INTRODUCTION: Post-traumatic arthritis (PTA) is one of the most frequent causes of disability following trauma of weight-bearing joints. While the exact etiology of the disease is not well understood, it is hypothesized that pro-inflammatory cytokines such as interleukin 1 (IL-1) or tumor necrosis factor alpha (TNF-α) may play a key role. Recent studies also suggest that chemokines and their receptors play important roles in rheumatoid arthritis and other forms of inflammatory arthritis. For example, the chemokine CXCL10, or interferon-inducible cytokine (IP-10), is upregulated in the serum and plasma of patients with these diseases. CXCL10 protein secretion is increased in normal and osteoarthritic (OA) human chondrocytes by IL-1 and TNF-α, but its effect on cartilage and its role in PTA has yet to be determined.

We hypothesized that CXCL10 is upregulated following intra-articular fracture and contributes to cartilage degeneration associated with PTA. The objectives of this study were to: (1) compare gene expression of CXCL10 in synovial tissue from knee joints of C57BL/6 mice that develop PTA following articular fracture and MRL/MpJ mice that are protected from PTA; (2) assess CXCL10 protein expression in normal, OA, and post-traumatic human cartilage; and (3) determine the effects of exogenous CXCL10 on IL-1 mediated catabolism of cartilage explants.

METHODS: CXCL10 Expression in Mouse Articular Fracture Model. All procedures were performed in accordance with an IACUC approved protocol. At 16 weeks of age C57BL/6 (Charles River Labs) and MRL/MpJ (Jackson Laboratory) male mice were sacrificed to represent the pre-fracture condition (n=6 per strain) or received moderate articular fractures of the left tibial plateau and then were sacrificed (n=6 per strain) at 0, 1, 3, 5, and 7 days post-fracture.

At sacrifice, joint capsule tissue was pooled from both knees of 6 animals at each time point. RNA was isolated, and RT-PCR was performed in duplicate using the mouse inflammatory cytokines and receptors RT Profiler PCR array (SA Biosciences). Relative mRNA expression was determined for both the difference from pre-fracture to each time point (threefold change considered significant).

CXCL10 Protein Expression in Human Articular Cartilage. Cartilage was collected from cadavers with no history of joint injury (Normal; n=7), patients with end stage OA undergoing joint replacement (OA; n=11), and from patients with traumatic joint injuries (Trauma; n=29). De-identified human surgical waste tissues were collected under an IRB waiver (Table 1).

Table 1. Patient demographics for human cartilage.

<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>Sex (M/F)</th>
<th>Joint (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>40-70</td>
<td>3 / 3</td>
</tr>
<tr>
<td>OA</td>
<td>53-69</td>
<td>5 / 6</td>
</tr>
<tr>
<td>Trauma</td>
<td>19-77</td>
<td>18 / 11</td>
</tr>
</tbody>
</table>

All tissue samples were formalin-fixed, paraffin-embedded, sectioned at 8 µm, and immunostained for CXCL10 (AF-266-NA, R&D Systems). Positive CXCL10 staining in each cartilage zone was noted.

Effects of Exogenous CXCL10 on Cartilage Explants. Explants (5 mm diameter) were harvested from the femoral condyles of skeletally mature female pigs. Explants (n=6 per group) were equilibrated for 72 hours and then incubated with combinations of 0 or 100 ng/ml of porcine CXCL10 (Abcam) and 0, 10, or 100 ng/ml of porcine IL-1α (R&D Systems). Media were assessed for total matrix metalloproteinase (MMP) activity, aggrecanase activity (Anaspec), sulfated glycosaminoglycan (S-GAG) release, and nitric oxide (NO) production. A two-factor ANOVA and Newman-Keuls post-hoc test were performed to determine significant differences.

RESULTS: CXCL10 Expression in Mouse Articular Fracture Model. Following fracture, C57BL/6 mice showed 8 to 25 fold increases in CXCL10 gene expression, as compared to 3 to 7 fold increases in MRL/MpJ mice (Figure 1). Prior to fracture there were no strain differences in CXCL10 expression, however following articular fracture C57BL/6 mice had 7 to 14 fold elevated CXCL10 gene expression, compared to MRL/MpJ mice at all time points.

DISCUSSION: CXCL10 gene expression is upregulated in synovium following articular fracture in the mouse knee. MRL/MpJ mice showed significantly lower levels of CXCL10 expression than C57BL/6 mice, consistent with reduced levels of IL-1 and TNF in these joints. In addition, following articular fracture, human chondrocytes showed the highest CXCL10 protein expression. However, exogenous CXCL10 did not induce a pro-inflammatory response and trended towards being anti-inflammatory in cartilage explants.

These data in combination suggest that although CXCL10 in joint tissues is upregulated following trauma or with inflammatory stimulus, CXCL10 does not directly induce catabolism of articular cartilage but may play a role in modulating inflammation. More studies are needed to determine the exact role of CXCL10 in arthritis, including PTA.

SIGNIFICANCE: CXCL10 is upregulated by pro-inflammatory mediators that are important in PTA. CXCL10 is not pro-inflammatory but may play a role in modulating inflammation during the progression of PTA.


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