Autoimmune antibody (IgG Kansas) against the fibrin stabilizing factor (factor XIII) system
(autoimmune bleeding/fibrin stabilization/factor XIII inhibitor)

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ABSTRACT Serum from a patient who died from massive hemorrhage within 4 months after onset of an acquired bleeding disorder at age 85 contained a potent inhibitor of fibrin stabilization. Other parameters of coagulation and fibrinolysis and his bleeding time were within normal limits. The inhibitor was shown to be an IgG with κ light chains (IgG Kansas); its specific target was the factor XIII system itself. Although IgG Kansas combined with the normal [αb] form of the zymogen, it did not block the thrombin-catalyzed conversion to [αb]. However, IgG Kansas prevented the subsequent Ca++-mediated activation of [αb] to αa + b, where αa denotes the catalytically competent factor XIIIa species. IgG Kansas, in contrast to a previously studied autoimmune antibody from a similar bleeding disorder (IgG Warsaw), could also inhibit the transamidating activity of the preactivated αa enzyme.

Autoimmune antibodies can cause the premature destruction of cells or connective tissue constituents. Catastrophic bleeding may occur following the appearance of antibodies capable of neutralizing one of the coagulation components in the circulation. In this latter category, investigations relating to inhibitors of the fibrin stabilizing factor (factor XIII) system are particularly challenging on account of the molecular complexity of the activation of the zymogen (1–3). Earlier reports (4, 5) described two patients with acquired antibodies specifically targeted against the thrombin and Ca++-regulated conversion of factor XIII to the XIIIa enzyme, which is normally required to strengthen the fibrin clot by Nε-(γ-glutamyl)lysine side-chain bridges (6). Nevertheless, the two inhibitors could be shown to differ from one another in terms of recognizing the α subunit of the zymogen, which carries the potential catalytic site. The present paper deals with yet another autoimmune inhibitor, which, like the previously studied examples, blocked the activation of factor XIII. However, it could be distinguished from them by virtue of the fact that it interfered also with the functioning of the factor XIII enzyme. This antibody was identified in the plasma and serum of an elderly individual who died of massive hemorrhage.

Case Report

An 85-year-old white male retired farmer was admitted to the hospital for evaluation of a bleeding tendency. He had been well until 3 months prior to admission when he developed a spontaneous intramuscular hematoma of his right shoulder and chest wall, which resolved spontaneously. One month prior to admission, he developed a spontaneous intramuscular hematoma of the right thigh, necessitating transfusion of 3 units of packed erythrocytes at an outside hospital. Gross hematuria was also noted at the time. Family history was noncontributory. He took occasional acetaminophen tablets and denied use of aspirin, tobacco, or ethanol. He had a history of allergy to penicillin diagnosed 27 years previously, when he developed "swelling and a skin rash" after taking penicillin G. There was, however, no record of him having taken penicillin since that time. There was no previous history of lung disease, tuberculosis, or treatment with isoniazid.

Physical examination revealed dullness to percussion at the right lung base, and edema and tenderness of the right thigh. Hemoglobin was 11.0 g/dl; leukocytes, 6800 per μl; and platelets, 548,000 per μl. Prothrombin time was 12.2 s (normal, 11.0–13.0 s); activated partial thromboplastin time, 28.7 s (normal, 24.0–38.0 s); fibrinogen, 533 mg/dl; and bleeding time, 5.5 min (normal, 2.5–9.5 min). A clot prepared from the patient’s citrated plasma (recalcified by mixing equal parts of plasma and 0.025 M calcium chloride) dissolved in 5 M urea in <24 hr (7). Samples containing various concentrations of patient’s plasma (ranging from 10% to 95%) were prepared by mixing patient’s citrated plasma with citrated pooled normal plasma (from 10 healthy donors) in various proportions. Recalified plasma clots were prepared from each sample by adding an equal volume of 0.025 M calcium chloride. Those clots prepared from samples containing 95%, 90%, 85%, and 80% patient’s plasma dissolved completely in 5 M urea in <24 hr, while those clots containing 75%, 50%, 25%, and 10% patient’s plasma showed partial dissolution. Commercial kits were used to assay euglobulin lysis time (Dade Data-Fi) and plasminogen activity (Dade Protopath) according to the manufacturers’ instructions. Euglobulin lysis time was 80 min (normal, >60 min), and plasminogen activity was 3.5 CTA units/ml (normal, 2.4–3.8 CTA units/ml) (CTA, Committee on Thrombotic Agents). Serum protein electrophoresis showed a polyclonal hypergammaglobulinemia, with IgG 2180 mg/dl, IgA 284 mg/dl, and IgM 235 mg/dl. Latex test for rheumatoid factor was positive at a titer of 1 in 80 (normal negative), anti-nuclear antibody titer was 1 in 20 (normal, <1 in 20), and a plasma reagin test performed by the method of the Venerable Disease Research Laboratory was negative. Urinalysis was normal and chest x-ray showed bilateral pleural thickening. The right thigh swelling, shown on CT scan to be due to an intramuscular hematoma, resolved spontaneously, and the patient was discharged.

