

Immunochemical Characterization of a Human Antibody to Factor XIII

By James E. Graham, William J. Yount, and Harold R. Roberts

Antibodies to factor XIII have not been previously characterized in detail. An acquired inhibitor to factor XIII, which developed in a patient following thrombectomy for a femoral artery occlusion, has been characterized as to immunoglobulin composition. A highly sensitive monodansylcadaverine assay for factor XIII was employed. The inhibitor was completely neutralized by antisera specific for the γ G class, γ G1 subclass, and λ light-chain type.

Antisera to γ G2, γ G3, γ G4, γ A, γ M, γ D, γ E, and k light-chains had no detectable effect. Preparative zone electrophoresis revealed the inhibitor to be in the cathodal portion of the gamma peak, with moderately restricted electrophoretic mobility. The factor XIII inhibitor is thus characterized as a γ G1, λ immunoglobulin and resembles other human antibodies to clotting factors that are restricted in immunoglobulin composition.

FACTOR XIII, when activated by thrombin, stabilizes the fibrin clot by catalyzing formation of γ -glutamyl- ϵ -lysine bridges between fibrin units.^{1,2} Fibrin, cross-linked by factor XIII, is stable and is insoluble in solutions of 1% monochloroacetic acid and 5 M urea. Fibrin not stabilized by factor XIII is unstable and is soluble in such solutions.

Unstable fibrin clots occur in patients with hereditary factor XIII deficiency³ and may be found in patients with liver disease and various hematologic malignancies.⁴ Recently, acquired inhibitors to factor XIII have been discovered in patients with and without congenital factor XIII deficiency.^{5,6} Some of these inhibitors appear to be specific antibodies to factor XIII,⁶ although one acquired factor XIII inhibitor has been thought to represent an unusual metabolite of isonicotinic acid hydrazide (INH).⁷ Factor XIII inhibitors that behave as antibodies have not been characterized in detail as to immunoglobulin content. The characterization of factor XIII inhibitors as antibodies is important from the standpoint of understanding the pathogenesis of their development, the bleeding syndrome resulting from their occurrence, and the possible modes of therapy. Human antibodies to clotting factors, including antibodies to fibrinogen⁸ and factors V,⁹ VIII,¹⁰ IX,¹¹ and XIII,^{5,6,12} have been described. Many of the acquired human antibodies to clotting factors have been restricted in

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immunoglobulin composition.^{10,11,13,14} Five of six antibodies to factor VIII have been characterized as γ G4 antibodies.^{13,14} Several of the anti-factor VIII antibodies have been homogenous in light-chain type as well. A recently described antibody to factor IX was found to be monoclonal γ G4, λ ¹¹ The present study relates to the immunochemical characterization of an acquired factor XIII antibody that developed in a patient whose clotting mechanism was apparently normal prior to the development of the factor XIII inhibitor.

MATERIALS AND METHODS

Patient

The factor XIII inhibitor was obtained from a patient who was not congenitally deficient in factor XIII activity. He had no hemorrhagic episodes until he began to bleed excessively following a left femoral thrombectomy. This patient has previously been reported in detail.¹² The patient's plasma (8 parts blood: 1 part 3.2% sodium citrate), containing the inhibitor, was collected and stored at -20°C for periods up to 2 yr prior to the present studies.

Factor XIII Assay

Factor XIII was assayed according to the method described by Lorand et al.⁶ Briefly, the method is based on the observation that activated factor XIII is capable of catalyzing the incorporation of monodansylcadaverine into casein, a reaction that can be measured fluorometrically. The amount of factor XIII present in the test system is proportional to the amount of amine incorporated into casein and can be expressed in arbitrary units.⁶ Mean levels in the normal adult plasma pools were found to be 17 ± 3 U of factor XIII/ml in our laboratory. The pool used for the present studies contained 17 ± 1 U.

