Induction of Stromelysin Gene Expression by Tumor Necrosis Factor α Is Inhibited by Dexamethasone, Salicylate, and N-Acetylcysteine in Synovial Fibroblasts

ISABELLE MORIN, WEN QING LI, SUMING SU, MUSHTAQ AHMAD, and MUHAMMAD ZAFARULLAH

Louis-Charles Simard Research Centre, Centre Hospitalier de L’Université de Montréal Campus Notre-Dame, and Department of Medicine, University of Montreal, Montreal, Quebec, Canada (I.M., W.Q.L., S.S., M.Z.); and Department of Medicine, Division of Cardiology, Emory University School of Medicine, Atlanta, Georgia (M.A.)

Accepted for publication January 19, 1999 This paper is available online at http://www.jpet.org

ABSTRACT
Proinflammatory cytokines, altered connective tissue metabolism, and overexpression of matrix metalloproteinases (MMPs) such as stromelysin compared to tissue inhibitors of metalloproteinases (TIMPs) result in synovial inflammation and erosion of arthritic cartilage. Tumor necrosis factor α (TNF-α) is a major synovial inflammatory mediator responsible for inhibiting extracellular matrix (ECM) synthesis and stimulating degradation of cartilage ECM by activated MMPs in arthritic joints. To suppress these effects and to gain insight into the mechanism of TNF-α action, we identified the inhibitors of TNF-α stimulation of stromelysin gene expression. In bovine synovial fibroblasts, TNF-α did not affect a recently identified inhibitor, TIMP-3, but induced stromelysin mRNA expression in a dose- and time-dependent fashion (3- to 5-fold) which required de novo protein synthesis. Stimulation by TNF-α was potently inhibited (99–100%) by the synthetic glucocorticoid, dexamethasone. Sodium salicylate dose-dependently inhibited (100%) the TNF-α action. Indomethacin and ibuprofen were partially inhibitory. Free radical scavenger antioxidant, N-acetylcysteine (but not other antioxidants) also suppressed the TNF-α induction (36–100%) of stromelysin suggesting involvement of reactive oxygen species in the induction process. TNF-α induction of stromelysin gene expression can therefore be inhibited at the gene expression level by several pharmacological agents which are likely to function via arachidonic acid metabolites, free radical scavenging or interference with the activator protein 1, polyoma virus enhancer A-binding protein 3, and nuclear factor κB classes of transcription factors. Our results may help to elucidate the mechanism of TNF-α action and explain the beneficial role of these agents in the treatment of inflammatory diseases.

Tumor necrosis factor α (TNF-α) is the major inflammatory mediator in arthritis that invokes multiple responses and regulates numerous genes implicated in inflammation (Vilcek and Lee, 1991). It is found at high levels in the synovial fluids of patients with arthritis, whose synovium exhibits different degrees of inflammation (Westacott and Sharif, 1996). TNF-α inhibits transcription of cartilage-specific type-II, -IX and -XI collagens in human chondrocytes and contributes to cartilage loss in joint diseases (Reginato et al., 1993). Osteoarthritic (OA) cartilage is more susceptible to stimulation by TNF-α due to increased p55 TNF-α receptor on the chondrocytes (Westacott and Sharif, 1996). TNF-α inhibits embryonal chondrocytic phenotype and turns them into fibroblast-like cells in vitro (Mohamed-Ali, 1995). Overall, TNF-α promotes tissue destruction by metalloenzymes and prevents tissue repair.

Matrix metalloproteinases (MMPs) consist of collagenases, stromelysins, gelatinases, and membrane-type MMPs, which can degrade extracellular matrix during its physiological (e.g., animal development) and pathological (arthritis, cancer, cardiovascular diseases) turnover. Activity of MMPs is regulated by the tissue inhibitor of metalloproteinase (TIMP) gene family which consists of four members named TIMP-1, -2, -3, and -4 (Birkedal-Hansen, 1995). MMPs not only cause erosion of arthritic joints but also process TNF-α into its mature secreted active form (Gearing et al., 1994). Inhibitors of MMPs prevent this processing. Stromelysin is a major MMP, responsible for degradation of cartilage aggrecan and activation of procollagenase (Birkedal-Hansen, 1995). The expression of this gene is increased in arthritic joints (Zafarullah et al., 1993) and in unstable atherosclerotic plaques.

