Cross-reacting material in genetic variants of haemophilia B

DOMINIQUE MEYER, ETHEL BIDWELL, AND MARIE JOSÉ LARRIEU

From the Coagulation Department, Institut de Pathologie Cellulaire, Hôpital Bicêtre, Paris, France, and the Churchill Hospital, Oxford

SYNOPSIS  Cross-reacting factor IX material (CRM) was immunologically detected in the plasma of 38 normal individuals and 21 out of 22 haemophilia B patients using a rabbit antibody to factor IX. The same reacting material was detected in only nine of these patients using a human antibody. These results indicate that the plasma of the majority of haemophilia B patients contains a protein-lacking biological activity but having antigenic determinants in common with normal factor IX.

Genetic variants of haemophilia A and B (factor VIII and IX deficiencies) have been recently demonstrated by the ability or inability of the patient's plasma to neutralize specific human antibodies against factor VIII or IX (Roberts, Gross, Webster, and Dejanov, 1966; Denson, Biggs, and Mannucci, 1968; Hoyer and Breckenridge, 1968; Roberts, Grizzle, McLester, and Penick, 1968; Feinstein, Chong, Kasper, and Rapaport, 1969; Denson, Biggs, Haddon, Borrett, and Cobb, 1969; Brown, Hougie, and Roberts, 1970; Meyer, Dray, and Larrieu, 1970; Hoyer and Breckenridge, 1970; Meyer and Larrieu, 1971). In most patients (haemophilia A− or B−) immunological cross-reacting material was absent, while in 10 to 15% of them an inactive protein could be demonstrated (haemophilia A+ or B+). However, conflicting data were obtained in haemophilia A when using factor VIII antibodies raised in different species (rabbit, goat) by immunization with partially purified factor VIII. Depending on the reactivity of the various antibodies, inactive cross-reacting material could be demonstrated in a highly variable percentage of haemophilia A patients: 100% (Zimmerman, Ratnoff, and Powell, 1971; Stites, Hershgold, Perlman, and Fudenberg, 1971; Meyer, Lavergne, Larrieu, and Josso, 1972), 90% (Bennett and Huehns, 1970), or around 15% (Denson et al., 1969; Gralnick, Abrell, and Bagley, 1971). The present study demonstrates a similar discrepancy when the reactivity of different haemophilia B plasmas was compared with two types of factor IX antibodies, namely, a human inhibitor which occurred in a transfused haemophilia B patient, and rabbit antibodies raised by immunization with a partially purified antigen.

Materials and Methods

The factor IX concentrate used as a source of antigen was a sample from a batch of human material (RD40) prepared at the Oxford Haemophilia Centre by batch adsorption on DEAE-cellulose followed by displacement elution in a column (Dike, Bidwell, and Rizza, 1972). This concentrate contained 62 U/ml factor IX, 50 U/ml factor II, 44 U/ml factor X, and a very low concentration of factor VII (1.7 U/ml). The protein concentration was 10 mg/ml and the purification with respect to factor IX was 400-fold.

Rabbit antisera were obtained by injection of equal parts of factor IX concentrate and complete Freund's adjuvant. Three injections (6 mg protein) were given at intervals of eight days, the first one in the popliteal lymph node and subsequent ones in foot pads. Blood was drawn 10 days after the last injection, and allowed to clot in glass tubes, which were kept at 37°C for six hours and at 4°C for 12 hours. Serum was obtained by centrifugation at 5000 g for 15 min, oxalated, adsorbed with barium carbonate (40 mg/ml), and heated at 56°C for 30 minutes. The antibody titres (Denson, 1967) were 5 U/ml and 18 U/ml (1 U was defined as the amount of serum destroying 75% factor IX after incubation at 37°C for 15 min). The anti-factor II and anti-factor X antibody titres were < 2 U/ml. These antibodies were not specific for factor IX, as by immunodiffusion four lines of precipitation were shown against normal plasma or factor IX concentrate.

Human factor IX antibody was a specific inhibitor which appeared in a haemophilia B patient after multiple transfusions. The antibody titre was 15 U/ml.

Received for publication 18 November 1971.
Factor IX activity was measured by a one-stage assay (Langdell, Wagner, and Brinkhous, 1953), and prothrombin time with human and ox-brain thromboplastin (Thrombotest) as previously described (Meyer and Larrieu, 1971).

