Modulation of TIMP-1 Synthesis by Antiinflammatory Cytokines and Prostaglandin E2 in Interleukin 17 Stimulated Human Monocytes/Macrophages

DRAGAN V. JOVANOVIC, JOHN A. DI BATTISTA, JOHANNE MARTEL-PELLETIER, PASCAL REBOUL, YULAN HE, FRANÇOIS-CYRIL JOLICOEUR, and JEAN-PIERRE PELLETIER

ABSTRACT. Objective. To examine the regulation of tissue inhibitor of metalloproteinase 1 (TIMP-1) synthesis by interleukin 17 (IL-17) stimulated human monocytes/macrophages in primary culture in the presence of prostaglandin E2 (PGE2) and antiinflammatory cytokines, and to compare this with the regulation of matrix metalloproteinase (MMP-9) production.

Methods. IL-17 stimulated human monocytes isolated from the peripheral blood of healthy donors were cultured in the presence of PGE2, cyclic adenosine monophosphate (cAMP) mimetics (IBMX, cAMP, forskolin, cholera toxin), or antiinflammatory cytokines (IL-4, IL-10, IL-13), or with protein kinase inhibitors of diverse specificity. MMP-9 and TIMP-1 were measured using specific ELISA, while expression of specific messenger RNA was determined by Northern blotting.

Results. IL-17 stimulated an increased level of MMP-9 production relative to TIMP-1 production in monocytes/macrophages. Stimulation was accompanied by upregulation of specific MMP-9 mRNA expression relative to TIMP-1 mRNA. Exogenous PGE2, cAMP, and cAMP-mimetics completely inhibited both basal and IL-17 induced MMP-9 synthesis, while only IL-17 induced TIMP-1 synthesis was abrogated. The same effect was found for the antiinflammatory cytokines. Both basal and IL-17 induced production of TIMP-1 involved p42/44 and p38 kinases and nuclear factor κB signaling pathways.

Conclusion. The excess of MMP-9 over TIMP-1 production, and decreased inhibition of MMP-9 activity in chronic rheumatoid diseases, may result in cartilage degradation and joint destruction.

Key Indexing Terms: INTERLEUKIN 17 REGULATION TISSUE INHIBITOR OF METALLOPROTEINASE 1 MATRIX METALLOPROTEINASE 9 MONOCYTES/MACROPHAGES

Rheumatoid arthritis (RA) is a chronic disease characterized by sustained inflammation in synovial joints and concomitant destruction of articular cartilage and bone. During chronic joint inflammation, considerable thickening of the lining is characteristic, and the subsynovial tissue becomes infiltrated by numerous macrophages and lymphocytes. In the early stages of RA, an unknown antigen activates T cells to release cytokines, which in turn activate macrophages and fibroblasts, triggering the immune-inflammatory cascade of synovitis. The high density of activated macrophages in rheumatoid synovium suggests that these cells also play an important role in RA. Synovial lining macrophages are of crucial importance in the development of arthritis. When these cells are selectively eliminated from the joint by local application of “toxic” liposomes, the joint becomes markedly resistant to subsequent arthritis induction.

Cartilage degradation and joint destruction are often found in RA. The underlying molecular basis for matrix degradation is believed to be dependent on the actions of a variety of proteolytic enzymes. The matrix metalloproteinases (MMP) are a family of related enzymes with different substrate specificities, which together are capable of degrading all the components of the extracellular matrix. They also contribute significantly to tissue damage in chronic inflammatory diseases such as RA. MMP are first secreted from the cells as inactive proenzymes. Activation in vivo is thought to occur by the proteolytic cleavage involving plasmin and/or MMP such as stromelysin. Once activated, the control of enzyme activity is dependent on the local concentration of tissue inhibitors of metalloproteinases (TIMP) and on nonspecific protease inhibitors such as α2-macroglobulin.

