Tumor Necrosis Factor-α (TNF-α) Converting Enzyme Contributes to Production of TNF-α in Synovial Tissues from Patients with Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** Expression and function of tumor necrosis factor-α (TNF-α) converting enzyme (TACE) in synovia of patients with rheumatoid arthritis (RA) were examined to investigate posttranslational regulation of TNF-α production by TACE in RA.

Methods. Expression of TACE protein was evaluated by immunohistochemistry. Cytokines and soluble cytokine receptors were measured by ELISA. TACE mRNA was detected by RT-PCR. The enzymatic activity of TACE was measured using TACE-specific fluorogenic substrate.

Results. Expression of TACE at protein level in synovial tissue (ST) of patients with RA was significantly stronger than that of patients with osteoarthritis (OA). In RA, TACE was mainly expressed in CD68+ macrophage-like synovial cells. ST from 9 of 9 RA and 3 of 8 OA patients expressed TACE mRNA. RA ST cells possessed significantly higher TACE-like enzymatic activity than OA ST. A synthetic TACE inhibitor significantly reduced the release of TNF-α and p75 TNF receptor from RA ST cells.

Conclusion. TACE is an important regulator of the secretion of TNF-α from synovia of patients with RA. (J Rheumatol 2001;28:1756–63)

Key Indexing Terms:
RHEUMATOID ARTHRITIS
METALLOPROTEINASE
TUMOR NECROSIS FACTOR-α
SYNOVIUM

In the synovia of patients with rheumatoid arthritis (RA), tumor necrosis factor-α (TNF-α) is produced excessively and contributes to the immunopathogenesis of the disease[1,2]. In RA synovia, TNF-α induces the production of other proinflammatory cytokines and chemokines, such as interleukin-1 (IL-1), IL-6, IL-8, and monocyte chemoattractant protein-1 (MCP-1)[3,4]. TNF-α also enhances the production of matrix metalloproteinases (MMP)[5], the expression of adhesion molecules that are important for recruitment of leukocytes[6,7] and proliferation of synovial fibroblasts[8]. The significant relevance of TNF-α in RA has been shown by the encouraging results of clinical trials with anti-TNF-α antibodies or soluble TNF-α receptors[9-11]. Some immunological mechanisms for the excessive production of TNF-α have been reported. We proved that CD40 ligand (CD154) on activated synovial T cells stimulates production of TNF-α by macrophage-like synovial cells of patients with RA[12]. It has also been reported that IL-15 activated T cells can enhance the production of TNF-α by synovial cells via CD69 molecule[13].

The gene for TNF-α encodes a polypeptide with a molecular weight of 26 kDa that is inserted into the cell membrane[14-16]. This transmembrane TNF-α is cleaved at the Ala76–Val77 bond by a metalloproteinase referred to as TNF-α converting enzyme (TACE), resulting in the release of soluble TNF-α of 17 kDa[17,18]. Thus, TACE plays an essential role in posttranslational regulation of TNF-α expression. TACE is a member of the ADAM (a disintegrin and metalloproteinase) family of proteins that bears characteristics of both cell surface adhesion molecules and proteinases. Some of the ADAM family proteins, including TACE, are now considered to be responsible for the proteolytic processing of extracellular domain (ectodomain) of various cell surface molecules such as cytokines, cytokine receptors, adhesion molecules, and enzymes[19-23].

To understand the molecular mechanism of posttranslational regulation of TNF-α production in RA synovial tissue (ST), we focused on this molecule, TACE, and investigated its role in the immunopathogenesis of RA. We investigated the expression of TACE in ST of patients with RA at both the mRNA and protein levels. We also found RA ST cells possessed significantly higher TACE-like enzymatic activity than those from patients with osteoarthritis (OA).

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Finally, a synthetic TACE inhibitor significantly reduced the release of both TNF-α and p75 TNF receptor (TNF-RII) from RA ST cells. The results are discussed in connection with the implication of TACE in synovial inflammation in RA.

MATERIALS AND METHODS

Subjects. Subjects consisted of patients with RA (n = 17) and OA (n = 8). Diagnosis of RA was in accordance with the 1987 criteria established by the American College of Rheumatology[24]. OA was diagnosed according to clinical manifestations, laboratory data, and typical radiological findings. Throughout the study, the Declaration of Helsinki principles were followed.

Reagents. Anti-human TACE monoclonal antibody (Mab) and a synthetic, hydroxamic acid based TACE inhibitor (a derivative of Immunex compound 2[17,25], in which the naphthyl-alanine side chain is replaced by a tertbutyl group, referred to here as TACE inhibitor) were kindly provided by Immunex Corporation (Seattle, WA, USA). Recombinant human TNF-α (rhTNF-α) and rhIL-1ß were purchased from R&D Systems (Minneapolis, MN, USA). Control mouse IgG and anti-human CD68 Mab were purchased from Dako Japan (Tokyo, Japan). Anti-hTNF-α antibody, biotinylated anti-hTNF-α antibody, anti-hIL-6 antibody, and biotinylated anti-hIL-6 antibody were purchased from Pharmingen (San Diego, CA, USA). A fluorogenic substrate specific for TACE, N-methylanthranilic acid-Leu-Ala-Gln-Ala-Val-Arg-Ser-Lys-(2,4-dinitrophenyl)-D-Arg-D-Arg-NH_2, was kindly provided by Dr. K. Inokuma (Wakunaga Pharmaceutical Co. Ltd., Hiroshima, Japan). The sequence of the peptide was based on the cleavage site of transmembrane TNF-α by TACE[17,18].

