Development of Antithrombin Antibodies Following Surgery in Patients With Prosthetic Cardiac Valves

By Raphael B. Stricker, Philip K. Lane, Jonathan D. Leffert, George M. Rodgers, Marc A. Shuman, and Laurence Corash

Although antibody inhibitors directed against blood coagulation factors are well known, antibody inhibitors directed against thrombin are rare. We describe three postsurgical patients with prosthetic cardiac valves who developed serum autoantibodies reactive with human and bovine thrombin, as demonstrated by coagulation studies and immunoblotting. Despite marked prolongation of the thrombin time in these patients, the inhibitors were not associated with significant clinical bleeding. The mechanism of antithrombin autoantibody formation following surgery in patients with prosthetic cardiac valves remains to be determined.

A CQUIRED ANTIBODY inhibitors directed against blood coagulation factors have been associated with surgery, malignancy, infection, and autoimmune diseases. 1,2 Autoantibodies against factor VIII and phospholipid (the lupus anticoagulant) appear to be the most common of these inhibitors. 3,4 Although anti-factor VIII antibodies are known to cause significant clinical bleeding, 1 lupus anticoagulants are often associated with thrombosis rather than hemorrhage. 5 In patients with the lupus anticoagulant, an acquired inhibitor of prothrombin has recently been described. 6 However, immunoglobulin inhibitors directed against prothrombin or thrombin have rarely been isolated, 7,8 and the clinical significance of these antibodies has not been defined.

We describe three patients with prosthetic cardiac valves who developed markedly prolonged thrombin times following surgery. We used a novel application of immunoblotting to identify serum antibodies reactive with prothrombin and thrombin in these cases.

MATERIALS AND METHODS

Case Reports

Patient 1. A 39-year-old Samoan woman had a history of mitral stenosis and insufficiency, presumably on the basis of rheumatic heart disease. In 1978, she underwent mitral valve replacement with a Carpenter-Edwards (porcine) valve. No excess bleeding occurred during surgery. In 1979, she developed subcutaneous (SC) nodules on her legs. Tuberculous leprosy was diagnosed by punch biopsy, and she was treated with Dapsone for 5 years (through 1984). In July 1986, she presented with increasing dyspnea on exertion. Clinical evaluation revealed stenosis of the mitral valve prosthesis, tricuspid insufficiency, and moderate pulmonary hypertension. On August 7, lower abdominal pain developed, and exploratory laparotomy revealed 1,500 mL blood in the pelvis; no bleeding source was identified. The hematoma was evacuated, but on August 20 abdominal pain recurred. Reexploration revealed 1,000 mL blood in the pelvis, and several small bleeding vessels were ligated. Hemostasis was described as “good,” and her hemoglobin level stabilized. She had received 18 U packed RBCs and 18 U plasma during the previous ten days.

On August 9, the patient developed fever and a pulmonary infiltrate (FTA-ABS) for antinuclear antibody (ANA), rheumatoid factor, rapid plasma reagin (RPR) and fluorescent treponemal antibody absorption (FTA-ABS) were negative. Progressive hypoxemia developed; in February 1987, acute hepatic decompensation occurred. The patient died on February 14, 1987. Permission for autopsy was not granted.

Patient 2. A 43-year-old black woman with a history of intravenous (IV) drug use had positive tests for RPR and FTA-ABS during her fifth pregnancy in 1976. She was treated with erythromycin. In 1977, she developed aortic valve endocarditis and underwent valve replacement with a Bjork-Shiley prosthesis. No excess bleeding occurred, and the patient was placed on warfarin. In 1983, she developed acute aortic insufficiency and valve dehiscence related to Streptococcus viridens endocarditis. She underwent valve replacement with a second Bjork-Shiley valve. At surgery, a large abscess cavity medial to the left coronary artery ostium was drained. Again, no excess surgical bleeding occurred. The patient was poorly compliant with her warfarin regimen after discharge.

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On December 29, 1986, she was admitted with fever, cough, and a new heart murmur. Chest radiograph revealed a pulmonary infiltrate, and she was treated with vancomycin, gentamicin, and metronidazole. Her PT and PTT were normal (Table 1). Heparin was started, and the PTT was maintained at 60 to 90 seconds during the next two weeks with no bleeding. Cardiac catheterization revealed a pseudoaneurysm adjacent to the left coronary sinus and a paravalvular leak. Heparin was stopped, and valve replacement with an aortic root homograft was performed on January 14. No excess surgical bleeding occurred, but a homograft leak had to be repaired on the following day. Autopsy revealed a defect in the homograft suture line and clotted blood in the graft suture line and anterior pericardial cavity. Patient 2: A 62-year-old Filipino woman who had a history of childhood acute rheumatic fever and progressive mitral stenosis underwent mitral valve replacement with a Bjork-Shiley valve in 1980. No excess surgical bleeding occurred. Subsequently, she was maintained on warfarin with PT levels of 20 to 25 seconds.