He was readmitted 2 weeks later with a recurrent spontaneous soft-tissue hemorrhage involving the right thigh, buttock, and hip area. The hemoglobin was 7.0 g/dl, and hemorrhage progressed, necessitating transfusion of 8 units of packed erythrocytes over the following 6 days. On the sixth hospital day, he developed progressive right lower quadrant abdominal pain, shown on CT scan to be due to retroperitoneal hemorrhage. He was transfused with fresh frozen plasma and cryoprecipitate, and received 100 mg of

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Cytosar orally. Hemorrhage progressed, and the patient died the following day. At autopsy, the cause of death was shown to be massive intraperitoneal and retroperitoneal hemorrhage. Other major findings included pneumonia oiosis of the mixed dust type, with bilateral fibrous pleural plaques and acute bilateral hemorrhagic bronchopneumonia.

Materials and Methods

Blood was collected into 3.8% sodium citrate with a blood/ anticoagulant ratio of 9:1. Platelet-poor plasma was prepared by centrifuging at 1500 × g for 20 min. For obtaining serum, blood was collected into plain glass tubes, allowed to clot at room temperature for 2 hr, and centrifuged at 1500 × g for 20 min.

IgG was purified on a ZetaChrom 60 D1 amine disk (AMF Laboratory Products, Meriden, CT) by applying, with the aid of a syringe, 1 ml of serum and/or plasma diluted with 10 ml of 0.015 M sodium phosphate buffer (pH 6.3) to the equilibrated disk at a flow rate of 3.5 ml/min. The disk was then washed with 30 ml of the above buffer and the IgG collected in the unbound fraction was concentrated by precipitation with an equal volume of saturated ammonium sulfate (pH 7.2) on ice for 30 min. The precipitate was recovered by centrifugation (3000 × g, 30 min, 4°C) and redissolved in 0.154 M sodium chloride/15 mM sodium azide. The IgG was stored at 4°C. Protein concentration was determined by absorbance at 280 nm (A1%cm = 13.5). IgG Kansas from the present patient was isolated from serum; control IgG was isolated from normal plasma; IgG Warsaw (5) was isolated from a mixture of serum and plasma.

Rabbit antibodies to human IgG γ heavy chain as well as to κ and λ light chains were purchased from Dako (Santa Barbara, CA) and were concentrated by Amicon CentriFree microparticulation system to titers of approximately 3000, 470, and 1400, respectively. Rabbit antibody to the α subunit of human factor XIII was purchased from Behring Diagnostics (San Diego, CA) and antibody to the β subunit was available in our laboratory. Protein A (bacterial absorbent; Miles) was taken up in 10 ml of water, which were washed with 30 mM Tris-HCl and centrifuging in between was exchanged for this buffer (pH 7.5). The final suspension (17 ml), stored at −20°C, had a binding capacity of ~1.2 mg of IgG per ml. Centriﬁgation of immunoprecipitates and protein A–IgG conjugates was carried out in a Brinkmann model 5414 Microcentrifuge for 5–10 min at 15,000 × g.

Human α-thrombin was a gift from J. W. Fenton III (New York State Department of Health, Albany, NY) and it was stored at −70°C in 0.75 M NaCl. Bovine thrombin (Parke, Davis) was dissolved in 25 mM Tris-HCl, pH 7.5/25% (vol/vol) glycerol to 500 units/ml and stored at −20°C. Hirudin (1000 units/ml; Sigma) and 0.2 mM Thromstop (American Diagnostica, Greenwich, CT) were dissolved in 50 mM Tris-HCl (pH 7.5) and stored at −20°C; either of these agents was used to terminate activation of factor XIII by thrombin.

