Development of Antitissue Factor Antibodies in Patients After Liver Surgery
By Hiroko Tsuda, Shouichi Higashi, Sadaaki Iwanaga, Takahiro Kubota, Takashi Morita, and Katsuhiko Yanaga

After liver surgery, two patients developed unexplainable prolonged prothrombin times (PT) that were not associated with bleeding tendency. The substitution of rabbit thromboplastin for either human or monkey thromboplastin in performing PT tests resulted in a normal clotting time. Tissue factor (TF) procoagulant activity assays and an immunoblotting analysis showed that these patients had developed IgG \( \lambda \)-type immediate anticoagulants directed against both rabbit and bovine TF that did not cross-react with either human or monkey TF. In a chromogenic assay, the patient IgG caused a decrease in both the \( K_m \) and the \( V_{max} \) of the factor X activation by rabbit TF-factor VIIa complex. The lack of reactivity of the patient IgG with human TF presumably explained why there was no clinical bleeding. Both patients had been treated earlier with a topical hemostatic agent prepared from bovine corium, microfibrillar collagen hemostat, while undergoing previous surgery. In an immunoblotting analysis, the patient IgG stained a 42-Kd band in the Triton extract of the collagen preparation under either reducing or nonreducing conditions. The Triton extract of the collagen preparation blocked the binding of the patient IgG to bovine TF. Thus, it is suggested that the iatrogenic immunization by intraoperative exposure of bovine TF retained in the collagen preparation may be responsible for the development of anti-TF antibodies in these patients. The anti-TF antibodies resulted in a clinical error in the evaluation of coagulation status after the use of rabbit thromboplastin.

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continued for about 2 months, and then gradually improved to a normal level. No bleeding problems were observed postoperatively.

In both patients, antinuclear antibody, rheumatoid factor, serological tests for syphilis, and a Treponema pallidum hemagglutination test were negative. In addition, lupus anticoagulant, as determined by a diluted activated partial thromboplastin time (APTT) test, was also found to be negative.

**MATERIALS AND METHODS**

**Materials.** Protein A-Sepharose CL-4B, concanavalin A-Sepharose, and diethyl aminoethyl (DEAE)-Sepharose CL-6B were obtained from Pharmacia-LKB (Uppsala, Sweden). Formyl-Cellulofine and protein A-Cellulofine were obtained from Seikagaku-Kogyo (Tokyo, Japan). The nitrocellulose membrane was from Toyo Roshi (Tokyo, Japan). The fluorogenic substrate Z-Pyr-Gly-Arg-4-methylcoumaryl-7-amide (Z-Pyr-Gly-Arg-MCA) was from Peptide Institute Inc (Osaka, Japan). Rabbit-brain cephalin was from Sigma Chemical Co (St Louis, MO). Nonimmunized rabbit IgG, rabbit antibodies against human γ-, μ- and α-heavy chains and against κ- and λ-light chains were obtained from Dakopatts (Glostrup, Denmark). Bio-beads SM-2 and horseradish peroxidase-conjugated goat antibody against rabbit IgG was from Bio-Rad Laboratories (Richmond, CA). Biotinylated sheep antibody against human Ig, biotinylated sheep F(ab)2, fragments against mouse Ig and streptavidin-biotinylated horseradish peroxidase complex were obtained from Amersham International plc (Amersham, UK). Thromboplastins of rabbit brain-lung, rabbit lung, monkey brain, and human placenta were obtained from Organon Teknika Co (Durham, NC), Mochida Pharmaceutical Co (Tokyo, Japan), Boehringer Mannheim (Mannheim, Germany), and Behringwerke AG (Marburg, Germany), respectively. Bovine brain acetone powder was provided by Eizai Co (Tokyo, Japan). Microfibrillar collagen hemostat was obtained from Alcon Inc (Humacao, Puerto Rico). Bicinchoninic acid (BCA) protein assay reagent was from Pierce Chemical Co (Rockford, IL). Triton X-100 was from Wako Pure Chemical Industries (Osaka, Japan). Tween 20 was obtained from Katayama Chemical Industries (Osaka, Japan). All other chemicals were the best analytical grade commercially available.

