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Soluble IL-6 Receptor Governs IL-6 Activity in Experimental Arthritis: Blockade of Arthritis Severity by Soluble Glycoprotein 130

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Studies in IL-6-deficient (IL-6−/−) mice highlight that IL-6 contributes to arthritis progression. However, the molecular mechanism controlling its activity in vivo remains unclear. Using an experimental arthritis model in IL-6−/− mice, we have established a critical role for the soluble IL-6R in joint inflammation. Although intra-articular administration of IL-6 itself was insufficient to reconstitute arthritis within these mice, a soluble IL-6R-IL-6 fusion protein (HYPER-IL-6) restored disease activity. Histopathological assessment of joint sections demonstrated that HYPER-IL-6 increased arthritis severity and controlled intrasynovial mononuclear leukocyte recruitment through the CC-chemokine CCL2. Activation of synovial fibroblasts by soluble IL-6R and IL-6 emphasized that these cells may represent the source of CCL2 in vivo. Specific blockade of soluble IL-6R signaling in wild-type mice using soluble gp130 ameliorated disease. Consequently, soluble IL-6R-mediated signaling represents a promising therapeutic target for the treatment of rheumatoid arthritis. The Journal of Immunology, 2003, 171: 3202–3209.

Although elevated IL-6 concentrations have been documented in the serum and synovium of arthritic patients (1–5), the molecular mechanism by which it contributes to the pathogenesis of rheumatoid arthritis (RA) is the subject of much debate (6, 7). A pivotal role for IL-6 is, however, suggested by several experimental models of arthritis, in which induction of disease in IL-6-deficient (IL-6−/−) mice results in no clinical signs or significantly reduced arthritis (8–12). Indeed, many of the systemic symptoms associated with RA, such as high fever, autoantibody production, and regulation of the acute-phase response, have been attributed to IL-6, while Abs directed against IL-6 or its cognate receptor (IL-6R) have proven to be clinically beneficial (5, 13–19). However, the manner by which localized IL-6 responses are regulated within an inflamed joint and the consequence of its action remain far from clear.

It is increasingly apparent that many of the activities assigned to IL-6 are mediated via soluble IL-6R (sIL-6R) (20, 21). This soluble receptor forms a stimulatory complex with IL-6, which triggers cellular events through the ubiquitously expressed signal-transducing subunit for all IL-6-related cytokines, gp130 (22). Soluble IL-6R-mediated signaling thus enables activation of cell types that would not normally respond to IL-6 itself (20, 21). Interestingly, the cellular distribution of the cognate IL-6R is largely restricted to hepatocytes, megakaryocytes, and leukocytes, which infer that sIL-6R-mediated signaling represents the prominent mechanism by which IL-6 elicits its effects in vivo. Analysis of synovial fluids from arthritic patients highlights that increases in synovial sIL-6R levels correlate with the degree of leukocyte infiltration and correspond with the more advanced stages of RA (5, 23). Synovial sIL-6R concentrations might therefore originate from infiltrating leukocytes and may ultimately control IL-6 signaling within an inflamed arthritic joint. This is supported by in vitro studies, which have implicated the involvement of sIL-6R in osteoclast formation, synovial fibroblast proliferation, and cartilage degradation (23–26).

Using a murine model of monoarticular Ag-induced arthritis, we have examined the significance of sIL-6R signaling within the synovium and established that blockade of sIL-6R activity by its natural antagonist soluble gp130 (sgp130) inhibits arthritis progression. Thus, use of the inhibitory properties of sgp130 may represent a novel therapeutic strategy for the treatment of RA.

**Materials and Methods**

**Synovial fluid**

Ethical approval was obtained from Bro-Taf Health Authority (Cardiff, Wales, U.K.) before commencing the study. All patients were diagnosed as having RA for at least 3 mo. Such patients used nonsteroidal anti-inflammatory drugs, corticosteroids, and disease-modifying antirheumatic drugs (with the exception of TNF inhibitors) either alone or in combination. These regimes are in line with conventional rheumatology clinical practice. Synovial fluids were collected from RA (n = 25) and osteoarthritis (OA; n = 15) patients during routine joint aspiration. Fluids were rendered cell free by centrifugation and supernatants stored at −80°C. Before ELISA determination, synovial fluids were diluted 50:50 (v/v) in PBS containing 1% BSA.