Factor XIII Inhibitor

Evidence that the inhibitor is specific for factor XIII has been previously reported. When the patient's plasma was mixed with normal plasma, factor XIII was virtually undetectable in the normal plasma. The inhibitor was present in the gamma globulin fraction of plasma and was stable at pH 4-9 and 56°C . The inhibitor was not dialyzable.¹²

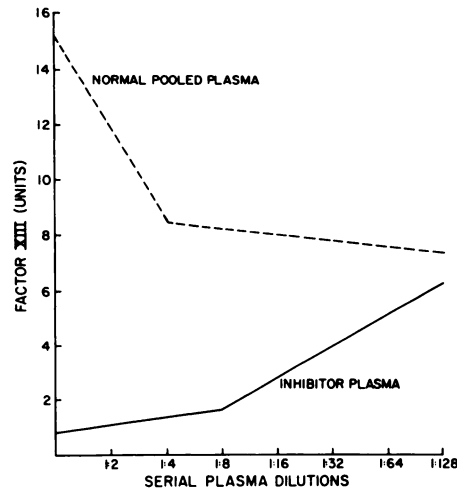
Inhibitor Titer

Serial dilutions of inhibitor plasma and normal plasma were prepared using citrated imidazole saline buffer, pH 7.2, as a diluent (Fig. 1). One part of the diluted plasma was added to one part of normal plasma, and residual factor XIII was determined. One unit of inhibitor was defined as that amount of plasma that would neutralize 1 U of factor XIII under the conditions of the test system. It can be seen that inhibitory activity of the patient's plasma could still be detected at a final dilution of 1:64. Further dilution obscured the effect of inhibitor, since the residual factor XIII approached that of the control normal pooled plasma diluted 1:128. The sensitive monodansylcadaverine factor XIII assay was essential, since inhibitor plasma had to be diluted 1:30 with heterologous antisera to human gamma globulins to carry out some of the specific neutralization experiments in the zone of antibody excess.

Isolation of Human Immunoglobulins

Purified immunoglobulins γ A, γ D, γ E, γ G, and γ M were isolated from serum of patients with multiple myeloma or macroglobulinemia by preparative zone electrophoresis and gel filtration on Sephadex G-200 or 1.5 M agarose beads (Biogel) (0.1 M Tris-HCl, 0.5 M NaCl, pH 8.0).¹¹ Antisera specific for immunoglobulin classes, heavy-chain subclasses, and κ and λ light-chain types were prepared in rabbits, goats, rhesus monkeys, and baboons as previously described.¹⁵ Globulins were precipitated by bringing the plasma to 50%

Fig. 1. Factor XIII inhibitor titer: Factor XIII is plotted on the ordinate in U/ml. Dilutions of normal plasma (control) and plasma containing factor XIII inhibitor is plotted on the abscissa. Each dilution of either factor XIII inhibitor plasma or normal plasma was then mixed in a 1:1 ratio with pooled normal human plasma, incubated at room temperature, and then the residual factor XIII activity was measured.



saturation with ammonium sulfate. The precipitates were dissolved in normal saline and dialyzed free of residual sulfate. The solution was oxalated and then absorbed with BaSO_4 (100 mg/ml) before heating to 56°C for 20 min to destroy residual clotting factor activity.

Neutralization of Factor XIII Inhibitor with Specific Antisera

Table 1 shows the method used to neutralize the factor XIII inhibitor. In mixture 1, specific antisera to immunoglobulin classes, subclasses, and light-chain types were added to the factor XIII inhibitor plasma in the amounts indicated. Mixture 1 was allowed to incubate for 1 hr at 37°C and for 12 hr at 4°C . After incubation, the mixture was centrifuged at 10,000 rpm in a Sorval RC2-B centrifuge for 30 min at 4°C . Residual antigen or antibody was determined using the Ouchterlony double-diffusion technique (Fig. 2). In all experiments, care was taken to insure that mixtures were in the zone of antibody excess. Mixture 1 was then assayed for residual inhibitor to factor XIII by adding an aliquot of this mixture to normal plasma and then determining the amount of factor XIII neutralized (mixture 2). Mixture 2 consisted of supernatant from mixture 1 and an equal volume of a standard normal plasma containing 17 ± 1 U of factor XIII. After incubation for 15 min the residual factor XIII in mixture 2 was determined. The amount of factor XIII remaining was directly proportional to the amount of factor XIII inhibitor neutralized in mixture 1 by the various antisera. Controls included: (1) normal plasma replacing inhibitor plasma, (2) buffer in place of specific antiserum, (3) normal globulin fractions in place of specific antiserum globulins, and (4) absorption of the specific antisera using purified immunoglobulins.