**Proteins.** TF was purified from bovine lung acetone powder according to the method of Broze et al with a slight modification. The purification procedure entailed 2% Triton X-100 extraction, concanavalin A-Sepharose chromatography, and bovine factor VII coupled formyl-Cellulofine affinity chromatography. Monospecific antisera against TF apoprotein was raised in a rabbit, from which IgG fraction was prepared by ammonium sulfate precipitation followed by protein A-Cellulofine column chromatography. The TF apoprotein was relipidated by incubation with rabbit brain cephalin in the presence of Bio-beads SM-2. The 2% Triton extract of thromboplastin of either rabbit lung, monkey brain, or human placenta was prepared as described previously. Factor VII and factor X were purified from bovine plasma according to previously published methods. Bovine factor X was activated by the factor X activator from Russell’s viper venom as described previously. Human factor VIIa was kindly provided by the Chemo-Sero Therapeutic Research Institute (Kumamoto, Japan). Monoclonal antibody (MoAb) against recombinant human TF was a generous gift from Dr K. Sueshi (Kyushu University, Fukuoka, Japan). The protein concentration of factor VIIa was determined by amino acid analysis while that of factor X was done by the extinction coefficient (E280) of 12.4. The active site of factor Xa was determined by titration. The protein concentration of TF was determined by a BCA assay using bovine serum albumin (BSA) as a standard.

**Solubilization and enrichment of microfibrillar collagen hemostat.** Four grams of microfibrillar collagen hemostat was suspended in 100 mL of 0.05 mol/L Tris-HCl, pH 7.4, 0.1 mol/L NaCl, 0.01% NaN3 (Tris buffered saline [TBS]) containing 0.25% Triton X-100 and stirred overnight at 4°C. After centrifugation at 10,000g for 30 minutes at 4°C, the supernatant was lyophilized. The lyophilized powder was extracted with 200 mL of acetone to remove Triton X-100 and air-dried. The protein pellet was solubilized in 3 mL of TBS containing 0.1% Triton X-100 and dialyzed overnight against the same buffer at 4°C.

**Plasma.** Blood was collected by venipuncture into 1/10 vol of 3.1% sodium citrate. Platelet-poor plasma was prepared by centrifugation at 1,500g for 10 minutes at 4°C. Samples were immediately assayed or divided into aliquots and stored at -80°C until use.

**Isolation of IgG.** IgG was isolated from patient plasma or normal pooled plasma either by protein A-Sepharose column chromatography or by ammonium sulfate precipitation followed by DEAE-Sepharose CL-4B column chromatography.

**Coagulation studies.** PT, individual clotting factors, and other coagulation tests were assayed by standard techniques. TF activity assays. After preincubating TF samples with an equal volume of IgG at 37°C, TF activity was assessed using either a clotting or a chromogenic assay. In a one-stage clotting assay, 200 μL of the mixture of TF sample and IgG was incubated with 100 μL of normal pooled plasma at 37°C for 2 minutes. Then 100 μL of 25-mmol/L CaCl2 was added and clotting time was determined by a Behring Fibrintimer (Behringwerke AG). The percent residual activity was calculated from a standard curve prepared with serial dilutions of each TF sample.

In the chromogenic assay, 100 μL of the mixture of TF sample and IgG was incubated with 40 μL of TBS containing 0.1 mg/mL BSA (TBSA), 10 μL of 0.1 mol/L CaCl2 and 25 μL of human factor VIIa at 37°C for 5 minutes. Then 25 μL of bovine factor X was added and the incubation at 37°C was continued. After 5 minutes, the reaction was terminated by the addition of 500 μL of TBSA containing 10 mmol/L EDTA and 100 μmol/L Z-Pyr-Gly-Arg-MCA, the fluorogenic substrate of factor Xa. After incubation at 37°C for 10 minutes, 25 μL of 20% acetic acid was added to stop the reaction and the released 7-amino-4-methylcoumarin was measured fluorometrically with excitation at 380 nm and emission at 440 nm. The amount of factor Xa generated was calculated from a calibration curve prepared with serial dilutions of purified bovine factor Xa.

