Interleukin-18 Promotes Joint Inflammation and Induces Interleukin-1-Driven Cartilage Destruction

Leo A.B. Joosten,* Ruben L. Smeets,* Marije I. Koesters,* Liduine A.M. van den Berselaar,* Monique M.A. Helsen,* Birgitte Oppers-Walgreen,* Erik Lubberts,* Yoichiro Iwakura,† Fons A.J. van de Loo,* and Wim B. van den Berg*

From the Rheumatology Research Laboratory and Advanced Therapeutics,* University Medical Center Nijmegen, Nijmegen, The Netherlands; and the Center for Experimental Medicine,† Institute of Medical Science, University of Tokyo, Tokyo, Japan

Interleukin (IL)-18 is a member of the IL-1 family of proteins that exerts proinflammatory effects and is a pivotal cytokine for the development of Th1 responses. The goal of the present study was to investigate whether IL-18 induces joint inflammation and joint destruction directly or via induction of other cytokines such as IL-1 and tumor necrosis factor (TNF). To this end we performed both in vitro and in vivo kinetic studies. For in vivo IL-18 exposure studies C57BL/6, TNF-deficient, and IL-1-deficient mice were injected intra-articularly with 1.10⁷ pfu mIL-18 adenovirus followed by histopathological examination. Local overexpression of IL-18 resulted in pronounced joint inflammation and cartilage proteoglycan loss in control mice. Of high interest, IL-18 gene transfer in IL-1-deficient mice did not show cartilage damage, although joint inflammation was similar to that in wild-type animals. Overexpression of IL-18 in TNF-deficient mice showed that TNF was partly involved in IL-18-induced joint swelling and influx of inflammatory cells, but cartilage proteoglycan loss occurred independent of TNF. In vitro cartilage degradation by IL-18 was found after a 72-hour culture period. Blocking of IL-1 with IL-1Ra or an ICE-inhibitor resulted in complete protection against IL-18-mediated cartilage degradation. The present study demonstrated that IL-18 induces joint inflammation independently of IL-1. In addition, we showed that IL-1β generation, because of IL-18 exposure, was essential for marked cartilage degradation both in vitro and in vivo. These findings implicate that IL-18, in contrast to TNF, contributes through separate pathways to joint inflammation and cartilage destruction. (Am J Pathol 2004, 165:959–967)
transforming growth factor-β. Furthermore, IL-18 expression in synovial tissue biopsies from rheumatoid arthritis patients with clinically active disease is associated with enhanced IL-1β and TNF-α levels. In addition, IL-18 induces the expression of CXC chemokines by synovial fibroblasts, stimulates angiogenesis, and is involved in leukocyte recruitment by up-regulation of vascular adhesion molecule-1 through nuclear factor-κB-dependent mechanisms.

Preclinical studies have shown that IL-18 is a primary cytokine that promotes both systemic and local cytokine production. Administration of IL-18 alone or in combination with IL-12 increased the severity of murine type II collagen (CIA) arthritis. Blockade of endogenous IL-18 during the onset of disease in an acute model of joint inflammation, significantly reduced local TNF-α and IL-1β levels. In line with these findings, blockade of IL-18 with antibodies or with the endogenous inhibitor IL-18BP suppressed the disease activity in murine CIA. Interestingly, intra-articular overexpression of IL-18BP using an adenoviral vector for murine IL-18BP ameliorated disease activity and suppressed joint destruction in CIA. Neutralization of local IL-18 activity was accompanied by reduction of TNF-α and IL-6 levels in the joint.

It was demonstrated previously that IL-18 induces chondrocyte proliferation; up-regulates mRNA expression of inducible nitric oxide synthetase, stromelysin (MMP-3), and cyclooxygenase 2 (COX2) in cultured chondrocytes; and increases cartilage glycosaminoglycan release in vitro. In contrast to these observations, several investigations have shown that IL-18 exposure of different cell types, such as peripheral blood mononuclear cells and macrophages, did not lead to the production of NO, COX-2, or PGE2. In the present study we examined whether IL-18 induces inhibition of chondrocyte proteoglycan synthesis and cartilage proteoglycan depletion directly or via induction of other mediators, such as IL-1 and TNF. Local gene transfer technology was used to explore the direct proinflammatory role of IL-18 in naïve murine knee joints. In vitro studies with cartilage explants were performed to get more insight in IL-18-driven cartilage destruction.

