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A Peptide From Thrombospondin 1 Modulates Experimental Erosive Arthritis by Regulating Connective Tissue Growth Factor

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Objective. **Rheumatoid arthritis (RA) is a chronic inflammatory disease associated with leukocyte adhesion to and extravasation through vascular endothelium into synovial tissue. Recent evidence indicates that the thrombospondin 1 gene is up-regulated in patients with RA. We have identified a region within the TSP-1 type 3 repeats that inhibits human neutrophil elastase (HNE) and binds to human neutrophils. The present study was undertaken to investigate the therapeutic benefit of this TSP-1–derived peptide sequence and its effect on connective tissue growth factor (CTGF), a protein involved in fibrotic disorders and in neovascularization, which is a hallmark of RA.**

Methods. **CTGF gene and protein expression, as well as protein levels of CTGF in the synovium, after treatment with the TSP-1–derived peptide were studied in the peptidoglycan–polysaccharide animal model of erosive arthritis.**

Results. **Peptide treatment prevented joint infiltration and inflammation and was associated with reduced circulating antigen levels of HNE and TSP-1. Additionally, CTGF was up-regulated in this experimental model of RA. Treatment with the TSP-1–derived peptide was associated with down-regulation of the message and protein levels of CTGF. Immunofluorescence studies showed that the mean area fraction of CTGF immunoreactivity in the peptide-treated group of animals was significantly less than that in the untreated group.**

Conclusion. **These results document a role for TSP-1 in regulating CTGF gene and protein expression in synovial tissue, suggesting a link with the disease course in this model of RA. This TSP-1–derived synthetic peptide may represent an important template for drug development in RA and other inflammatory conditions associated with neutrophil activation.**

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease characterized by leukocyte infiltration, neovascularization, articular cartilage destruction, and synovial membrane inflammation associated with pain and loss of joint function. Traditional paradigms for RA have implicated a variety of mechanisms that contribute to the initiation and perpetuation of synovial inflammation, including persistence of cytokine networks, production of proinflammatory molecules (1), and activation of T cells (2).

There is a large body of scientific evidence implicating thrombospondin 1 (TSP-1) in the pathophysiology of RA; however, its mechanism(s) of action remains obscure. Koch et al implanted human TSP-1–containing pellets into the ankle of Lewis rats with adjuvant-induced arthritis (AIA) and demonstrated that this treatment augmented the severity of the disease (3). TSP-1 is a globular homotrimer comprising a small N-terminal globular domain that binds heparin, followed by the type 1, 2, and 3 repeats, which are attached to a

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large terminal globular domain. It is capable of interacting with other ligands, including plasminogen, fibrinogen, fibronectin (4), and coagulation factor V (FV) (5). We have shown that binding of TSP-1 to FV on the surface of activated polymorphonuclear cells (PMNs) is associated with increased generation of activated coagulation factor X (FXa) and thrombin and that these reactions are triggered by human neutrophil elastase (HNE) expressed on the surface of the activated PMNs (5).

Studies have shown that thrombin, FXa (6), and FVIIa–tissue factor complex (7) have a strong effect on migration of cultured smooth muscle cells. There is evidence that thrombin and FXa can stimulate connective tissue growth factor (CTGF) messenger RNA (mRNA) levels in fibroblasts (an immediate early gene response) in vitro (8). CTGF is a novel, potent, cysteine-rich heparin-binding growth factor, originally isolated from human umbilical vein endothelial cells (8), that is also highly expressed by fibroblasts (9,10). It has been implicated in more complex biologic processes, including angiogenesis and embryonic development. CTGF acts as an angiogenic inducer in vivo (10). Angiogenesis is one of the earliest histopathologic findings in RA and appears to be necessary for pannus development (11).

TSP-1 contains amino acid sequences within its type 3 repeats that protect the molecule from degradation by neutrophil elastase (12). Purified TSP-1 binds to unactivated as well as activated neutrophils, which induces their chemotaxis. There are 2 separate and distinct regions on the TSP-1 molecule for binding PMNs. We have delineated the amino acid sequence of these 2 binding regions (13). The amino acid sequence for binding to HNE was found to be contiguous to an amino acid sequence responsible for interacting with the putative receptor for the TSP-1 molecule on PMNs (13). These characteristics of TSP-1 and CTGF prompted us to investigate the ability of human monocytes and neutrophils to synthesize CTGF and the therapeutic effect of a TSP-1–derived synthetic peptide in an experimental animal model of erosive arthritis. Our results suggest that CTGF is, at least in part, responsible for the joint swelling observed in this animal model.

