Biologically Active Thrombomodulin Is Synthesized by Adherent Synovial Fluid Cells and Is Elevated in Synovial Fluid of Patients With Rheumatoid Arthritis

By Edward M. Conway and Barbara Nowakowski

Thrombomodulin (TM) is a transmembrane glycoprotein that interacts with thrombin, thereby serving as a cofactor in the activation of protein C, a major physiologically relevant natural anticoagulant. Although initially described as a vascular endothelial cell receptor, TM has also been reported to be synthesized by several cells, including megakaryocytes, platelets, monocytes, neutrophils (PMN), mesothelial cells, and synovial lining cells. A prominent feature of rheumatoid arthritis (RA) is infiltration of PMN into the joint space. To determine whether TM might play a role in the inflammatory process, we examined synovial fluid for the presence of TM in 10 patients with RA and five patients with osteoarthritis (OA). We determined that the mean synovial fluid and plasma TM levels in the OA group were 23.5 ng/mL and 24.2 ng/mL, respectively, whereas those with RA had a significantly elevated mean synovial fluid TM level of 136.2 ng/mL as compared with the plasma TM concentration of 43.9 ng/mL ($P < .05$). Synovial fluid TM levels did not correlate with PMN counts ($r = .261$). Purified TM from synovial fluid was identical in molecular weight to plasma-derived TM and was biologically functional with respect to protein C cofactor activity. Using direct immunofluorescence, we determined that adherent cultured synovial fluid cells that are not monocytoid in origin express surface and cytoplasmic TM, thereby providing an alternative source of the protein. Biologic activity of the cell-surface TM was confirmed by acceleration of thrombin-dependent protein C activation. Northern analysis of RNA extracted from the cultured cells indicated that TM messenger RNA was present, suggesting local synthesis. Our results indicate that in RA-associated synovial effusions, biologically active TM is increased, the source of which may be from plasma, PMN, and/or synovial lining cells. TM may play a regulatory role either in fibrin deposition in the inflamed joint and/or in the progression of the inflammatory process.

RHEUMATOID ARTHRITIS (RA) is a chronic, systemic disorder characterized predominantly by articular joint inflammation and destruction. Several lines of evidence indicate that the hemostatic mechanism is closely linked to the inflammatory process in RA. Inman and Harpel examined components of the fibrinolytic system in the synovial fluid of patients with RA, osteoarthritis (OA), and septic joints and concluded that the system is intact in all cases, with generation of proteolytically active plasmin. Hyperfibrinogenemia is commonly associated with RA and deposition of fibrin in RA joints is often observed. In vitro, plasminogen activators (PAs) can be induced in both synoviocytes and vascular endothelial cells, and presumably are critical for the generation of plasmin in the inflamed joint. Plasmin or PAs may directly activate collagenase that is bound to collagen fibrils, leading to rapid destruction of collagen-containing tissues, thereby playing a major role in connective tissue turnover and remodeling in the arthritic joint.

From the Division of Hematology-Oncology, the Department of Medicine, The Toronto Hospital, University of Toronto, Toronto, Ontario, Canada.

Submitted August 14, 1992; accepted October 5, 1992.

Supported by The Heart and Stroke Foundation of Ontario and the Physicians’ Services Incorporated Foundation of Ontario. E.M.C. is supported by a Research Scholarship from the Heart and Stroke Foundation of Ontario.

Presented in part as an abstract at the American Federation of Clinical Research Meeting in May 1992, in Baltimore, MD.

Address reprint requests to Edward M. Conway, MD, The Toronto Hospital-General Division, 585 University Ave, Mulock Larkin 2-031, Toronto, Ontario, Canada M5G 2C4.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1993 by The American Society of Hematology.

Thrombomodulin (TM) is a vascular endothelial cell receptor that forms a 1:1 complex with thrombin. This interaction product forms the basis of a major physiologically relevant natural anticoagulant mechanism by rapidly converting protein C (PC) to its serine protease form. Activated PC (APC) suppresses further thrombin formation by proteolytically destroying cofactors of the coagulation mechanism and may also produce an increase in fibrinolytic activity. In baboon studies, APC and its cofactor, protein S, have been demonstrated to modulate the manifestations of Escherichia coli-induced sepsis, thereby providing a definitive link between the coagulation system and inflammation.