Received for publication October 2, 1997.

1 This work was supported by grants from the Medical Research Council of Canada (MT-12867), the Arthritis Society of Canada, and the Heart and Stroke Foundation of Quebec and a scholarship of the Fonds de la recherche en santé du Québec.

ABBREVIATIONS: AP-1, activator protein-1; FCS, fetal calf serum; PEA-3, polyoma virus enhancer A-binding protein 3; MMP, matrix metalloproteinase; NAC, N-acetylcysteine; NMA, N-monomethyl-L-arginine; PDTC, pyrrolidine dithiocarbamate; TIMP, tissue inhibitors of metalloproteinase; TNF-α, tumor necrosis factor α.
(Galits et al., 1994). Since synovial inflammation is the major discomfort in arthritis, and the synovial fibroblasts are the main target cell-type, this study was aimed at identifying inhibitors of stromelysin upregulation by TNF-α in the bovine synovial fibroblast model in order to understand the mechanism of TNF-α action. We tested the hypothesis that the inhibitors of protein synthesis, transcription factors, and reactive oxygen species may block TNF-α stimulation of stromelysin gene expression.

**Materials and Methods**

**Reagents.** Dulbecco’s modified Eagle’s medium (DMEM), fetal calf serum (FCS), antibiotic-antimycotic agents, and agarose were obtained from Canadian Life Technologies Inc. (Gibco-BRL, Burlington, Ontario, Canada). Plasticware such as 100-mm plates and T-75 flasks were purchased from Nunc, Inc. (Roskilde, Denmark). Tumor necrosis factor α was obtained from R&D systems (Minneapolis, MN) and Boehringer Mannheim (Laval, Quebec). Cytochrome c was obtained from U.S. Biochemical Corporation (Cleveland, OH). Collagenase type II, dexamethasone, sodium salicylate, indomethacin, ibuprofen, N-methyl-L-arginine, α-tocopherol, ascorbic acid, and pyrrolidine dithiocarbamate were purchased from Sigma Chemical Company (St. Louis, MO). Digoxigenin (DIG) RNA labeling and chemiluminescence systems were obtained from Boehringer Mannheim. Hybond nylon membrane was purchased from Amersham. RNA probe labeling kits were obtained from Promega (Madison, WI). Restriction endonucleases were purchased from Pharmacia Biotec. (Biotec, Madison, WI). The vector was linearized with EcoRI and XbaI and a probe was synthesized using T7 polymerase. The probes were labeled with the digoxigenin (DIG) RNA labeling kit containing DIG-11-UTP followed by prehybridization, hybridization, and detection of Northern blots with the DIG Luminescent Detection kit of Boehringer Mannheim according to their protocols. Alternatively the probes were labeled to high specific activity (1 × 10⁶ cpm/μg) with [α-³²P]dCTP (3000 Ci/mmol; DuPont-NEW (Boston, MA). Densitometric values from three experiments (obtained with Alpha Imager, Canberra-Packard, Meriden, CT) of stromelysin bands were divided by those of GAPDH bands to get an average quantitative estimate of inhibition as depicted by bar graphs.

**Results**

Before testing the potential inhibitors, we investigated whether the normal bovine synovial fibroblasts were responsive to TNF-α. To that end, these cells were exposed for 24 h to different concentrations (50–1000 U/ml) of TNF-α in medium containing either minimal 0.5% or 5% FCS. The RNA was hybridized with a human stromelysin probe which cross-hybridizes with the bovine stromelysin mRNA of the same size. Although the lower doses were effective, following normalization with the constitutive GAPDH or 28S control RNA, stromelysin mRNA was 3- to 4-fold inducible by TNF-α at 200 U/ml and 150 U/ml in 0.5% FCS and 4- to 5-fold inducible in 5% FCS (Fig. 1). The RNA levels of a housekeeping GAPDH gene (Fig. 1, lower panel) and those of another control gene, 28S rRNA (not shown) were not changed by the treatments. Therefore, TNF-α is a potent inducer of stromelysin gene expression in bovine synovial fibroblasts.