Human factor XIII was purified from citrated plasma by published procedures, yielding a product of 80% functional purity, defined by titration with iodol[14C]acetamide (8–11). Protein concentration was determined by absorbance at 280 nm (A1%cm = 13.8). Proteolysis of the zymogen by thrombin was examined by NaDODSO4/PAGE (3, 12), transfer to nitrocellulose (0.2 μm; Schleicher & Schuell) and reacted with the method of Towbin et al. (13) at 0.6 A for 2 hr at 5°C, and immunostaining with the rabbit anti-a subunit antibody. Unbound sites on the nitrocellulose were blocked with Biotto (1% nonfat dry milk in 10 mM Tris-HCl/0.5 M NaCl, pH 7.5) at 40°C for 1 hr, followed by incubation overnight at 25°C with the primary antibody diluted 1:2000 in Biotto. Labeling of the bound antibody utilized a Vectastain ABC kit specific for rabbit IgG (Vector Laboratories, Burlingame, CA): the blot was washed three times with TBS (10 mM Tris-HCl/0.5 M NaCl, pH 7.5), incubated with biotinylated antibody to rabbit IgG (diluted 1:600 in Blotto) for 90 min, washed three times with TBS, incubated with the avidin–biotinylated peroxidase complex (diluted 1:300 in Blotto; 30 min complex formation) for 90 min, and again washed three times with TBS. The peroxidase label was developed with a solution containing 10 ml of 4-chloro-1-naphthol (3 mg/ml; Sigma) dissolved in ice-cold methanol, 50 ml of TBS, and 30 μl of 30% hydrogen peroxide.

Human factor XIII activity was measured by the incorporation of [14C]putrescine into N,N′-dimethylasein using a filter paper assay (10. 14). [1,4-14C]Putrescine dihydrochloride was purchased from Amersham. A 250-μCi vial (109 mCi/mmol; 1 Ci = 37 GBq) was dissolved with 50 μl of 0.04 M unlabeled putrescine and 2.5 ml of 50 mM Tris-HCl (pH 7.5) to give a stock solution of 1.68 mM putrescine (58 mCi/mmol), which was stored at −20°C. When indicated, the stock solution was further diluted with an equal volume of 2 mM unlabeled putrescine in 50 mM Tris-HCl (pH 7.5) to give 1.84 mM putrescine (27 Ci/mmol). N,N′-Dimethylasein was dissolved in 50 mM Tris-HCl (pH 7.5) to 2% and stored at −20°C. Reaction mixtures are described in detail in the figure legends. Typically, factor XIII (0.5 μg) was converted to XIII′ by treatment with thrombin (2.5 units) for 20–30 min (approximately 25°C or 37°C) and, if necessary, thrombin was then quenched by adding hirudin (5 units) or Thromstop (1 μM). After addition of dithiothreitol and CaCl2, incorporation of [14C]putrescine (0.15 mM) into N,N′-dimethylasein (0.67%) was tested. Aliquots (10 μl) from the 30- to 60-μl mixtures were spotted onto filter paper after 30 min of reaction and were processed for measuring incorporated radioactivity; enzyme activity is expressed as 30-min incorporation of [14C]putrescine (cpm) at 37°C for 10 μl of reaction mixture.

Results

The urea solubility of clots (7) formed from recalcified mixtures of citrated normal plasma and patient’s plasma (see Case Report) was suggestive of the presence of an inhibitor to fibrin stabilization. When increasing amounts of the patient’s serum (0–5 μl) were added to purified factor XIII (0.5 μg) prior to activation by thrombin and Ca2+, marked decreases of enzyme activities were observed, as judged by the incorporation of [14C]putrescine into N,N′-dimethylasein (Fig. 1). It could also be shown that the patient’s serum interfered with expression of enzyme activity in the factor XIII test system regardless of whether it was admixed before or after thrombin treatment and before or after activation with Ca2+ (Table 1).

Pretreating the serum with rabbit antibodies against human IgG γ chain or κ light chain eliminated its inhibitory property (Table 2), but treatment with rabbit antibody to human light chain had no effect. Essentially all (93%) of the inhibitory capacity could be removed by incubation with rabbit antibody to human γ chain. Moreover, the inhibitor could be purified by passage of either the patient’s plasma or serum through a ZetaChrom 60 D1 amine disk.