Inhibitor-neutralizing activity was measured by a modified two-stage procedure (Denson et al, 1969). In the first step, 0·4 ml of normal or test plasma was incubated at 37°C for 15 min with 0·1 ml of an appropriate dilution of human or rabbit antibody. In the second step, 0·2 ml of normal plasma was added to an equal volume of the first mixture. After a second incubation at 37°C for 15 min residual factor IX was measured by a one-stage assay. Results were expressed as units of neutralized inhibitor.

Results

Inhibitor-neutralizing activity in control plasmas

The specificity of the factor IX assays ensures the specificity of the inhibitor-neutralizing technique for the antigenic determinants of factor IX. Other coagulation factor antibodies, such as anti-factor VIII (from human or rabbit origin) or rabbit anti-human factor II,1 tested in the same system, did not interfere with the final assay of factor IX after the two incubations.

Inhibitor-neutralizing activity was measured in normal plasma (38 experiments): it varied from 0·85 to 1·07 unit (mean 1·03 ± 0·07 unit) when using

1 kindly provided by J. M. Lavergne and F. Josso, Hôpital Necker, Paris.

human antibody (Fig. 1), and from 0·85 to 1·25 unit (mean 0·92 ± 0·05 unit) when using antihuman factor IX antiserum (Fig. 2). Cross-reacting material was present in normal serum as well as in the plasma of coagulation deficiencies other than factor IX. It was lacking in aluminium hydroxide or barium sulphate-adsorbed plasma or serum, the results being the same as those with citrated saline.

Inhibitor-neutralizing activity in patients with haemophilia B

Twenty-two haemophilia B patients were tested in the same way using both human and rabbit antibodies.

Human antibody

Plasma samples from 13 haemophilia B patients failed to neutralize the human antibody. These patients were classified as haemophilia B+. Five plasmas contained an amount of cross-reacting material identical to that in normal plasma, and four showed intermediate results (Fig. 1). These nine patients were classified as haemophilia B+.

Rabbit antiserum

The capacity of the same plasmas to neutralize the rabbit antihuman factor IX antibodies appeared entirely different. Only one patient out of the 22 tested lacked immunologically detectable cross-reacting material (Fig. 2). This patient had a moderate form of haemophilia B (factor IX activity 4%). In 14 patients plasma inhibitor neutralizing activity varied from 0·65 to 1·1 unit, i.e., within the normal

![Fig. 1](image1.png)

Fig. 1 Inhibitor-neutralizing activity in 22 cases of haemophilia B (human factor IX inhibitor).

■ Haemophilia B

![Fig. 2](image2.png)

Fig. 2 Inhibitor-neutralizing activity in 22 cases of haemophilia B (rabbit factor IX antiserum).

■ Normal range.
range, and seven patients had intermediate values, from 0.30 to 0.55 unit. The same results were observed using two different rabbit antisera. No correlation was found between the level of active factor IX and the amount of factor IX antigen.

OX BRAIN CLOTTING TIME IN PATIENTS WITH HAEMOPHILIA B

In five of the 22 patients tested, the Thrombotest time (or clotting time in the presence of ox-brain thromboplastin) was prolonged on repeated testing (62-85 sec) while the clotting time was normal in the presence of human brain thromboplastin. These patients were classified as haemophilia B_M (Hougie and Twomey, 1967). Cross-reacting material was present in three of these patients when tested by human antibody, and in all five when tested with rabbit antibody (Table I).

### Table I  Inhibitor-neutralizing activity in haemophilia B_M (five patients) (comparison of two types of antibodies)

<table>
<thead>
<tr>
<th>Factor IX Activity</th>
<th>Factor IX Antigen (INN Units)</th>
<th>Human Antibody</th>
<th>Rabbit Antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 1</td>
<td>0</td>
<td>0.50</td>
<td></td>
</tr>
<tr>
<td>&lt; 1</td>
<td>0</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td>&lt; 1</td>
<td>0.46</td>
<td>1.05</td>
<td></td>
</tr>
<tr>
<td>&lt; 1</td>
<td>1.05</td>
<td>0.95</td>
<td></td>
</tr>
<tr>
<td>1-5</td>
<td>0.92</td>
<td>0.95</td>
<td></td>
</tr>
<tr>
<td>Control (mean)</td>
<td>0.92</td>
<td>1.03</td>
<td></td>
</tr>
</tbody>
</table>