In addition to playing a critical role in the activation of PC, TM has several other distinct blood anticoagulant activities that provide the vascular endothelium with properties that prevent the initiation and promotion of clot formation. Finally and in contrast to the above, TM accelerates the neutralization of single-chain urokinase-type PA (scu-PA) by thrombin, thereby interfering in the generation of plasmin and fibrinolysis. The biologic significance of this effect is unknown.

Studies on rabbit and human articular tissue have indicated that synovial lining cells express TM on the cell surface. We have reported that neutrophils (PMN), which are a prominent feature of the synovitis associated with RA, express TM. Based on these observations and the fact that the molecule is essential for the activation of PC, we postulated that TM would be present in inflammatory joint effusions and may play a role in regulation of the inflammatory process. We report here that biologically active TM is found in the synovial fluid of patients with noninflammatory joint disease, but that it is significantly elevated in the inflamed joint fluid of RA. Our results further indicate that there may be several cellular sources of the protein, including neutrophils, monocytes, synovial lining cells, and adherent synovial fluid cells.
MATERIALS AND METHODS

Materials. Bovine PC was purchased from Enzyme Research Laboratories (South Bend, IN). Hirudin was obtained from Pentapharm Ltd (Basel, Switzerland) and the chromogenic substrate HD-Phe-Pip-Agpa-Na (S2238) was provided by Helena Laboratories (Beaumont, TX). Bovine thrombin was purified as previously detailed26 and has a specific activity of 1,900 U/mg. The murine monoclonal antibodies 24FM and 3E2 directed against human TM were a gift of Stago (Paris, France) and specifically immunoprecipitate human TM. Human TM cDNA was kindly provided by Drs R.W. Jackman and R.D. Rosenberg (Boston, MA). Immunologic reagents were obtained from Bio-Rad Laboratories (Mississauga, Canada). Restriction enzymes were bought from Boehringer Mannheim Canada (Dorval, Quebec).

Cell culture. Human umbilical vein endothelial cells (HUVEC) were harvested by the method of Jaife et al.29 and grown in M199 supplemented with 20% fetal bovine serum (FBS), porcine heparin 0.1 mg/mL, endothelial cell growth factor (Biomedical Technologies Inc, Stoughton, MA) 5 µg/mL, penicillin 100 µg/mL, and streptomycin 100 µg/mL. Cell monolayers demonstrated the typical cobblestone morphology of endothelial cells and were characterized by immunofluorescent staining for von Willebrand’s factor. Studies were performed on cells at passages 2 through 4. ASFC were obtained from the fresh synovial fluid of patients with arthritis by the method of Fraser and McCal12 with minor modifications: heparinized synovial fluid was dibuted 1:20 in Dulbecco’s modified Eagle medium (DMEM) with 10% FBS, and plated into 100-mm culture dishes. After 24 hours, nonadherent cells were removed and adherent cells were cultivated in DMEM with 20% FBS until confluent, at which time the cells were trypsinized and passaged at a 1:3 ratio. After adhesion and before first passage, less than 2% of the cells were monocytes or endothelial cells, as assessed by immunofluorescent staining with anti-Leu M3 and von Willebrand’s factor, respectively. All cell cultures were incubated at 37°C in a humidified atmosphere of 5% CO₂ and 95% air. Media was changed 24 hours before all experiments, which were conducted after cells were grown 2 to 3 days postconfluence.

PC activation. Thrombin-dependent activation of PC was performed as previously detailed.22,23 Briefly, confluent cell monolayers or purified TM, in a final volume of 200 µL, was incubated with bovine thrombin and bovine PC at final concentrations of 2.1 nmol/L and 267 nmol/L, respectively. After 90 minutes at 37°C, the reaction was quenched with antithrombin III and hirudin. Functional activity of TM was then evaluated by measuring the rate of change in absorbance at 405 nm when 200 µL of the reaction mixture was added to 500 µL of 0.4 mmol/L S2238. Controls containing thrombin and PC in the absence of cells or purified TM were treated similarly and no activation was seen. Coincubation of polyclonal anti-TM antibodies in the test reaction with purified TM or cells expressing surface TM, totally abrogated activation of PC.