Immunohistochemical staining for frozen sections. Frozen sections of ST were fixed in cold acetone and internal peroxidase activity was blocked by incubating in 0.03% H_2O_2-phosphate buffered saline (PBS) for 15 min at room temperature. TACE was detected by anti-human TACE Mab and the TSA-indirect kit (NEN Life Science Products, Boston, MA, USA) according to the manufacturer's instruction. CD68 antigen was detected by anti-human CD68 Mab and using a Vectastain ABC-alkaline phosphatase kit (Vector, Burlingame, CA, USA). For 2-color staining, frozen sections were initially stained for TACE and then stained for CD68. The degree of the expression of TACE and CD68 was scored blindly. The degree of TACE expression was scored as follows: none, 0; weakly positive, 1; moderately positive, 2; strongly positive, 3. The degree of CD68 expression was scored as follows: none, 0; positive, 1; strongly positive, 2. Synovial lining cells and sublining regions were separately scored and totaled for each patient. Hence total scores for TACE and CD68 ranged from 0 to 6 and 0 to 4, respectively.

Reverse transcription-polymerase chain reaction (RT-PCR). Total RNA was extracted using TriZol (Gibco BRL, Grand Island, NY, USA). One microgram of heat denatured total RNA was reverse transcribed with oligo-dT primer and Superscript II (Gibco BRL) in a volume of 20 μl. Two microliters of the cDNA were used as PCR templates and amplified by Taq DNA polymerase (Roche Diagnostics, Basel, Switzerland). PCR primers were as follows: TACE forward 5'-TTGGCACACCTTTTCACATAC-3'; TACE reverse 5'-CCTCATTCGGGGCACATTCTG-3'; ß-actin forward 5'-TACATGGCTGGGGTGTTGAA-3'; ß-actin reverse 5'-AAGAGAG-GCATCCTCACCCT-3'[26]. The lengths of the PCR products of TACE and ß-actin cDNA were 263 and 218 bp, respectively.

Preparation and culture of peripheral blood monocytes. Peripheral blood lymphocytes (PBL) were prepared by Ficoll-Conray density centrifugation (Lymphosepal I, Fujioka, Japan). CD14+ cells were separated from PBL by the MACS system using anti-human CD14 Mab coated beads and MS+ column (Miltenyi Biotec, Auburn, CA, USA) according to the manufacturer's instruction. The purity of CD14+ cells was > 98% by flow cytometry analysis. These CD14+ cells were used as peripheral blood monocytes. To investigate the effects of the TACE inhibitor, peripheral blood monocytes were cultured in the presence of varying concentrations of TACE inhibitor for 48 h. After centrifugation at 2000 rpm for 10 min at 4°C, supernatants were stored at –80°C until assayed for the production of cytokines and cytokine receptors.

Preparation and culture of synovial tissue cells. ST were obtained at the time of joint replacement or arthroplasty. ST cells were prepared as described[4,12]. In brief, the extensively washed ST were minced and then digested by DNase (800 units/ml; Sigma, St. Louis, MO, USA) and collagenase (130 units/ml; Sigma) for 1 h in a 37°C water bath with gentle agitation. The cells were collected by passing through stainless mesh and washed 5 times with RPMI 1640 (Nikken Kagaku, Tokyo, Japan)-10% fetal calf serum (Filtron Pty, Brooklyn, Australia). To investigate the effects of the TACE inhibitor on TNF-α production of ST cells, living cells (5×10^4) were cultured in RPMI 1640-10% FCS in 96 well flat-bottom plates (Costar, Cambridge, MA, USA) with or without various concentrations of TACE inhibitor for 12 h. Then the culture supernatants were aspirated and replaced by fresh RPMI 1640-10% FCS containing the same concentration of TACE inhibitor, and incubated another 12 h to collect supernatants. After centrifugation at 2000 rpm for 10 min at 4°C, supernatants were stored at –80°C until assayed for the production of cytokines and cytokine receptors.