**Materials and Methods**

**Animals**

Male C57/BL6 mice were obtained from Charles River, Sulzfeld, Germany. Breeder pairs of TNF-α-deficient mice were kindly provided by Prof. Dr. G. Kollias, Athens, Greece. IL-1β gene-deficient mice were a kind gift from Merck, Rahway, NJ. Breeder pairs of IL-1α,β-deficient mice were provided by Prof. Dr. Y. Iwakura, Tokyo, Japan. The breeder pairs were controlled for cytokine deficiency by genotyping, according standard protocols. The mice were housed in filter top cages, and water and food were provided *ad libitum*. The mice were used at the age of 10 to 12 weeks. Care was taken to house all of the deficient and control littermate mice under identical conditions. All animal experiments conducted in this study were cared for in accordance with the institutional ethics committee.

**Materials**

Bovine serum albumin was purchased from Sigma Chemical Co., St. Louis, MO. RPMI 1640 medium was obtained from Life Technologies, Breda, The Netherlands. Recombinant murine IL-1β, IL-1Ra, IL-18, and TNF-α were purchased from R&D Systems, Abingdon, UK. The ICE inhibitor Boc-Asp(Obz)-CMK (N-1430) was obtained from Bachem AG, Bubendorf, Switzerland. Recombinant human IGF-1 was purchased from Preprotech, Rocky Hill, NJ. Radioactive 35S-sulfate was purchased from NEN Life Sciences Products, Boston, MSA. Bioplex kits for multicytokine determination were purchased from Bio-Rad, Hercules, CA.

**Adenoviral mIL-18 Vector and Intra-Articular Gene Transfer**

Recombinant adenovirus AdmIL-18 was constructed with insertion of the murine pro-IL-18 cDNA in the early regions 1 (E1) and 3 (E3), respectively. Expression of cDNA was driven by the human cytomegalovirus immediate early gene promoter and terminated by the polyadenylation sequence of SV40. The virus was produced by co-transfection of 293 cells with the plasmid. Large scale production was performed in conjunction with Prof. Dr. J. Kolls from the Department of Medicine, Louisiana State University, New Orleans, LA. Transfection with this adenoviral construct results in active mIL-18 production, both *in vivo* and *in vitro*. As a control we used the empty recombinant replication-defective adenovirus Ad5del70-3. Gene transfer was performed by intra-articular injection of naïve mice with 107 pfu/μl of AdmIL-18 or Ad5del70-3. At different time points, patellae with adjacent tissue were dissected and patellae washouts were used for the determination of IL-18, IL-1β, or TNF-α levels. In addition, we examined joint swelling (days 2, 4, and 7) and histopathology (days 7 and 14) after mIL-18 gene transfer.

**Measurement of Joint Inflammation**

Joint inflammation was quantified by 99mTc-uptake method. This method measures by external gamma counting the accumulation of a small radioisotope at the site of inflammation because of local increased blood flow and tissue swelling. The severity of inflammation is expressed as the ratio of the 99mTc-uptake in the right (inflamed) over the left (control) knee joint. All values exceeding 1.10 were assigned as inflammation.

**Cytokine Measurements**

To determine levels of several cytokines, including IL-1β, IL-18, and TNF-α in patellae washouts, patellae were isolated from inflamed knee joints as previously de-
sensitivity of the multiplex kit was for IL-18/gp100 ng/ml) for either 24, 48, or 72 hours. Thereafter the levels IL-18, IL-1β, or TNF were determined by using the Luminex bead array system. The sensitivity of the kits was <10 pg/ml for each cytokine. The data represents the mean ± SD of six patellae washout per time point. ND, not detectable.