**Neutralization of patient IgG.** Patient IgG was mixed with rabbit antibodies against specific Ig classes and chain types. The mixture was incubated for 18 hours at 4°C and then again for 1 hour at 37°C. After the precipitate was removed by centrifugation, the supernatant was examined for the inhibitory effects on the procoagulant activity of rabbit lung thromboplastin.

**Immunolectron microscopic analysis.** Purified bovine-lung TF, the Triton X-100 extracts of thromboplastins and that of microfibrillar collagen hemostat were fractionated by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then were electrophoretically transferred to nitrocellulose membranes. The membranes were blocked with TBS containing 5% nonfat dry milk. Subsequently the membranes were incubated with patient IgG, normal human IgG, rabbit antiovine TF IgG, or murine MoAb against human recombinant TF overnight at room temperature. After extensive washing with TBS containing 0.05% Tween 20, the membranes were incubated in a second antibody (biotinylated sheep antibody against human IgG for patient IgG and normal human IgG; horseradish peroxidase-conjugated goat antibody against rabbit IgG for rabbit anti-TF IgG; and biotinylated sheep F(ab)2 fragments against mouse Ig for MoAb for 2 hours. The membranes that had been incubated with either patient IgG, normal human IgG, or MoAb as the first antibody were further incu-
bated with an indicator, streptavidin-biotinylated horseradish peroxidase complex, for 2 hours. After extensive washing, the membranes were developed with 4-chloro-1-naphthol.

RESULTS

Coagulation studies. In both patients, coagulation studies showed that factor VII activity was reduced postoperatively when PT was prolonged (patient 1: 13%; patient 2: 49%) whereas the activities of factors X, V, and II were normal. The mixing tests showed that the plasma from these patients were able to prolong the PT of normal plasma, indicating the presence of circulating inhibitors to the initial step in the extrinsic clotting cascade containing TF and factor VII.

Because neither patient showed bleeding tendency despite a markedly prolonged PT, we substituted the rabbit brain-lung thromboplastin routinely used in performing PT for the thromboplastins of varying species and tissues (Table 1). Irrespective of tissue origin, the PT of both patients measured by either rabbit or bovine thromboplastin were significantly prolonged, which became more apparent with the use of more diluted thromboplastins (data not shown). In contrast, when either monkey or human thromboplastin was used, the PT became normalized. These data suggest that the inhibitors present in the plasma of these two patients were directed primarily not against factor VII but against rabbit and bovine TF with little or no reactivity to either human or monkey TF.

TF activity assays. The IgG nature of the inhibitors was suggested by the disappearance of the inhibitory activity from the patient plasma after passing through a protein A-Sepharose column. The IgG fractions eluted from the column were analyzed for the effects on TF activity by both clotting and chromogenic assays. After incubating rabbit lung thromboplastin with the patient IgG, the residual TF activity was quantified in a one-stage clotting assay. The patient IgG inhibited TF activity in a dose-dependent manner (Fig 1A) with a maximal inhibition of more than 95%. The concentration of IgG producing half-maximal inhibition (ID50) was 40 pg/mL for patient 1 and 160 pg/mL for patient 2. The inhibition was rapid and it was completed within 1 minute for patient 1 and within 4 minutes for patient 2 (Fig 1B). In both patients, pretreatment of the patient IgG with rabbit antibody against either human γ-heavy chains or λ-light chains completely abolished anti-TF activity of the patient IgG. However, pretreatment with nonimmunized rabbit IgG and that with rabbit antibody against either μ-heavy chains, α-heavy chains or κ-light chains had no effect. The inhibitory activity of the IgG from patient 1 was further evaluated using both purified bovine TF reconstituted with phospholipid and thromboplastins of different species. The patient IgG inhibited the procoagulant activities of both bovine lung TF and a saline extract of bovine lung acetone powder to the same extent. The maximal inhibition was 80% and the ID50 was 600 μg/mL. In contrast, the patient IgG did not inhibit the procoagulant activity of thromboplastin of either monkey brain nor human placenta even at a concentration of 8.0 mg/mL.