To determine the time course of stromelysin mRNA expression, cells were treated with TNF-α (150 U/ml) for different time periods under 0.5% FCS (Fig. 2A) or 5% FCS (Fig. 2B). Cells were also treated for 16 h at a higher dose (1000 U/ml) as a positive control. As shown in Fig. 2A, the maximal (3.4-fold) induction of stromelysin mRNA expression occurred between 16 and 24 h. The pattern of induction was similar under 5% FCS conditions (2.5–3.75-fold), although overall levels of RNA were some what lower than those under 0.5% conditions. To investigate the inducibility of a recently produced, the RNA probe was synthesized with SP6 polynucleotide according to the protocols of Promega Biotec. The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA probe (from American Type Culture Collection, Rockville, MD) was described earlier (Zafarullah et al., 1993). The human 28S ribosomal RNA plasmid (American Type Culture Collection) was digested with XbaI and a probe was synthesized using T7 polymerase. The probes were labeled with the digoxigenin (DIG) RNA labeling kit containing DIG-11-UTP followed by prehybridization, hybridization, and detection of Northern blots with the DIG Luminescent Detection kit of Boehringer Mannheim according to their protocols. Alternatively the probes were labeled to high specific activity (1 × 10⁶ cpm/μg) with [α-³²P]dCTP (3000 Ci/mmol; DuPont-NEW (Boston, MA). Densitometric values from three experiments (obtained with Alpha Imager, Canberra-Packard, Meriden, CT) of stromelysin bands were divided by those of GAPDH bands to get an average quantitative estimate of inhibition as depicted by bar graphs.

**Synergial Fibroblast Culture.** Normal bovine synovial membranes were dissected from the freshly slaughtered adult animals from a local abattoir. The tissues were washed extensively in large volumes of 0.9% NaCl containing 5× antibiotic-antimycotic solution and further washed five times with PBS containing 5× penicillin-streptomycin and 1× fungizone (Gibco-BRL, Burlington, Ontario, Canada). Synovial fibroblasts were released by dissociation with trypsin and collagenase (Sigma type II) for 6 h in DMEM at 37°C. The cells were collected by centrifugation and washed three times with PBS and plated as primary monolayer cultures until adherence and confluent growth were attained. These cells were passaged several times to obtain a homogeneous population of synovial fibroblasts. Before different treatments, cells were kept in 0.5 or 5% serum containing DMEM for 24 h and then exposed to different reagents in the same medium for various periods of time described in the text.

**RNA Extraction and Northern Blot Hybridization Analysis.** Total RNA was extracted by a rapid procedure (Chomczynski and Sacchi, 1987) and aliquots of 5 μg were analyzed by electrophoretic fractionation in 1.2% formaldehyde-agarose gels. The integrity and quantity of applied RNA were verified by ethidium bromide staining of the gels and photography of the 28S and 18S ribosomal RNA bands. The RNA was electroblotted onto Hybond nylon membrane using a Bio-Rad Transblot in the presence of 1× standard saline citrate buffer at a current of 500 mAmp for 12 h. Complete transfer was ascertained by ethidium bromide staining of the gel. Northern blots were hybridized as described previously (Zafarullah et al., 1993) with a human stromelysin cDNA probe generously provided by Dr. Richard Breathnach (Muller et al., 1988). This probe which cross-hybridizes with bovine stromelysin RNA, was a 1.6-kbp EcoRI-EcoRI cDNA fragment cloned in the plasmid pGEM-4Z (Promega Biotec, Madison, WI). The vector was linearized with NruI and RNA probe synthesized with T7 polymerase according to the protocols of Promega Biotec. The bovine TIMP-3 probe (Su et al., 1996) was a 2.042-kbp EcoRI-EcoRI cDNA fragment cloned in the plasmid pGEM-4Z (Promega Biotec). The vector was linearized with SacI and