Although the macrophage seems to play a cardinal role in established RA, T lymphocytes are likely to be essential for the initiation of rheumatoid synovitis. T cell secreting interleukin 17 (IL-17) is among the first to be activated during the immune response, suggesting that this cytokine...
may play an important role in the early stages of inflammation. We and others have reported that biologically active IL-17 was highly produced by RA but not osteoarthritis (OA) synovium, and the concentration of this cytokine was significantly elevated in the synovial fluid (SF) of patients with RA compared with OA.\(^{14,15}\) We described the potential of macrophages to synthesize MMP-9 upon stimulation by IL-17.\(^{14}\) MMP-9 was found at high levels in the SF of patients with RA.\(^{16-19}\) Abnormally high levels of this MMP may contribute to destructive processes of the joints in patients with RA; a correlation was found between the increased level of 92 kDa gelatinolytic activity in RA SF and disease severity.\(^{17-19}\) The activity of MMP-9 is thought to be regulated by activation levels of the proenzyme and inhibition by TIMP-1. Not only is TIMP-1 a potent inhibitor for gelatinase B, but the progelatinase B/TIMP-1 complex is also highly resistant to activation by stromelysin.\(^{20,21}\) Latent MMP-9 and/or MMP-9/TIMP-1 complexes were shown to be elevated in RA SF compared with OA SF.\(^{19}\)

We observed that IL-17 stimulated macrophages to increase TIMP-1 secretion and that this production was differentially regulated by antiinflammatory cytokines and prostaglandin E\(_2\) (PGE\(_2\)) compared with the regulation of MMP-9.

**MATERIALS AND METHODS**

**Monocyte/macrophage isolation from human blood.** Peripheral blood mononuclear cells (PBMC) consisting of lymphocytes and monocytes were isolated from heparinized blood samples from healthy donors (n = 24; mean age 35 yrs, range 26–52) as described.\(^{11}\) Briefly, the samples were diluted 1:2 with phosphate buffered saline (PBS) containing 3 U/ml preservative-free heparin (Sigma Diagnostic, St. Louis, MO, USA), centrifuged over Ficoll/Hypaque (Amersham Pharmacia Biotech, Baie d’Urfé, Québec, Canada), and washed 3 times in RPMI 1640 (Gibco-BRL, Life Technologies Inc., Gaithersburg, MD, USA) containing penicillin and streptomycin (100 U/ml, 100 µg/ml, respectively), and supplemented with 2 mM L-glutamine (Gibco-BRL). All reagents were endotoxin tested. The cells were seeded in untreated plastic petri dishes (Flow Laboratories Inc., McLean, VA, USA) at a density of 4 × 10\(^5\) cells/cm\(^2\). They were allowed to adhere to plastic dishes (60 min at 37°C) in a humidified atmosphere and 5% CO\(_2\) in RPMI medium.\(^{22}\) The nonadherent cells (mainly lymphocytes) were removed by vigorous washes with PBS. Monocytes/macrophages represented > 92% of the cell population, determined by flux cytometry and 260 nm, and the OD\(_{260}/OD_{280}\) was between 1.7 and 2.0 with no detectable genomic DNA contamination as judged by agarose gel electrophoresis. For Northern blot experiments, generally 5 µg total RNA was resolved on 1.2% formaldehyde-agarose gels and transferred electrophoretically to nylon membranes (Hybond-N, Amersham) in 10 mM sodium acetate buffer, pH 7.8, 20 mM Tris, and 0.5 mM EDTA overnight at 4°C. The RNA was crosslinked to the membranes by exposure to ultraviolet light, and hybridized to 10 ng/ml of the 92 kDa gelatinase or TIMP-1 DNA probe overnight at 50°C.

The plasmid encoding the 92 kDa gelatinase gene (kindly provided by Dr. G. Goldberg, Washington University, St. Louis, MO, USA) was digested with BglII and XbaI to obtain a 0.56 kb probe, as described.\(^{24}\) The human TIMP-1 cDNA probe (0.8 kb inserted into EcoRI sites of Bluescript) was kindly provided by Dr. R.H.L. Pang (Creative Biomolecules, Hopkinton, MA, USA). The DNA probes were labeled with digoxigenin (Boehringer Mannheim, Laval, Québec, Canada) according to the manufacturer’s specifications. Stringent serial posthybridization washes were conducted at 68°C, with the final wash in 0.1 × SSC, 0.1% SDS. Detection was carried out by chemiluminescence with CDP-Star substrate (Boehringer Mannheim) and exposure to Kodak X-AR5 film.