**Animals**

Experiments were undertaken in 7- to 8-wk-old male inbred C57BL/6j wild-type (IL-6−/−) and IL-6−/− mice (27). Procedures were performed in accordance with Home Office-approved project license PPL-30/1820.

**Induction of murine Ag-induced arthritis (AIA)**

Briefly, male mice were s.c. immunized with 1 mg/ml of methylated BSA (mBSA) emulsified with an equal volume of CFA and injected i.p. with 100 μl heat-inactivated *Bordetella pertussis* toxin (all reagents were from...
Sigma-Aldrich, St. Louis, MO). The immune response was boosted 1 wk later. Twenty-one days after the initial immunization, AIA was induced by intra-articular (i.a.) administration of 10 mg/ml mBSA in the right hind limb. To modulate IL-6 signaling in vivo, AIA was induced by i.a. administration with mBSA in conjunction with 50 ng/ml IL-6, 50 ng/ml HYPER-IL-6, or 500 ng/ml sgp130 (R&D Systems, Minneapolis, MN). In all cases, the total volume administered i.a. was 10 μl. HYPER-IL-6 was prepared, as previously described (28).

Assessment of anti-mBSA titers

Anti-mBSA titers were monitored in sera from IL-6⁺/⁻ and IL-6⁻/⁻ mice immunized with 1 mg/ml mBSA. Briefly, microtiter plates were coated with 5 μg/ml mBSA and blocked with 5% (w/v) milk in PBS containing 0.05% Tween 20. Sera were diluted as indicated in PBS containing milk extract and Tween 20. Bound Abs were detected using HRP-conjugated goat anti-mouse IgG as a secondary Ab. Absorbance changes were monitored at 450 nm, and values were presented as the mean ± SD from four mice per condition.

Histological assessment of arthritis

Arthritis severity was judged by comparing the difference between the AIA (right hind limb) and control (left hind limb) joints. Animals were inspected daily for arthritis development by measuring the knee joint diameter using a digital micrometer. Inflammatory parameters were assessed in six animals (per condition) sacrificed at days 3 and 21 post-i.a. over two separate experiments. Joints were fixed in neutral buffered formal saline and decalcified with formic acid at 4°C before embedding in paraffin. Mid-saggital serial sections (of 7 μm thickness) were stained with hematoxylin/safranin-O. Two independent observers blinded to the experimental groups scored these sections. Synovial hyperplasia (pannus formation), cellular exudate, and cartilage depletion/bone erosion were scored from 0 (normal) to 3 (severe). Synovial infiltrate was scored from 0 to 5. All parameters were subsequently summed to give an arthritis index (mean ± SEM).

Immunohistochemistry

Localization of Abs in paraffin sections was detected using an HRP-DAB (diaminobenzidine) Cell & Tissue Staining kit from R&D Systems. To eliminate endogenous peroxidase activity, sections were treated with a peroxidase-blocking reagent (R&D Systems). Sections were subsequently treated with 10% normal donkey serum and an avidin/biotin blocking reagent (R&D Systems) to reduce nonspecific staining before labeling with either 2 μg/ml goat anti-CCL2, anti-CCR2, or isotype control Abs (Auto-gen Bioclear, Calne, U.K.). All reagents were diluted in PBS containing 1% BSA. Ab labeling was detected using a high sensitivity streptavidin-HRP conjugate and DAB chromogen (R&D Systems). Intensity of staining was measured in sections of synovium taken from three to four separate animals. Images were digitally captured using a Neotec ImagineGrubber PCI, and the DAB staining intensity was analyzed using Aequitas IA software (Dynamic Data Links, Cambridge, U.K.). Three randomly chosen areas of synovium were measured per section.