Immunofluorescent studies. Cells were grown on poly-L-lysine-coated, glass coverslip slides, washed in phosphate-buffered saline (PBS), and fixed with 3.0% paraformaldehyde in 0.1 mol/L sodium phosphate, pH 7.2, for 20 minutes and/or permeabilized with 0.2% Triton X-100 in PBS for 2 minutes. After further washes in PBS, the cells were treated for 30 minutes with blocking solution (PBS with 1% bovine serum albumin [BSA]), washed with PBS, and the first monoclonal murine antibody was added at 10 µg/mL for 30 minutes at 37°C in blocking solution. After further washes, rabbit antinmoum IgG (RAM) 25 µg/mL was incubated for 30 minutes. Detection was performed with goat-antirabbit-Fab’ bound to fluorescent isothiocyanate (FITC). Non-specific immunofluorescence was excluded in parallel experiments using an irrelevant first antibody.

Synovial fluid and plasma samples. Joint aspirations and venipunctures were performed under sterile conditions by a rheumatologist at The Toronto Hospital Rheumatic Diseases Unit for clinical indications only with the written consent of patients and approval of the institution’s Human Subjects for Research Committee. Synovial fluid was drawn directly into a heparinized syringe and put on ice. A sample was removed for cell count and the remainder was spun to remove the cell pellet. The synovial fluid was then aliquoted and stored at -70°C for subsequent use. Citrated venous blood was immediately spun to obtain platelet-poor plasma and aliquoted as above.

Enzyme immunoassay of TM. A double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) method ("Asserochrom Thrombomodulin"; Diagnostica Stago, Asnieres, France) was used to quantitate solubilized TM according to the manufacturer’s instructions. In this technique, microplate wells were coated with specific monoclonal anti-TM antibody (24FM) 0.5 µg/well, blocked with 200 µL of 10% normal rabbit serum for 2 hours, and washed extensively with PBS containing 0.1% BSA and 0.05% Tween-20 (PBS-BSA-Tween). Two hundred microliters of test sample or standard was added in duplicate to each well and incubated for 2 hours at room temperature. After five rapid washes, the second monoclonal anti-TM antibody (3E2) conjugated to horseradish peroxidase was added for 2 hours at room temperature. After further washes, color development was evaluated at an absorbance of 492 nm was accomplished with the substrate ortho-phenylene-diamine in the presence of hydrogen peroxide and the reaction was stopped with sulphuric acid. A standard curve was determined to be linear in the range of 2 to 100 ng/mL.

Purification of TM. TM from synovial fluid or plasma was purified by a combination of ion-exchange and affinity chromatography according to previously described methods.24,25 Protease inhibitors were added to all solutions during the glycoprotein’s preparation at the following concentrations: 10 µmol/L benzamidine, 1 µmol/L phenylmethylsulfonyl fluoride (PMSF), 5 µmol/L 1, 10 phenanthroline, 10 µmol/L leupeptin, 1 µmol/L pepstatin A, and 0.05 U/mL aprotinin. Synovial fluid or plasma was diluted 1:10 in 20 mmol/L Tris-HCl, pH 7.4, 0.15 mol/L NaCl and run through a 5-ML Q-Sepharose column (Pharmacia, Baie d’Urfé, Quebec, Canada). After extensive washing, the bound material was eluted with 2 mol/L NaCl, dialyzed in 20 mmol/L Tris-HCl, pH 7.4, with 50 mol/L NaCl, and passed through a 4-nL Affigel-10 column (Bio-Rad) that was previously conjugated with polyclonal anti-TM antibody 2 mg/mL of gel. After extensive washes with 20 mol/L Tris-HCl, pH 7.4, 10 mmol/L NaCl, elution of TM was accomplished with 50 mmol/L diethylamine, pH 11.3, into 1/10 volume of 1 mol/L Tris-HCl, pH 7.4. The peak fractions of TM were identified by absorbance at 280 nm or dot-blotting onto nitrocellulose paper for immunodetection using monoclonal anti-TM antibodies. TM purified in this manner was greater than 95% pure as evaluated by silver staining of 15% gradient SDS-polyacrylamide gels.

