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An IgM Circulating Anticoagulant with Factor VIII Inhibitory Activity

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An acquired IgM type L inhibitor factor VIII was identified in a 63-year-old man who presented with a 2-year history of arthralgia and occasional Raynaud's phenomenon. The patient has shown no evidence of underlying malignancy, drug sensitivity, collagen disease, Waldenström's macroglobulinemia, or other overt dysproteinemia. The inhibitor was initially present in high titer (1:1200) and was not corrected in vitro with either porcine factor VIII or human cryoprecipitate. However, inhibitor activity was suppressed by specific antiserums to anti-human IgM and type L light polypeptide chains and by reduction and alkylation of the purified IgM antibody. Treatment with chlorambucil was accompanied by a reduction in the patient's serum anti-factor VIII activity.

CIRCULATING anticoagulants inhibiting antihemophilic globulin (factor VIII) have been found in association with a variety of disorders (1-5). In addition to their spontaneous appearance in postpartum females (6, 7) and in otherwise healthy individuals (8), factor VIII inhibitors have been observed in patients with classical hemophilia A, (9, 10), systemic lupus erythematosus (9), rheumatic heart disease (11), temporal arteritis (12), and after penicillin reactions (2). Many of these conditions have been associated with immunologic abnormalities. A direct link between coagulation defects and immunologic disorders became apparent when circulating anticoagulant activity was demonstrated among certain abnormal immune globulins (13-16). All of the previously described idiopathic circulating anticoagulants have been proteins of the IgG class. When light-chain typing of these monoclonal circulating anticoagulants has been carried out, proteins with type K light polypeptide chains have predominated (13, 15).

Defects in hemostasis have long been recognized in association with dysproteinemias of various heavy-chain and light-chain types (17), but only rarely has significant anticoagulant activity been demonstrated. Glueck and Hong (18) have reported a patient whose IgA myeloma protein showed some anti-factor VIII properties. In addition, Castaldi and Penny (19) described a patient with IgM type L macroglobulinemia associated with a mild anti-factor VIII activity during the height of the disease. However, no spontaneous IgM anticoagulants have been documented that were not associated with a serum paraproteinemia.

We describe a patient who spontaneously acquired a unique IgM type L anticoagulant with factor VIII inhibitory activity in the absence of any overt dysproteinemia.

Case Report

Our patient, a 63-year-old white man, was first admitted to the Veterans Administration Research Hospital, Chicago, in December 1966 for a left herniorrhaphy. For 2 years before admission he had noted vague generalized arthralgia and occasional Raynaud's phenomenon, although no specific rheumatologic diagnosis could be established. Tests for rheumatoid factor, antinuclear factors, cryoglobulins, and lupus erythematosus cell activity had consistently been negative. His past history was otherwise unremarkable, and there was no record

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that the patient had ever received a blood transfusion. No coagulation defects were noted, and his operation was uneventful. The postoperative course was complicated by an episode of thrombophlebitis. His one-stage prothrombin time (20), using human brain extract, was 23.5 sec (23%); by the thrombotest method (21) it was 75 sec (20%). The partial thromboplastin time (22) was 78 sec (63%), normal in this laboratory being 70 sec (100%). His thromboplastin generation test (20) was normal, with clotting times, after a 6-min incubation, of 14 sec for both the patient's plasma and the patient's serum. Although one-stage prothrombin times, done on three occasions over the following 6 months, showed values of 27 sec (18%), 15.9 sec (58%), and 16.3 sec (55%), respectively, no defect in the intrinsic coagulation system was detected.

In October 1967 the patient was admitted for the elective resection of a benign gastric polyp. His prothrombin time was 60%; however, the partial thromboplastin time was abnormal (18%). Studies were therefore undertaken to document the nature of his clotting defect.

Coagulation Studies

The results of tests performed on this last admission included a partial thromboplastin time of 175 sec (18%), a factor VIII level of 14%, and a prothrombin time of 14 sec (60%). The patient's thrombin time (11 sec) and fibrinogen level (285 mg/100 ml) were within normal limits. Plasma concentrations of factor V, factor IX, and factor X were all 100% of normal. Therefore, the only two significant abnormalities were the prolongation of his partial thromboplastin time and a decreased level of factor VIII. This low level of factor VIII activity was found to be caused by a specific circulating anticoagulant, because small quantities of the patient's serum were capable of inhibiting the coagulation of normal plasma. When the thromboplastin generation test was performed, the presence of the patient's serum or the patient's absorbed plasma in the mixtures resulted in prolongation of clotting times (Table 1). The addition of 1.0 ml porcine factor VIII* (800% strength) or 1.0 ml factor VIII cryoprecipitate (800% strength) to a mixture of 0.1 ml patient's plasma and 1.0 ml normal plasma did not significantly alter the partial thromboplastin time (Table 2).

