findings may help to clarify the apparently contradictory reports of CRM in hemophilia A.\textsuperscript{7,12} Goudemand and co-workers concluded that hemophilia A plasma “consumes” the anticoagulant to a greater extent than a buffer control.\textsuperscript{9} Their data were expressed as an average of 13 plasmas and may represent a mixture of patients having or lacking CRM. Similar results were obtained by Uszynski when he tested 20 plasmas from patients with moderate (1-12 units AHF activity/100 ml) hemophilia A.\textsuperscript{10} Familial hemophilia with CRM may have been overlooked in both series. The studies done...
Table 3.—Neutralization of Anticoagulant to AHF by Hemophilia A Plasmas

<table>
<thead>
<tr>
<th>Family</th>
<th>Patient</th>
<th>Plasma AHF (Units per ml.)*</th>
<th>Clotting Time (Seconds)</th>
<th>% AHF activity retained†</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. D.D.</td>
<td>&lt;1</td>
<td>138</td>
<td>5.7</td>
<td></td>
</tr>
<tr>
<td>I. R.Go.</td>
<td>&lt;1</td>
<td>134</td>
<td>7.5</td>
<td></td>
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<tr>
<td>II. W.A.</td>
<td>&lt;1</td>
<td>128</td>
<td>9.6</td>
<td></td>
</tr>
<tr>
<td>II. Dan.A.</td>
<td>&lt;1</td>
<td>134</td>
<td>7.2</td>
<td></td>
</tr>
<tr>
<td>II. Dav.A.</td>
<td>&lt;1</td>
<td>130</td>
<td>9.0</td>
<td></td>
</tr>
<tr>
<td>III. J.M.</td>
<td>&lt;1</td>
<td>130</td>
<td>9.0</td>
<td></td>
</tr>
<tr>
<td>IV. T.Wo.</td>
<td>&lt;1</td>
<td>135</td>
<td>6.3</td>
<td></td>
</tr>
<tr>
<td>V. R.Wa.</td>
<td>&lt;1</td>
<td>128</td>
<td>9.6</td>
<td></td>
</tr>
<tr>
<td>VI. G.Al.</td>
<td>&lt;1</td>
<td>135</td>
<td>7.8</td>
<td></td>
</tr>
<tr>
<td>VII. J.We.</td>
<td>&lt;1</td>
<td>140</td>
<td>5.4</td>
<td></td>
</tr>
<tr>
<td>VIII. R.B.</td>
<td>&lt;1</td>
<td>133</td>
<td>7.8</td>
<td></td>
</tr>
<tr>
<td>IX. M.R.</td>
<td>&lt;1</td>
<td>140</td>
<td>5.4</td>
<td></td>
</tr>
<tr>
<td>X. W.T.</td>
<td>&lt;1</td>
<td>132</td>
<td>8.4</td>
<td></td>
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<tr>
<td>XI. D.Sc.</td>
<td>&lt;1</td>
<td>134</td>
<td>7.2</td>
<td></td>
</tr>
<tr>
<td>XII. C.G.</td>
<td>&lt;1</td>
<td>138</td>
<td>5.7</td>
<td></td>
</tr>
<tr>
<td>XIII. W.Ke.</td>
<td>&lt;1</td>
<td>140</td>
<td>5.1</td>
<td></td>
</tr>
<tr>
<td>XIV. D.Sm.</td>
<td>&lt;1</td>
<td>135</td>
<td>9.5</td>
<td></td>
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<tr>
<td>XV. S.S.</td>
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<td>134</td>
<td>9.5</td>
<td></td>
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<td>XVI. S.Co.</td>
<td>1.0</td>
<td>135</td>
<td>9.5</td>
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<td>XVII. A.La.</td>
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<td>7.9</td>
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<tr>
<td>XVIII. H.P.</td>
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<td>131</td>
<td>9.9</td>
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<tr>
<td>XIX. D.Cu.</td>
<td>1.5</td>
<td>135</td>
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<tr>
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<td>2.5</td>
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<td>7.0</td>
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<tr>
<td>XXI. M.Ho.</td>
<td>3.0</td>
<td>141</td>
<td>6.2</td>
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<tr>
<td>XXII. R.He.</td>
<td>5.0</td>
<td>131</td>
<td>7.1</td>
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<tr>
<td>XXIII. T.B.</td>
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<td>127</td>
<td>7.6</td>
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<td>XXIV. D.Bu.</td>
<td>10</td>
<td>130</td>
<td>8.0</td>
<td></td>
</tr>
<tr>
<td>XXV. W.Se.</td>
<td>10</td>
<td>123</td>
<td>8.2</td>
<td></td>
</tr>
<tr>
<td>XXVI. W.Ka.</td>
<td>1.0</td>
<td>115</td>
<td>9.0</td>
<td></td>
</tr>
<tr>
<td>XXVII. Z.L.</td>
<td>2.5</td>
<td>110</td>
<td>28.4</td>
<td></td>
</tr>
<tr>
<td>XXVII. G.C.</td>
<td>3.5</td>
<td>111</td>
<td>27.0</td>
<td></td>
</tr>
<tr>
<td>XXVII. K.E.</td>
<td>5.0</td>
<td>107</td>
<td>27.0</td>
<td></td>
</tr>
<tr>
<td>XXVII. D.E.</td>
<td>5.0</td>
<td>114</td>
<td>17.0</td>
<td></td>
</tr>
<tr>
<td>XXVII. S.E.</td>
<td>2.5</td>
<td>108</td>
<td>28.8</td>
<td></td>
</tr>
</tbody>
</table>