Table 1. Local Cytokine Production after IL-18 Gene Transfer

<table>
<thead>
<tr>
<th></th>
<th>IL-18</th>
<th>IL-1β</th>
<th>TNF-α</th>
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<tbody>
<tr>
<td>Day 1</td>
<td>AdmIL-18 820 ± 122</td>
<td>AdmIL-18 45 ± 15</td>
<td>AdmIL-18 100 ± 34</td>
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<tr>
<td>Day 2</td>
<td>AdmIL-18 512 ± 78</td>
<td>AdmIL-18 76 ± 34</td>
<td>AdmIL-18 74 ± 23</td>
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<tr>
<td>Day 4</td>
<td>AdmIL-18 325 ± 56</td>
<td>AdmIL-18 178 ± 65</td>
<td>AdmIL-18 56 ± 41</td>
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<tr>
<td>Day 7</td>
<td>AdmIL-18 200 ± 49</td>
<td>AdmIL-18 137 ± 41</td>
<td>AdmIL-18 34 ± 29</td>
</tr>
<tr>
<td>Day 14</td>
<td>AdmIL-18 125 ± 23</td>
<td>AdmIL-18 32 ± 28</td>
<td>AdmIL-18 21 ± 18</td>
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Male C57BL6 mice were intra-articularly injected at day 0 with 1.107 pfu of either AdmIL-18 or Ad5Del70-3. At several time points, patellae with adjacent synovial tissue were isolated and cultured for 1 hour at room temperature in RPMI 1640 medium (completed with 1% bovine serum albumin). Thereafter the levels IL-18, IL-1β, or TNF were determined by using the Luminex bead array system. The sensitivity of the kits was <10 pg/ml for each cytokine. The data represents the mean ± SD of six patellae washout per time point. ND, not detectable.

In Vitro Proteoglycan Degradation Assay
Patellae with minimal surrounding tissue were isolated and cultured in RPMI 1640 medium containing 0.1% bovine serum albumin (200 µl/patella) for 24, 48, or 72 hours. Thereafter supernatant was harvested and centrifuged for 5 minutes at 1000 × g. Cytokine levels were determined using the Luminex multiplex system with 50 µl of patella washout medium. The sensitivity of the multiplex kit was for IL-1β, IL-18, or TNF-α 5, 20, and 5 pg/ml, respectively.

Histology
Mice were sacrificed by ether anesthesia. Thereafter, whole knee joints were removed and fixed for 4 days in 4% formaldehyde. After decalcification in 5% formic acid the specimens were processed for paraffin embedding. Tissue sections (7 µm) were stained with hematoxylin and eosin (cell influx) or Safranin O (cartilage proteoglycan depletion). Histopathological changes were scored using the following parameters. Infiltration of cells was scored on a scale of 0 to 3, depending on the amount of inflammatory cells in the synovial cavity and synovial tissues. The loss of proteoglycans was scored on a scale of 0 to 3, ranging from full stained cartilage to destained cartilage. Histopathological changes in the knee joints were scored in the patella/femur region on five semiserial sections of the joint, spaced 70 µm apart. Scoring was performed on decoded slides by two observers, as described previously.

Chondrocyte Proteoglycan Synthesis
Patellae with minimal surrounding tissue were isolated from knee joints of naive C57/BL6 mice. Thereafter, patellae were cultured in RPMI 1640 medium, glutamax, and gentamycin (50 µg/ml) supplemented with rhIGF-1 (250 ng/ml) with or without IL-1 (10 ng/ml) or IL-18 (10 ng/ml) for either 24, 48, or 72 hours. Thereafter the patellae were placed in RPMI 1640 medium with glutamax, gentamycin (50 µg/ml), and 35S-sulfate (0.74 MBq/ml). After 3 hours of incubation at 37°C in a CO2 incubator, patellae were extensively washed in sterile saline three times and were cultured for 24, 48, or 72 hours in either RPMI 1640 medium or 0.1% bovine serum albumin and 250 ng/ml of recombinant human IGF-1. Patellae were exposed to IL-1β or IL-18 with or without inhibitors/antagonists. Thereafter, patellae were fixed in 4% formaldehyde and subsequently decalcified in 5% formic acid for 4 hours. Patellae were punched out of the adjacent tissue, dissolved in 0.25 ml of LumaSolve at 65°C (Ominlabo, Breda, The Netherlands), and after addition of 1 ml of Lipoluma (Ominlabo) the 35S content was measured by liquid scintillation counting (Trilux 1450 microbeta; EG&G Wallac, Turku, Finland). Values are presented as percentage of 35S incorporation of the left control joint.