**Data analysis.** Values were expressed as mean ± SEM, where “n” refers to the number of different individuals. Statistical significance was assessed using Student’s t test. Significant differences were confirmed only when p < 0.05.

**RESULTS**

**Effects of IL-17 on macrophage TIMP-1 and MMP-9 synthesis.** To compare the effect of IL-17 on TIMP-1 and MMP-9 synthesis, dose and time response experiments were performed. There was a spontaneous (basal) release of MMP-9 and TIMP-1 by nonstimulated monocytes/macrophages that increased over time (Table 1). Of note, the level of spontaneously secreted proMMP-9 in 72 h culture supernatants (19.13 ± 2.28 ng/ml) was far less than the level...
of spontaneously secreted total TIMP-1 (137.42 ± 6.22 ng/ml). For proMMP-9, the spontaneous secretion was always < 50% of IL-17 stimulated proMMP-9. The possible effect of low levels of endotoxin on this spontaneous MMP-9 and TIMP-1 production was overcome by using polymixin B (10 µg/ml; data not shown). In 12 h cultures there was a 4.7-fold increase in TIMP-1 production by IL-17 stimulated cells compared with the basal value. MMP-9 was not detected. In 24, 48, and 72 h cultures, increased MMP-9 production occurred (4.1, 2.6, and 3.0-fold, respectively) relative to TIMP-1 production (2.0, 1.8, and 1.4-fold, respectively).

The TIMP-1 synthesis induced by IL-17 was dose dependent (Figure 1). Previously14 we had found that IL-17 at the highest concentration (200 ng/ml) induced about a 3-fold increase in the level of MMP-9 compared with the basal value. In the present work, 200 ng/ml IL-17 increased basal TIMP-1 production by 1.7-fold. TIMP-1 increased synthesis was related to upregulation at the gene expression level, as indicated by the increased levels of TIMP-1 messenger RNA (Figure 2). The maximal levels of TIMP-1 mRNA were found after 3 h stimulation with IL-17. At that time, TIMP-1 mRNA and MMP-9 mRNA (data not shown) were 1.4-fold and 1.5-fold higher than in the nonstimulated cells, respectively.

Effect of PGE2 and cAMP-mimetics on MMP-9 and TIMP-1 synthesis.

We previously reported that IL-17 stimulated the expression of cyclooxygenase 2 (COX-2) in a time dependent manner14, and strongly induced PGE2 secretion25. We have also shown that IL-17 induced MMP-9 production in human monocytes/macrophages was dependent on endogenous PGE2 synthesis14. In these experiments, we examined the effect of exogenous PGE2 and cAMP-mimetics on MMP-9 and TIMP-1 production. We found that exogenous PGE2 (2 µg/ml) almost completely inhibited both IL-17

### Table 1. Time course of MMP-9 and TIMP-1 secretion by IL-17 stimulated macrophages (100 ng/ml). MMP-9 and TIMP-1 in culture supernatants were measured by ELISA. Mean ± SEM (n = 6).

<table>
<thead>
<tr>
<th>Incubation Time, h</th>
<th>Nonstimulated Cells MMP-9, ng/ml</th>
<th>IL-17 Stimulated Cells MMP-9, ng/ml</th>
<th>Nonstimulated Cells TIMP-1, ng/ml</th>
<th>IL-17 Stimulated Cells TIMP-1, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>0</td>
<td>0.07 ± 0.07</td>
<td>7.94 ± 2.04</td>
<td>37.43 ± 3.35</td>
</tr>
<tr>
<td>24</td>
<td>0.88 ± 0.13</td>
<td>3.61 ± 0.52</td>
<td>31.05 ± 0.71</td>
<td>61.67 ± 8.70</td>
</tr>
<tr>
<td>48</td>
<td>10.50 ± 1.42</td>
<td>27.58 ± 1.05*</td>
<td>80.27 ± 5.96*</td>
<td>146.34 ± 3.88*</td>
</tr>
<tr>
<td>72</td>
<td>19.13 ± 2.28</td>
<td>58.29 ± 5.29*</td>
<td>137.42 ± 6.22*</td>
<td>194.36 ± 7.40*</td>
</tr>
</tbody>
</table>