* Courtesy of Dr. David Green.

Table 1. Thromboplastin Generation Test in October 1967

Platelets	Serum	Absorbed Plasma	Time of Incubation		
			2 Min	4 Min	6 Min
			←——— *sec* ———→		
Normal	Normal	Normal	69	34	15
Normal	Patient	Patient	172	154	125
Normal	Patient	Normal	125	115	75
Normal	Normal	Patient	160	110	58

Table 2. Partial Thromboplastin Time of Mixtures of Patient's Plasma, Normal Plasma, and Factor VIII Preparations

Reaction Mixture*	Partial Thromboplastin Time
	sec
Barbital buffer + normal: patient plasma mixture	175
Porcine factor VIII + normal plasma	55
Porcine factor VIII + normal: patient plasma mixture	140
Cryoprecipitate + normal plasma	60
Cryoprecipitate + normal: patient plasma mixture	125

* The reaction mixture contained 1.0 ml of porcine factor VIII, cryoprecipitate, or barbital buffer; 1.0 ml normal plasma; and 0.1 ml of patient's plasma. Partial thromboplastin times were measured after 15 minutes of incubation at 37 °C.

The strength of this factor VIII inhibitor was titrated as follows. The patient's plasma was diluted with increasing amounts of barbital buffer (0.05 M, pH 7.4). One part of these respective dilutions of patient's plasma was added to nine parts of normal plasma and incubated at 37 °C for 1 hour. Partial thromboplastin times were measured for the various incubated mixtures. A mixture of one part of buffer and nine parts of normal plasma was used as a control. A 1:1200 dilution of the patient's plasma was capable of prolonging the partial thromboplastin time.

The kinetics of the inactivation of factor VIII by the patient's plasma is shown in Figure 1. Two reaction mixtures of patient's plasma and normal plasma were prepared. In the first mixture one part of the patient's plasma was added to nine parts of normal plasma (100% factor VIII). In the second mixture one part of patient's plasma was added to nine parts of a plasma sample containing 50% factor VIII activity. These mixtures were incubated at 37 °C for various lengths of time, and the residual factor VIII activity was measured by Koller's one-stage assay (23). It can be seen that the residual factor VIII activity is a function of the initial factor VIII concentration and the incubation time. Most of the factor VIII inactivation occurred within the first 15 minutes of incubation, with only slight changes in residual factor VIII concentrations thereafter.

Immunologic Studies

In an attempt to characterize the patient's factor VIII inhibitor, concentrated serum was fractionated by starch block electrophoresis in barbital buffer, pH 8.6, and ionic strength, 0.05 (Figure 2). The

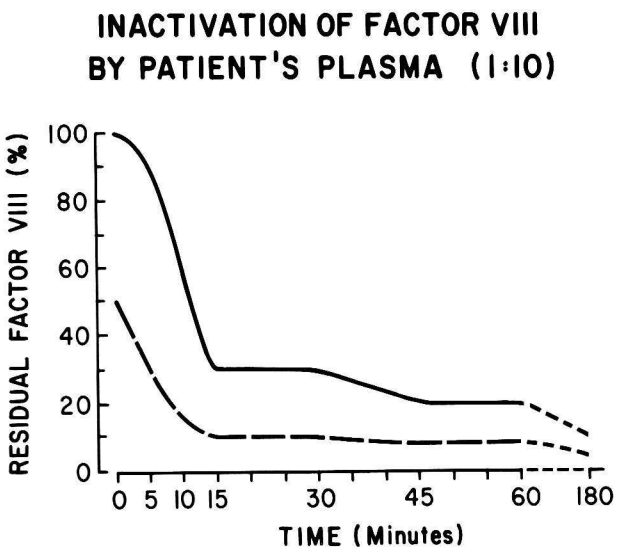

Figure 1. Kinetics of factor VIII inactivation by the patient's plasma. Residual factor VIII activity is plotted against time. The upper curve shows the kinetics when the initial factor VIII concentration equalled 100% of normal plasma. The lower curve is the kinetics for an initial factor VIII level of 50%.

anticoagulant activity was found for each block segment by measuring the effect of the eluted protein on the normal plasma partial thromboplastin time. Factor VIII inhibitory activity was found exclusively among serum proteins of gamma electrophoretic mobility.

These serum gamma globulins were then concentrated by ultrafiltration and separated by Sephadex G-200 chromatography on a 2.5 × 45-cm column with 0.01 M, pH 8.0 phosphate buffer (Figure 3). Anticoagulant activity again showed a localized distribution, being confined to the first macroglobulin effluent peak. On immunodiffusion testing this active fraction gave a precipitin reaction only with anti-IgM antiserum; no reaction was obtained with specific antiserum against the other major immunoglobulin classes. Conversely, no precipitin reaction could be identified between either the patient's serum or the active Sephadex G-200 fraction and concentrated factor VIII, Cohn's fraction I, or normal plasma.