Statistical Analysis
Differences between experimental groups were tested using the Mann-Whitney U-test unless stated otherwise.

Results
Intra-Articular Overexpression of IL-18 Results in Delayed Joint Swelling and Suppression of Chondrocyte Metabolic Function
To investigate the effect of prolonged IL-18 exposure in vivo, an adenovirus coding for murine IL-18 was injected in the right knee joint of naive C57/BL6 mice. Table 1 shows that IL-18 was highly expressed in a mouse knee joint after adenoviral gene transfer, using 1.107 pfu of AdmIL-18. Enhanced levels of IL-18 could be found up to day 14 after injection of the adenovirus coding for IL-18. Although not vehemently, levels of both IL-1β and TNF-α
were locally elevated after injection of AdIL-18 (Table 1). In contrast, injection of control adenovirus (Ad5del70-3) did lead to slightly enhanced levels of IL-1β, IL-18, or TNF-α, only detectable at day 1 after virus injection (Table 1). Local overexpression of IL-18 in wild-type mice resulted in protracted joint inflammation, as determined by joint swelling assessment (Figure 1A). A gradually increased joint swelling was observed after local injection of AdIL-18. At day 2 we noted a right/left ratio of 1.2 ± 0.05 that increased to 1.4 ± 0.08 at day 14. In addition, we analyzed the suppressive effect of high IL-18 levels on chondrocyte proteoglycan metabolism. Despite high IL-18 levels at days 1 and 2, no inhibition of chondrocyte proteoglycan synthesis was noted (Figure 1A). Significant suppression of chondrocyte proteoglycan synthesis was found after day 4. This was in line with the increasing IL-1β and TNF-α levels found in patellar washouts after injection of adIL-18 virus.

To examine the role of either IL-1 or TNF in the IL-18-driven joint swelling we injected 1.10^7 pfu of adIL-18 in IL-1- or TNF-α-deficient mice. Lack of TNF-α resulted in suppressed joint swelling after local injection of the IL-18 adenoviral vector, as visualized in Figure 1B. In contrast to TNF-deficient mice, mice lacking both forms of IL-1 did not differ from the wild-type animals at all examined days after application of the IL-18 vector, indicating no role of IL-1α or IL-1β in the IL-18-driven joint swelling (Figure 1B).

**IL-18 Drives Influx of Inflammatory Cells and Cartilage Degradation by Separate Pathways**

Because extended IL-18 exposure in vivo caused enhanced joint swelling we examined whether IL-18 overexpression initiated influx of inflammatory cells and cartilage degradation. Figure 2B shows clearly that IL-18 generated an influx of cells in the joint cavity of wild-type C57/BL6 mice. Both at days 7 and 14 enhanced cell influx was noted, although the number of cells was less pronounced at day 14 (data not shown). Remarkably lower numbers of inflammatory cells were found in the joint cavity of TNF-deficient mice compared to wild-type animals (Figure 2D). In contrast to TNF gene knockout mice, overexpression of IL-18 in IL-1α,β-deficient mice resulted in a similar influx of inflammatory cells in synovial tissue as in wild control mice (Figure 2C). These data indicate that influx of inflammatory cells, induced by local IL-18 application, is driven mainly by TNF-α.