MATERIALS AND METHODS

Materials. Peptidoglycan–polysaccharide (PG-PS 10S) was obtained from Lee Laboratories (Grayson, GA). TSP-1 type 3 repeats–derived peptide D793–P824, as well as a scrambled sequence, were synthesized and provided in a sterile injectable solution by Commonwealth Biotechnologies (Richmond, VA). PMN separation medium for isolation of human neutrophils and monocytes was obtained from Robbins Scientific (Sunnyvale, CA). Hanks' balanced salt solution and TRIzol reagent were obtained from Life Technologies (Grand Island, NY). The Pro Star first-strand reverse transcriptase–polymerase chain reaction (RT-PCR) kit was obtained from Stratagene (La Jolla, CA), the PMN elastase kit was obtained from EM Science (Gibbstown, NJ), and the TSP-1 enzyme-linked immunosorbent assay (ELISA) kit was obtained from Chemicon (Temecula, CA). Anti-CTGF and anti–β-actin antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA) and Abcam (Cambridge, MA). Cy3-conjugated antibody was purchased from Jackson ImmunoResearch (West Grove, PA).

Characterization of the TSP-1 peptide. In previously published work (13), we have demonstrated that residues D793–P824 within the type 3 repeats of TSP-1 comprehend a binding region on TSP-1 which interacts with its putative receptor on the neutrophil surface. Binding of this TSP-1 peptide interferes with the signal transduction and migration induced by intact TSP-1 on human neutrophils.

RT-PCR. Specific primers for CTGF were synthesized by Sigma-Genosys (Woodland, TX). The CTGF primers used were 5′-CCC-TGA-CCC-AAC-TAT-GAT-GC-3′ (sense) and 5′-GGG-ACA-GTT-GTA-ATG-GCA-GG-3′ (antisense). Human neutrophils and monocytes from healthy donors were separated from whole blood using PMN Separation Media as recommended by the manufacturer. Total RNA was isolated from the cells using TRIzol reagent. Reverse transcription was performed with the Pro Star First Strand RT-PCR Kit, using either oligo(dT) primers or gene-specific primers. Complementary DNA was amplified by PCR at an annealing temperature of 58°C, for 30–35 cycles. The PCR products were separated on a 1% agarose gel containing ethidium bromide in Tris–acetate–EDTA buffer at 90 volts for 45–60 minutes at room temperature and visualized using ultraviolet light.

Experimental animal protocol. In the experimental model of erosive arthritis, injection of PG-PS into genetically susceptible Lewis rats is associated with features of inflammation, including an increase in joint diameter, neutrophil migration, and increased levels of circulating neutrophil elastase. Inflammatory changes induced by a single intraperitoneal (IP) injection of PG-PS are biphasic, with an acute phase during days 1–5 and a chronic phase starting at approximately days 10–12. This chronic phase is progressive and irreversible, with development of ankylosis or loss of joint function.

A total of 48 female inbred Lewis rats (Charles River, Raleigh, NC) were used. Animals were randomly divided into 4 groups. Group 1 (negative control; n = 8) received a single IP injection of saline. Group 2 (disease untreated positive controls; n = 12) received a single IP injection of PG-PS. Group 3 (disease treated; n = 12) received a single IP injection of PG-PS and daily intravenous (IV) injections of TSP-1–derived peptide at a concentration of 13.1 μM during the acute phase of the disease and IP injection of the peptide every other day during the chronic phase. Group 4 (disease treated scrambled peptide; n = 16) received a single IP injection of PG-PS and daily IV injections of scrambled TSP-1–derived peptide during the acute phase of the disease and IP injections of the scrambled peptide every other day during the chronic phase. For this study the peptide was produced in a sterile form for injection into the animals. The length of the experimental protocol was predetermined to be 16 days, representing the acute and chronic phases of this experimental model of erosive arthritis.

Body weight and rear extremity diameters were evaluated daily during the acute phase and every other day during the chronic phase. The severity of arthritis was graded based on ankle joint diameter, measured with a pediatric caliper as previously described (14). Blood was collected in sodium citrate by cardiac puncture at the beginning of the experimental protocol (day 0), on day 3, and at the end of the acute phase (day 5), for determination of hematologic parameters and for evaluating circulating levels of neutrophil elastase and TSP-1. The ankle joints were processed for histopathology and immunochemistry studies.

Determination of circulating antigen levels of neutrophil elastase and TSP-1 in plasma. Circulating antigen levels of neutrophil elastase and TSP-1 in the plasma of the rat blood was determined using a PMN elastase or TSP-1 ELISA kit according to the instructions of the manufacturers. In addition, differential white blood cell counts were determined using an automated method.