The effect of patient IgG on the factor X activation by TF-factor VIIa (TF-VIIa) complex was evaluated using rab-

| Table 1. PT Measured by Thromboplastins of Varying Species, and Tissues |
|-------------------------|----------------|----------------|
| Thromboplastins         | Patient 1     | Patient 2     | Normal   |
| Rabbit brain-lung       | 19.9           | 17.1           | 12.0     |
| Rabbit lung             | 29.7           | 22.0           | 10.7     |
| Bovine lung             | 63.4           | 50.7           | 32.7     |
| Bovine brain            | 47.6           | 42.7           | 33.5     |
| Monkey brain            | 13.9           | 13.6           | 12.4     |
| Human placenta          | 12.3           | 11.7           | 12.5     |

PT was examined for plasma obtained from patient 1 on January 31, 1991 as well as that from patient 2 on June 6, 1991. In addition, PT was also examined for normal pooled plasma.
but could not be completely blocked even at a concentration of 4.0 mg/mL IgG (residual activity: 50%). The ID₉₀ was 550 μg/mL in both patients, which was much higher than that required in a clotting assay. However, the patient IgG had no effect on the amidolytic activity of factor Xa (data not shown). The kinetic parameters of factor X activation were determined by varying concentrations of factor X either in the presence or in the absence of IgG from patient 1 (Table 2). When the concentration of factor VIIa was saturated (4.4 nmol/L), the patient IgG caused a decrease in both the Km and the Vmax, showing noncompetitive inhibition. Both parameters remained unchanged after incubation with normal human IgG (data not shown). The extent of the decrease of Vmax by the patient IgG was not affected by decreasing the concentration of factor VIIa to 0.2 nmol/L, which may be explained by the fact that the antibodies are not directed against factor VIIa.

**Immunoblotting analysis.** To assess the binding of patient IgG to TF apoprotein, an immunoblotting analysis was performed. When the Triton X-100 extract of rabbit lung thromboplastin was fractionated by SDS-PAGE under reducing conditions (Fig 2A), the IgG obtained from both patients just after surgery with a significantly prolonged PT showed several bands including a band of 42 Kd (lanes 1, 3, and 4). The 42-kD band was also seen in the Triton extract electrophoresed under nonreducing conditions (data not shown). However, the IgG obtained late after surgery when PT was normalized (lanes 2 and 5) as well as normal human IgG (lane 6) failed to stain this 42-Kd band. Both the patient IgG and normal human IgG did not stain any band with the Triton X-100 extract of thromboplastin of either human placenta (Fig 2B, lanes 1 through 6) or monkey brain (data not shown), although the MoAb against recombinant human TF identified a band of 43 Kd (lane 7). With the Triton X-100 extract of bovine lung acetone powder, the IgG prepared from the patient plasma with prolonged PT showed several bands including a band of molecular weight of 42 Kd (Fig 3A, lanes 1 and 2), and a similar band was identified by rabbit antivovine TF IgG (lane 5). The binding of the patient IgG to TF apoprotein was confirmed by the fact that the patient IgG stained a 42-Kd band with purified bovine TF (Fig 3B, lanes 1 and 2). A band of the same molecular weight was seen by rabbit antivovine TF IgG (lane 5). However, normal human IgG did not stain any band with either the Triton extract of bovine lung acetone powder (Fig 3A, lane 4) or purified bovine TF (Fig 3B, lane 4). Thus, it appears that after surgery both patients transiently developed antibodies against TF apoprotein and several other proteins present in the Triton X-100 extract of bovine and rabbit thromboplastins.