In addition, we analyzed the catabolic effect of extended IL-18 exposure in vivo on cartilage. Figure 3B demonstrates nicely that IL-18 gene transfer resulted in loss of matrix proteoglycans from the cartilage of a wild-type mouse. Both at days 7 and 14 enhanced cartilage proteoglycan degradation was seen, although the strongest proteoglycan depletion was found at 14 days after intra-articular injection of adenoviral vector coding for IL-18. In contrast to the role of TNF-α in the attraction of proinflammatory cells, provoked by IL-18 overexpression, we noted no differences in cartilage proteoglycan depletion between wild-type and TNF-α-deficient mice (Figure 3D). Of high interest, IL-1α,β gene-deficient mice were almost completely protected against cartilage proteoglycan loss, induced by extended IL-18 exposure in vivo (Figure 3C).

**IL-18 Induces Delayed Cartilage Degradation in Vitro**

To obtain more insight in the mechanism of IL-18-mediated cartilage damage we investigated the effect of IL-18 on both chondrocyte proteoglycan synthesis and cartilage matrix degradation. Therefore, we exposed patellar cartilage explants with minimal adjacent synovial tissue to IL-18 in an in vitro culture system. Figure 4A shows that
IL-18 did not induce significant inhibition of chondrocyte proteoglycan synthesis in vitro after a 72-hour culture period, even at a concentration of 100 ng/ml of IL-18. In contrast to IL-18, IL-1β strongly inhibits chondrocyte proteoglycan synthesis (50% inhibition compared to IGF-1 control) already after 24 hours of culture. Previous reports indicated that IL-18 induces catabolic responses in chondrocytes, but we found no suppressive effect on chondrocyte metabolic function, determined as chondrocyte proteoglycan synthesis. In additional studies, we examined the catabolic effect of IL-18 on in vitro cartilage degradation. Sulfate-prelabeled patellar cartilage explants were cultured up to 72 hours with either IL-18 or IL-1β. Figure 4B shows late cartilage degradation after exposure to both 10 and 100 ng/ml of IL-18. We found that nearly 40% of the prelabeled cartilage was released after a 72-hour culture period. Interestingly, no cartilage proteoglycan-loss was observed after 24 or 48 hours of exposure with IL-18. In contrast to IL-18, IL-1β already induced cartilage degradation after 24 hours of stimulation (Figure 4B). Interestingly, the degree of cartilage proteoglycan release was comparable at 72 hours between IL-18 (100 ng/ml) and IL-1β (10 ng/ml).

**IL-18-Driven Cartilage Degradation in Vitro is IL-1β-Dependent**

To investigate whether IL-1α or IL-1β was involved in the IL-18-driven cartilage degradation, we blocked IL-1 receptor signaling and de novo production of IL-1β. To this end we added either IL-1Ra or an IL-1-converting enzyme (ICE) inhibitor to the in vitro cultures. Figure 5A shows that addition of 1 μg/ml of human IL-1Ra completely blocked the IL-18-driven cartilage proteoglycan loss as found after 72 hours of culture. Inhibition of IL-1β-induced cartilage proteoglycan degradation in vitro by IL-1Ra confirmed the efficacy of used concentration of IL-1Ra. Using the ICE inhibitor we demonstrated that newly processed IL-1β was responsible for the observed IL-18-driven cartilage degradation in vitro (Figure 5B).

To exclude that TNF-α, induced by IL-18 exposure, was involved in the observed proteoglycan degradation we used cartilage explants from TNF-α-deficient mice. Figure 5C indicates that TNF-α was not a crucial cytokine in the IL-18-induced cartilage proteoglycan loss. We found no difference in IL-18-mediated cartilage degradation between the wild-type and TNF-α-deficient mice after
72 hours of IL-18 exposure. Using patellae from IL-1β knockout mice confirmed the findings with the ICE inhibitor, that IL-18-induced cartilage degradation is mediated by IL-1β generation (Figure 5C).