Histologic analysis. Tissues were harvested, fixed, decalcified (for bone), and embedded in paraffin. Tissues were cut into 5–10–μm sections and mounted into slides. Hematoxylin and eosin (H&E) and Safranin O staining were used for evaluation of morphologic changes of articular cartilage, including inflammatory infiltration, thickening of the synovial membrane, and bone erosion.

Immunofluorescence analysis. Isolated human monocytes and neutrophils were stained for localization of CTGF. Cells were incubated with donkey serum in phosphate buffered saline (PBS) (1:3), then incubated with goat anti-CTGF primary antibody (1:100) overnight at 4°C, followed by Cy3-conjugated anti-goat secondary antibody (1:2,000) for 1 hour at room temperature. Five-micrometer tissue sections were stained for CTGF as follows. Sections were deparaffinized, washed with PBS, quenched with 3% H_2O_2 in methanol, incubated in pepsin–0.01% HCl, blocked with 10% donkey serum, and incubated with chicken anti-CTGF primary antibody (6 μg/ml) overnight at 4°C followed by Cy3-conjugated anti-chicken secondary antibody (1.6 μg/ml) for 1 hour at room temperature. Control experiments were performed without primary antibody or with preadsorption with the peptide used to generate the primary antibody. The signal was visualized with a fluorescence microscope (Nikon, Melville, NY).

To determine changes in CTGF immunoreactive product, immunofluorescence-stained slides were analyzed using the video count area and the field mode features of a bioquantitation system (Bioquant TCW 98; R&M Biometrics, Nashville, TN) attached to a Nikon microscope. Video count area is the number of pixels in a field that meet a user-defined criterion (threshold) multiplied by the area of a pixel at the selected magnification (40× in our analyses). The mean area fraction of immunoreactive product in a 0.0768-mm^2 field was determined by dividing the video count area of pixels above background threshold by the video count area of the entire field. This determination was made at 4 locations for each slide analyzed. Group means and SEM were plotted.

RT-PCR and Western blot analysis of synovial tissue. Using TRIzol reagent, total RNA and protein were extracted from synovial tissue that had been snap-frozen by immersion in liquid nitrogen. Message for CTGF was detected as described above. G3PDH was used as a loading control for this analysis. Expression of CTGF and β-actin protein (control protein) was determined by Western blot analysis. Spot densitometries were used to evaluate the integrated density value ratios, using Alpha Imaging System software.

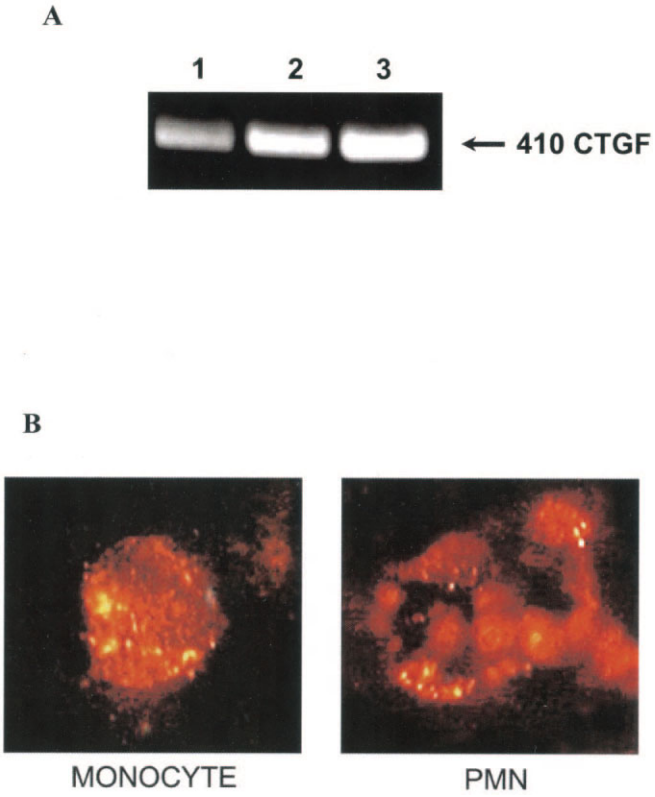

Figure 1. Presence of connective tissue growth factor (CTGF) mRNA and protein in human polymorphonuclear cells (PMNs) and monocytes. **A,** Results of reverse transcriptase–polymerase chain reaction studies for CTGF (410 bp). Lane 1, Rat long bone (positive control). Lane 2, Human monocytes. Lane 3, Human PMNs. **B,** Immunofluorescence staining of human monocyte and PMN, showing CTGF localization.

Statistical analysis. One-way analysis of variance and the Student-Keuls procedure were used to evaluate the difference between the negative controls and treated groups at each interval. *P* values less than 0.05 were considered significant.