### Table II  Classification of patients with haemophilia B according to the results of Thrombotest and inhibitor-neutralizing activity

<table>
<thead>
<tr>
<th>Antibody</th>
<th>No. of Cases</th>
<th>Haemophilia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B_M</td>
<td>B+</td>
</tr>
<tr>
<td>Human</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>+</td>
</tr>
<tr>
<td>III</td>
<td>2</td>
<td>+</td>
</tr>
<tr>
<td>IV</td>
<td>3</td>
<td>+</td>
</tr>
<tr>
<td>Rabbit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>II</td>
<td>16</td>
<td>+</td>
</tr>
<tr>
<td>III</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>IV</td>
<td>5</td>
<td>+</td>
</tr>
</tbody>
</table>

This work was supported by a grant from INSERM. We wish to thank G. W. R. Dike (Oxford Haemophilia Centre) for providing factor IX concentrate. The technical help of B. Obert is gratefully acknowledged.

### References


Reports and Bulletins prepared by the Association of Clinical Biochemists

The following reports and bulletins are published by the Association of Clinical Biochemists. They may be obtained from The Administrative Office, Association of Clinical Biochemists, 7 Warwick Court, Holborn, London, WC1R 5DP. The prices include postage, but air mail will be charged extra. Overseas readers should remit by British Postal or Money Order. If this is not possible the equivalent of 50p is the minimum amount that can be accepted.

**SCIENTIFIC REPORTS**

1. **Automatic Dispensing Pipettes.** An assessment of 35 commercial instruments 1967 P. M. G. BROUGHTON, A. H. GOWENLOCK, G. M. WIDOWSON, and K. A. AHLQUIST 85p ($2)

2. **An Evaluation of five Commercial Flame Photometers suitable for the Simultaneous Determination of Sodium and Potassium** March 1970 P. M. G. BROUGHTON and J. B. DAWSON 85p ($2)

**SCIENTIFIC REVIEWS**

1. **The Assessment of Thyroid Function** March 1971 F. V. FLYNN and J. R. HOBBIS 62p ($1.50)


**TECHNICAL BULLETINS**

9. **Determination of Urea by AutoAnalyzer** November 1966 RUTH M. HASLAM 42p ($1)

11. **Determination of Serum Albumin by AutoAnalyzer using Bromocresol Green** October 1967 B. E. NORTHAM and G. M. WIDOWSON 42p ($1)

13. **An Assessment of the Technicon Type II Sampler Unit** March 1968 B. C. GRAY and G. K. MCGOWAN 42p ($1)

14. **Atomic Absorption Spectroscopy. An outline of its principles and a guide to the selection of instruments** May 1968 J. B. DAWSON and P. M. G. BROUGHTON 42p ($1)


16. **A Guide to Automation in Clinical Chemistry** May 1969 P. M. G. BROUGHTON 62p ($1.50)

17. **Flame Photometers** (2nd edition) 1969 P. WILDING 62p ($1.50)


19. **Spectrophotometers. A comparative list of low-priced instruments readily available in Britain May 1970 C. E. WILDE and P. SEWELL 62p ($1.50)

20. **Quantities and Units in Clinical Biochemistry** June 1970 P. M. G. BROUGHTON 62p ($1.50) More than 30 copies in units of 10 at 20p


23. **Interchangeable Cells for Spectrophotometers and Fluorimeters September 1971 E. S. BROWN and A. H. GOWENLOCK 62p ($1.50)

24. **Simple Tests to Detect Poisons** March 1972 B. W. MEADE t L. 62p ($1.50)
Cross-reacting material in genetic variants of haemophilia B

Dominique Meyer, Ethel Bidwell and Marie José Larrieu

doi: 10.1136/jcp.25.5.433

Updated information and services can be found at:
[http://jcp.bmj.com/content/25/5/433](http://jcp.bmj.com/content/25/5/433)

**Email alerting service**

Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

**Notes**

To request permissions go to:
[http://group.bmj.com/group/rights-licensing/permissions](http://group.bmj.com/group/rights-licensing/permissions)

To order reprints go to:
[http://journals.bmj.com/cgi/reprintform](http://journals.bmj.com/cgi/reprintform)

To subscribe to BMJ go to:
[http://group.bmj.com/subscribe/](http://group.bmj.com/subscribe/)