Measurement of TACE-like enzymatic activity in cell lysates. TACE-like enzymatic activity was measured by a modified method[18]. Briefly, 5 million synovial tissue cells were washed twice with buffer A (10 mM sodium phosphate buffer, pH 7.4, 1 mM $MgCl_2$, 30 mM NaCl, 0.02% NaN_3, 0.2 μg/ml DNase, 1 μM phenylmethylsulfonylfluoride), suspended in buffer A in a concentration of 1×10^6/ml, and homogenized with a Teflon homogenizer (Ikemoto, Tokyo, Japan). The homogenate was centrifuged at 12,000 rpm for 20 min at 4°C and washed twice with buffer A. After centrifugation at 12,000 rpm for 20 min at 4°C, 1 ml of buffer B (10 mM Tris-HCl, pH 8.0, 1 mM $MgCl_2$, 30 mM NaCl, 1% octylglucoside) was added to the pellet and rotated 1 h at 4°C. The lysates were collected by centrifugation at 12,000 rpm for 20 min at 4°C and stored at –80°C until assayed. ST cell lysate was incubated with 20 μM of TACE-specific fluorogenic substrate for varying periods at 37°C in 50 μl of buffer D (50 mM Tris-HCl, pH7.4, 5 mM $CaCl_2$, 0.002% NaN_3, 0.002% Brij 35). Fluorescence intensity was measured with an FL500 fluorescence plate reader (Bio-Tek Instruments, Winooski, VT, USA) using an excitation filter for 340 nm and an emission filter for 430 nm.

ELISA for TNF-α and IL-6. ELISA procedures for TNF-α and IL-6 were developed in our laboratory[4,12]. Microtiter plate wells (Costar) were coated with 50 μl of anti-human TNF-α antibody or anti-IL-6 antibody (1.0 μg/ml; Pharmingen) in monocarbonate buffer (100 mM $NaHCO_3$, pH 8.2) at 4°C overnight. After discarding the antibody, the plates were washed 4 times with PBS-Tween 20 (137 mM NaCl, 2.68 mM KCl, 7.04 mM NaH_2PO_4, 1.15 mM KH_2PO_4, 0.05% Tween 20). Subsequent additions of the reagents with 4 PBS-Tween 20 washes were as follows: (1) 150 μl of PBS-10% FCS (room temperature, 2 h); (2) 100 μl of rhTNF-α, rhIL-6 (R&D Systems), or samples diluted in RPMI 1640-10% FCS (4°C overnight); (3) 100 μl of biotinylated anti-human TNF-α antibody or anti-human IL-6 antibody (0.5 μg/ml; Pharmingen) diluted in PBS-10% FCS (room temperature, 45 min); (4) 100 μl of avidin-peroxidase diluted in PBS-10% FCS (2.5 mg/ml; Sigma), room temperature, 30 min; (5) 100 μl of ABTS peroxidase substrate mixture (Kirkegaard & Perry Laboratories, Gaithersburg, MD, USA). The absorbance at 414 nm was measured by an ELISA plate reader (Immuno Mini NJ-2300, Nippon Intermed, Tokyo, Japan). The detection limit of these assay systems was 7.8 pg/ml for both cytokines.

ELISA for soluble p60 TNF receptor (sTNF-RI) and soluble p75 TNF receptor (sTNF-RII). Soluble TNF-RI and sTNF-RII were measured by ELISA kit (Immunotech, Marseille, France) according to the manufacturer's instructions.

Statistical analysis. Mann-Whitney U test was used for comparison of expression scores by immunohistochemistry and TACE-like enzymatic activity. Spearman rank correlation coefficient was used for analysis of correlation among the expression scores. Student's t test was used for the

comparison of production of cytokines and cytokine receptors. Differences were considered significant if $p \leq 0.05$.

RESULTS

Localization of TACE in synovial tissues by immunohistochemistry. We initially examined the expression of TACE in ST from patients with RA or OA by immunohistochemistry. TACE was strongly expressed in lining cells as well as in sublining regions of RA ST (Figure 1A, small arrows). In the sublining region of RA ST, TACE was also expressed in the infiltrating cells around microvessels (Figure 1A, large arrows). TACE was found by double staining to be colocalized with CD68 antigen, suggesting that activated macrophages or macrophage-like synovial cells express TACE (Figure 1B, asterisk). In contrast, ST from patients with OA showed weak or marginal expression of TACE (Figure 1C). "Expression scores" for TACE and CD68 antigens were quantified in each RA and OA ST (Figure 2A). Expression scores of these molecules in RA ST were significantly higher than in OA ST (Figure 2A; $p < 0.05$ for both molecules, Mann-Whitney U test). We found significant correlation between expression scores of TACE and CD68 antigen (Figure 2B; Spearman rank correlation coefficient), which is consistent with the result of double staining with anti-TACE and anti-CD68 antibodies (Figure 1B).

Expression of TACE mRNA in synovial tissues. Total RNA was extracted from ST of 9 patients with RA and 8 with OA, and the expression of TACE and ß-actin mRNA was determined by RT-PCR as described in Materials and Methods (Figure 3). TACE mRNA was detected in ST from all RA patients and 3 of 8 OA patients. ß-actin mRNA was detected in all samples.

