RAPID COMMUNICATION

Thrombomodulin Expression by Human Blood Monocytes and by Human Synovial Tissue Lining Macrophages

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Thrombomodulin is an essential cofactor for the activation of the anticoagulant protein C by thrombin. We have identified the expression of thrombomodulin messenger RNA (mRNA) and protein in peripheral blood monocytes. While untreated monocytes expressed thrombomodulin mRNA by Northern blot analysis, lipopolysaccharide-treated cells had decreased mRNA expression. Thrombomodulin antigen was shown in the cytoplasm and on the surface of monocytes by immunohistochemical staining, and thrombomodulin activity was shown on the surface of intact monocytes. One population of synovial lining cells that normally expressed mononuclear phagocyte antigens also expressed thrombomodulin in both noninflamed osteoarthritic synovium and in inflamed rheumatoid arthritis synovium. However, these cells did not express another endothelial protein, von Willebrand factor. We conclude that both circulating and tissue mononuclear phagocytes are capable of expressing thrombomodulin.

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**MATERIALS AND METHODS**

**Reagents.** Radioisotopes were from Amersham Corporation (Arlington Heights, IL) or New England Nuclear Chemicals (Boston, MA). All molecular biology grade materials and tissue culture media were from Gibco-BRL (Gaithersburg, MD). All chemicals were from Sigma Chemical Co (St Louis, MO), unless otherwise specified.

**Cell isolation.** Human peripheral blood mononuclear cells were isolated by ficoll-hypaque density gradient centrifugation and monocytes were prepared as described. Monocytes were then deposited on glass microscope slides using a cytocentrifuge (Shandon Southern Instruments, Sewickly, PA) or cultured overnight.

**RNA isolation and analysis.** RNA was isolated from mononuclear cells in suspension as described. Northern blot analysis was performed as described using random hexamer primed labeled TM probe to measure thrombomodulin RNA levels. A probe detecting 28S ribosomal RNA (T24) was used to show comparable amounts of RNA in each lane.

**Synovial tissue.** Human synovial specimens were obtained as described. Specimens from joint trauma, osteoarthritis, and rheumatoid arthritis were available. Normal synovial specimens were obtained from the tissue bank of the Duke University Comprehensive Cancer Center. Tissues were snap frozen and stored over liquid nitrogen until use.

**Immunocytochemistry and immunohistology.** Immunohistochemical procedures, staining, and photography were performed as described. Immunohistochemistry was performed using an ABC-Elite kit (Vector Laboratories, Burlingame, CA). Adjacent sections of synovium were analyzed for the presence of von Willebrand factor (vWF), TM, and mononuclear phagocyte antigens. Rabbit anti-TM IgG was used in a dilution of 1:1,000 to detect human thrombomodulin. Antibody LeuM3 (anti-CD14) and antibody HAM56 (ENZO Diagnostics, New York, NY) were used to identify mononuclear phagocytes. Anti-vWF was from Dako Corporation (Carpenteria, CA). For the monoclonal antibodies, murine myeloma proteins of appropriate isotype were used as negative controls.

**TM assay.** Peripheral blood mononuclear cells were isolated as described above and washed three times in 4°C phosphate-buffered saline. TM activity was determined in resuspended cells as previously described.

**RESULTS**

**TM RNA and antigen expression.** Freshly isolated mononuclear cells were incubated in culture medium alone or in the presence of 1 μg/mL lipopolysaccharide for 4 hours. Northern blot analysis showed the presence of a 3.7-kb band corresponding to TM messenger RNA (mRNA) in untreated cells, while incubation with lipopolysaccharide...
decreased expression of TM mRNA (Fig 1). To determine if TM RNA expression was accompanied by protein expression, purified monocytes were analyzed by immunocytochemistry using rabbit antihuman TM IgG. Figure 2 shows the expression of TM antigen on the majority of purified peripheral blood monocytes. No staining was seen with nonimmune rabbit IgG. Expression was most pronounced on the cell surface, although some cytoplasmic staining was noted. The TM recognized by the antibody was functional. Determination of TM activity on the surface of monocytes showed activity at 19% of the activity on intact early passage human umbilical vein endothelial cells (5.73 ± 1.98 pmoles protein C activated/min-mg-mL v 30.7 ± 2.0 pmoles protein C activated/min-mg-mL; W.A.D. and S.S.M., submitted).

Analysis of TM in synovium. Serial sections of synovium from patients with osteoarthritis, rheumatoid arthritis, and trauma were analyzed for expression of TM antigen. All synovia tested expressed TM in endothelium and along areas lining the synovial space. Adjacent sections were stained for mononuclear phagocyte antigens (anti-CD14 or HAM56), vWF, and TM. Figure 3 shows the immunohistochemical staining in a representative sample of relatively noninflammatory osteoarthritic synovium. In Fig 3A, TM is located in blood vessel walls throughout the synovium, in a distribution similar to that for vWF (Fig 3C). TM is also found along the synovial lining areas, a location that is not reactive with vWF, but that is stained by anti-CD14 (Fig 3B). No staining was seen with control antibodies (Fig 3D).

Figure 4 shows the immunohistochemical localization of TM in a case of inflammatory rheumatoid synovitis. TM staining is seen predominantly in the synovial lining areas and in vessel walls. The vascular localization is similar to that for vWF (Fig 4C), although the staining is less intense. There is a mononuclear cell infiltrate in this specimen, and many of the infiltrating cells are stained by anti-CD14, as are cells along the synovial lining (Fig 4B). Although there appears to be an increase in TM staining deep within the synovium in Fig 4A, detailed observation has thus far failed to convincingly show the expression of TM in tissue.

