Effects of soluble interleukin-1 type II receptor on rabbit antigen-induced arthritis: clinical, biochemical and histological assessment


Arthritis Biology, Department of Arthritis and Bone Metabolism and Biomolecules Production, Core Technology Area, Novartis Pharma AG, Basel, Switzerland

Abstract

Objectives. To investigate the effects of soluble interleukin-1 (IL-1) type II receptor (sIL-1RII) on a number of clinical, biochemical and histological parameters in rabbit antigen-induced arthritis.

Methods. Arthritis was induced by intra-articular injection of methylated bovine serum albumin (mBSA) into rabbits pre-sensitized to the same antigen. An initial i.v. bolus of sIL-1RII was administered, followed by s.c. mini-pump dosing for 14 days, starting at the time of the arthritis induction. Animals received vehicle (saline 500 µl + 5 µl/h), low-dose sIL-1RII (13.4 µg + 1.34 µg/h) or high-dose sIL-1RII (40.2 µg + 4.02 µg/h).

Results. Marked, dose-related inhibition of joint diameter, plasma prostaglandin E₂ (PGE₂), and synovial fluid IL-1α and IL-1β concentrations were seen after administration of sIL-1RII. However, synovial fluid PGE₂ concentrations and synovial fluid cell counts were not affected. A significant inhibitory effect was also seen histologically on soft-tissue swelling and joint damage with high-dose sIL-1RII.

Conclusions. These results demonstrate that IL-1 plays an important role in the pathogenesis of rabbit antigen-induced arthritis, thus confirming it as an excellent animal model with respect to evaluating anti-cytokine therapies for rheumatoid arthritis.

Key words: Arthritis, Inflammation, IL-1α, IL-1β, IL-1ra, sIL-1RII, Rabbit, Histology.
was prepared, purified and tested for biological activity as described previously (paper submitted).

Rabbits were sensitized intradermally at two sites to methylated bovine serum albumin (mBSA) homogenized 1:1 with complete Freund’s adjuvant on days −28 and −14 (0.5 ml containing 4 mg/ml mBSA). On day −7, a skin test was performed to assess the immune status of the animal by examining the delayed-type hypersensitivity (DTH) reaction to the soluble antigen on days −6 and −5. The results of this test were used to assign the animals to the three matched treatment groups. On day 0, a blood sample was taken from each animal and the initial right and left joint diameters measured. The rabbits were then sedated with 1 mg/kg s.c. acepromazine before anaesthesia with an ultra-short-acting barbiturate (methohexitol sodium, 10 mg/kg i.v.) for the intra-articular injections and s.c. implantation of the mini-pumps (Alzet Corp.). The right knee received 0.5 ml of 2 mg/ml mBSA in 5% glucose solution (antigen-injected knee), while the left knee received 0.5 ml of 5% glucose solution alone (vehicle-injected knee). Mini-pumps were aseptically implanted between the shoulder blades into a s.c. pouch produced by blunt dissection. At the same time, the rabbits received a bolus i.v. injection of either vehicle (500 μl), low-dose sIL-1RII (13.4 μg) or high-dose sIL-1RII (40.2 μg) to cover the initial period until the mini-pumps started accurate s.c. drug delivery. The knee joint diameters were again measured on days 1, 2, 7, 9 and 14 after the intra-articular injection and further blood samples taken at the same time points, except on day 1. Blood samples were centrifuged, the cell-free plasma aliquoted and stored at −20°C prior to assay.