TACE-like enzymatic activity in RA synovial tissues. We next compared TACE-like enzymatic activity of ST cells from RA and OA patients. TACE-specific fluorogenic substrate was incubated with ST cell lysate from a patient with RA at various protein concentrations for 2 h (Figure 4A) or at 25 μg/ml for different lengths of time (Figure 4B). ST cell lysate from a patient with RA cleaved TACE-specific fluorogenic substrate in dose and time dependent fashion (Figures 4A, 4B). We chose a 2 h incubation period and 25 μg/ml of protein concentration for the following experiments. To examine the specificity of TACE-like enzymatic activity, RA ST cell lysate was incubated with TACE-specific fluorogenic substrate in the presence or absence of the TACE inhibitor. The TACE inhibitor reduced the cleavage of TACE-specific fluorogenic substrate in a dose dependent fashion (Figure 4C). The remaining cleavage activity of RA ST cell lysate for TACE-specific fluorogenic substrate in the presence of 10^{-4} M of the TACE inhibitor may indicate the involvement of other protease than TACE in this assay. We prepared ST cell lysates from 5 RA and 3 OA patients and measured TACE-like enzymatic activity using this assay system. ST cell lysate from RA patients possessed significantly higher TACE-like enzymatic activity than that from OA patients (Figure 4D).

Function of TACE in RA synovial tissue. We next investigated the function of TACE in RA ST by using the TACE inhibitor, which has been reported to specifically inhibit the

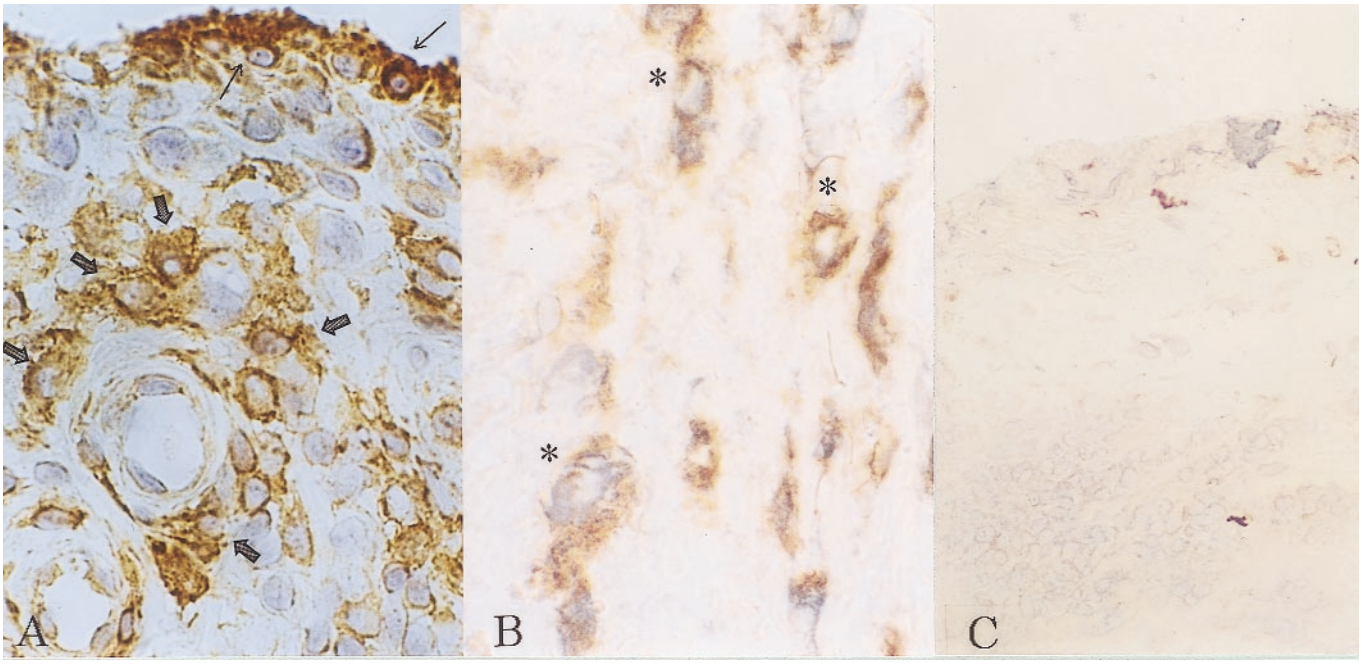

Figure 1. Immunohistochemical staining of TACE in synovial tissue (ST). ST from patients with RA (A, B) and OA (C) were stained with anti-TACE Mab. RA ST was double stained with anti-TACE Mab (brown) and anti-CD68 Mab (purple) (B). In RA, TACE was expressed in lining cells (thin arrows) and the sublining region, including the cells around microvessels (thick arrows) (A). TACE was mainly expressed in CD68+ cells (*, B). Original magnification: A, B 400×, C 200×.