Affinity of the inhibitor toward the [ab] zymogen itself could be demonstrated by binding the patient’s IgG to protein A and using this solid-phase adsorbent to remove factor XIII from solutions with the same initial concentrations of the zymogen (14 μg/ml). As the proportion of the specific adsorbent was increased, the residual amount of factor XIII decreased (Fig. 2). Removal of the zymogen from solution was monitored by testing for [14C]putrescine incorporating activity after activation by thrombin and Ca2+. Adding
nonspecific IgG bound to protein A did not result in significant depletion of factor XIII.

In spite of the demonstrated ability to bind to the zymogen, the patient's IgG, just like that of another patient (IgG Warsaw), did not block the thrombin-catalyzed transition from [a]b to [a' b]. The change from the a subunit to the a' species was examined by NaDodSO₄/PAGE (12) and analyzed by immunoblotting against a rabbit polyclonal antibody that recognized the parent a subunit as well as its hydrolytically modified a' form (Fig. 3).

When added to the factor XIII activating system prior to Ca²⁺, the IgG preparation from the present patient (hence-

Table 1. Patient’s serum mixed with the factor XIII-generating system before or after thrombin, before or after Ca²⁺ activation, inhibits expression of enzymatic activity

<table>
<thead>
<tr>
<th>Set</th>
<th>Serum</th>
<th>Added to factor</th>
<th>30-min incorporation, cpm*</th>
<th>% of corresponding control</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Control</td>
<td>XIII</td>
<td>6709 (6700, 6717)</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Patient</td>
<td>XIII</td>
<td>866 (838, 873)</td>
<td>13</td>
</tr>
<tr>
<td>B</td>
<td>Control</td>
<td>XIII'</td>
<td>5553 (5330, 5775)</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Patient</td>
<td>XIII'</td>
<td>721 (721, 721)</td>
<td>13</td>
</tr>
<tr>
<td>C</td>
<td>Control</td>
<td>XIII</td>
<td>4829 (4956, 4701)</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Patient</td>
<td>XIII</td>
<td>1170 (1144, 1195)</td>
<td>24</td>
</tr>
</tbody>
</table>

The enzyme-generating system comprised 10 μl of factor XIII (0.5 μg), 5 μl of dithiothreitol (0.2 M), 5 μl of bovine thrombin (0.05 unit); after 20 min at 25°C, 5 μl of hirudin (0.1 unit) was added, followed by 5 μl of CaCl₂ (40 mM) for 5 min at 37°C. At that time, enzyme activity was measured by admixing [¹⁴C]putrescine (5 μl, 1.84 mM) and N,N'-dimethylcasein (20 μl, 2%). The patient’s serum (5 μl, diluted 2:3 with 50 mM Tris-HCl) or normal serum as a control, was added at different points to the above sequence in enzyme generation. For set A, serum was added immediately to the factor XIII zymogen, before dithiothreitol and thrombin; for set B, serum was mixed with the thrombin-activated zymogen — i.e., factor XIII — after hirudin and before CaCl₂; and, finally, for set C, the serum was added to the factor XIII species, just prior to testing with [¹⁴C]putrescine and N,N'-dimethylcasein.

*Average of duplicate samples; individual values are in parentheses.

Table 2. Neutralization of the inhibitory activity of patient's serum by rabbit antibodies specific for the γ and κ chains of IgG

<table>
<thead>
<tr>
<th>Serum</th>
<th>Rabbit antibody</th>
<th>30-min incorporation, cpm*</th>
<th>% of corresponding control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Anti-γ</td>
<td>12,127 (12,276, 11,977)</td>
<td>100</td>
</tr>
<tr>
<td>Patient</td>
<td>Anti-γ</td>
<td>11,615 (11,590, 11,639)</td>
<td>96</td>
</tr>
<tr>
<td>Control</td>
<td>Anti-κ</td>
<td>10,986 (10,830, 11,141)</td>
<td>100</td>
</tr>
<tr>
<td>Patient</td>
<td>Anti-κ</td>
<td>10,180 (10,262, 10,098)</td>
<td>93</td>
</tr>
<tr>
<td>Control</td>
<td>Anti-λ</td>
<td>8,693 (9,747, 7,638)</td>
<td>100</td>
</tr>
<tr>
<td>Patient</td>
<td>Anti-λ</td>
<td>3,254 (3,268, 3,240)</td>
<td>37</td>
</tr>
<tr>
<td>Control</td>
<td>—</td>
<td>11,781 (11,884, 11,678)</td>
<td>100</td>
</tr>
<tr>
<td>Patient</td>
<td>—</td>
<td>4,059 (4,092, 4,026)</td>
<td>34</td>
</tr>
</tbody>
</table>