*AHF activity of plasma tested.†

†Five one-hundredth ml. of a 1:25 dilution of the anticoagulant plasma was incubated with 0.5 ml. of the test plasma and 0.1 ml. of normal plasma for 90 minutes at 37 C. At the end of this period the mixture was placed in a melting ice waterbath, a suitable dilution made with cold buffer, and the residual AHF activity determined.

The AHF activity was determined using incubated normal plasma as standard. The percentage AHF present after incubation was then calculated comparing the units AHF present with the total AHF activity added in the 0.5 ml. test plasma and 0.1 ml. normal plasma. Controls run with each neutralization assay included normal plasma (clotting times of 92-94 seconds with 17-23 percent of AHF activity retained) and buffer in place of test plasma (clotting times of 134-138 seconds with 5.4-7.2 percent AHF activity retained) in place of test plasma.

with incompletely characterized rabbit anti-human AHF are difficult to interpret in that the assays may have been sensitive to contamination with antifibrinogen activity in the anticoagulant serum. While they are consistent with CRM in hemophiliacs, they do not provide evidence for such material.

It is not possible to estimate the incidence of circulating CRM in the 40
hemophiliacs Uszynski studied for the details of assay reproducibility are not given and it is unclear how many of these patients definitely have CRM. Abildgaard and co-workers found no examples of hemophilia A with CRM among 20 patients tested by methods similar to those we have used. As CRM was present in only two of the 27 families in our series, the combined data suggest that this genetic variant is uncommon.

All of the patients in whom CRM has been detected have measurable AHF activity by standard assay methods. Uszynski separated patients with severe (less than 1 unit AHF/100 ml.) and moderate (1-12 units AHF/100 ml.) hemophilia and identified CRM only in the later group. Our findings are consistent with this distinction in that the six patients with CRM had detectable AHF procoagulant activity (1 to 5 units/100 ml.). No CRM activity was found in patients (14 families) with less than 1 unit AHF activity/100 ml.

A genetic variant of hemophilia B (Christmas disease) has also been recognized. Roberts and co-workers demonstrated PTC (Factor IX Christmas factor)-inhibitor-neutralizing activity in plasmas of seven patients of four families with hemophilia B. About 10 per cent of the hemophilia B patients studied had CRM; the presence of CRM was not correlated with PTC level or clinical severity.