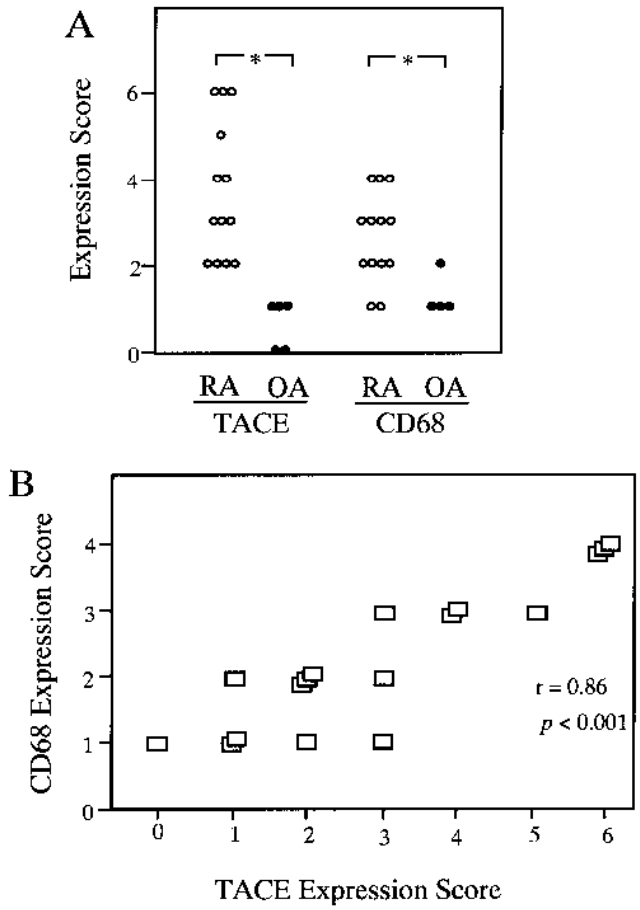

Figure 2. Expression scores of TACE and CD68 in synovial tissues from RA and OA patients. A: TACE and CD68 scores were determined as described in Materials and Methods. The scores were compared between RA and OA; *p < 0.05, Mann-Whitney U test. B: Correlation between TACE and CD68 scores of patients with RA (n = 13) and OA (n = 4). There was significant correlation between these scores (p < 0.001, Spearman rank correlation coefficient).

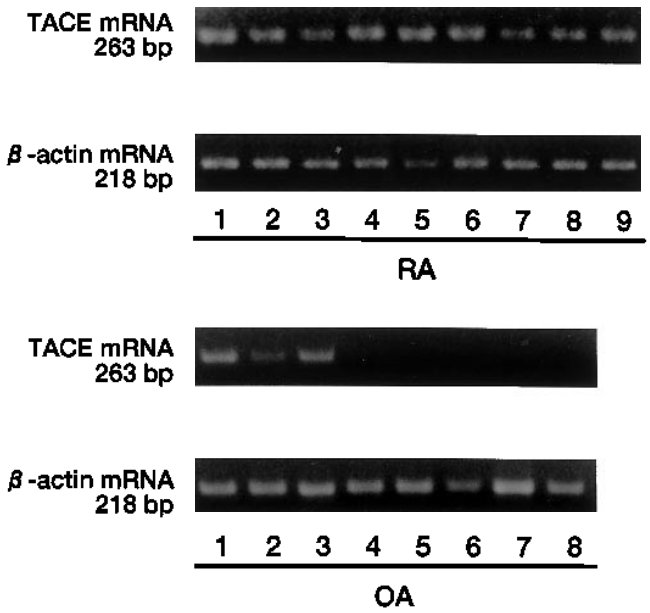

Figure 3. Expression of TACE mRNA in synovial tissue (ST) from patients with RA and OA. Total RNA was extracted from ST, and TACE and ß-actin mRNA were amplified by RT-PCR. TACE mRNA was detected in ST from all RA patients and 3 of 8 OA patients. ß-actin mRNA was detected in all samples.

enzymatic activity of TACE[17,25]. We initially confirmed the effects of the TACE inhibitor on TNF-α secretion by peripheral blood monocytes from healthy controls. TACE inhibitor significantly inhibited secretion of TNF-α, but not of IL-6 from normal peripheral blood monocytes, in a dose dependent fashion. TACE has been shown to be involved in the ectodomain processing not only of TNF-α but also of other molecules such as TNF-RII and transforming growth factor-α (TGF-α)[20]. Since sTNF-RII is an important immunomodulator in RA, we measured this molecule in the supernatants of normal peripheral blood monocytes. As expected, the TACE inhibitor significantly inhibited the secretion of sTNF-RII in a dose dependent fashion (Figure 5A). We also measured sTNF-RI in the same culture supernatants, but the concentration was below the sensitivity of the ELISA system (data not shown). These data indicate the specificity of the TACE inhibitor in *in vitro* cultures and the presence of TACE activity in unstimulated peripheral blood monocytes. We prepared cell lysate from unstimulated peripheral blood monocytes and measured TACE-like enzymatic activity. The cell lysate from unstimulated normal peripheral blood monocytes contained a significant amount of TACE-like enzymatic activity (2331 ± 302, mean ± SD of fluorescence intensity, n = 3). Similar results were obtained when peripheral blood monocytes from patients with RA were treated with TACE inhibitor (data not shown). We then examined the effects of the TACE inhibitor on freshly isolated ST cells from patients with RA. The TACE inhibitor significantly inhibited spontaneous secretion by ST cells not only of TNF-α (Figure 5B) but also of sTNF-RII (Figure 5C) in a dose dependent fashion. These data as well as the augmented TACE-like enzymatic activity in RA ST cells strongly indicate that TACE or TACE-like metalloproteinase expressed in ST contributes to the ectodomain processing of both TNF-α and TNF-RII. We also tried to measure cell associated TNF-α in the lysates of synovial tissue cells, but the concentration was below the sensitivity of our ELISA system (data not shown).