Ultracentrifuge analysis of the active Sephadex G-200 fraction indicated that it was composed entirely of high-molecular-weight material with a 19 S sedimentation coefficient.

Absorption studies were carried out to block the patient's anti-factor VIII activity by specific immune globulin antiserums. Both commercial antiserum and specific rabbit antihuman-immunoglobulin antiserum prepared in this laboratory (24) were used in these absorption tests. Equal parts of the patient's serum and the antiserum preparation were incubated for 1 hour at 37 °C and assayed for anticoagulant activity by the normal plasma partial thromboplastin test. Appropriate controls were used to exclude a direct coagulant effect in any of the specific antiserums.

We found that the factor VIII inhibitory activity in the patient's plasma could be abolished by prior incubation with anti-IgM antiserums. Anti-IgG and anti-IgA antiserums did not alter the anticoagulant effect. These absorption tests were then repeated with rabbit anti-human light polypeptide chain antiserum prepared in our laboratory (24). The inhibitor was blocked by absorption with specific antiserum to type L light polypeptide chains, but no reduction in anticoagulant activity could be detected with anti-type K antiserum. We therefore concluded that the patient's circulating anticoagulant was an immune globulin of the IgM class that contained exclusively type L light polypeptide chains.

Solutions of the patient's purified IgM with a high degree of anticoagulant activity were treated with 0.2 M cysteine and 0.2 M mercaptoethanol to determine whether dissociation of the IgM molecule would alter the inhibitor activity. As in the previous investigations, the anticoagulant activity was evaluated by the normal plasma partial thromboplastin

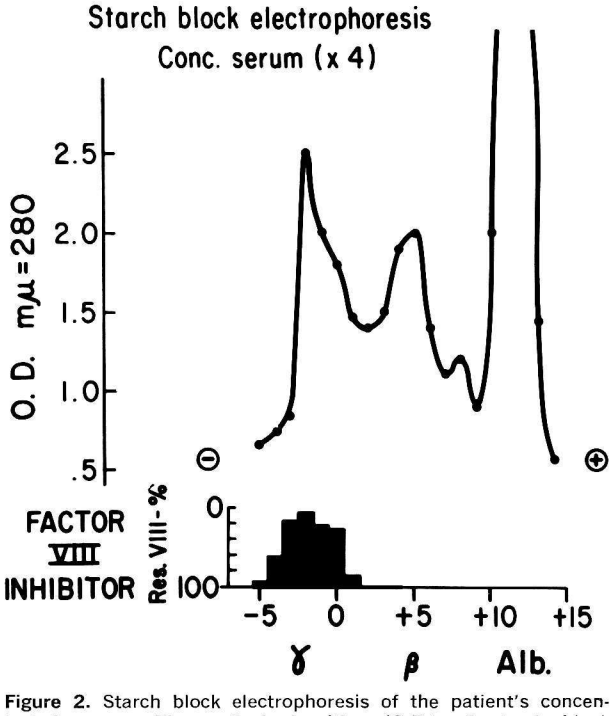

Figure 2. Starch block electrophoresis of the patient's concentrated serum. The optical densities (*O.D.*) of starch block washings are shown for block segments at various distances from the application point. Anticoagulant activity, as measured by prolongation of the normal plasma partial thromboplastin time, was found exclusively among the gamma globulins. Alb. = albumin.

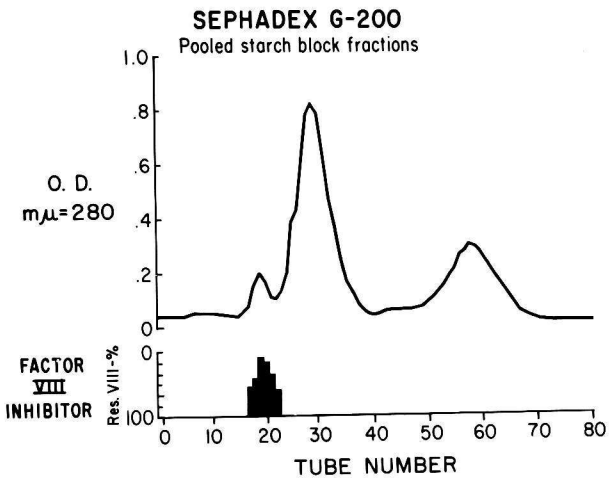

Figure 3. Sephadex G-200 column chromatography of the patient's serum gamma globulins. The effluent optical density (O.D.) and the level of factor VIII inhibition are shown for each fraction. Anticoagulant activity was confined to the first peak, which consists exclusively of serum macroglobulins.

test. Although the anticoagulant effect continued after treatment with cysteine and mercaptoethanol, it was clear that these reagents alone were capable of prolonging normal coagulation, as measured by our test system, and that the resulting anticoagulant activity could not be clearly ascribed to the IgM protein. Accordingly, purified preparations of the patient's IgM were reduced with mercaptoethanol and alkylated with iodoacetamide according to the method of Fleischman, Pain, and Porter (25). The reduced and alkylated IgM fragments were then separated according to molecular size by Sephadex G-75 chromatography. On subsequent testings these reduced and alkylated fractions had lost virtually all of their anticoagulant property, indicating that intact IgM globulin is required for the anticoagulant activity.