The only material of animal origin that the patients had been exposed to during surgery was microfibrilar collagen hemostat. We hypothesized that microfibrilar collagen hemostat contains TF apoprotein by which the patients postoperatively developed inhibitors against bovine TF cross-reactive to rabbit TF. The microfibrilar collagen hemostat was solubilized in Triton X-100, concentrated by lyophilization, and then reconstituted with phospholipid. However, this preparation did not show any TF activity either by clotting or chromogenic assays (data not shown). An immunoblotting analysis showed that the patient IgG stained a 42-Kd band with the Triton X-100 extract of microfibrilar collagen hemostat both under reducing (Fig 3C, lanes 1 and 2) and nonreducing (data not shown) conditions, although no band was stained by either normal human IgG (lane 4) or rabbit antivovine TF IgG (lane 5). To confirm the hypothesis, we examined whether the Triton extract of the collagen preparation was able to block the binding of the patient IgG to bovine TF. After incubation with the Triton extract of the collagen preparation, the IgG from patient 1 lost not only the reactivity to the Triton extract of the collagen preparation (Fig 3C, Lane 3) but also the reactivity to either the 42-Kd band in the Triton extract of bovine lung acetone powder (Fig 3A, lane 3) or purified bovine TF (Fig 3B, lane 3). However, the incubation with 0.1% Triton X-100 produced no difference in the reactivity of the patient IgG (data not shown).

**DISCUSSION**

The present report is the first to describe the acquired antibody inhibitors to TF that developed postoperatively in two patients undergoing liver surgery. The patients showed isolated prolonged PT measured by rabbit and bovine thromboplastins, which were markedly enhanced by diluting the thromboplastins; however, neither of them were associated with bleeding tendency. The inhibitors immediately abolished the procoagulant activities of rabbit and bovine TF with no effect on those of monkey and human TF. The inhibitors appeared to be exclusively of the IgG type and were completely neutralized by antibody against either γ-heavy or λ-light chains. Some inhibitors to factor VIII and IX developed in hemophilia patients after replacement therapy also tend to show restricted heterogeneity, mostly of a single light-chain type and the same heavy-chain subclass. The characteristic of inhibition by the patient IgG was further evaluated by kinetic analysis. The patient IgG inhibited the factor X activation by TF-factor VIIa complex with decreasing both the Km and Vmax. The extent of the decrease of Vmax was not affected by the con-

Table 2. Effect of Patient IgG on the Kinetic Parameters of the Factor X Activation by TF-VIIa Complex

<table>
<thead>
<tr>
<th>Factor VIIa (nmol/L)</th>
<th>Addition</th>
<th>Km (nmol/L)</th>
<th>Vmax (nmol/L/min)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>Buffer</td>
<td>189 ± 20</td>
<td>1.91 ± 0.14</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Patient IgG</td>
<td>87 ± 30</td>
<td>0.65 ± 0.14</td>
<td>5</td>
</tr>
<tr>
<td>4.4</td>
<td>Buffer</td>
<td>73 ± 10</td>
<td>0.60 ± 0.04</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Patient IgG</td>
<td>75 ± 32</td>
<td>0.17 ± 0.06</td>
<td>3</td>
</tr>
</tbody>
</table>

Rabbit lung thromboplastin was incubated with TBSA alone or IgG from patient 1 (4.0 mg/mL) at 37°C for 10 minutes, followed by the addition of factor VIIa (0.2 or 4.4 nmol/L) and factor X (12.5 to 500 nmol/L). The rates of factor Xa formation were determined as described under Materials and Methods. The kinetic parameters of factor X activation were calculated by fitting the data to the double-reciprocal ploting of the rates of factor Xa formation versus the factor X concentrations. Data are expressed as means ± SD for the number of experiments given by n.
Fig 2. Immunoblotting analysis of the patient IgG binding to the Triton X-100 extract of rabbit or human thromboplastin. The Triton X-100 extract of thromboplastin of either rabbit lung (A) or human placenta (B) was fractionated by SDS-PAGE under reducing conditions, transferred to nitrocellulose membrane, and reacted with 5 mg/mL patient IgG (lanes 1-5), 5 mg/mL normal human IgG (lane 6), and 3.3 μg/mL murine MoAb against human recombinant TF (lane 7). IgG were obtained from patient 1 just after the first surgery on January 25, 1991 (lane 1); 6 months after the first surgery on July 12, 1991 (lane 2); just after the second surgery on January 24, 1992 (lane 3); from patient 2 just after the second surgery on June 6, 1991 (lane 4); 4 months after the second surgery on September 19, 1991 (lane 5). The molecular weights of the marker proteins run under reducing conditions are indicated at the left.

centration of factor VIIa. Therefore, it is suggested that some mechanism other than that interfering with substrate assembly to the complex must exist in the rapid inhibition by the patient IgG. The MoAbs against human TF that are noncompetitive inhibitors as the patient IgG have been reported to inhibit preformed TF-factor VIIa complex by dissociating factor VIIa from the cell surface TF.