Preparative Zone Electrophoresis of Inhibitor Plasma

Preparative zone electrophoresis¹⁶ of 3 ml of inhibitor plasma on polyvinyl copolymer (Pevikon) was carried out in pH 8.6 barbital buffer, ionic strength 0.1, at 400 V for 16 hr. Total protein was determined by the Folin-Lowry method; γG concentration was determined by single radial diffusion,¹⁷ and factor XIII inhibitor activity was determined on individual 0.5 cm fractions as described above.

RESULTS

Antibody Class of Inhibitor

The initial experiments employed class-specific antisera for neutralization. Factor XIII inhibitor plasma was incubated with antisera to γA , γD , γE , γM ,

Table 1. Neutralization of Factor XIII Inhibitor With Specific Antisera

Mixture 1: neutralization phase	
(1) Inhibitor plasma + specific antisera → ppt.	
Ratio	1:19 immunoglobulin class
	1:29 γ G subclass
	1:29 light-chain type
(2) Assay supernatant for residual antigen and antibody to insure that mixture was in zone of antibody excess.	
Mixture 2: assay for residual inhibitor	
(1) Supernatant from mixture 1 + normal plasma assay residual factor XIII	
Ratio	1:1
(2) Residual factor XIII is directly proportional to amount of inhibitor neutralized.	

and γ G at a ratio of 1:19 (v/v). The residual inhibitor was determined by incubating this mixture with normal plasma. The results are shown in Table 2. As can be seen, the only antisera that neutralized the factor XIII inhibitor were antisera to γ G. Although not shown in Table 2, three other antisera specific for γ G also completely neutralized the inhibitor. When the γ G antisera were absorbed with purified γ G myeloma protein prior to neutralization, the effect on the factor XIII inhibitor was lost. This indicated that neutralization was due to the designated specificity of the antisera. When inhibitor plasma was incubated with control rabbit globulin, there was no effect on the factor XIII inhibitor. Normal plasma controls showed no inhibitory activity.

γ G Subclass of Inhibitor

Further experiments were carried out to determine the γ G subclass of the inhibitor. The results are shown in Table 3. It can be seen that antisera to γ G1 subclass completely neutralized the inhibitor, whereas no detectable inhibitor was neutralized by antisera to the other subclasses. Furthermore, the inhibitor-neutralizing properties of the γ G1 antisera could be abolished by prior absorption with purified γ G1 myeloma proteins, which again demonstrate the specificity of neutralization.

In each experiment antibody excess was demonstrated as shown in Fig. 2. No unprecipitated immunoglobulin present in the supernatant could be detected by specific antisera placed in the adjacent well.

Table 2. Immunoglobulin Class of Factor XIII Inhibitor

Antisera	Expected With Inhibitor Present	Factor XIII (U/ml)	Observed
		Expected With Inhibitor Neutralized	
γ A	6.3	14.1	7.0
γ D	6.3	14.1	6.3
γ E	6.3	14.1	7.4
γ G	6.3	14.1	14.8
γ G (absorbed with purified γ G)	6.3	14.1	5.2
γ M	6.3	14.1	7.4

Table 3. γ G Subclass of Factor XIII Inhibitor

Antisera	Expected With Inhibitor Present	Factor XIII (U/ml) Expected With Inhibitor Neutralized	Observed
γ G ₁	7.0	12.0	11.8
γ G ₂	7.0	12.0	8.1
γ G ₃	7.0	12.0	7.8
γ G ₄	7.0	12.0	7.8
γ G ₁ (absorbed with purified γ G ₁)	7.0	12.0	7.4