*p < 0.05 versus control (12 hour cultured cells), Student's t test.
induced and basal MMP-9 production, while in the case of TIMP-1, only induced levels were inhibited (Figure 3). Of note, the concentration of exogenous PGE$_2$ used in these experiments is unphysiologically high. Additional experiments are being conducted in which dose dependent studies with physiological concentrations of PGE$_2$ are tested to elucidate the clinical relevance of this effect. Similar results were obtained using the cAMP-mimetics IBMX plus forskolin and cholera toxin. These results showed that the increased endogenous PGE$_2$ or cAMP concentration in IL-17 stimulated human monocytes/macrophages resulted in complete inhibition of MMP-9 protein synthesis, and inhibited stimulated TIMP-1 secretion. Spontaneous TIMP-1 production was not altered. These findings were confirmed by experiments in which the cAMP was added to cultures of IL-17 stimulated monocytes/macrophages (Figure 4). A dose-response inhibition of both MMP-9 and TIMP-1 was observed. Once again, spontaneous TIMP-1 production could not be inhibited by the increased intracellular cAMP concentration, indicating different mechanisms of MMP-9 and TIMP-1 regulation by PGE$_2$ and cAMP.

**Effects of antiinflammatory cytokines on TIMP-1 synthesis.** Ambient cytokines may influence the IL-17 effect on macrophages in vivo. We investigated whether antiinflammatory cytokines could modulate the IL-17 stimulated synthesis of TIMP-1. As illustrated in Figure 5, all 3 antiinflammatory cytokines inhibited IL-17 induced TIMP-1 secretion and, slightly but not significantly, the basal TIMP-1 production.

**Effects of protein kinase inhibitors on TIMP-1 synthesis.** We used different inhibitors of intracellular protein kinases to delineate postreceptor signaling pathways activated by IL-17 and involved in TIMP-1 synthesis (Figure 6). We found that specific inhibition of mitogen activated protein kinases (MAPK), the MEK-1/MEK-2 and p38, abrogated basal and IL-17 induced TIMP-1 synthesis, implicating these cascades in both basal and IL-17 stimulated signal transduction in
spontaneous and cytokine induced TIMP-1 synthesis. Interestingly, the transcription factor NF-κB was found to be involved in these regulations, since the inhibition of NF-κB by PDTC also decreased TIMP-1 production significantly.

**DISCUSSION**

Erosive synovitis, cartilage degradation, and joint destruction are often seen in chronic rheumatoid diseases such as RA. A variety of proteolytic enzymes elicited by both soft and hard tissue elements, as well as by inflammatory cells, are thought to be responsible for matrix degradation. Monocytes are recruited to the site of tissue injury or chronic inflammation by cell derived cytokines and chemotactic factors. Once at the site, activation of monocytes mediates the destruction of connective tissue. The activated monocytes/macrophages orchestrate connective tissue destruction either directly by the secretion of elevated MMP or indirectly by the production of proinflammatory cytokines including tumor necrosis factor-α (TNF-α) and IL-1. We and others have reported that IL-17 concentration was significantly elevated in RA SF and IL-17 strongly stimulated the secretion of the proinflammatory cytokines TNF-α and IL-1β in human macrophages and increased the production of MMP-9. Secreted cytokines can activate other cell types, for example fibroblasts, amplifying the pathogenetic cascade. On the other hand, the increased level of MMP-9 in RA SF may contribute to destructive joint processes.