Human synovial fibroblasts (HSF)

HSF were isolated from synovial tissue obtained after synovectomy from RA patients at the time of total knee joint replacement. HSF were isolated and maintained, as previously described (29). Briefly, HSF were digested for 1 h at 37°C with 1 mg/ml collagenase (Sigma-Aldrich). Cells were cultured in DMEM medium containing 100 U/ml penicillin/streptomycin; 1 μM hydrocortisone, 1 μl insulin-transferrin-selenium-X, and 20% heat-inactivated FCS (from Life Technologies Invitrogen, Paisley, U.K.). All stimulations were performed on growth-arrested HSF, and cells were exclusively used between the third and seventh passage. Cells were stimulated with 20 ng/ml IL-6 and 0–100 ng/ml PC-sIL-6R (R&D Systems), or DS-sIL-6R, or 0–100 ng/ml HYPER-IL-6, as indicated in the figure legends. DS-sIL-6R was prepared, as previously described (30, 31). In control experiments, 2 μg/ml of a blocking anti-gp130 Ab was added (mAb-228; R&D Systems).

**ELISA**

Matched Ab pairs and protein standards for use in IL-6, sIL-6R, CCL5, CXCL8, CXCL9, and CXCL10 ELISA were obtained from R&D Systems. CCL2, CCL3, CCL4, and CCL11 were quantified using matched Ab pairs from BD PharMingen (San Diego, CA) and Amersham Pharmacia Biotech (Piscataway, NJ). Assays were performed in accordance with the manufacturer’s instructions. Detection of DS-sIL-6R was conducted, as previously described (31).

**RT-PCR**

RNA was extracted from HSF with 500 μl TRI-reagent (Sigma-Aldrich) and processed according to the manufacturer’s protocol. Total RNA (0.5 μg) was reverse transcribed using random hexanucleotide primers and Superscript II Reverse Transcriptase (Life Technologies Invitrogen). β-Actin oligonucleotide primers were used as a normalization control. RT-PCR for CCL2 and β-actin was performed, as described (32).

**FACS**

Analysis of gp130 and IL-6R on HSF was performed, as previously described (27).

**Statistical methods**

Statistical analysis was performed using one-way unpaired ANOVA. Values of p < 0.05 were considered statistically significant.

**Results**

Detection of sIL-6R isoforms in synovial fluids of arthritic patients

Examination of human synovial fluids obtained from 40 independent donors (25 RA; 15 OA) showed that synovial IL-6 levels were significantly elevated (p < 0.05) in RA patients (51.17 ± 9.46 ng/ml) as compared with those diagnosed with OA (6.39 ± 3.35 ng/ml). Synovial concentrations of sIL-6R were also significantly more elevated in individuals with RA (28.54 ± 2.39 ng/ml) than OA (14.78 ± 2.11 ng/ml), and these levels directly correlated (r = 0.57) with those of IL-6 (p < 0.001; Fig. 1). Moreover, when appreciating the significance of sIL-6R during disease, it is noteworthy to consider that differential mRNA splicing (DS) and proteolytic (shedding) cleavage (PC) of the cognate receptor both lead to the generation of sIL-6R (termed DS-sIL-6R and PC-sIL-6R) (20). These isoforms can be distinguished using Abs against the...
Reconstitution of sIL-6R signaling in IL-6\(^{-/-}\) and IL-6\(^{+/+}\) mice by i.a. administration of mBSA in PBS. a, Joint swelling after i.a. injection. Data are mean ± SEM of a single measurement of swelling from all mice remaining in the experiment before sacrifice for histology. Data from IL-6\(^{-/-}\) mice injected (i.a.) with HYPER-IL-6 alone, without prior immunization with mBSA are also included (open boxes). b, Histopathological analysis of hematoxylin/safranin-O-stained sections from experimental mice sacrificed on day 3. Six mice were sacrificed in each group over two separate experiments. Two blinded independent observers scored each slide. Synovial hyperplasia, synovial infiltrate, cellular exudate, and joint erosion values were totaled to give an arthritis index. **, p < 0.01; ***, p < 0.001. Hematoxylin/safranin-O-stained mid-sagittal sections from IL-6\(^{+/+}\) and IL-6\(^{-/-}\) mice sacrificed on day 3 of AIA are shown in c–e. Representative micrographs are shown from mBSA-challenged IL-6\(^{+/+}\) mice (c); IL-6\(^{-/-}\) mice (d); and IL-6\(^{-/-}\) mice administered with HYPER-IL-6 (e). Femoral and tibial heads are on the left and right, respectively. The synovium is at the top of each micrograph, and the meniscus is in the center. Scale bar represents 100 \(\mu\)m.