DISCUSSION

TACE has been cloned as a metalloproteinase that specifically cleaves transmembrane TNF-α[11,12]. T cells homologous for a targeted mutation in TACE that deletes the Zn^+ binding domain ($tace^{\Delta Zn/DZn}$) showed 80–90% reduction in soluble TNF-α release and increase in transmembrane TNF-α expression[11]. It has been reported that soluble TNF-α as

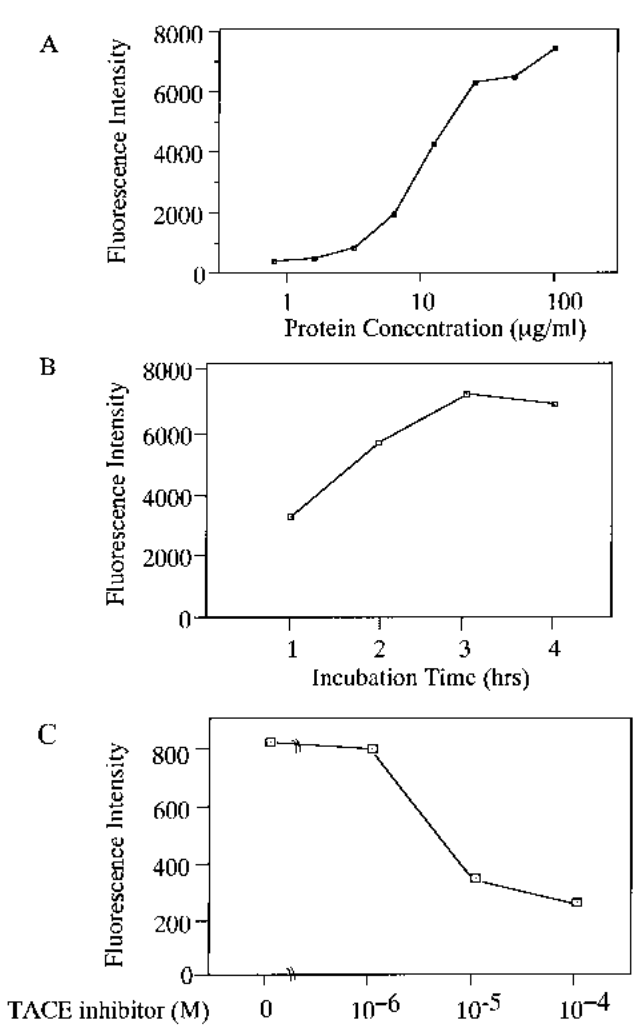

Figure 4. TACE-like enzymatic activity in synovial tissue cell lysate. A and B: TACE-specific fluorogenic substrate (20 μM) was incubated with RA synovial tissue cell lysate at various protein concentrations for 2 h (A) or at 25 μg/ml for different lengths of time (B) in 50 μl of buffer D. As a control, TACE-specific enzymatic substrate was incubated in buffer D without cell lysate. Fluorescence intensity of the control reaction was subtracted from that of each sample. The synovial tissue cell lysate cleaved TACE-specific fluorogenic substrate in dose and time dependent fashion. Results are expressed as mean values of duplicate assays. C: TACE-specific fluorogenic substrate (20 μM) was incubated with RA synovial tissue cell lysate (25 μg/ml) in the presence of various concentrations of TACE inhibitor for 2 h. TACE inhibitor reduced the cleavage of TACE-specific fluorogenic substrate in a dose dependent fashion. Results are expressed as mean values of duplicate assays. D: TACE-specific fluorogenic substrate (20 μM) was incubated with synovial tissue cell lysates (25 μg/ml) from 9 RA and 3 OA patients for 2 h to measure TACE-like enzymatic activity. All samples were measured on the same plate. RA synovial tissue cell lysate possessed significantly higher TACE-like enzymatic activity than OA lysate. Results are expressed as mean values of duplicate assays. *$p < 0.05$, Mann-Whitney U test.

