A Macroglobulin with Inhibitory Activity Against Coagulation Factor VIII

By P. A. CASTALDI AND R. PENNY

HEMOSTATIC DISORDERS with a varying incidence of bleeding, are common in the dysproteinemias, and are characteristic of the macroglobulinemia of Waldenström. These abnormalities are most frequently attributed to abnormal platelet function,\(^1\) and depression of specific clotting factors appears to be a rare occurrence. Nile\(^2\) includes four instances of antihemophilic globulin (factor VIII) deficiency in a survey of macroglobulinemia among other disorders, but does not elaborate on the mechanism. In contrast, other authors\(^3\)\(^4\) have found high levels of factor VIII in the dysproteinemias, including macroglobulinemia. The present report is concerned with acquired deficiency of factor VIII occurring in a patient with macroglobulinemia, in whom clinical and laboratory studies indicate that the macroglobulin behaved as an inhibitor of this factor.

MATERIALS AND METHODS

Venous blood, collected from the antecubital vein, was anticoagulated with one part 3.8 per cent sodium citrate to nine parts of blood and collected and processed in nonwettable polyvinyl tubes. Platelet-rich and platelet-poor plasmas were prepared by centrifugation at 180g. for five minutes and 1000g. for 15 minutes at \(4^\circ\) C, respectively.

The bleeding time was determined by the method of Ivy, platelet adhesiveness in vivo by the method of Borchgrevink\(^5\) and in a glass bead column by the method of Salzman\(^6\) with the modification that blood was collected with 40 mm. mercury compression of the upper arm during a period of 60 seconds in a volume of 2–4 ml. Tests of clot retraction, platelet aggregation with adenosine diphosphate and thrombin, and of platelet-factor-3 activity were performed as previously described.\(^7\) Thrombin time, thrombin titre, one-stage prothrombin time with rabbit brain thromboplastin,\(^*\) thromboplastin generation test and plasma fibrinogen level were estimated using standard procedures.\(^8\) The partial thromboplastin time with kaolin\(^9\) and assays of coagulation factors II, V, and VII–X were performed by standard methods\(^8\) and coagulation factors VIII and IX were assayed by one-stage procedures using the partial thromboplastin time technique. The standard of reference was a freeze-dried pool of normal plasma.\(^**\) In addition, assays of factor VIII in mixtures of concentrated preparations of the clotting factor and test plasma or protein preparations, were performed as described by Pool and Robinson.\(^10\)

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* Commonwealth Serum Laboratories, Melbourne.
** Hyland Laboratories, U.S.A.
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TEST OF INHIBITOR ACTIVITY

Mixtures of normal plasma or semipurified factor VIII* with saline, patient’s plasma or isolated macroglobulin were made in different proportions as shown in the results. Following incubation for 60 minutes at 37°C, the presence of inhibitor activity was determined by assay of factor VIII.

Immunoelectrophoresis of serum was performed as described by Grabar and Burtin** using antisera specific for gamma-G, A and M, and Kappa and Lambda light chains. The macroglobulin was isolated from aged serum or aluminum hydroxide absorbed citrated plasma by gel filtration on Sephadex G.200 and elution with 0.1M phosphate buffer, pH 7.2. Protein fractions were identified by absorbance at 280 μm; the first peak contained gamma-M macroglobulin as demonstrated by immunoelectrophoresis. Refiltration was performed if contaminating gamma-G globulin was present. Repeated euglobulin precipitation of whole serum, followed by washing and suspension of the precipitate in saline also produced immunologically pure gamma-M macroglobulin. Purified gamma-G globulin from the patient’s serum was obtained from DEAE cellulose chromatography and elution with 0.01M phosphate buffer, pH 8.0. The purified protein fractions were stored in the freeze-dried state at −20°C. Reduction of the macroglobulin was achieved with mercaptoethanol at a final concentration of 0.1M and incubation at 37°C for one hour.