novel COOH-terminal sequence (GSRRRGSCGL) of DS-sIL-6R, which is introduced through the splicing process (30, 31). Using such an approach, we observed that expression of DS-sIL-6R was confined to patients with RA (0.07 ± 0.03 ng/ml) and was not detected in any of the synovial fluids obtained from individuals with OA (Fig. 1). These increases in DS-sIL-6R did not reflect systemic changes in sIL-6R levels (data not shown; see also Ref. 31), and implies that DS-sIL-6R production occurs locally within the inflamed synovium during active disease.

Reconstitution of sIL-6R signaling in IL-6\(^{-/-}\) mice restores experimental arthritis

The monoarthritis AIA model has previously been used to show that IL-6 has an effect on both the induction and effector phase of arthritis (9). To examine the role of sIL-6R signaling within an arthritic joint, we have monitored AIA in wild-type (IL-6\(^{+/+}\)) and IL-6\(^{-/-}\) mice. As shown in Fig. 2a, IL-6\(^{+/+}\) mice displayed substantial joint swelling upon induction of arthritis with mBSA, which continued for ~14 days post-i.a. administration. Conversely, the degree of joint swelling observed in IL-6\(^{-/-}\) mice was markedly reduced, and by day 3 showed no significant difference from the contralateral nonarthritic limb (Fig. 2a). To confirm that the swelling response was the product of local IL-6 activity, signaling was reconstituted within the joint space of IL-6\(^{-/-}\) mice by administration of mBSA in combination with 50 ng/ml IL-6 or HYPER-IL-6 (i.a.). HYPER-IL-6 is a unimolecular sIL-6R-IL-6 fusion protein, which prevents the natural dissociation-association of the ligand receptor complex and ensures signaling only occurs via sIL-6R (28). Administration of IL-6 only marginally increased joint swelling over the first 3 days of arthritis induction, while HYPER-IL-6 restored the profile of joint swelling to that encountered in IL-6\(^{+/+}\) mice (Fig. 2a). In agreement with previous studies, anti-mBSA titers were lower in IL-6\(^{-/-}\) than IL-6\(^{+/+}\) mice (9, 10); however, i.e. administration of HYPER-IL-6 did not affect the Ab titer detected in IL-6\(^{-/-}\) mice, emphasizing that joint swelling in response to HYPER-IL-6 was not Ab dependent (Fig. 3). Finally, to ensure that...
the joint swelling associated with HYPER-IL-6 treatment was not simply inherent to this chimeric protein, HYPER-IL-6 was administered i.a. to IL-6−/− mice that had not received prior challenge from mBSA, and were therefore not prone to Ag-induced arthritis. Under these conditions, no joint swellings were encountered over the course of the experiment, which infers that restoration of arthritis severity by HYPER-IL-6 is a product of mBSA-mediated Ag-mediated arthritis (Fig. 2a).

To establish the severity of disease in IL-6−/− and IL-6+/+ mice, histopathological analysis of joint sections was performed on samples collected on day 3 postarthritis induction. Arthritis severity was graded as an index of synovial hyperplasia, synovial infiltrate, cellular exudate, and joint erosion. Fig. 2b shows that arthritis was significantly more advanced in IL-6−/− mice than that observed in IL-6+/+ mice. Indeed, all of the histological parameters assessed were markedly reduced in IL-6+/+ mice (Table I). However, reconstitution of sIL-6R-mediated signaling in IL-6−/− mice with HYPER-IL-6 restored the arthritic index to that encountered in IL-6+/+ mice (Fig. 2b and Table I). Representative micrographs from each condition as seen on day 3 of arthritis are presented in Fig. 2, a–c. Administration (i.a.) of HYPER-IL-6 to mBSA-challenged IL-6−/− mice also significantly (p < 0.05) increased the degree of joint erosion seen on day 21 in IL-6−/− mice; however, restoration of arthritis severity was not comparable to the extent observed in IL-6+/+ mice (Table II). Consequently, sIL-6R-mediated signaling may primarily influence early inflammatory processes; however, it is unclear whether a single administration of HYPER-IL-6 is sufficient to elicit events 21 days later.