On June 21, 1987, the patient was admitted with a two-day history of severe headache and weakness. A CT brain scan revealed a right paraventricular hemorrhage. Her PT was 29.3 seconds and her PTT was 36.3 seconds. Tests for ANA, rheumatoid factor, and RPR were negative. The FTA-ABS was positive.

The patient was stable until February 2, when she had sudden onset of cardiac tamponade. A computed tomographic (CT) scan showed a large pericardial hematoma compressing the left atrium, right ventricle, and circumflex coronary artery. Her PT was 15.0 seconds, PTT was 44.5 seconds, and thrombin time was >120 seconds (Table 1). Liver function tests were normal, and there was no evidence of DIC. She experienced a rapid downhill course and died on the following day. Autopsy revealed a defect in the homograft suture line and clotted blood in the anterior pericardial cavity.

Patient 3: A 65-year-old woman who had a history of severe headache and weakness. A CT brain scan revealed a right paraventricular hemorrhage. Her PT was 40.4 seconds and her PTT was 120 seconds (Table 1). Liver function tests were normal, and there was no evidence of DIC. Tests for ANA and RPR were negative. The patient's hemoglobin was stable, and no bleeding was observed. Liver function tests were normal, and there was no evidence of DIC. Tests for ANA and RPR were negative. The patient was placed on warfarin with PT levels of 15 to 20 seconds, and her thrombin time decreased to 68.2 seconds. August 18, the thrombin time had also become normal. The patient was placed on warfarin with PT levels of 15 to 20 seconds, and her thrombin time stayed in the normal range. She remained comatose without evidence of bleeding until her death on September 1. An autopsy was not performed.

Coagulation studies and purified coagulation proteins. The PT, activated PTT, thrombin time, reptilase time, fibrinogen, and fibrin monomer were assayed by standard techniques. The thrombin time with addition of protamine or mixing with normal plasma was performed as described. Individual factor assays were also performed by standard techniques. Human prothrombin, factor IX, and factor X were purified as previously described. Purified human a-thrombin was the gift of Dr John W. Fenton, II (New York State Department of Health, Albany). Human fibrinogen (grade L) was purchased from Kabi Vitrum, Stockholm. Bovine thrombin was purchased from Behring Diagnostics, San Diego.

Antibody preparations and immunoblotting. Rabbit anti-thrombin antibody was obtained as previously described. Serum

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Table 1. Coagulation Studies in Three Patients

<table>
<thead>
<tr>
<th>Test</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Preoperative</td>
<td>Postoperative</td>
<td>Preoperative</td>
</tr>
<tr>
<td></td>
<td>(8/3/86)</td>
<td>(8/11/86)</td>
<td>(12/29/86)</td>
</tr>
<tr>
<td>PT</td>
<td>11.6</td>
<td>33.5</td>
<td>12.3</td>
</tr>
<tr>
<td>PTT</td>
<td>36.1</td>
<td>73.5</td>
<td>30.0</td>
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<tr>
<td>TT</td>
<td>&gt;120</td>
<td>&gt;120</td>
<td>&gt;120</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>—</td>
<td>391</td>
<td>—</td>
</tr>
<tr>
<td>Fibrin monomer</td>
<td>—</td>
<td>Negative</td>
<td>—</td>
</tr>
<tr>
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<td>—</td>
<td>10.9</td>
</tr>
<tr>
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<td>&gt;30</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>PTT Mix</td>
<td>&gt;70</td>
<td>—</td>
<td>35.7</td>
</tr>
<tr>
<td>TT Mix</td>
<td>&gt;120 &gt;120</td>
<td>—</td>
<td>64.6</td>
</tr>
<tr>
<td>TT + Protamine</td>
<td>&gt;120 &gt;120</td>
<td>—</td>
<td>&gt;120</td>
</tr>
<tr>
<td>Factor II</td>
<td>—</td>
<td>*</td>
<td>—</td>
</tr>
</tbody>
</table>

PT, prothrombin time; PTT, activated partial thromboplastin time; TT, thrombin time; Mix, mixing study using control and patient plasma (1:1); (—), not done.

*Indeterminate owing to presence of inhibitor.

Fig 1. PT, PTT, and thrombin time in relation to clinical course in patient 1. Arrowheads indicate surgical procedures.
samples were obtained from the patients before surgery and during the course of their hospitalization. The samples were frozen at -70°C until use. IgG fractions were prepared from the sera by affinity chromatography with Protein A-Sepharose-CL6B. Immunoblotting was performed as described previously. Purified coagulation proteins (10 μg) were electrophoresed in sodium dodecyl sulfate (SDS) 12% or 5% polyacrylamide gels under nonreducing conditions. The proteins were transferred to nitrocellulose membranes and incubated with patient serum or purified IgG (2 mg/mL) diluted 1:50 in Tris-buffered saline. Antibody binding was detected using a biotin-avidin-peroxidase system.