---

**Fig. 1. Stromelysin mRNA expression in response to different doses of TNF-α.** Lanes 0, DMEM containing 0.5% or 5% FCS-treated bovine synovial fibroblasts. Other lanes represent cells treated in the same medium with different concentrations of TNF-α (in U) for 24 h. The GAPDH RNA serving as loading and constitutively expressed control is shown at the bottom.
characterized metalloproteinase inhibitor, TIMP-3, by TNF-α, the same blots were hybridized with the bovine TIMP-3 cDNA probe. This factor had very little effect on TIMP-3 gene expression (middle panels of Fig. 2). The application of RNA in different lanes was even. The RNA levels of GAPDH (Fig. 2, lower panel) and those of 28S rRNA (not shown) were not changed by the treatments. Therefore, TNF-α induces stromelysin but not TIMP-3 gene expression.

These time points were considered adequate for further inhibitor experiments.

The mechanism of TNF-α induction of stromelysin gene expression was investigated by treatment of synovial fibroblasts with a nontoxic dose (10 μg/ml) of an inhibitor of protein synthesis, cycloheximide. The representative Fig. 3 demonstrates that this treatment inhibited stromelysin induction by TNF-α. Densitometric scanning of the stromelysin RNA levels normalized with the GAPDH control RNA from three separate experiments revealed an average inhibition of 100%.

To examine the effect of antiinflammatory corticosteroids on the induction of stromelysin expression, cells were treated with a synthetic glucocorticoid, dexamethasone (1–2 μg/ml). In three separate experiments, this agent potently suppressed (99–100%) stromelysin induction by TNF-α, reducing its mRNA to basal level (Fig. 4). GAPDH RNA levels were not inhibited.

Aspirin-like agents or salicylates are commonly used drugs to treat inflammation in arthritis. They can induce heat shock transcription factor and inhibit TNF-α-induced nuclear factor κB (NF-κB) activation (see Discussion). We examined whether this drug influenced the induction of stromelysin gene by TNF-α. Cells were treated with sodium salicylate (2–20 mM) alone or in combination with TNF-α. It dose-dependently down-regulated TNF-α induction of stromelysin gene expression in three separate experiments (Fig. 5, upper panel). Following correction with the consistent GAPDH control RNAs, 100% inhibition was observed (Fig. 5, lower panel).

Since glucocorticoids and salicylates can influence prostaglandin metabolism, we further examined the latter's involvement by exposing cells to other cyclooxygenase inhibitors, indomethacin and ibuprofen alone or with TNF-α. Indomethacin and ibuprofen partially inhibited (48–50%) stromelysin RNA induction by TNF-α in a reproducible fashion (Fig. 6). Since nitric oxide (NO) activates stromelysin, and salicylates can inhibit inducible nitric oxide synthase (iNOS), we examined whether iNOS inhibitor could reduce TNF-α-
induced stromelysin induction. N-methyl-L-arginine (NMA), an inhibitor of iNOS did not decrease but further increased this induction (1 mM). Thus, arachidonic acid metabolites (but not NO) appear to partially mediate this induction in our system.

To investigate the role of reactive oxygen species in TNF-α induction of stromelysin gene, synovial fibroblasts were treated either with TNF-α or with a thiolic antioxidant, N-acetylcysteine (NAC) alone or in combination. GAPDH and 28S RNA (not shown) levels were unaffected by the treatments. In three experiments, this agent, at concentrations of 20 to 30 mM, strongly down-regulated stromelysin mRNA induction by TNF-α in a dose-dependent fashion as depicted in the representative Fig. 7 (upper panel). An inhibition of 36% (at 10 mM) to 100% (at 20–30 mM) was observed with NAC (Fig. 7, lower panel). Other antioxidants such as α-tocopherol, ascorbate, and pyrrolidine dithiocarbamate (PDTC) did not block TNF-α induction of stromelysin. PDTC by itself induced stromelysin mRNA and actually further enhanced induction by TNF-α (Fig. 8).

Discussion

In this report, we demonstrated that the proinflammatory cytokine, TNF-α, up-regulates stromelysin gene without affecting TIMP-3 gene expression in mammalian synovial fibroblasts. The induction process requires de novo protein synthesis and may involve activation of transcription factors. We further showed that the induction can be inhibited or down-regulated by dexamethasone, an aspirin-like drug and antioxidant activity, and interference with the activator protein 1 (AP-1) and NF-κB classes of transcription factors.