**HYPER-IL-6 controls mononuclear cell infiltration in vivo through induction of CCL2**

Based on the histological observations presented in Table I, we next tested whether HYPER-IL-6-6 orthologues mononuclear leukocyte infiltration during experimental AIA. Immunohistochemistry using Abs against the CC-chemokine CCL2 and its receptor CCR2 was performed on sequential joint sections prepared from IL-6−/− mice, in which arthritis had been induced for 3 days. As shown in Fig. 4, CCL2 staining within the synovial tissue of IL-6−/− mice was poorly defined, whereas the intensity of staining was dramatically increased in IL-6+/+ mice receiving HYPER-IL-6. Expression of CCL2 colocalized with staining for CCR2 and supported a role for sIL-6R in regulating mononuclear leukocyte infiltration (Fig. 4).

**The sIL-6R isofoms regulate CCL2 expression in synovial fibroblasts in vitro**

The immunolocalization of CCL2 within joint section emphasized that synovial-lining cells may be major producers of this inflammatory chemokine. To address this issue, and to substantiate the involvement of sIL-6R in leukocyte recruitment, we tested the ability of the sIL-6R isofoms to regulate chemokine expression in human synovial fibroblasts (HSF). FACs analysis of HSF using Abs against the cognate IL-6R and the signal-transducing element gp130 confirmed that these cells express gp130, but show no detectable levels of sIL-6R (Fig. 5a). Consequently, HSF only respond to IL-6 in the presence of sIL-6R. As shown in Fig. 5b, stimulation of HSF with IL-6 in combination with either PC- or DS-sIL-6R significantly increased the expression of CCL2 in both a time- and dose-dependent manner. Similar data were also obtained using HYPER-IL-6 (Fig. 5b), while sIL-6R-mediated CCL2 production was specifically inhibited (70 ± 2%; n = 3 independent experiments) by inclusion of a blocking anti-gp130 Ab at the time of stimulation. Stimulation of HSF with IL-6 and it soluble receptor isoforms, however, failed to induce expression of the chemokines CCL3, CCL4, CCL5, CCL11, CXCL8, CXCL9, and CXCL10, while the cellular expression of ICAM-1 and VCAM-1 remained unaltered.

**Blockade of experimental arthritis in IL-6+/+ mice by sgp130**

To confirm a role for sIL-6R in the progression of AIA, IL-6+/+ mice were coadministered on the day of arthritis induction with mBSA and 500 ng/ml sgp130 (i.a.). This naturally occurring antagonist selectively inhibits the cellular events elicited by the sIL-6R/IL-6 complex and does not affect IL-6 signaling via its cognate IL-6R (33). Histological analysis of day 3 joint sections showed that all parameters of disease severity were suppressed in response to sgp130, and no significant difference was observed between the histological scores attributed to IL-6−/− mice and those assigned to IL-6+/+ mice exposed