RESULTS

The results of coagulation studies and factor assays are shown in Table 1. Before surgery, all three patients had normal PT, PTT, and platelet counts with no evidence of bleeding. Following her initial two procedures, patient 1 showed a significant rise in the PT, PTT, and thrombin time (Table 1), with massive pelvic bleeding. Further evaluation revealed an inhibitor against factor V (data not shown) that became undetectable within ten days, with normalization of the PT and PTT and resolution of bleeding. However, the thrombin time remained persistently elevated, and the reptilase time was normal. Patient 2 had a mildly prolonged PT and PTT two weeks after surgery, and her thrombin time was markedly prolonged with a normal reptilase time (Table 1). Patient 3 had been taking warfarin and had a prolonged PT that returned to normal when the anticoagulant was discontinued. One week after craniotomy, she developed a prolonged PT, PTT, and thrombin time with a normal reptilase time. In all three patients, the thrombin time did not correct into the normal range on mixing with normal plasma or protamine (Table 1), confirming the presence of an inhibitor other than heparin. Furthermore, factor II (prothrombin) levels could not be measured owing to the presence of inhibitory activity in each patient’s plasma (Table 1).

The clinical course in patient 1 is shown in Fig 1. Despite relatively normal PT and PTT values, her thrombin time remained persistently prolonged throughout her hospitalization, with no further evidence of bleeding. Thus, clinical hemorrhage occurred only when the factor V inhibitor was present in this patient but not when the thrombin inhibitor alone was present (Fig 1).

Figure 2 shows the results of immunoblotting using serum from patient 1. Rabbit antiserum raised against human thrombin bound to a protein of ~39,000 daltons corresponding to purified thrombin (lane A) and to a protein of ~70,000 daltons corresponding to purified prothrombin (lane B). The additional bands apparent in the thrombin and prothrombin lanes (in Figs 2 through 6) probably represent degradation products of thrombin or prothrombin. Serum antibody from patient 1 (obtained in the postoperative period) also bound to thrombin and prothrombin, as shown in lanes C and D. The antibody did not bind to purified factor IX or factor X, as shown in lanes E and F. Serum obtained before surgery did not contain antibody against either thrombin or prothrombin, whereas serum obtained just before the patient’s death showed persistent antibody binding to these coagulation proteins (data not shown). Antibody binding to fibrinogen was not detected (data not shown).

Figure 3 shows the results of immunoblotting with patient 1 serum and purified factor V. Serum obtained before surgery did not contain antibody against factor V, as shown in lane A. However, the postoperative serum sample contained an antibody that bound to a protein of apparent mol wt 325,000, corresponding to purified factor V (lane B). In contrast, serum obtained 1 month later (September 7, 1986) no longer showed antibody binding to factor V (lane C). However, this sample still showed antibody binding to thrombin and prothrombin (data not shown). Thus, the presence of antibody against factor V correlated with clinical bleeding in patient 1. In contrast, prolongation of the thrombin time persisted in the absence of bleeding when the factor V inhibitor was no longer detected.

Figure 4 shows the results of immunoblotting using serum from patient 2. Rabbit antibody binding to thrombin and
prothrombin is apparent in lanes A and B. Serum obtained before surgery from patient 2 failed to bind to these proteins, as shown in lanes C and D. However, serum obtained when the patient's thrombin time was >120 seconds contained antibody that bound to both proteins (lanes E and F). Antibody binding to factor V, factor IX, factor X, and fibrinogen was not detected (data not shown).

Figure 5 shows the immunoblot results when purified IgG was incubated with human and bovine thrombin. Rabbit anti-thrombin antibody bound to both human thrombin (lane A) and bovine thrombin (lane B). Purified IgG obtained from postsurgical sera of patients 1 and 2 also bound to human thrombin (lanes C and E) and bovine thrombin (lanes D and F). Serum antibody from a patient with systemic lupus erythematosus and a lupus anticoagulant did not react with either human thrombin (lane G) or bovine thrombin (lane H).

**DISCUSSION**

We have described three patients with prosthetic cardiac valves who developed an unusual coagulopathy. The essential features of the coagulopathy included a markedly prolonged thrombin time that did not correct on mixing with normal plasma or protamine and a normal reptibase time (Table 1). Immunoblotting showed all three patients to have serum IgG antibodies that bound to thrombin and prothrombin (Figs 2 and 4 through 6). In addition, patient 1 had a transient IgG antibody that bound to factor V and was associated with clinical bleeding (Figs 1 and 3). Although patient 2 ultimately died of a pericardial hemorrhage related to a mechanical defect in her graft, none of the patients appeared to have significant bleeding related solely to the antithrombin antibody. Indeed, the inhibitors might have been missed if a thrombin time had not been performed in each case, since the PT and PTT corrected over time in patients 1 and 3, whereas the PT and PTT were only mildly prolonged in the context of an acute fatal event in patient 2. Patient 3 was diagnosed on a routine coagulation screen after having a normal PT and PTT four days earlier. Her inhibitor slowly disappeared during the ensuing six weeks.