Discussion

Since circulating anticoagulants appear in such a variety of clinical settings, it is understandable that on many occasions their underlying etiology remains obscure. It is therefore particularly important to document as fully as possible the biologic properties of these anticoagulants and to study their mechanisms of action. The characteristics of the inhibitor in this case were similar to those in previously described cases of idiopathic, acquired circulating anticoagulants (13, 16). Our patient's plasma could inhibit the factor VIII activity in up to 1200 times its volume of normal plasma. Paradoxically, as has been reported with other factor VIII inhibitors (20), the factor VIII activity was not 100% inhibited. Irrespective of the initial factor VIII concentration in the reaction mixtures, a fraction of activity was always left. This accounts for a measurable factor VIII activity in the patient's plasma in spite of his high anticoagulant titer.

The association of anti-factor VIII anticoagulants with certain IgG immunoglobulins provided a link between immunologic disorders and coagulation defects. Because some of these anticoagulants were IgG globulins with monoclonal type K light polypeptide chains, a specific immune globulin disorder was suggested, rather than a peculiar host sensitization. It was not clear, however, whether the anticoagulant properties were unique to either the gamma heavy polypeptide chain or the kappa light polypeptide chain. The documentation of an IgM type L inhibitor of factor VIII indicates that anticoagulant properties may be found in more than one class of immunoglobulin and that these properties are not confined to proteins of one type of light polypeptide chain.

Monoclonal proteins with factor VIII inhibitory activity have been clearly identified (13, 15, 16), but anticoagulant activity is not commonly associated with serum monoclonal gammopathies (18). Our patient did not show a monoclonal pattern on serum protein electrophoresis, and his serum protein concentrations were within normal limits (albumin, 3.2 g/100 ml; globulin, 3.0 g/100 ml). Quantitative analysis of the serum immunoglobulins by the antibody agar plate technique (26) showed a slight increase in IgM (IgG, 11.0 mg/ml; IgA, 1.1 mg/ml; and IgM, 3.5 mg/ml), although his serum immunoelectrophoresis was entirely normal. No monoclonal pattern could be demonstrated with specific for anti-human light polypeptide chain antiserums. One may speculate that if our patient could be followed for a sufficient length of time, a pathologic immune globulin would appear. Monoclonal antibodies and serum monoclonal gammopathies (M-components) may, however, be two distinctly different alterations of immune globulin production.

Since our patient's factor VIII inhibitor was an IgM globulin, and a slightly increased concentration of IgM was noted in the serum, therapy was begun with chlorambucil, 6 mg/day, to try to reduce his circulating anticoagulant activity. Although no significant change was noted among the serum immunoglobulin concentrations, after 4 months of chlorambucil therapy the titer of the patient's factor VIII inhibitor activity fell from 1:1200 to 1:80. The patient's symptoms improved, and his arthritis cleared. Thereafter, moderate variation of inhibitor activity was noted, although the inhibitor titer never again reached pretreatment levels. Chlorambucil therapy was discontinued after 1 year of treatment.

Serum anticoagulant activity has subsequently increased, and his inhibitor titer is currently 1:640. Although the patient has never developed symptoms of a bleeding disorder, no further surgery has been attempted. This absence of serious clinical bleeding is consistant with other reported cases of idiopathic, acquired circulating anticoagulants (27, 28). Although there certainly was a temporal association between chemotherapy and the loss of anticoagulant activity, it is not clear how much of the observed response should be ascribed to chlorambucil. Green (29) has subsequently reported a patient with an IgG factor VIII inhibitor whose activity decreased after intravenous therapy with cyclophosphamide. No change in antifactor VIII activity had been noted when his patient was treated with a combination of methotrexate, azathioprine, and cyclophosphamide.

These studies provide insight into the problem of treatment of circulating anticoagulants. Clearly, various immunosuppressive agents affect the individual immune globulin classes differently. Although chlorambucil is favored for suppression of IgM, other drugs may be preferable for IgG suppression. Accordingly, careful documentation of the effects of specific immunosuppressant drugs on both the autoimmune activity and on overall antibody and immune globulin production should provide fundamental information for the rational choice of therapy.

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