### Table I. Joint inflammation and cartilage damage on day 3 of AIA

<table>
<thead>
<tr>
<th>Species</th>
<th>Treatment</th>
<th>Hyperplasia</th>
<th>Synovial infiltrate</th>
<th>Synovial exudate</th>
<th>Erosions</th>
<th>Arthritis Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6−/−</td>
<td>PBS only</td>
<td>1.50 ± 0.23</td>
<td>2.58 ± 0.23</td>
<td>2.50 ± 0.19</td>
<td>0.42 ± 0.15</td>
<td>7.00 ± 0.46</td>
</tr>
<tr>
<td>IL-6+/+</td>
<td>PBS only</td>
<td>1.07 ± 0.19†</td>
<td>1.78 ± 0.18*</td>
<td>1.24 ± 0.19***</td>
<td>0.00 ± 0.00**</td>
<td>4.07 ± 0.43***</td>
</tr>
<tr>
<td>IL-6−/−</td>
<td>HYPER-IL-6</td>
<td>1.50 ± 0.25†</td>
<td>2.12 ± 0.18†</td>
<td>2.25 ± 0.21†</td>
<td>0.25 ± 0.11†</td>
<td>6.13 ± 0.43†</td>
</tr>
<tr>
<td>IL-6+/+</td>
<td>sgp130</td>
<td>0.90 ± 0.41†</td>
<td>1.70 ± 0.42†</td>
<td>0.80 ± 0.33†</td>
<td>0.00 ± 0.00†</td>
<td>3.4 ± 1.07†</td>
</tr>
</tbody>
</table>

* Synovial hyperplasia, cellular infiltrate, and exudate into the synovial cavity were observed in hematoxylin/safranin-O-stained sections. Comparisons were made against control IL-6+/+ mice values by one-way unpaired ANOVA. † NS; *p < 0.05; **p < 0.01; ***p < 0.001.

### Table II. Joint inflammation and cartilage damage on day 21 of AIA

<table>
<thead>
<tr>
<th>Species</th>
<th>Treatment</th>
<th>Hyperplasia</th>
<th>Synovial infiltrate</th>
<th>Synovial exudate</th>
<th>Erosions</th>
<th>Arthritis Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6−/−</td>
<td>PBS only</td>
<td>2.00 ± 0.30</td>
<td>2.70 ± 0.26</td>
<td>1.20 ± 0.20</td>
<td>1.60 ± 0.27</td>
<td>7.50 ± 0.78</td>
</tr>
<tr>
<td>IL-6+/+</td>
<td>PBS only</td>
<td>0.43 ± 0.14****</td>
<td>0.86 ± 0.18***</td>
<td>0.07 ± 0.07***</td>
<td>0.00 ± 0.00**</td>
<td>1.35 ± 0.17***</td>
</tr>
<tr>
<td>IL-6−/−</td>
<td>HYPER-IL-6</td>
<td>0.67 ± 0.18*</td>
<td>1.33 ± 0.14***</td>
<td>0.17 ± 0.11**</td>
<td>0.25 ± 0.13***</td>
<td>2.14 ± 0.29***</td>
</tr>
<tr>
<td>IL-6+/+</td>
<td>sgp130</td>
<td>0.80 ± 0.36*</td>
<td>1.50 ± 0.45*</td>
<td>0.70 ± 0.30†</td>
<td>0.80 ± 0.29†</td>
<td>3.80 ± 1.34*</td>
</tr>
</tbody>
</table>

* Synovial hyperplasia, cellular infiltrate, and exudate into the synovial cavity were observed in hematoxylin/safranin-O-stained sections. Comparing criteria were as described in Table I. Comparisons were made against control IL-6+/+ mice values by one-way unpaired ANOVA. † NS; *p < 0.05; **p < 0.01; ***p < 0.001.
to sgp130 (Fig. 6, Table I). Indeed, treatment of IL-6−/− mice with sgp130 significantly reduced the intensity of CCL2 staining to levels comparable with that seen in IL-6+/+ mice (Fig. 6, e and f). This suppression of arthritis severity in response to sgp130 was not due to changes in anti-mBSA levels because sgp130 did not affect anti-mBSA titers in IL-6+/+ mice (Fig. 3).