The patient's serum or control serum (5 μl) was added to rabbit antibodies (45 μl) specific for γ heavy chains and κ or λ light chains. After 1 hr at 25°C, overnight at 4°C, precipitates were removed by centrifugation and the supernatants were tested for residual inhibitory potencies. Aliquots (20 μl) of the treated sera were mixed at 37°C with 5 μl each of factor XIII (0.5 μg), dithiothreitol (0.2 M), CaCl₂ (40 mM), and bovine thrombin (2.5 units) for 20 min. At that time, 20 μl of N,N'-dimethylcasein (2%) and [¹⁴C]putrescine (5 μl, 1.84 mM) were added to assay enzyme activities.

*Average of duplicate samples; individual values are in parentheses.

Fig. 2. Depletion of factor XIII from solutions of the zymogen by IgG Kansas bound to protein A. Normal IgG or patient's IgG (~1.2 mg in 0.2 ml of 0.154 M NaCl) was mixed with 0.5 ml of protein A adsorbent for 30 min (~25°C); the suspensions were centrifuged, washed with 50 mM Tris-HCl (pH 7.5), centrifuged again, and resuspended with 1 ml of the buffer. Increasing volumes of the IgG-protein A complexes (50-150 μl) were centrifuged and the pellets were incubated with 0.5 μg of factor XIII (35 μl) for 30 min (25°C). After centrifugation, the residual amount of factor XIII was measured in the supernatants. Aliquots (20 μl) of supernatant were added to a 15-μl mixture of dithiothreitol (40 mM), bovine thrombin (2.5 units), and CaCl₂ (20 mM) in 50 mM Tris-HCl (pH 7.5). After 20 min at 37°C, N,N'-dimethylcasein (20 μl, 2%) and [¹⁴C]putrescine (5 μl, 1.84 mM) were admixed and isotope incorporation was allowed to proceed for 30 min at 37°C.
tested by admixing CaC\(_2\)\([14C]\)putrescine (1.84 mM).

IgG Kansas, IgG Warsaw, pretreated (10 min, 15°C) with rabbit anti-a subunit. Treatment of the immunoprecipitate with thrombin and Ca\(^{2+}\) releases the a\(^*\) enzyme into the supernatant where its activity on the incorporation of \([^{14}C]\)putrescine into \(N,N'\)-dimethylcasein can be measured (15). When the two patients' IgGs were compared in regard to interference with the transamidase itself, it was found that IgG Warsaw did not inhibit the activity of the a\(^*\) species, but IgG Kansas was a rather potent inhibitor (Fig. 5).

**Discussion**

Fibrin stabilizing factor or factor XIII is the precursor of the enzyme responsible for strengthening the clot network by \(N^\epsilon-(\gamma\text{-glutamyl})\)lysine side-chain bridges. It is unique among the zymogens of the coagulation cascade in that it has a heterologous [ab] protomeric composition and that its conversion to factor XIII\(_a\) under physiological conditions occurs in two distinct time-resolvable biochemical steps (3). Limited hydrolysis by thrombin generates the [a'\(b\)] or factor XIII\(_a\) ensemble, which, however, is still devoid of enzymatic activity. Under the influence of Ca\(^{2+}\) ions, [a'\(b\)] dissociates into a' + b and, in a concerted manner, the a' subunit undergoes a conformational change to a\(^*\). The latter is the catalytically competent factor XIII\(_a\) species. While all other enzymes produced on the coagulation cascade belong to the trypsin family of serine proteases, factor XIII\(_a\) is a transamidase that functions through a cysteine thiol group in its active center (8, 9).