RESULTS

Expression of CTGF mRNA and protein in PMNs and monocytes. By RT-PCR using gene-specific primers for CTGF, we found that human neutrophils, as well as human monocytes, expressed CTGF message (Figure 1A). RT-PCR was also performed using specific primers for β-actin and G3PDH, 2 common housekeeping genes, as controls. CTGF protein expression was verified by immunofluorescence staining of the cells using anti-CTGF primary antibody and Cy3-conjugated anti-IgG as the secondary antibody (Figure 1B). These

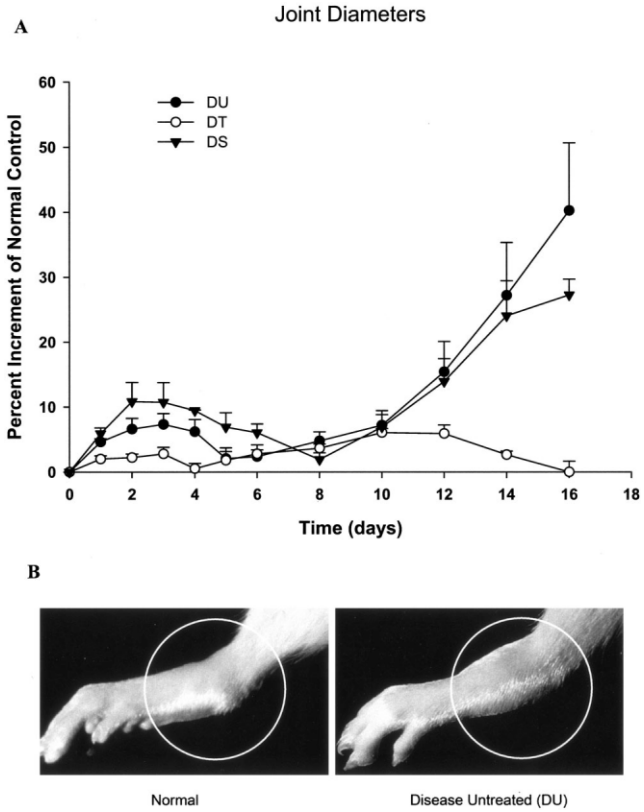

Figure 2. Percent increase in joint diameter after a single injection of peptidoglycan–polysaccharide, with and without synthetic thrombospondin 1 (TSP-1) peptide treatment. **A,** In contrast to findings in rats with erosive arthritic disease but without TSP-1 peptide treatment (disease untreated [DU]), the inflammatory response was either blunted or significantly attenuated in the peptide-treated disease group (DT). The disease course was not affected in rats with erosive disease treated with scrambled peptide (DS). Values are the mean and SEM. $P < 0.05$, peptide-treated versus disease untreated and scrambled peptide–treated groups; P not significant, scrambled peptide–treated group versus disease untreated group. **B,** Representative photographs showing the increase in joint diameter in a disease untreated rat compared with a normal rat.

results demonstrate that human neutrophils and monocytes are capable of synthesizing and expressing CTGF.

Prevention of joint swelling by TSP-1 peptide treatment. The percent increase in joint diameter among rats in the disease untreated group represents the natural course of disease during the acute phase in this experimental animal model. The acute phase reached a peak by day 3, with spontaneous reversal followed by the chronic phase, which peaked by day 16. TSP-1 peptide treatment was associated with blunting of the increase in joint diameter; the degree of increase in joint diameter was significantly lower in treated animals ($P < 0.05$) compared with the disease untreated group (Figure 2). In animals treated with a scrambled peptide, there was no significant difference in joint diameter compared with that in disease untreated animals, indicating that the peptide was responsible for preventing the increase in joint diameter (Figure 2).

Reduction of HNE and TSP-1 levels in plasma by TSP-1 peptide treatment. Peptide treatment was associated with a decrease in circulating levels of HNE and TSP-1, measured by ELISA ($P < 0.001$ and $P < 0.03$, respectively), compared with the disease untreated group (Figures 3A and B). Levels of circulating neutrophil elastase and TSP-1 in diseased animals treated with the peptide were not significantly different from those in the negative control group. A 26% increase in the neutrophil count was observed in the peptide-treated group compared with the negative control group. However, the increase in the disease untreated group compared with negative controls was 46%, which was significantly higher ($P < 0.05$) than that in the peptide-treated group. Thus,