To establish whether blockade of the cellular processes seen early in AIA development was also evident in the latter stages of the model,

**FIGURE 5.** The sIL-6R isoforms stimulate CCL2 expression in vitro. a, Expression of IL-6R subunits (IL-6R and gp130) on HSF was analyzed by flow cytometry, and the data were acquired from 5000 gated events. b, HSF were growth arrested for 48 h and stimulated as indicated with 20 ng/ml IL-6 and increasing doses of the sIL-6R isoforms over a period of 24 h. Time-dependent release of CCL2 in response to IL-6 and 50 ng/ml PC-sIL-6R or DS-sIL-6R. CCL2 levels were quantified using ELISA. Data represent the mean ± SEM from five independent donors. Inset, RT-PCR analysis performed using CCL2 and β-actin-specific oligonucleotide primers. Data are shown for HSF stimulated with IL-6 in combination with PC-sIL-6R (PC) and DS-sIL-6R (DS). The time- and dose-dependent release of CCL2 in response to HYPER-IL-6 is also shown.
arthritis was induced in IL-6−/− and IL-6+/+ mice, and evidence of cartilage/bone erosion and synovial hyperplasia was monitored 21 days later (Fig. 7). IL-6−/− mice displayed a highly significant reduction (vs IL-6+/+ mice) in all parameters of joint inflammation and cartilage damage, and this was paralleled by data obtained from sgp130-treated IL-6+/+ mice. Indeed, sgp130 significantly reduced synovial hyperplasia, synovial infiltrate/exudate, and the degree of joint destruction observed on day 21 (Table II).
Discussion

Although high IL-6 concentrations have been documented in the serum and synovial fluids of patients with various arthritides, cell types resident to the joint (chondrocytes, synoviocytes, fibroblasts, and endothelial cells) lack expression of a cognate IL-6R (5). Consequently, these cells remain unresponsive to IL-6 itself. IL-6 signaling through its soluble receptor may therefore represent the prominent mechanism by which IL-6 elicits its effect during arthritis. Analysis of synovial fluids from arthritic patients has highlighted that increases in sIL-6R correlate with the extent of joint destruction, and correspond with more advanced stages of RA (23, 34). In this respect, in vitro studies have implicated an involvement for sIL-6R in synovial fibrosis, bone resorption, and other inflammatory processes (23–26). Using an experimental model of arthritis, we now conclude that sIL-6R governs the IL-6 signal within an inflamed joint and contributes to an exacerbation of the disease process. Specifically, i.a. reconstitution of sIL-6R-mediated signaling within IL-6−/− mice led to a thickening of the synovial lining, cellular hyperplasia, and increased leukocyte infiltration and joint destruction.

The absence of a cognate IL-6R on the major structural cells of the joint infers that these are unlikely to contribute to synovial sIL-6R concentrations. Because expression of IL-6R is largely restricted to hepatocytes and leukocytes (20), increases in synovial sIL-6R levels might originate from infiltrating leukocytes. In this respect, leukocytes release sIL-6R following challenge with inflammatory mediators (27, 35–37), while the degree of leukocyte infiltration into an arthritic joint correlates with synovial concentrations of sIL-6R (5). Consequently, sIL-6R might act as a paracrine mediator localized to its site of generation, and in this capacity influences leukocyte recruitment during inflammation (21). Several studies have highlighted a role for sIL-6R in the regulation of chemokine and adhesion molecule expression (27, 36–41), while sIL-6R-mediated signaling appears to control transition between the early neutrophilic stage of acute inflammation and the more sustained mononuclear cell influx (27). Such a scenario may be relevant to arthritis, in which neutrophils play an essential role in the initiation and progression of RA, and chronic maintenance of mononuclear leukocytes within an arthritic joint exacerbates the disease (42, 43). Through analysis of AIA in IL-6−/− and IL-6+/− mice, we observed that IL-6 deficiency is associated with reduced synovial infiltration and is accompanied by a defect in both CCL2 expression and the recruitment of leukocytes bearing the CCL2 receptor CCR2. Leukocytes expressing CCR2 (monocytes and activated T cells (44)) play a central role in the persistence of chronic inflammation in RA, and CCL2 may invariably represent an important factor in the progression of arthritis. Examination of our pool of synovial fluids indicates that CCL2 levels are significantly more elevated in RA (3.61 ± 0.92 ng/ml) than OA (1.66 ± 0.41 ng/ml) patients, while blockade of CCL2 activity prevents onset of autoimmune arthritis in MRL−1pr mice (45). Significantly, development of this arthritis model has been associated with elevated sIL-6R levels (46).