well as transmembrane TNF-α can bind to both types of TNF receptors[8]. Several reports indicate, however, that their biological function may differ[27,28]. Recently, Grell, *et al*[28] reported that TNF-RII can be more strongly stimulated by transmembrane TNF-α than by soluble TNF-α. Since transmembrane TNF-α can also stimulate TNF-RI, the resulting cooperativity of both receptors leads to much stronger cellular responses than those achievable with soluble TNF-α alone. Moreover, the signal through TNF-RII can switch the cellular response pattern to TNF-α, such as rendering resistant tumor cells sensitive to TNF mediated cytotoxicity. Thus, ectodomain processing of TNF-α does have some effects on the quality and quantity of TNF-α signaling. Peschon, *et al*[20] reported that the ectodomain shedding by TACE plays an essential role during mouse development and that TACE cleaves not only TNF-α but also transforming growth factor-α, TNF-RII, and L-selectin. The ability of TACE to cleave molecules other than TNF-α is conserved in humans, because in this study we were able to show that the TACE inhibitor reduced the shedding of not only TNF-α but also TNF-RII from both peripheral blood monocytes and synovial tissue cells. Taking our data and the enzymatic specificity of TACE into account, it is obvious that TACE plays a crucial role in the excessive production of TNF-α of ST cells in RA. TACE has also been reported to cleave IL-6 receptor (IL-6R) gp80[23] and TNF related activation induced cytokine/receptor activator of nuclear factor-κB ligand (TRANCE/RANKL)[21]. Elevated levels of soluble IL-6R gp80 in RA synovial fluid[29,30] would be related to the expression of TACE in synovial tissue. It is intriguing that soluble TRANCE/RANKL, which is released by TACE or TACE-like metalloproteinase, has potent dendritic cell survival and osteoclastogenic activity[21]. Since the TRANCE/RANKL-TRANCE receptor/RANK system plays a central role in osteoclastogenesis in the joints of patients with RA[31,32], TACE or TACE-like metalloproteinase may be an important regulator of osteoclastogenesis through the release of TRANCE/RANKL as a soluble cytokine.

