Thrombomodulin levels in the plasma and joint fluid from patients with rheumatoid arthritis

Yukinobu ICHIKAWA, Masatoshi TAKAYA, Hiroaki SHIMIZU, Junko MORIUCHI, Mitsuaki UCHIYAMA, Kazuyuki MORITA, Yuichi HOSHINA and Terumi HORIKI

Department of Internal Medicine, Tokai University School of Medicine, Isehara, Kanagawa, 259-11, Japan

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The hemostatic mechanism is thought to contribute to the inflammatory process of rheumatoid arthritis (RA). Thrombomodulin (TM), an inhibitor of blood coagulation, is synthesized by various cells which are recognized in the inflammatory lesions of RA.

To elucidate a possible relation of TM with the process of RA, therefore, we measured soluble forms of TM in the plasma and joint fluid (JF) from RA patients by a recently developed sandwich enzyme immunoassay using monoclonal antibodies.

TM levels in the plasma and JF were not significantly elevated in RA patients, although TM levels in plasma were positively correlated with those in JF. The plasma TM levels were related to renal functions (serum creatinine levels), but the levels showed no connection with systemic inflammatory indices of RA such as erythrocyte sedimentation rates, serum C-reactive protein levels and Lansbury's activity index. In the JF, TM levels were not correlated with the numbers of neutrophils or monocytes/macrophages associated with articular inflammations.

Our results indicate that TM levels in the plasma and JF do not reflect systemic and articular inflammations of RA, and suggest that TM molecules in JF are mainly recruited from circulating TM.

(Key Words: thrombomodulin, joint fluid, rheumatoid arthritis)

INTRODUCTION

Thrombomodulin (TM) was originally described as a membrane-associated glycoprotein of the vascular endothelial cells (ECs). TM inhibits coagulation processes, and modulates fibrinolytic systems. Six and seven soluble forms of TM have been discovered in plasma and urine, respectively, by Ishii et al and their molecular weights range from 28 KDa to 105 KDa (5). The circulating TM molecules are considered as degradation products of tissue TM and their plasma levels might be used as a marker of EC damage. However, the exact sources of circulating TM are still obscure, and TM has recently been shown to be synthesized by various kinds of cells including neutrophils (PMNs) and synovial lining cells (2, 7). We previously reported that certain groups of patients with systemic lupus erythematosus (SLE) have increased circulating TM levels (10), but the TM levels have not been extensively examined in patients with other rheumatic diseases such as rheumatoid arthritis (RA). Chronic articular inflammations of RA are accompanied by proliferation of ECs and synovial cells, accumulation of inflammatory cells and EC injuries. In the inflammatory process, the hemostatic mechanism also seems to be involved (9), and elevated levels of soluble TM have recently been reported in the joint fluid (JF) from RA patients (8).

This is the second report which measured soluble forms of TM in the plasma and JF from RA patients by an enzyme immunoassay (EIA), but we further compared the TM levels with various clinical indices of inflammation to identify a possible association of soluble TM levels with systemic and/or articular inflammations of RA.

MATERIALS AND METHODS

1. Patients: Thirty-one patients with RA (9 males and 22 females, mean age: 51.7 ± 13.8
years old), who fulfilled the criteria for RA (1), were included in the present study.

Plasma was separated from heparinized peripheral blood in 22 of the RA patients. JF samples were aspirated from 17 RA patients (knee joints) as a procedure for treatment and were immediately transferred into heparinized glass tubes. The JF samples were diluted with an equal volume of phosphate buffered saline and centrifuged at 2,500 r.p.m. for 20 minutes at room temperature to eliminate cell components. Both plasma and supernatant of JF were preserved at -80°C until measurement. In 8 RA patients, we could simultaneously obtain plasma and JF samples.

Plasma collected from 8 healthy adults (6 males and 2 females, mean age: 32.3 ± 7.1 years old) were used as controls.

2. Determination of TM levels: Soluble forms of TM in the plasma and JF were measured by sandwich EIA (MGC-01-001, Mitsubishi Gas Chemical Company, Inc., Tokyo) as described previously (10). Briefly, each well of the microtiter plate was coated with 200 µl of monoclonal antibody (mAb) to human TM (TM mAb 20; 10 µg/ml). One hundred microliters of two horseradish peroxidase (HRP)-labeled mAbs to human TM (TM mAbs 2 and 11) and an equal volume of test sample were added to the well. After incubation for 2 hours and then washing, 200 µl of substrate solution containing 0.015% H2O2 and o-phenylenediamine (1.0 mg/ml) was placed in each well. After 15 minute-incubation, the reaction was terminated with 100 µl of 4N H2SO4 and optical absorbance was measured at 490 nm. Purified human placental TM was used to obtain a standard curve for TM levels.

3. Clinical parameters: Leukocyte counts of JF and their differentials (PMNs, lymphocytes and monocytes/macrophages) were determined by a routine laboratory method. Erythrocyte sedimentation rates (ESR), serum levels of C-reactive protein (CRP) and creatinine, and modified Lansbury's activity index (LAI) (6) were also determined at the time of blood- and/or JF-sampling.