The demonstrated stromelysin gene induction by TNF-α in bovine synovial fibroblasts is in agreement with other cell types where stromelysin was inducible by this factor. In bovine nasal chondrocytes, TNF-α induced both proteinases and prostaglandin E₂ (Smith et al., 1992). This factor transcriptionally induced collagenase and stromelysin expression in porcine chondrocytes by mediation of staurosporine- and H7-sensitive protein kinases distinct from protein kinase C.
The two major cell types in joints are clearly the targets of TNF-α action. A lack of TIMP-3 mRNA induction by TNF-α observed here is in contrast with a previous report where TNF-α induced TIMP-3 expression in mouse cells in the presence of 5% FCS serum (Sun et al., 1995). In our cells, TIMP-3 mRNA could not be markedly induced in the presence of 5% or 0.5% serum with different brands of TNF-α. The lack of TIMP-3 induction by TNF-α is in accord with another study where TIMP-1 (another family member) was not induced but was suppressed by this agent in synovial cells (Shingu et al., 1993). MMP induction and TIMPs inhibition (or noninduction) by TNF-α can contribute to enzyme-inhibitor imbalance commonly observed in arthritic tissues (Dean et al., 1989).

Suppression of TNF-α induction of stromelysin by cycloheximide is similar to that of collagenase inhibition by this agent in human fibroblasts (Sciavolino et al., 1994). Therefore, up-regulation is through indirect mechanisms. The prolonged induction of the c-jun protooncogene by TNF-α was proposed as one of the intermediate transcription factors that is part of the AP-1 complex comprising of c-fos/c-jun factors. This occurs by upstream prolonged activation of c-jun kinase which phosphorylates the transactivation domain of c-Jun (Westwick et al., 1994). The promoter DNA-binding activities of AP-1 proteins are increased by TNF-α (Sciavolino et al., 1994). AP-1 binding sites are found in the human collagenase and stromelysin 1 promoters (Buttice et al., 1991). Contrary to a previous report, we did not observe induction of stromelysin by cycloheximide alone (Otani et al., 1990) in bovine cells.

The inhibition of TNF-α action by the anti-inflammatory glucocorticoid in synovial fibroblasts is particularly relevant to arthritis as synovial inflammation is a major discomfort for the patients, and glucocorticoids are used to relieve the symptoms in patients and in animal models of arthritis. These agents stop further erosion of cartilage in vivo by metalloproteinases (Pelletier et al., 1994). Inhibition of collagenase and stromelysin mRNA by dex was also observed in human diploid FS-4 fibroblasts (Sciavolino et al., 1994). We showed that TIMP-3 gene induction by transforming growth factor β is also inhibited by dexamethasone (Su et al., 1996). The molecular mechanism of inhibition possibly involves direct interaction between c-jun and glucocorticoid receptor...
Inhibition of TNF-Induced Stromelysin Expression 1639

Proteins resulting in prevention of c-jun binding to the AP-1 sequences in stromelysin promoter (Yang-Yen et al., 1990). Another recently proposed mechanism of immunosuppressive and anti-inflammatory action of glucocorticoids is the induction of IkBa gene product which associates with TNF-α-activated NF-κB (dissociated NF-κB from IkBa factor), thus preventing its translocation to the nucleus (Scheinman et al., 1995). The applicability of this model for stromelysin inhibition is possible, since NF-κB is commonly found in joints of OA and RA patients (Marok et al., 1996). AP-1 and polyoma virus enhancer A-binding protein 3- (PEA-3) (but not NF-κB) binding sites have been detected in the known stromelysin promoters. An additional possibility is that at higher concentrations, salicylate acted by its antioxidant type of activity (Dinis et al., 1994) and scavenged TNF-α-induced hydroxyl radicals. This ability alone could be sufficient to inhibit stromelysin induction by TNF-α.