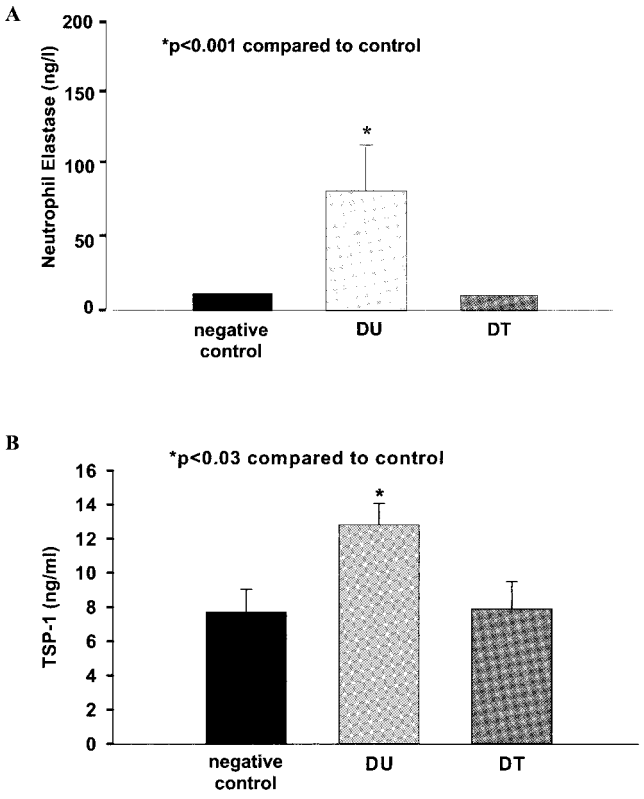

Figure 3. Effect of TSP-1–derived synthetic peptide on circulating levels of neutrophil elastase (**A**) and TSP-1 (**B**). Plasma levels of neutrophil elastase and TSP-1 were determined by enzyme-linked immunosorbent assay. Values are the mean and SEM. See Figure 2 for definitions.

peptide treatment significantly blunted the granulocytosis associated with this model of erosive arthritis.

Reduction of joint inflammation by TSP-1 peptide treatment. H&E staining indicated that TSP-1 peptide treatment was associated with amelioration of the thickening of the synovial lining, edema, and leukocyte infiltration in rat joints, compared with findings in the disease untreated group. There were no differences observed between the negative control and disease treated groups (Figure 4A). Safranin O staining for cartilage and mucin revealed bone erosion in the disease untreated group, whereas erosion was not observed in the disease treated and negative control groups (Figure 4B). Thus, peptide treatment modulated macroscopic, biochemical, and histochemical features in this experimental model of erosive arthritis.

Reduction of immunofluorescence localization of CTGF expression by TSP-1 peptide treatment. Inflammation and swelling of the paw are features of this model of erosive arthritis, as evidenced in Figure 4A. Immunofluorescence localization of CTGF showed high levels of CTGF expression in the paws of the disease untreated animals compared with the paws of the negative control and peptide-treated animals (Figure 4C). Immunohistochemical quantification indicated a decrease in the expression of CTGF in the paws of the peptide-treated animals compared with the disease untreated animals (Figure 4D). The mean ± SEM area of CTGF (calculated as described in Materials and Methods) was 1.64 ± 0.65 in the disease treated group, 7.56 ± 1.68 in the disease untreated group, and 1.90 ± 0.40 in the negative control group (Figure 4D). Peptide treatment was associated with significantly reduced CTGF expression ($P < 0.05$) compared with that in the disease untreated group. These results show that CTGF expression correlates with the severity of erosive arthritis, and its expression is modulated by TSP-1 peptide treatment.

Reduction of CTGF mRNA and protein expression by TSP-1 peptide treatment. Messenger RNA and protein were extracted from the ankles of negative control rats, disease untreated rats, and disease TSP-1 peptide–treated rats. By PCR with gene-specific primers for CTGF, we found that there was a 65% reduction in CTGF mRNA expression in the disease treated rats compared with the disease untreated rats. Integrated densitometry value ratios were used to compare the expression of CTGF mRNA and G3PDH mRNA (Figure 5A). Protein expression was determined by Western blot analysis using anti-CTGF antibody, and anti–β-actin antibody as a control. Integrated densitometry value ratios were used to compare the expression of CTGF protein with that of β-actin protein (Figure 5B). The results indicate a significant reduction in the levels of CTGF mRNA and protein expressed in the tissue of animals treated with the TSP-1 peptide.