Although it is evident from our studies that sIL-6R-mediated signaling regulates the influx of mononuclear leukocytes, IL-6 may also contribute to their maintenance at sites of inflammation. IL-6 has previously been reported to rescue T cells from apoptosis through induction of Bcl-2 and Bcl-xL, while blockade of sIL-6R signaling in experimental colitis prevents disease onset by suppressing expression of these antiapoptotic regulators (47, 48). Such processes may be relevant to arthritis, because T cells extracted from RA tissue remain resistant to apoptosis and show increased Bcl-xL expression (43, 49). Indeed, development of autoimmune arthritis in mice possessing a mutated gp130 subunit has been related to the inability of the T cell population to undergo apoptosis-induced cell death (50). Consequently, sIL-6R signaling may not only regulate leukocyte infiltration (as shown in this study), but might also exacerbate disease through maintaining leukocytes within the inflamed joint.

The inability of IL-6 to solely restore experimental arthritis in IL-6−/− mice is intriguing. This infers that sIL-6R must be generated within the synovium, but that the source of its production is unlikely to originate for cell types resident to the joint. Activation of leukocytes is known to liberate sIL-6R (27, 35–37), and leukocyte recruitment may be the rate-limiting event in regulating IL-6-mediated responses. In this respect, i.a. administration of IL-6 had no effect on the degree of leukocyte influx seen in IL-6−/− mice (data not shown). Consequently, IL-6 may not restore the arthritic severity because synovial sIL-6R concentrations within the joint are not sufficiently elevated to ensure optimal IL-6 signaling. For example, formation of the sIL-6R/IL-6 complex is known to prolong the circulating t1/2 of IL-6 (51). In this respect, specific blockade of sIL-6R-mediated signaling with the antagonist splg130 in IL-6−/− mice was shown to suppress arthritis severity, while the unimolecular nature of HYPER-IL-6 ensures that reconstituted signaling does not occur via the cognate IL-6R (21). Overall, these data emphasize that sIL-6R controls IL-6 responses within the inflamed joint, which in humans uses two distinct sIL-6R isoforms (DS- and PC-sIL-6R). At present, the importance of each form is unclear; however, release of these isoforms in vivo appears to depend on the individual’s age, disease condition, and stage of inflammation (20, 27, 31). During acute inflammation, initial increases in sIL-6R correlate with neutrophil infiltration and are released via proteolytic cleavage, whereas increases in DS-sIL-6R coincide with the influx of mononuclear leukocytes (27). We now report that detection of DS-sIL-6R is exclusively confined to synovial fluids from RA patients, which did not reflect changes in systemic sIL-6R levels. This implies that DS-sIL-6R is secreted within the inflamed synovium during active disease. In this respect, DS-sIL-6R has been found to be released from mononuclear cells and T lymphocytes (30, 31), and as such may represent a clinical marker of disease activity. Whether this means that DS-sIL-6R activity is associated with mononuclear leukocyte responses remains to be determined.

The observation that sIL-6R-mediated signaling contributes to the degree of arthritis severity emphasizes that specific therapeutic targeting of this soluble receptor may represent a novel strategy for the treatment of chronic inflammatory conditions, such as RA. In this respect, splg130 has been shown to block experimental colitis (48), while neutralizing Abs against either IL-6 or its cognate receptor have proven to be effective in alleviating RA progression (15–19). In this present study, specific inhibition of sIL-6R-mediated signaling by splg130 effectively suppressed the histopathological hallmarks of arthritis in IL-6−/− mice. Significantly, several isoforms of splg130 have been described (21). However, one form known as gp130-RAPS (RA antigenic peptide-bearing soluble form) has recently been identified as an RA autoantigen, and serum Abs against gp130-RAPS not only correlate with indices of disease activity, but also neutralize gp130-RAPS activity (52). Although the significance of these findings needs to be clarified, splg130 may symbolize a valuable addition to the current arsenal of therapeutics that have proven so effective in the management of chronic conditions such as RA.

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