In contrast, no patients with von Willebrand’s disease have been found to have CRM. Our six patients (four families) are like the ten reported by Goudemand et al. and the eight reported by Uszynski in this respect. If any patients with von Willebrand’s disease have CRM, they must comprise a small proportion of all those with the disease.

Another hereditary deficiency of procoagulant protein, Hageman trait, is similarly free of plasma antigenic material cross-reacting with the affected protein. Passive hemagglutination inhibition, procoagulant inhibition, and double diffusion in agar gel suggested that synthesis of Hageman factor is depressed in individuals with Hageman trait.

Anticoagulants to AHF are immunoglobulins and several have been recognized as having restricted antigenic characteristics. An example of heavy chain homogeneity (γ4) has recently been reported, and nine acquired AHF anticoagulants have been found to contain only kappa light chains. These findings, taken with the previous demonstrations that the activity of the weaker anticoagulants can be absorbed with AHF, establish that the anticoagulants are antibodies to AHF. Earlier rate studies had suggested that the anticoagulants might be enzymes, but it has become apparent that antibodies to enzymes may have similar temperature and time dependent characteristics, as originally suggested by Biggs and Bidwell in their studies of an anticoagulant which inactivated AHF.

The structural homogeneity of anticoagulants recognized in classic hemophiliacs after repeated transfusions, as well as those anticoagulants which have developed without recognized immunization, suggests a restriction in antibody structure imposed by the antigenic properties of AHF. We
identified AHF-like CRM using a well characterized IgG anticoagulant which
developed in a patient after a penicillin reaction. This anticoagulant may
have a different specificity from anti-AHF anticoagulants observed after re-
peated transfusions in AHF deficient patients or from antibodies pre-
pared in rabbits immunized with AHF. The CRM-positive familial
variant of hemophilia A will allow a more detailed consideration of the spe-
cificities of different anti-AHF anticoagulants.

The cross-reacting material is structurally altered AHF which lacks en-
zyme (procoagulant) activity but which retains antigenic properties. Other
structural changes might affect this antigenic site as well, and such molecules
would not be detected by our methods. The CRM-negative patients may in-
deed be a heterogeneous group with different molecular defects. At the
present time this possibility can not be distinguished from an alternative
hypothesis: markedly reduced synthesis of normal AHF.

Although AHF activity is destroyed by prolonged storage or clotting, aged
normal plasma and normal serum have material with the antigenic charac-
teristics of AHF. Our findings emphasize the distinction which has
been made between the procoagulant activity of AHF and its antigenic
characteristics. Isolation and characterization of AHF have been difficult
and remain incomplete. The immunologic methods which demonstrate this
genetic variant provide another means by which this elusive protein may be
recognized and characterized.

SUMMARY

Immunologic studies have identified two genetically distinct types of hemo-
philia A. While a majority of patients apparently fail to synthesize AHF, a
variant has been recognized in which a nonfunctional but antigenically cross-
reacting AHF-like protein is present. Plasmas from two of twenty-seven
families with hemophilia A have a cross-reacting material which inactivates
an anticoagulant to AHF. Nonfunctional cross-reacting material was not pre-
sent in plasmas from six patients with von Willebrand’s disease.

SUMMARIO IN INTERLINGUA

Studios immunologic ha resultate in le identification de duo geneticamente distincte
typos de hemophilia A. Durante que un majoritate del patientes pare esser incapace de
synthetisar factor antihemophilic (FAH), un variante esseva recognoscite in le qual es
presente un proteina FAH-simile le qual es nonfunctional sed capace de reaction cruciate
antigenic. Le plasmas ab duo de vinti-septe familias con hemophilia A habeva materiales
a reaction cruciate le quales inactivava un anticoagulante anti FAH. Nonfunctional
material a reaction cruciate non esseva presente in le plasmas ab sex patientes con
morbo de von Willebrand.

ACKNOWLEDGMENTS

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