RNA isolation and Northern analysis. Cytoplasmic RNA was isolated by the guanidium isothiocyanate/CsCl method of Chirgwin et al. Total cytoplasmic RNA was fractionated on a 1% agarose/formaldehyde gel and transferred onto Genescreen filters in 10× SSC (1× SSC: 0.15 mol/L NaCl, 0.015 mol/L sodium citrate, pH 7.4) for Northern analysis.27 After vacuum baking of the filters at 80°C, the filter was prehybridized for 4 to 6 hours in a buffer containing 50%...
formamide, 5× SSC, (1× SSC: 0.15 mol/L NaCl, 0.015 mol/L sodium citrate, pH 7.4), Denhardt’s solution (12.5×), 5% SDS, 5× SSPE (1× SSPE: 0.15 mol/L NaCl, 10 mmol/L NaH2PO4H2O, 1 mmol/L EDTA, pH 7.4), and 1.0 mg/mL herring sperm DNA. Detection of TM messenger RNA (mRNA) was accomplished using a Xho I/Xba I 3.1-kb DNA fragment from the TM gene that includes the entire coding region for the protein. The random primer synthesis method29 was used for radiolabelling with α-32P dCTP, and specific activity was approximately 1.5 × 106/μg of DNA. Hybridization with the 32P-labeled probe was performed overnight at 42°C. The RNA blots were sequentially washed with 2× SSC/1% SDS at room temperature twice for 20 minutes each, and with 0.5× SSC/1% SDS at 65°C for 15 minutes. Filters were then exposed to Kodak XAR-5 film (Eastman Kodak, Sargent Welch Scientific, Weston, Ontario, Canada) using an intensifying screen at −70°C for 1 to 4 days.

**Analysis of data.** Statistical analyses of data were conducted by standard techniques. In most instances, the means are provided with associated standard deviations (SD).

**RESULTS**

**Synovial fluid analysis in arthritis.** We analyzed the synovial fluid from five patients with OA and 10 patients with RA (Table 1). Renal function, reported to be a clearance mechanism for plasma TM,30 was normal in both groups. Synovial fluid was obtained from knee joints in all patients except for patient 6, in whom the fluid was taken from the elbow. The group of patients with OA had a mean plasma TM level of 24.2 ng/mL (SD = 5.1), which was not significantly different from a group of seven normal controls (P > .5). The glycoprotein receptor was detected in the joint effusions of those with OA at a mean concentration of 23.5 ng/mL (SD = 4.8), which is not statistically different from the plasma TM levels in the same patients (P > .4). In contrast, those with RA had markedly elevated synovial fluid TM levels, with a mean of 136.2 ng/mL (SD = 111.1), whereas the mean plasma TM level remained significantly lower at 43.9 ng/mL (SD = 40.0) (P < .05). It was considered that the TM in the synovial fluid in those patients with RA might simply reflect elevated plasma levels of the protein. However, this was not the case in those seven individuals with RA (patients 4 through 10) whose plasma TM levels were examined. In these patients, the mean plasma TM level was significantly lower than the synovial fluid TM level (P < .025); this concentration gradient argues strongly for local synthesis and release of TM. The association of RA with a large influx of PMN into inflamed joints, and our recent finding that PMN are capable of synthesizing TM,18 prompted us to determine whether these cells might be the source of synovial fluid TM. However, Table 1 indicates that there is no correlation (r = .261) between the PMN counts and synovial fluid TM levels in the group with RA, suggesting that these cells were not likely to be the exclusive origin of TM in the joint fluid.

**Characterization of synovial fluid TM.** To further characterize synovial fluid TM, we determined by immunoblotting with a monoclonal antibody specific for human TM that the molecule has an apparent molecular weight of approximately 69 Kd in the nonreduced state. This was identical to plasma-derived TM and smaller than affinity-purified plasm
cental TM (approximately 77 Kd) (Fig 1). The TM from the synovial fluid was subsequently isolated by a series of ion-exchange and affinity chromatography steps, as detailed in Materials and Methods. Silver stain analysis after SDS-PAGE under reducing conditions confirmed that the material was composed primarily of prominent bands with apparent molecular weights of 65 Kd and 75 Kd, the same size as seen with plasma-derived TM (Fig 2).