Light-Chain Type of Inhibitor

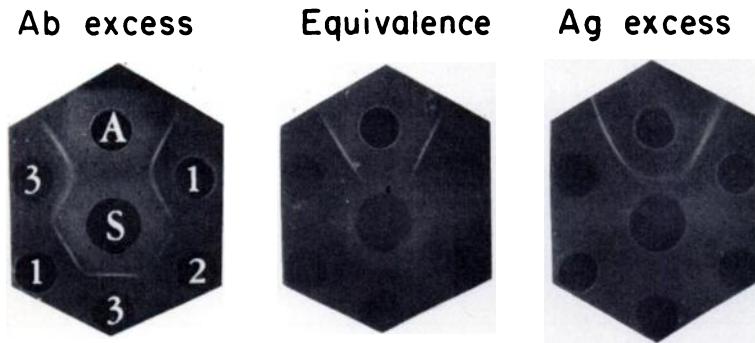
Antisera specific for κ and λ light-chains were incubated with inhibitor plasma. When antisera to κ light-chains were mixed with the factor XIII inhibitor no inhibitor was neutralized (Table 4). When anti- λ antisera were mixed with inhibitor, complete neutralization occurred. Ability of anti- λ antisera to block inhibitor activity could be removed by prior absorption with purified λ Bence Jones protein.

Further Characterization of the Factor XIII Inhibitor

Preparative zone electrophoresis of inhibitor on Pevikon is shown in Fig. 3. The inhibitor activity was found in the fractions migrating in the cathodal portion of the gamma peak with an electrophoretic mobility that corresponds to γ G1 and γ G3 subclasses. The distribution of fractions containing factors XIII inhibitor was relatively broad from fractions 13 to 25, indicating a somewhat less electrophoretically homogenous population of antibody molecules than previously described for a factor IX antibody.¹¹

DISCUSSION

The studies show that an acquired inhibitor to factor XIII could be neutralized by antisera specific for γ G heavy chains. Further immunochemical characterization of the inhibitor by specific neutralization revealed it to consist predominantly of γ G1 heavy chains and λ light chains. The γ G1 subclass accounts for $66\% \pm 8\%$ (± 1 SD) of total γ G.¹⁸ The factor XIII antibody resembles the acquired antibodies to factor VIII and factor IX in that it is restricted as to immunoglobulin class and γ G subclass. Five of six antibodies to factor VIII^{13,14} and one antibody to factor IX¹¹ have been γ G4 antibodies, which is the most minor of the γ G subclasses. The factor XIII assay was reproducible within ± 1 U. Thus, γ G2 antibody in its normal percentage of $23\% \pm 8\%$ ¹⁸ would have been detected by the assay system. The presence of the minor subclasses, γ G3 ($7.0\% \pm 3.8\%$) and γ G4 ($4.2\% \pm 2.6\%$), in their normal proportions^{15,18} cannot be excluded with certainty. Based on the sensitivity and reproducibility of the factor XIII assay and the dilutions required in order that neutralization was carried out in the zone of antibody excess, we estimate that a minimum of 80% of the factor XIII antibody was γ G1 subclass. γ G1 molecules have the capacity to fix complement, both by



- A Specific antiserum e.g. anti lambda
 S Supernatant of A after ppt with inhibitor plasma
 1 Inhibitor plasma 1:8
 2 γ G1, λ
 3 λ BJ

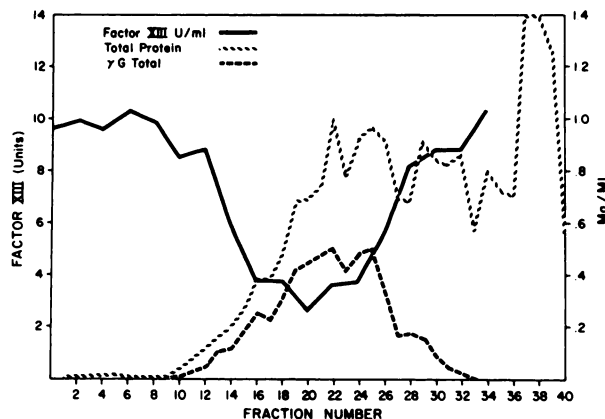
Fig. 2. Demonstration of antibody excess using the Ouchterlony double-diffusion technique. The figure is representative of that used to insure that studies on mixture 1 were conducted in the zone of antibody excess. Antiserum specific for λ light-chains is employed. Well A contains the λ antiserum. Well S contains supernatant from mixture 1 after precipitation with the plasma containing factor XIII inhibitor. Well 1 contains an appropriate dilution of inhibitor plasma. Well 2 in this example contains a purified γ G1, λ myeloma, and Well 3 contains a purified λ Bence Jones protein. All experiments were conducted in the zone of antibody excess, as illustrated in left-hand portion of the figure where, after mixing, antibody remains to react with purified proteins and no excess antigen occurs in S that would form a precipitate between S and A.