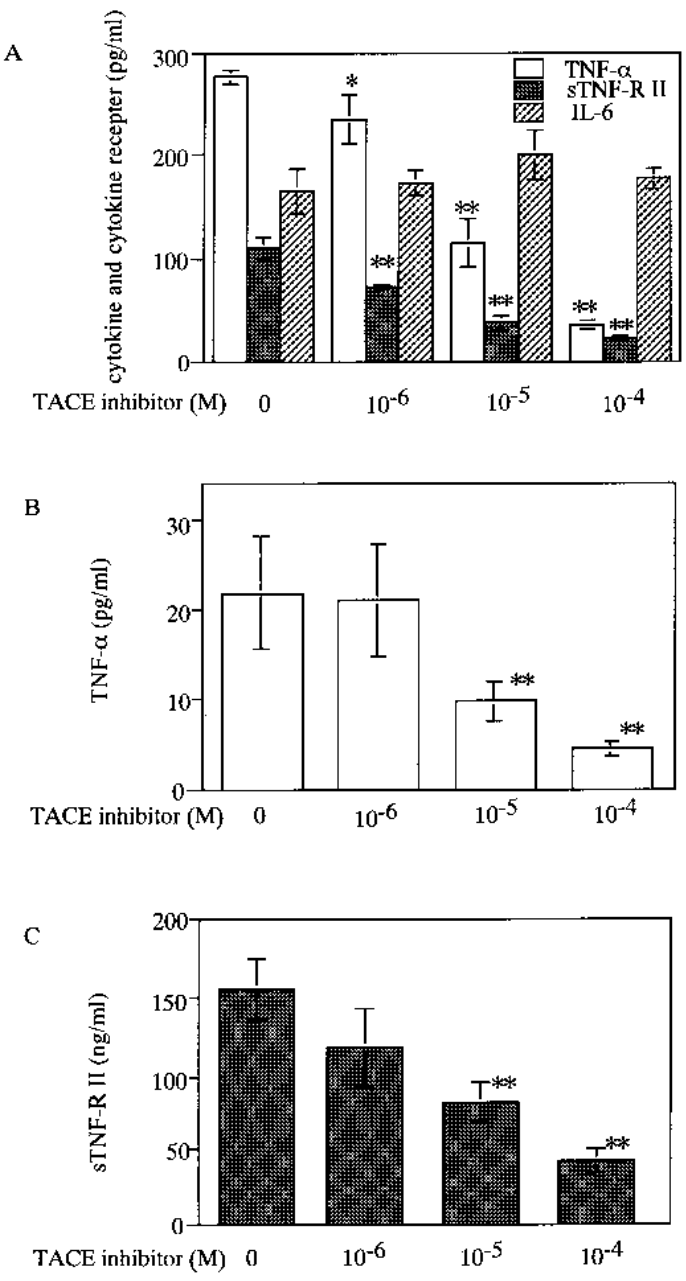

Figure 5. Effects of TACE inhibitor on normal peripheral blood monocytes and synovial tissue cells from a patient with RA. A: Normal peripheral blood monocytes were cultured in the presence of various concentrations of TACE inhibitor as described in Materials and Methods. TNF-α, sTNF-RII, and IL-6 in culture supernatants were measured by ELISA. TACE inhibitor significantly reduced secretion of TNF-α and sTNF-RII but not IL-6 by peripheral blood monocytes. Peripheral blood monocytes from 2 other healthy controls and 3 RA patients showed similar results. B, C: Synovial tissue cells were cultured in the presence of various concentrations of TACE inhibitor. TNF-α (B) and sTNF-RII (C) in the culture supernatants were measured by ELISA. TACE inhibitor significantly reduced secretion of TNF-α and sTNF-RII by synovial tissue cells. Experiments with synovial tissue cells from 2 other RA patients showed similar results. *$p < 0.05$, **$p < 0.01$, Student t test.

Regarding therapeutic values of TACE-specific inhibitor for RA, however, there are 2 major reasons that TACE-specific inhibitor may not ameliorate synovial inflammation of RA: (1) it increases the expression of transmembrane TNF-α in ST cells, which will induce stronger cellular responses[28]; and (2) it inhibits the secretion of sTNF-RII, the negative regulator of TNF-α signaling[33]. This possibility is supported by the report of Williams, *et al*[34] describing the effect of BB-2275, a broad spectrum MMP inhibitor, on RA ST cells. Although the addition of BB-2275 to the culture of RA ST cells reduced the levels of soluble TNF-α, sTNF-RI, and sTNF-RII, BB-2275 did not inhibit the production of IL-1ß, IL-6, and IL-8, which had been shown to be TNF-α dependent[2-4]. To the contrary, TACE-specific inhibitor may slow joint destruction by preventing the release of TRANCE/RANKL. The therapeutic value of TACE inhibitor for RA will be clarified, at least in part, by treatment of experimental arthritides with various kinds of TACE-specific inhibitors.

Recently, transgenic mice with transmembrane TNF-α have been reported[35,36]. These mice were found to develop chronic arthritis at 3 to 4 weeks with 100% incidence, which is similar to transgenic mice expressing wild-type TNF-α genes. These data indicate that overexpressed transmembrane TNF-α is sufficient to induce arthritis in transgenic mice. However, transgenic mice with transmembrane TNF-α did not show severe systemic TNF mediated pathology characterized by cachexia and by ischemic and extensive tissue necrosis, indicating that soluble TNF-α is important for these pathologic processes[35]. As far as we know, there is no report of transgenic mice in which the cleaving site of TNF-α by TACE is mutated with no modification of other portions of TNF-α gene so that transmembrane TNF-α alone, but not soluble TNF-α, can be induced by various inflammatory stimuli. Such transgenic mice would be useful for studying the significance of ectodomain processing of TNF-α in an inflammatory disease model such as collagen induced arthritis.

We compared the expression of TACE in ST as well as TACE-like enzymatic activity of ST cells between RA and OA. The expression of TACE and TACE-like enzymatic activity was significantly higher in patients with RA. TACE is produced as a proenzyme and the N-terminal prodomain is removed by proteolysis to generate the active enzyme[17,18]. Doedens and Black[37] reported that only the processed form of TACE was detected on the surface of THP-1 cells and that basal level of TACE activity may be sufficient for the release of TNF-α from lipopolysaccharide stimulated cells. Therefore, the augmented expression of TACE protein in RA ST could explain the significantly higher TACE-like enzymatic activity of RA synovial cells. Patel, *et al* reported the expression of TACE in cartilage from patients with RA and OA, on which spontaneous production of TNF-α may depend[38]. Although TACE-like enzymatic activity of

arthritis cartilage has not been compared between RA and OA, their data indicated that RA cartilage expressed TACE mRNA more strongly than OA cartilage[38]. Hence, TACE would be involved in TNF-α production of both synovium and cartilage in RA joints.

There have been few reports about the regulation of ectodomain shedding. PMA is a potent inducer of shedding of various molecules including TNF-α and TNF-RII. Phorbol 12-myristate 13-acetate rapidly induces ectodomain shedding of these molecules and then decreases the expression of active TACE on the cell surface, indicating negative feedback control for shedding[37]. Another important regulator of ectodomain shedding is nitric oxide (NO). It has been shown that NO activates TACE by nitrosation of inhibitory motif of the TACE prodomain and enhances the release of TNF-α and TNF-RII[39]. Since increased levels of NO have been reported in RA synovial fluids, NO could be relevant to the augmented TACE activity in RA ST[40].

We observed the augmented expression of TACE in RA synovial tissue and its contribution to the excessive release of TNF-α and sTNF-RII by RA synovial tissue. Since TACE is responsible for ectodomain shedding of both proinflammatory and antiinflammatory molecules, it serves as an important regulator of synovial inflammation in RA.

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