Discussion

The present study was performed to investigate the direct role of IL-18 in joint inflammation and/or cartilage damage. To this end we overexpressed murine IL-18 locally and analyzed joint inflammation and cartilage damage. The role of either IL-1 or TNF in IL-18-driven joint inflammation was studied by using mice deficient for either IL-1 or TNF. The in vivo observations were confirmed by in vitro cartilage degradation studies. Therefore, we exposed murine cartilage explants to IL-18 and analyzed the catabolic effect of IL-18. Using IL-1 inhibitors and/or cartilage explants from IL-1β or TNF-deficient mice we clearly demonstrated that IL-1β was the pivotal second mediator in IL-18-driven cartilage destruction.

Prolonged overexpression of IL-18 in naive murine knee joint, using an adenovirus coding for murine IL-18, resulted in mild joint inflammation and cartilage proteoglycan loss (Figures 1, 2, and 3). Recent reports indicated a role of IL-18 in the pathogenesis of several human inflammatory diseases. Elevated IL-18 levels can be found in psoriasis, inflammatory bowel disease, and sarcoidoses. Several preclinical studies have shown that co-administration of IL-18 enhanced the inflammatory response to antigens such as collagen type II. This is the first study that showed that IL-18 overexpression causes joint inflammation that accumulates in time (Figures 1A and 2). In line with previous findings, IL-18 attracts predominantly neutrophils into the joint tissues, although at later stages (day 14) for the most part monocytes/macrophages were seen in the synovial lining (data not shown). By using TNF-deficient mice we found that IL-18-driven joint inflammation was partly TNF-dependent. Joint swelling was completely absent and the influx of inflammatory cells was reduced in TNF gene-deficient mice. We have shown previously that TNF is the pivotal cytokine that drives swelling in acute SCW-induced joint inflammation. IL-18 itself can induce chemokines and chemoattractant factors (eg, IL-8 and LTB4) that may explain the partly TNF-independent cell influx. Interestingly, overexpression of IL-18 in a naive knee joint leads to cartilage damage, determined as loss of matrix proteoglycans. The loss of matrix proteoglycans was IL-1-dependent because IL-18 overexpression in IL-1α,β-deficient mice did not result in cartilage damage. It is well
known that IL-1 is the crucial cytokine that promotes cartilage destruction via induction of several catabolic mediators in synovial lining cells as well as in chondrocytes. IL-1 was originally identified as catabolin because it could induce cartilage destruction.\textsuperscript{49,50} Blocking studies with antibodies or IL-1 receptor antagonist (IL-1Ra) in models of arthritis clearly showed that IL-1 drives cartilage and bone destruction.\textsuperscript{43,51} Recently, it was demonstrated that intra-articular injection of IL-1Ra in patients with painful knee osteoarthritis had a dramatic therapeu-

Figure 4. Prolonged exposure to IL-18 induced cartilage degradation in vitro. A: Patellar cartilage explants were isolated from naive knee joints of C57/Bl6 mice and cultured for 24 to 72 hours in RPMI 1640 medium supplemented with recombinant hIGF-1 (250 ng/ml) with or without mIL-18. Thereafter chondrocyte proteoglycan synthesis was determined by \textsuperscript{35}S-sulfate incorporation. For details see Materials and Methods. B: For cartilage degradation studies cartilage explants were prelabeled with \textsuperscript{35}S-sulfate. Data expressed the mean ± SD of six patellae per group. The experiments were repeated twice with similar outcome. *, P < 0.01, Mann-Whitney U-test compared to IGF-1 control group.

Figure 5. In vitro cartilage degradation by IL-18 is mediated by IL-1. Patellar cartilage explants were prelabeled as indicated in Materials and Methods. Thereafter, cartilage explants were cultured for 72 hours with IGF-1-containing medium with either IL-18 (10 or 100 ng/ml) or IL-1β (10 ng/ml). To block IL-1 we added either recombinant mIL-1Ra (A, 10 μg/ml) or ICE-inhibitor (B, 2.5 μmol/L) to the culture medium. C: To confirm the ICE data and examine the role of TNF in IL-18-driven proteoglycan loss we use patellar explants from both IL-1β- and TNF-α-deficient mice. For details see Figure 4. *, P < 0.01, Mann-Whitney U-test compared to C57/Bl6 × 129Sv wild-type mice control group.