CASE HISTORY

R.B. (No. 106398), a woman aged 49 years, presented in February, 1967, with a two-year history of progressive weakness, lethargy and menorrhagia. There was no past or family history of bleeding. Physical examination showed bruising, mild cervical lymphadenopathy and retinal vein congestion. Venipunctures led to local bruising, and bone marrow aspiration from the iliac crest resulted in extensive subcutaneous bleeding and she was admitted to hospital. Initial investigations showed a hemoglobin level of 7.8 Gm./100 ml., white cell count of 5600/cu.mm. with 56 per cent neutrophils, 26 per cent lymphocytes and 18 per cent monocytes, a platelet count of 240,000/cu.mm. and erythrocyte sedimentation rate of 154 mm./hr. The morphology of the bone marrow was normal. Radiographs of chest, skull and skeletal survey were normal, but a lymphangiogram revealed groups of abnormal iliac and paraaortic nodes. Serum proteins were 11.2 Gm./100 ml., the Sia test was positive and electrophoresis and immunoelectrophoresis revealed a gamma-migrating monoclonal gamma-M macroglobulin with antigenic type-L light chains in a concentration of 5.4 Gm./100 ml. No urinary Bence–Jones protein was detected. Ultracentrifugal analysis of the serum revealed a large 17S peak. The results of tests of hemostasis are shown in Table 1. These tests revealed an abnormality in intrinsic coagulation with a factor VIII level of 10 per cent. The Ivy bleeding time was slightly prolonged to 14 minutes, but tests of platelet function performed at that time were not abnormal, although clot retraction and platelet adhesiveness were not assessed until later.

Treatment was commenced with a transfusion of 900 ml. of fresh-frozen plasma with transient elevation of the factor VIII level to 20 per cent, but early return to the pre-transfusion level of 10 per cent two hours later. Plasmapheresis was then continued for five days with daily exchange of two to three liters. This therapy resulted in a decrease of gamma-M concentration to 3.5 Gm./100 ml., and a rise in factor VIII to 18–21 per cent throughout the period of plasma exchange with amelioration of bruising. Three weeks after the first course of plasmapheresis, there occurred deterioration of vision with gross venous distension and retinal hemorrhage but this was reversed with further plasma exchange.

* Porcine AHF, Diagnostic Reagents. Oxon.
Following plasmapheresis, treatment included a course of intravenous penicillin in a daily dose of 12,000,000 units for five days and then penicillamine 2 Gm./day for 14 days, but neither influenced the level of factor VIII. Nitrogen mustard was given as a single dose of 0.4 mg./Kg. and subsequent maintenance treatment was with chlorambucil in a dose of 2–5 mg./day. During the initial period of treatment, factor VIII levels increased to a maximum of 70 per cent and subsequently remained in the range of 30–50 per cent, the most recent result in April 1969 being 45 per cent. Early gamma-M levels fluctuated about 5.5 Gm./100 ml., but have subsequently been less than 4.0 Gm./100 ml.

COAGULATION STUDIES

The plasma abnormality in the partial thromboplastin time was corrected by the addition of equal parts, or less, of normal plasma. The thromboplastin generation test was abnormal with the patient’s absorbed plasma, with substrate clotting times not less than 30 seconds, but gave normal results with the patient’s serum, with clotting time at six minutes of 11 secs. Minimal factor VIII levels were 10 per cent and other factors were present in normal concentrations (Table 1). Incubation of the patient’s plasma with normal plasma 1/9 (v/v) for 60 minutes at 37° C reduced the factor VIII concentration to 78 per cent compared with a saline control. A similar degree of change in factor VIII concentration was found in experiments with concentrates of the clotting factor incubated with the purified macroglobulin in concentrations of 10–50 mg./ml., prepared from aged serum collected prior to treatment. Reduction of the macroglobulin with mercaptoethanol removed this effect on factor VIII, and gamma-G isolated from the patient’s serum had no anti-factor VIII activity.