binding to Clq^{19,20} and by the C3 proactivator bypass mechanism.²¹ However, despite the capacity of γ G1 antibodies to fix complement, our patient showed no evidence of serum sickness or deposition of immune complexes despite multiple transfusions.

Table 4. Light Chain Type of Factor XIII Inhibitor

Antisera	Factor XIII (U/ml)		Observed
	Expected With Inhibitor Present	Expected With Inhibitor Neutralized	
κ	7.0	12.0	6.3
λ	7.0	12.0	12.5
λ (absorbed with purified λ B. J.)	7.0	12.0	7.8

Fig. 3. Preparative zone electrophoresis of factor XIII inhibitor. Fraction number is shown on the abscissa. Factor XIII activity and protein concentrations are plotted on the ordinates. Decreased factor XIII activity reflects the presence of a factor XIII inhibitor, which, as can be seen, migrates as a γ G protein. A relatively broad band of inhibitor activity was recovered in the area where γ G1 normally migrates.



The factor XIII antibody was also restricted in light-chain type; λ antisera completely neutralized the inhibitor, whereas κ antisera had no effect. The presence of at least 80% λ chains also indicates that the factor XIII antibody was restricted in immunoglobulin composition.

On preparative zone electrophoresis the factor XIII antibody was found in a relatively broad band, but the antibody activity could be recovered in the same area in which γ G1 normally migrates. Although the electrophoretic pattern suggests greater heterogeneity than that observed for the factor IX antibody recently described,¹¹ the experiments strongly suggest that factor XIII antibody is homogeneous (at least within the limits of our assay system) as to immunoglobulin class, γ G subclass, and light-chain type. Electrophoretic heterogeneity would suggest that the antibody is not monoclonal, unless such heterogeneity might be due to the presence of soluble complexes of factor XIII and antibody.

Factor XIII is thought to be composed of four polypeptide chains, two identical a chains and two b chains.²² The specificity of the factor XIII antibody for a or b chains on the factor XIII molecule is unknown.

The site of action for the factor XIII antibody has been postulated to be one of the following: (1) blocking the action of factor XIII at receptor sites of fibrin(ogen), (2) inhibition of precursor factor XIII, or (3) inhibition of activated factor XIII. It seems unlikely that the inhibitor blocks receptor sites on fibrinogen or fibrin products, since it also blocks the incorporation of monodansylcadaverine into casein, which is specific for factor XIII. It appears that the antibody blocks factor XIII itself, but whether it blocks both the precursor and activated forms cannot be ascertained from current experiments.

The reason for the development of such specific antibodies to clotting factors is unknown, but it may be related to relatively discrete antigenic sites on the clotting factor molecule. Antibodies to complex antigens and even to haptens are usually heterogeneous with respect to γ G subclasses and light-chain types. Only when the antigenic stimulus is highly limited in determinants, e.g., with antibodies to carbohydrate polymers, is the antibody response likely to be homogeneous.²³ In patients with "abnormal" clotting factors, transfusion of

the normal factor may stimulate the formation of antibody, but our patient had no known previous transfusions. Those patients with normal clotting mechanisms who acquire antibodies to clotting factors may be reacting to molecules structurally altered, perhaps at a site distant from that required for biologic activity. There is no evidence that the factor XIII inhibitor was related to the administration of isonicotinic acid hydrazide (INH) in our patient, although this drug has been incriminated in the development of factor XIII inhibitors in other patients.⁷ Whatever the mechanism of development, the studies reported here clearly demonstrate that an acquired factor XIII inhibitor, which developed in a patient without a congenital deficiency of factor XIII, was an antibody, and predominantly if not exclusively, γ G1, λ in immunoglobulin composition.

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