Aspirin is an old wonder drug that reduces the risk of heart attacks and colon cancer (Thun et al., 1991). Aspirin and sodium salicylate have neuroprotective abilities against glutamate toxicity by inhibiting NF-κB activation (Grilli et al., 1996). Stromelysin is involved in arthritic cartilage erosion, instability of atherosclerotic plaques (Galis et al., 1994), and metastatic invasion of cancer cells. Our demonstration of inhibition of stromelysin gene expression by aspirin-like drugs suggests a possible beneficial effect of this drug in these disorders and may explain the results of epidemiological studies on aspirin. Inhibition of TNF-α action is a major target for suppressing inflammation. In experimentally induced arthritis in rabbits, neutralization of TNF-α with its antibody suppressed inflammatory but not the cartilage reparative aspect of arthritis, suggesting its key role in inflammation (Lewthwaite et al., 1995). Our study, utilizing stromelysin as a target, has demonstrated alternative inhibitors of TNF-α action.

Inhibition of stromelysin mRNA induction by a reactive oxygen species scavenger NAC suggests that reactive oxygen species are involved in stromelysin induction by TNF-α. Oxidant by-products are used by phagocytes to combat infections during immune response, but are also a source of oxidative damage in degenerative diseases of aging (Ames et al., 1993). Indeed, a lower serum antioxidant level has been recognized as a risk factor for rheumatoid arthritis (Heliövaara et al., 1994). Stromelysin mRNA inhibition by NAC may be a result of upstream inhibition of redox-sensitive c-fos and c-jun transcription factor expression by this antioxidant (Lo et al., 1996). These AP-1 factors along with PEA-3 binding factors are important regulators of the transcription of human stromelysin promoter (Buttice et al., 1991). Other antioxidants such as α-tocopherol, ascorbate, and PDTC were drugs which are commonly prescribed for the management of arthritis. Aspirin-like drugs inhibit COX-2 induction by TNF-α and constitutive COX-1 (Geng et al., 1995), which through some unknown mechanism may inhibit stromelysin gene expression observed here. Other nonsteroidal anti-inflammatory drugs such as Naproxen and Tenidap also inhibit collagenase mRNA expression in a canine model of arthritis (Fernandes et al., 1995). Our results suggest that prostaglandins are partly involved in TNF-α induction of stromelysin. Alternatively, TNF-α could induce nitric oxide synthase (NOS) responsible for the synthesis of NO which could activate collagenase and stromelysin (Murrell et al., 1995). Aspirin-like drugs at pharmacological (1–3 mM) and suprapharmacological (IC50 = 20 mM) concentrations can inhibit inducible NOS (Amin et al., 1995). Treatment with NOS inhibitor, NMA, however, failed to block TNF-α action in our system. A third possibility is that TNF-α-induced activation of NF-κB and its translocation from cytoplasm to the nucleus is inhibited by high doses of sodium salicylate and aspirin (Kopp and Ghosh, 1994) which some how influences stromelysin gene expression. Activated NF-κB is commonly found in joints of OA and RA patients (Marok et al., 1996). AP-1 and polyoma virus enhancer A-binding protein 3- (PEA-3) (but not NF-κB) binding sites have been detected in the known stromelysin promoters. An additional possibility is that at higher concentrations, salicylate acted by its antioxidant type of activity (Dinis et al., 1994) and scavenged TNF-α-induced hydroxyl radicals. This ability alone could be sufficient to inhibit stromelysin induction by TNF-α.

Aspirin is an old wonder drug that reduces the risk of heart attacks and colon cancer (Thun et al., 1991). Aspirin and sodium salicylate have neuroprotective abilities against glutamate toxicity by inhibiting NF-κB activation (Grilli et al., 1996). Stromelysin is involved in arthritic cartilage erosion, instability of atherosclerotic plaques (Galis et al., 1994), and metastatic invasion of cancer cells. Our demonstration of inhibition of stromelysin gene expression by aspirin-like drugs suggests a possible beneficial effect of this drug in these disorders and may explain the results of epidemiological studies on aspirin. Inhibition of TNF-α action is a major target for suppressing inflammation. In experimentally induced arthritis in rabbits, neutralization of TNF-α with its antibody suppressed inflammatory but not the cartilage reparative aspect of arthritis, suggesting its key role in inflammation (Lewthwaite et al., 1995). Our study, utilizing stromelysin as a target, has demonstrated alternative inhibitors of TNF-α action.