Acquired antibody inhibitors against thrombin have rarely
become identified.4-10 Hawiger and colleagues6 described a patient with systemic lupus erythematosus who developed epistaxis, gum bleeding, and hematuria. The patient had prolonged PT, clotting and thrombin times, and normal levels of factors V and VII. Gel diffusion showed that the patient had an antibody that reacted with bovine thrombin. However, he was also severely thrombocytopenic and had positive Coombs’ and Ham’s tests, suggesting other etiologies for his bleeding and hematuria. More recently, Scully et al6 described an elderly woman with no underlying disease who had diffuse bleeding after a dental extraction and subsequent gastrointestinal hemorrhage. The patient had a prolonged PT, PTT, and thrombin time with a normal reptibase time and bound both thrombin and prothrombin on an affinity column. Assays of other clotting factors were not performed, however, and an additional inhibitor could not be excluded as the cause of the patient’s bleeding. Barthels and Heimburger18 recently reported the case of a woman with cirrhosis, gum bleeding, and massive hematuria who had prolonged PT, PTT, and thrombin time measurements. The patient’s IgG fraction prolonged the thrombin time, whereas fibrin polymerization was normal. However, the patient had severe thrombocytopenia as well as low levels of most of her clotting factors. Thus, her bleeding could not be ascribed specifically to the antithrombin antibody.

Based on these reports, the prevalence of antibody inhibitors directed against thrombin is difficult to determine. Because bleeding may occur only when other coagulation abnormalities are associated with the antibody, thrombin inhibitors may often go undetected. For example, antibodies against prothrombin have recently been described in “prothrombin-deficient” patients with the lupus anticoagulant.6,7 and a prolonged thrombin time has been reported in 24% to 38% of these patients.15-17 However, patients with the lupus anticoagulant and a prolonged thrombin time do not bleed.15-17 Possibly because the antibody in these patients reacts with a “non-neutralizing” epitope on prothrombin.7 Alternatively, the discrepancy between a thrombin inhibitor’s pronounced in vitro effect and its minimal effect in vivo could result from the fact that prothrombin activation occurs at the cell surface.18 Under these conditions, prothrombin may be protected from antibody binding and thrombin activity may remain intact.

The relationship between the antithrombin antibody and cardiac valve prostheses is unclear. Antibody inhibitors directed against animal coagulation proteins have previously been described in a patient with a prosthetic heart valve.19 However, that patient had a normal thrombin time, and his inhibitors failed to react with porcine proteins even though he had received a porcine xenograft. All three of our patients received their prosthetic valves 7 to 10 years before the thrombin inhibitors occurred. Therefore, chronic, subclinical activation of prothrombin on the surface of the valves may have exposed thrombin-related antigenic determinants that stimulated autoantibody formation. Evidence in favor of this hypothesis is lacking, however. Surgery and antibiotics have been associated with development of other clotting factor inhibitors,20,21 and each of our patients had undergone some type of surgery (valve replacement, craniotomy) just before the development of the thrombin inhibitor. Thus, the surgical procedure may have triggered autoantibody formation in each case. Two of our patients received multiple antibiotics, but there was no apparent correlation between a specific antibiotic and the thrombin inhibitor. The third patient received no antibiotics. Of interest is the history of leprosy in patient 1 and the positive test for syphilis in patient 2. Leprosy has been associated with numerous autoimmune phenomena,22,23 and Mycobacterium leprae has an adjuvant effect similar to that of M tuberculosis.24 Moreover, autoantibodies against factor V have been associated with M tuberculosis infection.20 Although multiple autoantibodies (including antibodies against phospholipids) have been described in Treponema pallidum infection,25 circulating anticoagulants have not been associated with this organism.26,27 Furthermore, patient 1 had received a full course of treatment with Dapsone and had no evidence of active leprosy, whereas patient 2 had no evidence of active syphilis. Patient 3 had no infection of any kind. Thus, the relationship between previous mycobacterial or treponemal infection and the factor V and thrombin inhibitors remains uncertain.

In summary, we have described three postsurgical patients with prosthetic heart valves and acquired antibody inhibitors directed against prothrombin and thrombin, as demonstrated by coagulation studies and immunoblotting. The clinical significance and prevalence of antithrombin antibodies in patients with prosthetic valves remains to be determined.

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REFERENCES


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Development of antithrombin antibodies following surgery in patients with prosthetic cardiac valves [see comments]

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