Table 1.—Results of Tests of Hemostasis on Patient R.B. with Waldenstrom’s Macroglobulinemia

<table>
<thead>
<tr>
<th>Test</th>
<th>Before Treatment</th>
<th>After Treatment</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleeding Time, Ivy (min.)</td>
<td>14</td>
<td>18</td>
<td>2–11</td>
</tr>
<tr>
<td>Platelet Count × 10^9/cu. mm.</td>
<td>240</td>
<td>320</td>
<td>150–400</td>
</tr>
<tr>
<td>Clot Retraction (Per cent)</td>
<td>67</td>
<td>65–95</td>
<td></td>
</tr>
<tr>
<td>Prothrombin Consumption Index (Per cent)</td>
<td>28</td>
<td>18</td>
<td>1–20</td>
</tr>
<tr>
<td>Platelet Factor 3 (sec.)</td>
<td>16</td>
<td>11–20</td>
<td></td>
</tr>
<tr>
<td>(Kaolin–Stypven)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelet Adhesiveness</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>in vivo (per cent)</td>
<td>34</td>
<td>20–80</td>
<td></td>
</tr>
<tr>
<td>in vitro (per cent)</td>
<td>78</td>
<td>30–95</td>
<td></td>
</tr>
<tr>
<td>Platelet Aggregation</td>
<td>Normal</td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>Prothrombin Time (sec.)</td>
<td>15</td>
<td>13</td>
<td>11–15</td>
</tr>
<tr>
<td>Thrombin Time (sec.)</td>
<td>10</td>
<td>10</td>
<td>8–16</td>
</tr>
<tr>
<td>Kaolin Partial Thromboplastin Time (sec.)</td>
<td>60</td>
<td>46</td>
<td>25–40</td>
</tr>
<tr>
<td>Fibrinogen mg./100 ml.</td>
<td>300</td>
<td>270</td>
<td>200–450</td>
</tr>
<tr>
<td>Coagulation Factor (Per cent)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>62</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>120</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VII–X</td>
<td>57</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IX</td>
<td>66</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VIII</td>
<td>10</td>
<td>32</td>
<td>45–180</td>
</tr>
</tbody>
</table>
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Table 2.—Effects of Patient Plasma and Isolated Proteins (Before and After Treatment) on Factor VIII Levels of Normal Plasma and Semipurified Porcine Factor VIII

<table>
<thead>
<tr>
<th>Test Mixture</th>
<th>Residual Factor VIII After 60 Mins. (Per Cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>One-Stage Assay</td>
</tr>
<tr>
<td>Normal Plasma + Saline 9 : 1</td>
<td>100</td>
</tr>
<tr>
<td>+ Patient Plasma 9 : 1</td>
<td>78</td>
</tr>
<tr>
<td>1 : 1</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>Two-Stage Assay</td>
</tr>
<tr>
<td>Factor VIII + gamma-M 5 mg./ml.</td>
<td>100</td>
</tr>
<tr>
<td>10</td>
<td>74</td>
</tr>
<tr>
<td>22</td>
<td>70</td>
</tr>
<tr>
<td>50</td>
<td>68</td>
</tr>
<tr>
<td>+ gamma-M reduced ME</td>
<td>100</td>
</tr>
<tr>
<td>+ gamma-G 4.4 mg./ml.</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Two-Stage Assay</td>
</tr>
<tr>
<td>Plasma + Post-treatment gamma-M 19 mg./ml.</td>
<td>100</td>
</tr>
</tbody>
</table>

when tested at a concentration of 4.4 mg./ml. Gamma-M samples collected during the period of remission when plasma levels of factor VIII had increased to 30–50 per cent could not be shown to possess inhibitory activity in similar incubation studies (Table 2).