For statistical evaluations, the mean values were analyzed by the Wilcoxon rank sum test or Wilcoxon matched-pairs signed-ranks test, and linear regression analysis was used to determine the correlation coefficients.

RESULTS

TM levels in plasma: Mean TM levels in plasma did not differ between RA patients (23.6 ± 10.0 ng/ml, n=22) and controls (20.7 ± 3.5 ng/ml, n=8, Figure 1). Five of the RA patients showed abnormal plasma TM levels (higher than the mean of controls ± 2 standard deviations), but three (two patients with renal amyloidosis) of them had increased levels of serum creatinine (1.5, 1.6, and 3.7 mg/dl, respectively). The plasma TM level in one patient with a slightly abnormal creatinine level (1.1 mg/dl) was within the normal range. Thus, plasma TM
levels were significantly higher in the patients with abnormal serum creatinine levels (38.3 ± 14.5 ng/ml, n=4) than in those with normal renal function (20.4 ± 5.0 ng/ml, n=18, p<0.05). In the 22 RA patients, plasma TM levels (X) were positively correlated with serum creatinine levels (Y; Y=−0.53+0.60X, r=0.855, p<0.001). However, plasma TM levels showed no relation with ESR (r=0.423, n=22), CRP levels (r=0.035, n=22) and LAI (r=0.047, n=18).

TM levels in the JF from 17 RA patients (23.1 ± 12.2 ng/ml) did not differ from the plasma TM levels in 22 RA patients. Three of the 5 JF samples with relatively high TM levels were obtained from RA patients with normal serum creatinine levels, but the TM levels in JF were not correlated with the numbers of leukocytes (r= 0.034, n=16), PMNs (r=0.053, n=16) or monocytes/macrophages (r=−0.267, n=16) in the JF. When eight paired samples were compared, the TM levels showed a positive correlation between JF (X) and plasma (Y; Y=0.75+1.14X, r=0.751, p<0.05). However, no significant difference was found in the mean TM levels between plasma (29.1 ± 14.1 ng/ml, n=8) and JF (24.9 ± 9.3 ng/ml, n=8). Furthermore, the TM levels in JF were low in 5 of the 8 patients when compared with the corresponding plasma levels.

**DISCUSSION**

TM on the ECs inactivates excess thrombin by forming a 1:1 complex with thrombin. The thrombin-TM complex further activates protein C which also inhibits coagulation processes. On the other hand, through neutralization of plasminogen activators (PAs) or PA-inhibitors, TM regulates the generation of plasmin which appears to be associated with articular destruction in RA (3, 9).

In addition to ECs, TM is now known to be synthesized by megakaryocytes, platelets, monocytes, PMNs, mesothelial cells, placental syncytiotrophoblasts, synovial lining cells and adherent cells in synovial fluid (2, 3, 7). Circulating TM, therefore, may have various origins other than EC injuries. Plasma TM levels have been found to be elevated in several diseases, including disseminated intravascular coagulation, thrombotic thrombocytopenic purpura, chronic renal failure, chronic myelogenous leukemia, and SLE (8, 10, 11), although their origins are not always clear. In addition, biological roles of soluble forms of TM are still unknown, but TM in plasma is known to be functionally active. Inflammatory cytokines such as interleukin-1 and tumor necrosis factor alpha, which are produced in inflammatory tissues of RA, are also known to down-regulate the expression of TM on the ECs (4). One recent report described elevated TM levels in the JF from 10 RA patients and suggested local synthesis of TM in the inflammatory lesions (3).

In contrast to previous reports (3), however, the present study showed that TM levels in the plasma and JF are not significantly elevated in RA patients. We also demonstrated that TM levels in both compartments were correlated with each other. We further compared plasma TM levels with clinical parameters of RA: the TM levels were not associated with systemic inflammatory indices of RA such as ESR, CRP levels and LAI, but they were closely related to serum creatinine levels. The correlation of circulating TM levels with renal functions was found in previous studies, including ours (10).

The EIA method employed in the present study can detect almost all TM molecules in plasma and urine (5). In addition, no significant differences in circulating TM levels determined by this method have been recognized by gender, or between plasma and serum, although the number of healthy adults used as controls in our study was relatively small and their gender ratio was different from that of RA patients. Indeed, the plasma TM levels were very close to serum TM levels of the healthy controls used in our previous study (10).

A double-antibody sandwich EIA using mAbs to human TM was also employed in a study by Conway and Nowakowski to quantify TM levels in plasma and JF (3). Their normal levels of plasma TM were similar to our results. All of their patients with RA had normal renal function, but they had significantly high TM levels in JF. We could not find any specific reasons for the discrepancies of TM levels in JF between the two results. However, inevitable technical differences such as the JF separation procedure and differences in patient populations may contribute to the difference. In agreement with their results, TM levels in JF
were not correlated with the numbers of PMNs in JF which indicate degrees of local inflammation.

Our study showed that TM levels in plasma and JF do not reflect systemic or local inflammations of RA. Our results also suggest that the TM molecules detected in JF are mainly derived from circulating TM (plasma TM) since plasma TM levels were higher than those in JF in most patients, and TM levels in both compartments showed good correlation. These considerations appear to be compatible with the evidence that molecular weights of TM in JF were identical to those in plasma (3).

REFERENCES
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