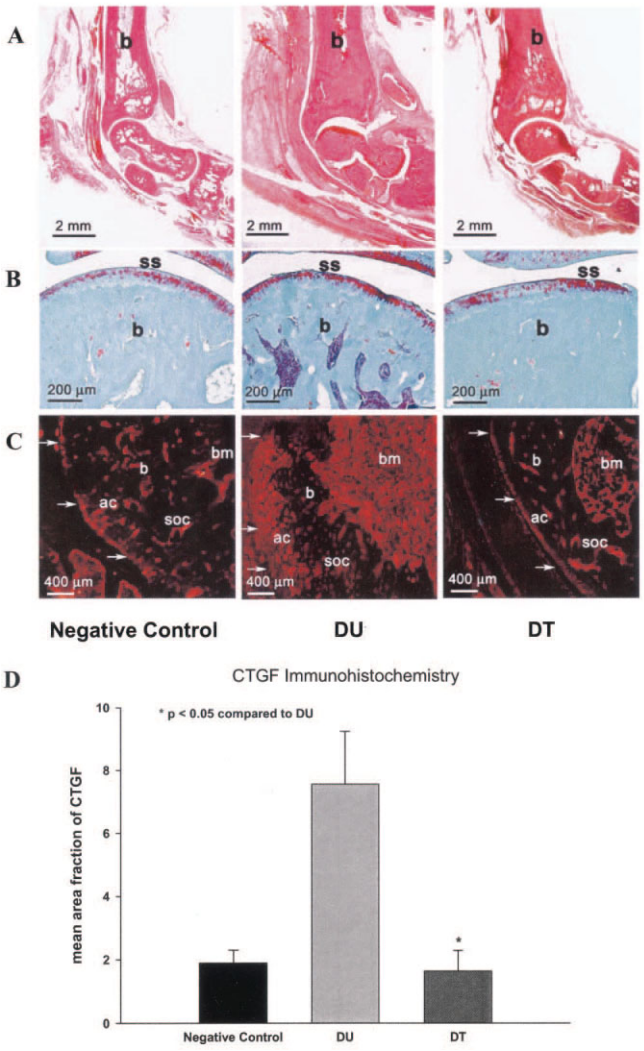

Figure 4. A–C, Hematoxylin and eosin (H&E) (**A**), Safranin O (**B**), and Cy3 immunofluorescence (**C**) localization of connective tissue growth factor (CTGF) expression in the joints of rats from the negative control, disease untreated, and disease treated groups. **Arrows** indicate the articular surface. **b** = bone; **ac** = articular cartilage; **bm** = bone marrow; **soc** = secondary ossification center (original magnification × 10 in **A;** × 40 in **B;** × 100 in **C**). **D,** Immunohistochemical quantification of CTGF expression (calculated as described in Materials and Methods). The amount of CTGF immunostaining in the synovial tissue of the TSP-1 peptide–treated animals is significantly less than that in the disease untreated animals. Values are the mean and SEM. See Figure 2 for other definitions.

DISCUSSION

This study documents the clinical efficacy of intravenous and intraperitoneal administration of a