tic response, indicating once more the pivotal role of IL-1 in cartilage catabolism.\textsuperscript{52}

Here we showed that prolonged exposure to IL-18 \textit{in vitro}, up to 72 hours, did not result in inhibition of chondrocyte proteoglycan synthesis. Even at high concentrations (100 ng/ml) no suppressive effects of IL-18 were noted (Figure 2B). In contrast, IL-1β induced substantial inhibition of chondrocyte metabolic function already at 24 hours and at low concentrations (1 ng/ml). Because it is known that NO is the causative agent for suppression of chondrocyte proteoglycan synthesis\textsuperscript{51} we analyzed NO production of cartilage explants after IL-18 exposure. In contrast to IL-1, we found low levels of NO in supernatants after 48 hours or 72 hours of culture with 10 ng/ml or 100 ng/ml IL-18 (data not shown). This is in line with a previous study indicating that IL-18 could produce NO in cultured chondrocytes,\textsuperscript{15} although the levels of NO were higher in cultured chondrocytes then in cartilage explants. Despite the enhanced NO levels in IL-18-exposed cartilage explants, no inhibition of chondrocyte proteoglycan synthesis was found. This indicates that although the signaling pathway of IL-18 and IL-1 are similar, IL-18 seems to generate a protective mechanism against NO-mediated suppression of chondrocyte metabolic function. It is demonstrated that heme oxygenase-1 (HO-1) plays a crucial role in NO production, because this enzyme catalyzes heme protein, which is a co-factor for the NOS-2 enzyme. More recently, it was shown that IL-1 and IL-17 down-regulate HO-1 activity, which may explain the increased
NO production by chondrocytes and the induction of inhibition of chondrocyte proteoglycan synthesis.\(^\text{5,3}\) IL-18 regulates induction of HO-1 expression via both Erk/MAPK and PI3K/Akt pathways that can be activated by IL-18 receptor signaling.\(^\text{5,4,5}\) At the moment, investigations are performed to unravel the IL-18-induced protection on chondrocyte anabolic metabolism.

IL-18 induces degradation of cartilage matrix molecules after extended exposure (Figure 4B). Olee and colleagues\(^\text{15}\) showed that IL-18 could stimulate release of proteoglycans from human articular cartilage. Whether this was because of increased degradation or enhanced synthesis of proteoglycans was not investigated. Although IL-18 exposure of cartilage resulted in rapidly increased mRNA levels of several catabolic mediators, such as MMP-3 and MMP-9, no degradation of proteoglycans was noted up to 48 hours of culture. Here we report that production of a second messenger molecule was responsible for the delayed cartilage destruction by IL-18. Blockade of IL-18 or the activation of pro-IL-18 was sufficient to protect the cartilage explants from proteoglycan release (Figure 5, A and B). It is known that IL-18 initiates production of several cytokines, including IL-1 and TNF, in monocytes and neutrophils.\(^\text{26,56}\) In addition, very recently it was shown that IL-18 stimulates monocyte IL-1 and TNF production, induced by contact to activated T cells isolated from rheumatoid arthritis synovium.\(^\text{57}\) Although IL-18 can induce TNF mRNA production in chondrocytes (data not shown), TNF is not involved in IL-18-mediated cartilage proteoglycan loss in vitro (Figure 5C).

In conclusion, this study indicates that IL-18 promotes cartilage proteoglycan loss, both in vitro and in vivo, mediated by IL-1 generation. Although IL-18 can induce both IL-1 and NO production in chondrocytes it is not competent to generate inhibition of chondrocyte proteoglycan synthesis. Local overexpression of IL-18 resulted in a delayed influx of inflammatory cells that is partly TNF-dependent. These data implicate that IL-18 can contribute to joint inflammation and cartilage destruction by separate pathways. Targeting of IL-18 during inflammatory joint diseases, such as rheumatoid arthritis, may provide a novel therapy because IL-18 promotes development of immunity and contributes to cartilage destruction via IL-1 production.

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**References**