Inhibition of stromelysin mRNA induction by a reactive oxygen species scavenger NAC suggests that reactive oxygen species are involved in stromelysin induction by TNF-α. Oxidant by-products are used by phagocytes to combat infections during immune response, but are also a source of oxidative damage in degenerative diseases of aging (Ames et al., 1993). Indeed, a lower serum antioxidant level has been recognized as a risk factor for rheumatoid arthritis (Heliövaara et al., 1994). Stromelysin mRNA inhibition by NAC may be a result of upstream inhibition of redox-sensitive c-fos and c-jun transcription factor expression by this antioxidant (Lo et al., 1996). These AP-1 factors along with PEA-3 binding factors are important regulators of the transcription of human stromelysin promoter (Buttice et al., 1991). Other antioxidants such as α-tocopherol, ascorbate, and PDTC were...
not inhibitory, but rather stimulatory (particularly PDTC), suggesting distinct mechanisms of action for different antioxidants. This may be due to the reported ability of PDTC to induce AP-1 transcription factors whose targets are found in the stromelysin promoter (Yokoo and Kitamura, 1996). Interestingly, induction of cartilage catabolism by fibronectin fragments by MMPs is also inhibited by antioxidants (Hom-andberg et al., 1996). NAC also blocks nuclear translocation of NF-κB in rheumatoid synovial fibroblasts (Sakurada et al., 1996).

In summary, we have demonstrated that stromelysin gene expression is upregulated by TNF-α. Inhibition of this augmentation by cycloheximide, glucocorticoids, salicylates, and NAC suggests that the induction is mediated by de novo protein synthesis and intermediate steps such as prostaglan-
dins, reactive oxygen species, and AP-1, PEA-3, or NF-κB transcription factor activation. These results also give a better insight into the mechanism of action of these drugs, some of which are beneficial in the treatment of arthritis, cardio-
vascular diseases, and cancer.

Acknowledgments

We are grateful to Dr. Richard Breathnach (Nantes, France) for the human stromelysin cDNA, Jean Maher (Abattoir les Cèdres) for supplying bovine joints, and Anna Chelchowska for preparing figures.

References


Evidence for metalloproteinase and metalloproteinase inhibitor imbalance in hu-


Dean DD, Martel-Pelletier J, Pelletier J-P, Howell DS and Woessner JF Jr (1989) Evidence for metalloproteinase and metalloproteinase inhibitor imbalance in hu-


Pelletier JP, Mineau F, Raynaud J-P, Woessner JF Jr, Gunja-Smith Z and Martel-
Pelletier J (1994) Intraarticular injections with methylprednisolone acetate reduce osteoarthritic lesions in parallel with chondrocyte stromelysin synthesis in exper-

Reginato AM, Sanz-Rodriguez C, Diaz A, Dharmavaram RM and Jimenez SA (1993) Transcriptional modulation of metalloproteinase-9-specific collagen gene expression by inter-


Scheinman RI, Cogwell PC, Logofut AK and Baldwin AS Jr (1995) Role of tran-
scriptional activation of IkBα in mediation of immunosuppression by glucocorti-
coids. Science (Wash DC) 270:283–289.


Shingu M, Nagai Y, Isayama T, Naono T, Nobunaga M and Nogai Y (1993) The effects of cytokines on metalloproteinase inhibitors (TIMP) and collagenase pro-
duction by human chondrocytes and TIMP production by synovial cells and endo-

Smith RJ, Justen JM, Ulrich RG, Lund JE and Sam LM (1992) Induction of neutral proteinase and prostatic proteinase in bovine nasal chondrocytes by interleuk-


Send reprint requests to: Dr. M. Zafarullah, K-5255 Maillon, CHUM Campus Notre-Dame, 1560 Sherbrooke est, Montréal, Québec, Canada H2L 4M1. E-mail: Zafarulm@ere.umontreal.ca

We are grateful to Dr. Richard Breathnach (Nantes, France) for the human stromelysin cDNA, Jean Maher (Abattoir les Cèdres) for supplying bovine joints, and Anna Chelchowska for preparing figures.