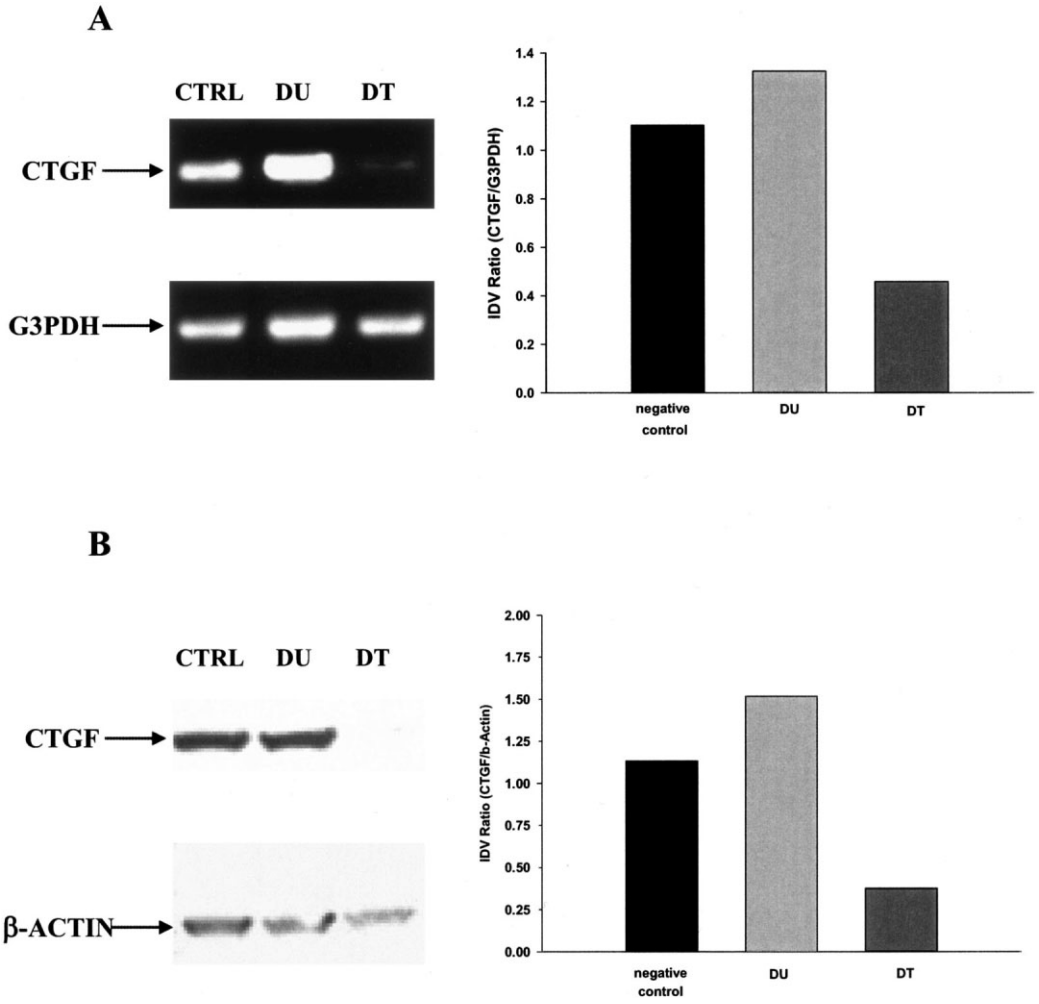

Figure 5. Comparison of the expression of connective tissue growth factor (CTGF) message and protein in synovial tissue of rats with erosive arthritis. **A,** Expression of mRNA was determined by comparing the integrated density values (IDV) of the bands from the reverse transcriptase–polymerase chain reaction products of CTGF and G3PDH. There was a 50% reduction in mRNA expression in disease TSP-1 peptide–treated rats compared with disease untreated rats. A representative normal Lewis rat was studied as a negative control. **B,** CTGF protein expression was determined by comparing the IDV of the bands from the Western blot analysis of CTGF and β-actin. The results indicate that CTGF protein expression is inhibited by treatment with the TSP-1 peptide. Values are the mean. See Figure 2 for other definitions.

thrombospondin-derived synthetic peptide in an animal model of erosive arthritis. In vitro studies have demonstrated that TSP-1 expressed on the surface of a monocytic cell line leads to FXa and thrombin generation (5). TSP-1 may function as an adhesive glycoprotein which is expressed on the neutrophil cell membrane, as well as on other cell membranes, and is colocalized with elastase and factor V to promote generation of factor Xa and thrombin. These latter 2 serine proteases have the ability to induce up-regulation of mRNA for CTGF, a potent mediator of angiogenesis (6). Our results suggest that CTGF participates in the series of events leading to joint destruction.

Recently, the TSP-1 gene has been among those identified as being up-regulated in humans with RA (15). These findings are not the first to link TSP-1 with the pathophysiology of RA. In 1997, Gotis-Graham et al reported a significant correlation between TSP-1 and expression of serine proteinases, including HNE, in human rheumatoid synovium (16). Initially, the potent antiangiogenic properties of TSP-1, in addition to its ability to bind and reversibly inhibit the active site of HNE, may be viewed as a benefit during the inflammatory response in RA. However, studies both in animals and in humans have demonstrated that this is not the case.

Stuhlmuller et al, using a combined strategy of gene subtraction and semiquantitative RT-PCR, defined a pattern of monocyte activation in patients with RA. Their work demonstrated that the gene for TSP-1 was up-regulated along with other genes well known to be up-regulated in RA, including interleukin-1α, tumor necrosis factor α, and interleukin-6 (15).

Koch and colleagues, in 1998, implanted Hydron pellets containing TSP-1 in one ankle of genetically susceptible rats with AIA, while the contralateral ankle received sham implants (3). The TSP-1 implants augmented the severity of the disease. Our data obtained using a monocytic cell line in vitro may explain, in part, the findings of Koch et al, since exogenous addition of TSP-1 enhanced the rate and amount of thrombin (and thus FXa) generated on the cell surface, thereby providing a mechanism by which CTGF gene and protein expression can be up-regulated (5). More recently, McLaughlin et al demonstrated that thrombin up-regulates the TSP-1 gene in human microvascular endothelial cells, which provides evidence of a positive feedback mechanism between thrombin and TSP-1 (17).