Fig 1. Northern blot analysis of TM mRNA levels in human mononuclear cells. Cells were harvested for analysis 4 hours after mock treatment or addition of endotoxin (lipopolysaccharide) 4 μg/mL. The blot was hybridized to a probe for TM and rehybridized to a probe for 28S rRNA.

Fig 2. Immunocytochemical detection of TM antigen in human monocytes. Cells were incubated with control antibody (A) or with anti-TM antiserum (B and C). Original magnification: ×200 (A and B), ×400 (C). TM reactivity is shown on the periphery and in the cytoplasm of the monocytes.
macrophages in this area. Lymphoid cells in the synovium did not express TM.

Specimens from six rheumatoid arthritis patients and six osteoarthritis patients have been examined for TM expression. Samples from two patients with joint trauma and two from autopsy cases without joint abnormalities have been tested. TM was found in the synovial vessels and synovial lining cells in each specimen. The specimens varied greatly in their intensity of inflammation, as measured by a previously described inflammation score. No correlation was noted between inflammation intensity and either vascular or synovial lining TM expression using these semiquantitative techniques (data not shown).

DISCUSSION

Although TM has been considered to be primarily an endothelial protein functioning as part of the protein C pathway, it has also been found on mesothelial surfaces lining body cavities. Based on observations of TM inducibility in U937 monoblastic cells (W.A.D. and S.S.M., submitted) we investigated the expression of TM in human mononuclear phagocytes. TM was detected in circulating human monocytes and in synovial lining cells bearing surface antigens characteristic of mononuclear phagocytes.

In normal blood monocytes, both TM mRNA and protein were shown. The majority of the circulating monocytes...
expressed the protein, but it is not yet known how this population differs from those not expressing TM. The function of monocyte TM is unknown, but as the TM on the cell surface is active, and present at levels near that on endothelial cells, monocyte TM may play a role in preventing clotting. Mononuclear phagocytes play important roles in both inflammation and coagulation. During inflammation (especially immunologically induced inflammation such as delayed-type hypersensitivity reactions), there is extravascular fibrin formation. Mononuclear phagocytes produce a number of factors involved in both the formation and dissolution of fibrin, including tissue factor, prothrombinase complex, factor XIII and tissue transglutaminase, and urokinase. Monocyte TM might function as an inhibitor of the procoagulant activities of mononuclear phagocytes. The finding that lipopolysaccharide reduces monocyte TM mRNA expression suggests that inflammatory stimuli can alter the balance in monocytes between procoagulant and anticoagulant activities, much as in endothelial cells. Interleukin-1 has been shown to be elevated in rheumatoid synovitis, and is known to decrease TM expression in endothelial cells in culture. Our studies show that TM expression is still maintained in rheumatoid synovitis. Future studies will further define the regulation of mononuclear phagocyte TM and its role in coagulation and inflammation.

In a survey of the tissue distribution of TM, Boffa et al noted that the mesothelial lining of various body cavities, including synovium, expressed TM. They did not, however, determine the cell type expressing the protein. The synovial lining is a heterogeneous tissue containing several cell types, but is composed in part of cells expressing mononuclear phagocyte antigens even in the normal uninfamed joint. We show here that these monocyte/macrophage-like cells are the likely source of synovial lining TM expression in both noninflamed and inflamed synovium. TM was present in normal synovium (not shown), in osteoarthritis, and in inflammatory rheumatoid arthritis. TM was seen in endothelium of vessels deep within the synovium, in addition to the synovial lining cells. We have not convincingly shown the expression of TM in tissue macrophages deep within the synovium. Inflammatory synovitis is a heterogeneous group of disorders including systemic immune disorders such as rheumatoid arthritis and more localized, less inflammatory conditions such as osteoarthritis. Especially in rheumatoid synovitis (and to a lesser degree in osteoarthritis), there is evidence for both increased extravascular fibrin formation and dissolution. Given the recognized increase in overall coagulation and fibrinolytic activity in inflammatory synovitis, one might hypothesize a role for synovial lining TM in countering the procoagulant activity in rheumatoid synovitis. Normal synovial fluid lacks fibrinogen, but in the setting of joint trauma or inflammation local release of fibrinogen into the synovial fluid may occur. Subsequent activation of coagulation pathways as a result of the trauma or inflammation would result in fibrin formation within the synovial space, potentially leading to adhesion formation. Synovial lining TM may therefore play an important role in normal and inflamed joints by inhibiting the formation of adhesions between synovial surfaces.

TM is an anticoagulant protein that has potential usefulness in thrombotic disorders. The extravascular coagulation/fibrinolysis in inflammatory disorders such as rheumatoid arthritis presents another potential therapeutic target for TM. Anticoagulant therapy modifies immunologically mediated inflammation in experimental animals and in humans. Defibrination with ancrod has been shown to reduce experimental nephritis and lupus erythematosus in animals, and glomerulonephritis in humans. These disorders are all characterized by increased extravascular fibrin deposition. Warfarin and heparin have been shown to diminish delayed-type hypersensitivity reactions and experimental nephritis. These findings suggest that TM may play a role in limiting inflammatory reactions in addition to serving as an anticoagulant. Once the regulatory features of mononuclear phagocyte TM have been determined, means of selectively altering its expression will provide insights into its roles in coagulation and inflammation.

REFERENCES


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