Biologic activity of the synovial fluid TM was tested by its ability to augment thrombin-dependent PC activation. Table 2 demonstrates that the activity of this TM was similar to that from plasma and less than that from placenta. The soluble plasmaderived receptor has previously been reported to have diminished PC cofactor activity as compared with the intact receptor, despite both moieties presumably having the critical epidermal growth factor (EGF)-like domains necessary for interaction with PC and thrombin.31-33

<table>
<thead>
<tr>
<th>Sample</th>
<th>ΔAOD/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer</td>
<td>0.00</td>
</tr>
<tr>
<td>Placental TM (0.25 µg)</td>
<td>0.20</td>
</tr>
<tr>
<td>Plasma TM (0.25 µg)</td>
<td>0.11</td>
</tr>
<tr>
<td>Synovial fluid TM (0.25 µg)</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Affinity-purified TM was incubated with PC and thrombin for 90 minutes as detailed in Materials and Methods. After quenching with antithrombin III and hirudin, activation of PC was evaluated using the amidolytic substrate S2238. The rate of change in absorbance at 405 nm is a measure of functional activity of the TM. The results reflect the means of three independent experiments each performed in duplicate.

Synovial fluid cells and TM expression. To examine whether the ASFC express TM, we cultured the cells from the synovial fluid of patients with RA, OA, and chlamydia-associated arthritis (latter cells kindly provided by Dr R. Inman, Toronto, Canada). These cells were different from the synovial lining cells examined by McCachren et al6 in that their cells were characterized as being monocytoid by anti-CD14 (anti-Leu M3) immunostaining, whereas the adherent cells in our experiments were greater than 98% negative for the same marker and morphologically similar to those derived from synovial explants and enzymatically dissociated synovial tissue.31,34 The synovial fluid cells were also negative for the neutrophil marker anti-CD15.

The presence of TM in the synovial fluid cells was first determined by immunoblotting electrophoretically separated membrane preparations using specific monoclonal antibodies that recognize human TM. Immunodetection indicated that TM derived from these cells has an apparent molecular weight of approximately 77 Kd, similar to that from HUVEC (Fig 3).

Table 2. Cofactor Activity of Purified TM

![Fig 2. SDS-PAGE analysis of antibody affinity-purified TM. TM from synovial fluid (1) or plasma (2) was purified by ion exchange and antibody affinity chromatography as outlined in Materials and Methods. After separation of the products by SDS-PAGE under reducing conditions in the presence of β-mercaptoethanol, the gel was silver stained.](image URL)
tured cells after three passages. Specific mRNA for TM was present as a single band at approximately 3.8 kb, identical in size to that from HUVEC (Fig 5). In view of our previous data suggesting that functional cell surface TM expression by ASFC exceeds that of HUVEC per cell, it was surprising to note that the intensity of the band representing TM mRNA was greater in HUVEC. This may be due to (1) variations in translational efficiency in different cell types under varying culture conditions; (2) differences in relative distribution of TM vis a vis intracellular versus cell surface expression; or (3) variability in PC cofactor activity of TM per molecule between cell types. The latter two possibilities are exemplified by our recent demonstration that neutrophils synthesize TM that is both nonfunctional and largely excluded from the cell surface.18

**DISCUSSION**

During the first few days to weeks of onset of RA, the subsynovial tissues become edematous, whereas the synovial cell lining begins to proliferate. PMN adhere to the vascular endothelium, accumulate in and around the microvasculature (the latter which often becomes obliterated with thrombus), and infiltrate the synovium.33,36 Inflammatory cells ultimately enter the joint space as vascular endothelial cell permeability increases. In the chronic state of RA, the synovial lining cells continue to proliferate and vascular changes again predominate as the synovium protrudes into the joint cavity forming a pannus, often considered one of the most destructive elements of the disease.