DISCUSSION

There are few examples of specific activity as antibodies, of proteins produced during the course of such disorders as myeloma and macroglobulinemia of Waldenström. Harboe drew attention to the biological activities of monoclonal gamma-M globulins and cited cold hemagglutination, complement fixation, rheumatoid factor and agglutination of stored red blood cells as the antibody activities then recognized. Subsequently, anti-beta lipoprotein and anti-2,4-dinitrophenol activities have been described. Nilehn had previously mentioned the finding of decreased factor VIII levels in four of six patients tested with macroglobulinemia, although an anticoagulant effect was not detected and this author did not further investigate the defect. Glueck and Hong described a circulating anticoagulant in a patient with gamma-A myeloma and thereby provided the first report of acquired factor VIII deficiency in myeloma. The coagulation defect was corrected transiently in vivo by large doses of penicillin and in vitro by disulfide reducing agents, such as penicillamine and 2-mercaptoethanol, and by a specific antiserum. A similar type of factor VIII deficiency was found in the patient described in this report with macroglobulinemia. The features of this case were similar to those reported by Glueck and Hong in that there was incomplete inhibition of clotting factor activity which, however, was directly attributable to the effect of the monoclonal gamma-M protein.

The anticoagulant effect was transiently reduced by transfusion of plasma.
and by plasmapheresis, as may be expected from the observation that correction could be obtained in vitro if sufficient normal plasma was added. The effect of the patient’s plasma and of the isolated protein on normal plasma or semipurified factor VIII showed an inhibitory activity of only mild degree which required a certain minimal protein concentration for detection and which was reversible after reduction with mercaptoethanol. The inhibitor was active against both porcine and human factor VIII. These findings suggested a mechanism different from that found with other factor VIII inhibitors that occurred spontaneously, in association with pregnancy, in association with some dermatological disorders, penicillin reactions and in hemophilia, where the activity was found in the gamma-G globulins and behaved as an antibody with a progressive stoichiometric reaction. There has been a recent report in abstract referring to an anti-factor VIII activity residing in the gamma-M fraction in a patient with arthritis, but apparently not macroglobulinemia. In the case of this macroglobulin, as well as the gamma-A myeloma protein described by Glueck and Hong, inhibition of factor VIII activity was incomplete and may have resulted from an affinity between the paraprotein and clotting factor, resulting in absorption and masking of the active part of the molecule. This effect was overcome by reduction in vitro with mercaptoethanol and in vivo by lowering of the concentration and perhaps alteration in the protein by plasmapheresis and chemotherapy.

Other acquired inhibitors of factor VIII were all characterized by kappa-type light chains. The light chain characteristics of the case reported by Glueck and Hong were not determined, and the present case was found to have lambda-type light chains. It was also of interest that most authors detected high levels of factor VIII in myeloma and macroglobulinemia, in contrast to the finding in the present case. Although this observation did not particularly aid the explanation of the mechanism, it did emphasize the variability and possible specificity of these proteins as already suggested by studies of their antigenicity.

A variety of different hemostatic disorders may occur in protein abnormalities. The most frequent involve abnormalities in platelet behavior with prolongation of the bleeding time and decreased adhesiveness, although others attributed bleeding to masking of platelet factor 3 liberation. Other coagulation disorders, such as increased antithrombin activity, also occur not infrequently, especially in myeloma, but depression of other clotting factors is a rare occurrence. The beneficial effect of plasmapheresis on the bleeding tendency with correction of the bleeding time and platelet adhesiveness has been previously documented and a similar, although transient effect has also been found on the factor VIII levels of this patient.

CONCLUSION AND SUMMARY

Deficiency of coagulation factor VIII occurred in a patient with a monoclonal gamma-M protein and characteristic features of Waldenström’s macroglobulinemia. The protein, found to contain lambda light chains, had specific activity against normal factor VIII that was reversible by reduction with 2-mercaptop-
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ethanol. Spontaneous correction of the clotting factor deficiency occurred during treatment. It is suggested that the macroglobulin in this patient removed or masked factor VIII by adsorption and that alteration of the protein in vitro by mercaptoethanol and in vivo by treatment removed this capacity.

ACKNOWLEDGEMENTS

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REFERENCES

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