In 2005, Jou and colleagues, by directly administering adenoviral vectors encoding TSP-1 into male Sprague-Dawley rats with collagen-induced arthritis (CIA), demonstrated that TSP-1 is an effective gene therapeutic strategy (18). Although the potent antiangiogenic properties of TSP-1 were responsible for the clinical efficacy of treatment in their experiments, such properties were not evident in our studies or those of Koch et al. Our explanation for these discrepancies relates to the difference in the animal models. The streptococcal cell wall model of arthritis in female Lewis rats is one of the most reliable and best-characterized experimental models of RA (19). A single IP injection of PG-PS suspended in an aqueous phase will induce a chronic, severe, erosive arthritis in female Lewis rats, unlike the CIA model, which lacks a chronic phase (20). As in human RA, female rats develop arthritis more readily than male rats. During the first 24 hours, a T cell–independent acute phase develops, followed by visible joint swelling by day 3. The chronic phase is T cell dependent. The CIA model is an extensively studied animal model of RA and primarily an autoimmune disease of joints, requiring both T and B cell immunity to autologous type II collagen (CII) for disease manifestation. Unlike the PG-PS model, the CIA model is governed by a complex set of genes in the induction phase, involving interaction of both B and CD4+ T cells, cytokines, and non–major histocompatibility complex genes for the development of disease. Although T cells play a prominent role in the regulation and development of CIA, autoimmunity to murine CII appears to be the primary mechanism of immunopathogenesis in this model.

In rheumatoid synovium, follicular structures resembling peripheral lymph nodes invariably develop. These structures consist of lymphocytic aggregates maintained on a stroma of macrophages and fibroblast-like synoviocytes. The tissue parenchyma also includes various extracellular matrix proteins, which profoundly influence cytokine gene expression by T cells. There is evidence that TSP-1 is persistently present in the rheumatoid synovium (3). There is also a characteristic focal expression of TSP-1 in the follicular structures, among fibroblast-like synoviocytes and macrophages of the tissue parenchyma, and on endothelial cells (2). This expression pattern of TSP-1 suggests a functional role beyond passive scaffolding of the synovial tissue architecture. Our findings indicate that peptide treatment is capable of reducing granulocytosis, a feature that may explain the decrease in the circulating levels of TSP-1 and HNE and the potential disposition of TSP-1 at sites of inflammation. Vallejo et al (2) and Yabkowitz et al (21) have presented evidence supporting our finding that TSP-1 plays a central role in the recruitment, activation, and retention of T cells in the inflammatory lesions.

CTGF is a novel, potent, angiogenic growth factor, originally isolated from human endothelial cells and highly expressed by fibroblasts. To our knowledge, this is the first documentation of the up-regulation of CTGF mRNA in an experimental model of erosive

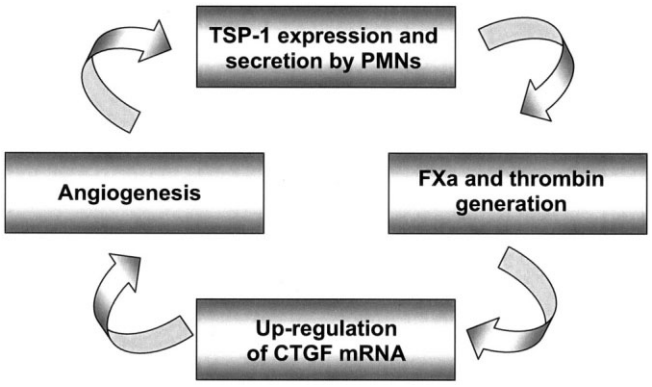

Figure 6. Graphic representation of the working model in which thrombospondin 1 (TSP-1) synthesis and cell membrane surface expression by polymorphonuclear cells (PMNs) (5) promotes generation of serine proteases, namely, activated coagulation factor X (FXa) and thrombin, which have been shown to induce expression of connective tissue growth factor (CTGF) mRNA (6,7). In turn, up-regulation of CTGF gene and protein expression induces angiogenesis (10), the hallmark of rheumatoid arthritis.

arthritis. In addition, our data directly link this up-regulation to TSP-1 since peptide treatment prevented the up-regulation of CTGF mRNA in the disease untreated group of animals. Although our observations are derived from the animal model, experimental data utilizing human monocytes and neutrophils from normal donors and from patients with RA provide evidence that these cells have CTGF message. In an extensive search of the literature, we found no descriptive clinical studies linking CTGF to the pathogenesis of RA.

One potential mechanism linking TSP-1 and CTGF in this model of erosive arthritis relates to the ability of FXa and thrombin to induce CTGF mRNA in neutrophils, monocytes, and fibroblasts (6,7). In summary, we present evidence for the existence of a novel pathway in which CTGF overexpression occurs and becomes associated with disease progression (Figure 6). The overexpression of CTGF seems to be linked to TSP-1 since it was prevented by treatment with a synthetic peptide derived from the TSP-1 type 3 repeats. This process is made possible by the ability of activated PMNs to synthesize and express TSP-1 on their activated membrane surface. Further studies should delineate the pathways involved in this process and provide potential avenues for novel adjuvant treatment of RA.

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