Alterations in synovial fluid composition under pathologic conditions will reflect a variety of dynamic events, including

<table>
<thead>
<tr>
<th>Sample</th>
<th>ΔOD₅₀₅/min/10⁶ Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>COS cells</td>
<td>0.0</td>
</tr>
<tr>
<td>COS-TM.CR</td>
<td>190</td>
</tr>
<tr>
<td>HUVEC</td>
<td>2.4</td>
</tr>
<tr>
<td>ASFC</td>
<td>56</td>
</tr>
</tbody>
</table>

Confluent monolayers of cells were grown in 24-well dishes, washed, and incubated with PC and thrombin as detailed in Materials and Methods. Activation of PC was evaluated colorimetrically using the substrate S2238 and corrected for the number of cells. The results reflect the mean of two independent experiments each performed in duplicate. COS cells are known not to express TM. COS-TM.CR is a cell line stably transfected with TM cDNA.29
THROMBOMODULIN IN SYNOVIAL FLUID

28s

the purified protein was lacking in PC activation cofactor activity. This is worthy and intriguing and suggested several potential explanations with respect to the source of the protein. We have recently demonstrated that PMN synthesize TM, but that the purified protein was lacking in PC activation cofactor activity.

If PMN, which are abundant in the synovial fluid of these patients, are the major source of synovial fluid TM, then conditions must be appropriate in the joint to render the otherwise inactive TM functional. The presence of several mediators of inflammation in the arthritic joint could hypothetically induce alterations in PMN processing of TM to affect its biologic function. Alternatively, other sources of biologically active TM may be present in the joint, as suggested by the lack of correlation of PMN count with synovial fluid TM levels and the large gradient between plasma and synovial fluid TM levels in patients with RA.

TM is synthesized by several cells, including vascular endothelial cells, platelets, megakaryocytes, mesothelial cells, neutrophils, and the syncytiotrophoblast of placenta. McCachren et al. have recently demonstrated that synovial lining macrophages express TM on the cell surface. Boffa et al. previously had made a similar observation using immunoperoxidase staining of rabbit joints, although the specific cell of origin was not defined. Based on these studies, we hypothesized that the synovial lining cells may provide an alternative source for the soluble form.

To isolate “synovial lining cells,” we used a previously described method whereby adherent cells from fresh synovial fluid are selected and cultured. Although the derivation of these cells is unknown, they are morphologically similar to those derived from synovial explants and enzymatically dissociated synovial tissue. However, they are clearly different from those isolated and examined by McCachren et al. which were monocytoid in origin. According to Burmester et al. and Shiozawa and Tokuhisa, cultured synovial cells may be divided into three types. In type I, the cells possess monocyte antigens and morphologically are monocytoid or fibroblastic. Type II cells are mostly dendritic in morphology and lack monocyte antigens. Type III cells are fibroblastic appearing and lack monocyte markers. We suspect that the cells studied in this report are type III synovial cells; however, for clarity, we have chosen to refer to these cells as ASFC.

When cultured, the human ASFC express TM diffusely on the cell surface and throughout the cytoplasm, and the protein is biologically functional and identical in apparent molecular weight to that from HUVEC. Conclusive evidence of the potential for local synthesis of TM by these cells was provided by Northern analysis of RNA from the ASFC, which showed specific mRNA for the receptor that was identical to that from HUVEC. We cannot rule out the possibility that TM expression was induced during culture. However, the detection of TM in primary cultures, and the lack of change in expression during early passage, suggests that this was not an in vitro effect.

Although we have not directly ascertained the source of the biologically active, soluble form of TM in the joint, and have not tested chondrocytes or dendritic cells for expression of the receptor, we postulate that the ASFC make a significant contribution. The monocytoid synovial lining cells from the study of McCachren et al. had markedly diminished PC cofactor activity, and we recently reported that human circulating PMN have no biologic activity. However, we hypothesize that these cells may all, under the appropriate inflammatory conditions, contribute to the pool of synovial fluid TM. Studies are ongoing to evaluate TM expression by ASFC from patients with OA and RA as affected by mediators such as dibutyryl cAMP, tumor necrosis factor, and others that are commonly found in the inflamed joint to determine if there are significant differences in response that may explain why synovial fluid TM is not increased above plasma levels in OA.

Although the process of inflammation in RA is likely driven by a variety of interactions between cells, cell-surface receptors, growth factors, cytokines, and lymphokines, available evidence supports the critical role of proteases in the destructive process. Among others, the serine protease plasmin appears to play a major part. The synovial fluid of patients with RA and OA contains PA, which may be produced by synovial lining cells, chondrocytes, or microvascular endothelial cells, or could also be derived from the circulation. Plasmin, once formed from its precursor plasminogen, may directly degrade extracellular matrix of the joint, or alter-
natively activate otherwise inactive collagenase that then leads to rapid destruction of collagen-containing tissues.