An immunoblotting analysis showed that the patient IgG was observed to bind to purified bovine TF apoprotein. Although the patient IgG stained several bands, including the band probably corresponding to TF in the Triton extracts of both rabbit thromboplastin and bovine acetone powder, no band was shown in the Triton extract of either monkey or human thromboplastin. The species specificity of the antibodies in both the inhibition of TF activity and the binding to TF apoprotein may reconcile the discrepancy between the presence of markedly prolonged PT and the absence of hemorrhagic problems during the clinical courses of both patients. Recently, regarding the amino acid sequence of bovine TF, 74.1% has been reported to be identical to rabbit TF, and 70.4% is identical to human TF. However, there is a highly variable sequence among these TF near the N-terminus of the extracellular domain. Therefore, the patient IgG may be associated with bovine and rabbit TF near this region and inhibit the interaction between TF and human factor VIIa.

Each of the two patients had undergone liver surgery twice for metastatic cancer originating from either the sigmoid colon or the rectum. The time of appearance of the antibodies against bovine and rabbit TF relative to the time of surgery suggests that the patients may have been exposed to antigen during surgery. In our hospital, microfibrillar collagen hemostat has been used as a topical hemostatic agent particularly in liver surgery, being applied to cover the raw surface of the liver, followed by saline irrigation for removal. Microfibrillar collagen hemostat is prepared from bovine corium by washing extensively with ethanol in the presence of HCl, milled into a flour-like form, and sterilized at 120°C for 28 hours. It is conceivable that the bovine corium used to prepare the collagen preparation could be contaminated by epidermis where high levels of TF expression has been reported. Although in the Triton extract of the collagen preparation we could neither detect TF activity nor identify the band corresponding to TF by immunoblotting with rabbit antibovine TF IgG, the patient IgG stained a 42-Kd band in the Triton extract electrophoresed under either reducing or nonreducing conditions. In addition, the Triton extract of the collagen preparation blocked the binding of the patient IgG to bovine TF. Therefore, we suggest that the patients were indeed immunized during surgery with the bovine TF present in microfibrillar collagen hemostat, which was probably denatured by the sterilization
Fig 3. An immunoblotting analysis of the patient IgG binding to the Triton X-100 extract of bovine lung acetone powder, that of microfibrillar collagen hemostat, and the purified bovine lung TF. The Triton X-100 extract of bovine lung acetone powder (A), that of microfibrillar collagen hemostat, and the bovine lung TF (450 ng/ lane; B) were fractionated by SDS-PAGE under reducing conditions, and immunoblotting was performed. Lane 1. IgG from patient 1 on January 24, 1992 (5 mg/mL); lane 2. IgG from patient 2 on June 6, 1991 (5 mg/mL); lane 3. IgG from patient 1 incubated with the Triton extract of microfibrillar collagen hemostat for 3 hours at room temperature (5 mg/mL); lane 4. normal human IgG (5 mg/mL); lane 5. rabbit antitissue TF IgG (1 mg/mL for A and C. 200 μg/mL for B).

procedure, and postoperatively developed inhibitory antibodies against both bovine and rabbit TF. The antibodies caused a biologic “false positive” in PT measured by rabbit thromboplastin. In the course of preparing this article, we have encountered four additional patients who developed anti-TF antibodies of the same specificity as that observed in the reported cases. All of them had previously undergone either liver or spleen surgery during which time a low dosage of microfibrillar hemostat (1 g) had been used. Therefore, anti-TF antibody should be considered when postoperative patients show an unexplainable prolongation of PT measured by either rabbit or bovine thromboplastin.

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REFERENCES

12. Morita T, Jackson CM: Preparation and properties of derivatives of bovine factor X and factor Xa from which the γ-carboxyglutamic acid containing domain has been removed. J Biol Chem 261:4015, 1986
16. Austen DEG, Rhymes IL: Laboratory diagnosis of blood co-


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