We observed that monocytes/macrophages spontaneously produced measurable levels of TIMP-1. The spontaneous secretion of TIMP-1 by unstimulated macrophages is likely to have a physiological role in maintaining tissue homeostasis. This basal production was increased when cells were stimulated with IL-17. At 72 h, IL-17 induced 3-fold and 1.7-fold increases in the level of MMP-9 and TIMP-1, respectively, compared with basal values. Many cytokines are present in arthritic joints, and it seems reasonable that they could stimulate the production of both MMP and TIMP. Spontaneous production of MMP-9 and TIMP-1 is likely to be IL-1β and TNF-α independent, while these cytokines are not present in the cultures of unstimulated cells. The stimulatory effect of IL-17 on MMP-9 synthesis has been shown to be TNF-α dependent, whereas blocking of IL-1β had no effect. Additional experiments with neutralizing antibodies against IL-1β and TNF-α will determine whether the IL-17 effect on TIMP-1 was also secondary to the release of these inflammatory cytokines. MMP-9 is secreted by macrophages as a complex with TIMP-1. TIMP-1 is the best known of the 4 closely related TIMP. TIMP-1 secretion was abrogated. Although basal MMP-9 secretion was inhibitable by PDTC also decreased TIMP-1 production significantly.

**Figure 6.** Effect of NF-κB and protein kinase inhibitors on human monocyte/macrophage TIMP-1 synthesis. Cells were preincubated with inhibitors of NF-κB (PDTC), MEK-1/ERK-2 (PD98059), and p38 (SB202190) prior to addition of 100 ng/ml of IL-17 for 72 h. For details see Figure 1 legend. Mean ± SEM (n = 3). *p < 0.05 versus control (IL-17 stimulated cells). Statistical analysis by Student t test.
reported that PGE suppressed the production of proMMP-9 in rabbit articular chondrocytes, and PGE-induced cAMP downregulated IL-1-induced synthesis of MMP in human uterine cervical fibroblasts. Pharmacological concentrations of COX inhibitors and exogenous PGE suppressed TIMP-1 and proMMP-1. On the other hand, it has been shown that indomethacin suppressed the agonist induced production of TIMP and collagenase in human macrophages, but induced collagenase in synovial fibroblasts to a high degree. Thus, the effects of COX inhibitors and PGE on the production of TIMP and MMP appear to vary with the cell type.

It is now well known that cytokines function in conjunction with positive and negative feedback loops acting through pro and antiinflammatory mediators. We have reported that IL-4 and IL-13 inhibited both basal and IL-17 induced MMP-9 production by human monocytes, while IL-10 inhibited only the former. We report the absence of control of basal TIMP-1 production by antiinflammatory cytokines; those antiinflammatory cytokines downregulated only the IL-17 induced TIMP-1 synthesis. Even in the presence of IL-4, IL-10, or IL-13, substantial basal TIMP-1 synthesis occurred. These results once again suggest that basal TIMP synthesis may proceed via a pathway that is regulated differently by prostaglandin and antiinflammatory cytokines, compared to the basal MMP-9 synthesis. However, because specific inhibitors of mitogen activated protein kinases, namely MEK-1/MEK-2 and p38 as well as the NF-xB inhibitor, abrogated basal and IL-17 induced TIMP-1 synthesis, it is believed that these cascades are involved in both IL-17 stimulated signal transduction and basal activation.

MMP play a significant role in tissue morphogenesis and in chronic inflammatory lesions. In normal tissue, the levels of TIMP and other proteinase inhibitors are sufficient to inhibit any MMP activity. We have shown that TIMP can inhibit the autoactivation of MMP, and it is now accepted that the balance between MMP and TIMP is important in maintaining joint cartilage homeostasis. It has been shown that the proMMP concentration in RA SF is much higher than that of TIMP. We found that, compared to the upregulation of MMP-9, TIMP-1 was modestly stimulated by IL-17 in human monocytes/macrophages. However, both basal and IL-17 stimulated MMP-9 production was found to be sensitive to downregulation by prostaglandin and antiinflammatory cytokines, while only induced TIMP-1 synthesis responded to this type of control. Therefore, in a chronic rheumatoid disease such as RA, excessive MMP activity over TIMP activity in the invading pannus, periartricular tissue, or SF occurs as a result of increased MMP production relative to TIMP secretion. This may be the consequence of alteration in the balance between the proinflammatory and antiinflammatory cytokines and cytokine inhibitors in affected tissue.

REFERENCES