Recent data indicate that the PC-TM anticoagulant mechanism may play an important role in regulation of the fibrinolytic system and plasmin generation. TM, when complexed with thrombin, supports the conversion of PC to its activated form, whereupon the newly formed serine protease not only suppresses further thrombin formation, but also is reported to enhance the fibrinolytic system by neutralizing PA inhibitor-1 (PAI-1). This inhibitor, which is present in synovial fluid, provides a major regulatory mechanism for the transformation of plasminogen to plasmin, and presumably therefore serves to protect the joint tissues from destruction. This would suggest that excess TM in the presence of adequate thrombin and PC might lead to further destructive processes within the acutely inflamed arthritic joint. However, in addition to several direct anticoagulant properties, recent in vitro and in vivo studies suggest that TM may also suppress fibrinolytic activity by accelerating thrombin’s inactivation of scu-PA. Therefore, the alternative scenario would be that TM directly interferes with plasmin generation, thereby protecting the joint from further deterioration.

Our observation that biologically functional TM is increased in the synovial fluid of patients with RA would therefore suggest that the molecule may play one or more of the following roles in the inflamed joint. (1) As stated above, TM may interfere with the fibrinolytic system, thereby suppressing extracellular joint destruction by plasmin or collagenase. (2) The known anti-inflammatory role of APC in the baboon model supports the hypothesis that TM may also be critical in regulating the inflammatory response in RA. By enhancing the activation of PC, TM may therefore act indirectly to limit damage due to inflammation. We have determined by Western immunoblotting that PC is present in synovial fluid from patients with OA and RA (unpublished observations). However, a quantitative analysis has not been performed, nor has there been an examination of synovial fluid for the presence and/or biologic significance of APC or the cofactor protein S. (3) Little is known as to the role of fibrin deposition in the highly vascular synovial tissue as the proliferating lesion develops into a destructive pannus; however, areas of thrombosis are commonly seen. Lack of TM in the microvasculature caused by either PMN-derived elastase proteolysis or cytokine-induced downregulation of the surface-bound receptor could lead to further fibrin clot formation. Mechanisms to enhance TM expression may therefore provide a means to attempt to constrain the overwhelming forces to form intravascular clots in the expanding pannus. (4) As previously indicated, APC also has been reported to augment fibrinolysis via the protease’s action to neutralize PAI-1. Consequently, the activation of PC by the thrombin-TM complex may actually lead to enhanced plasmin generation and joint destruction. (5) Finally, soluble TM may have other properties not yet elucidated. The putative multidomain structure of this molecule, composed partly of six EGF-like domains and a lectin-like amino-terminus region supports this hypothesis.

In summary, we have demonstrated that biologically functional TM is present in large quantities in the synovial fluid from joints of patients with RA. Our data indicate that there may be several sources of the protein, including local synthesis by synovial lining cells, adherent synovial fluid cells, and accumulated neutrophils. The precise role of TM in the inflamed joint is currently largely a matter of speculation; however, the fact that the protein is known to have multiple distinct structural domains suggests that it probably has several yet to be defined functions. With the use of well-defined in vivo models of RA, our observations hopefully will lead to further elucidation of the links between coagulation and inflammation.

ACKNOWLEDGMENT

We express our appreciation to Drs Robert Inman and Duncan Gordon for their help in recruiting study participants, to Dr Marie-Claire Boffa for providing the TM ELISA kits, and to all those patients who volunteered to partake in this study.

REFERENCES

42. Moore KL, Esmon CT, Esmon NL: Tumor necrosis factor leads to the internalization and degradation of thrombomodulin from the surface of bovine aortic endothelial cells in culture. Blood 73:159, 1989
46. Petersen T: The amino-terminal domain of thrombomodulin and pancreatic stone protein are homologous with lectins. FEBS Lett 231:51, 1988
Biologically active thrombomodulin is synthesized by adherent synovial fluid cells and is elevated in synovial fluid of patients with rheumatoid arthritis

EM Conway and B Nowakowski