Severe and, as in the present case, even fatal internal bleeding may ensue on account of the sudden appearance in the circulation of an acquired inhibitor against fibrin stabilization (1). In some instances, the inhibitor is an autoimmune antibody specifically directed against component(s) of the factor XIII → XIII\(_a\) sequence, the proper function-
ing of which is essential for normal clotting. The molecular analysis of such hemorrhagic conditions offers unusual possibilities for validating and extending our knowledge into this important aspect of blood coagulation.

In the present case, a general inhibitor to fibrin stabilization was suspected from the urea solubility of clots, which were obtained from recalculated mixtures of normal plasma in different proportions with the patient’s plasma (7). However, to demonstrate that the actual target of the inhibitor was the factor XIII system itself, it was necessary to show that the patient’s plasma or serum interfered with the ability of the purified human zymogen to mediate amine incorporation into protein upon activation by thrombin and Ca²⁺. A filter paper assay, based on the incorporation of [¹⁴C]putrescine into N,N’-dimethylcasein (10, 14), was used to measure the reaction and, as seen from the data in Fig. 1, the patient’s serum was indeed highly inhibitory.

An unusual feature of the inhibitor, in relation to two other cases previously examined in detail (4, 5), was that it interfered with expression of enzyme activity irrespective of whether it was added before or after treatment with thrombin (see sets A and B in Table 1), before or after activation with Ca²⁺-ions (set C in Table 1). These findings suggested that the inhibitor recognized the [a] and/or the [a’b] zymogen(s), and also the a* enzymatic species, although inhibiting the latter seemed to be somewhat weaker.

Assay values with the patient’s serum could be restored to 96% and 93% of normal after treatment with rabbit antibodies to the γ heavy chain and the κ light chain of human IgG. However, the inhibitory effect of the patient’s serum could not be abolished by an antibody against λ light chains (Table 2). These observations indicated that the circulating inhibitor was possibly a monoclonal IgG molecule with κ light chains. It could be isolated (Fig. 4) by passing the patient’s serum through a ZetaChrom 60 D1 amine disk, a conventional preparative procedure for purifying IgG.

As seen from the electrophoretic analysis (3, 12) in Fig. 3, the patient’s IgG—called IgG Kansas—did not block the thrombin-catalyzed conversion of [a] to the [a’b] allozymogen species. Nevertheless, the results in Fig. 2 clearly show that IgG Kansas is effective in binding the [a] form of the zymogen. Therefore, the attachment of the antibody to this ensemble must be of an orientation that does not present a significant steric hindrance for forming the Michaelis complex between thrombin and the combined [a]-IgG Kansas structure.

Because of the limited amount of serum available, the ancillary question of whether the antibody might have interfered with the binding of factor XIII to fibrinogen—fibrin was not addressed. Binding of factor XIII to the clotting substrates (16) is known to enhance considerably the rate of thrombin attack on the a subunit of the zymogen (17–20) and to bring the Ca²⁺ requirement of the a’b→a’+ b dissociation step into the physiological 1.5 mM Ca²⁺ ion range (21, 22).

It was deemed more significant to follow up on the finding, referred to earlier (Table 1), that IgG Kansas could actually inhibit the transamidating activity of the a* enzyme itself. If so, it might be distinguished from IgG Warsaw, which was equally potent in blocking enzyme generation (Fig. 4). When tested on the preactivated factor XIIIa, IgG Warsaw had no effect, but IgG Kansas was quite inhibitory (Fig. 5); thus, the two antibodies must be directed against different epitopes of the a subunit of factor XIII.

The origin of this antibody, similar to the others that prevent fibrin stabilization (1), remains unknown. In other cases, treatment with isoniazid (4) or penicillin (5) could be invoked as possible causative agents in producing “hit and run” type of accidental protein modifications, which might have precipitated a breakdown of immune tolerance against factor XIII. Isoniazid is known to be an amine substrate for factor XIII (23), usable even for self-incorporation into this protein; penicillin can react nonenzymatically with many proteins modifying mainly ε-lysine side chains (24). The 85-year-old patient from Kansas, like the 12-year-old boy from Warsaw (5), in fact had a history of allergy to penicillin. However, by contrast to the very severe episode in the Warsaw case, the allergic reaction was a relatively mild one and the onset of bleeding, rather than following soon, occurred some 3 decades later. It would seem to be unrealistic to suggest that the appearance of IgG Kansas and the fatal hemorrhage of the patient was in any way related to penicillin.

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