On day 14, the rabbits were killed by an overdose of thiopentone barbiturate, and a sample of synovial fluid was lavaged from each knee with 1 ml of heparinized buffered saline. Total cell numbers were counted on a Z1 Coulter Counter, the samples centrifuged and the cell-free synovial fluid aliquoted and frozen at −20°C prior to assay. The knees were then processed for undecalcified histology using a Histodur plastic embedding method (Leica AG, Germany). Sections (5 μm) from both the control and arthritic knees were cut on a Polycut S heavy-duty sledge microtome (Leica AG, Germany). After staining adjacent sections with toluidine blue (metachromatic stain for cartilage proteoglycans) and haemotoxylin and eosin (general morphology stain), the slides were number coded as left knee/ right knee pairs from each animal. The slides were read in a blinded fashion (JD). The left knee joint from each animal was examined for comparison, immediately prior to scoring the arthritic right knee joint from the same animal, to establish the anatomy of the non-arthritic knee in each case. A subjective scoring system was used to assess inflammatory cell infiltrate/hyperplasia, cartilage proteoglycan loss and erosive damage to bone and cartilage, where 1 is the minimum score and 5 is the maximum score for each parameter compared to the control sections (left knee) in each case. A detailed explanation of the scoring system used for each of the individual histological parameters is given in Table 1. The tissue swelling, observed between the outer ends of both menisci and the joint capsule, was measured on the joint sections using a ruled graticule and expressed in millimetres.

Rabbit IL-1α and IL-1β were measured in the synovial fluid samples using commercially available RIA kits from Endogen, following the manufacturer’s instructions. Concentrations of PGE₂, in both plasma and synovial fluid samples, were determined using a Biotrak EIA kit from Amersham.

Table 1. Description of the scoring system used to assess the histological parameters

<table>
<thead>
<tr>
<th>Score</th>
<th>Cell infiltrate/hyperplasia</th>
<th>Joint damage/erosions</th>
<th>Loss of proteoglycan*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Minimal infiltration/hyperplasia, lining cell layer still only 2–3 cells</td>
<td>Minimal (size and number) erosions of hard tissues at margins and in central region of joint</td>
<td>Minimal loss of surface metachromasia in &lt;10% of articular cartilage</td>
</tr>
<tr>
<td>2</td>
<td>Mild infiltration/hyperplasia with lining layer 5–10 cells, some pannus formation</td>
<td>Small erosions in notch region of femur and centre of tibia and at marginal cartilage–pannus junctions</td>
<td>Moderate loss of surface metachromasia in 10–30% of articular cartilage or marked loss &lt;10%</td>
</tr>
<tr>
<td>3</td>
<td>Moderate infiltration/hyperplasia, obvious intimal and subintimal layers, &gt;10 cells, definite pannus formation</td>
<td>Larger deeper erosions in same areas as in 2</td>
<td>Moderate loss of metachromasia in 30–60% of articular cartilage or severe loss &lt;30%</td>
</tr>
<tr>
<td>4</td>
<td>Marked infiltration/hyperplasia, organized pannus and lining layers</td>
<td>Erosions starting to break into the subchondral trabecular structure of epiphyses, definite marginal erosions</td>
<td>Marked loss of metachromasia in &gt;60% of articular cartilage or severe loss in 30–60% especially at the margins</td>
</tr>
<tr>
<td>5</td>
<td>Severe infiltration/hyperplasia with lining layers, pannus and distinct mononuclear cell foci</td>
<td>Erosions breaking into full depth of the epiphyses and deeply into the marginal sub-chondral bone, ‘hook erosions’</td>
<td>Severe loss of metachromasia in &gt;60% of articular cartilage</td>
</tr>
</tbody>
</table>

*The degree of loss of articular cartilage metachromasia staining can easily be determined by comparison to the metachromasia observed in the growth plate cartilages.
Results

Injection of mBSA in 5% glucose intra-articularly resulted in an arthritis which was observed clinically as a measurable increase in joint diameter and reluctance to put weight on the antigen-injected leg. This limping is most likely to result from pain in the injected knee, but the pain could not be clinically investigated further, in an objective way, during this experiment.

The effects of sIL-1RII on the degree and time course of joint swelling are shown in Fig. 1. In the control animals, the rapid increase in joint diameter was maximal on day 2, declining gradually thereafter to day 14. In sIL-1RII-treated animals, there was dose-related inhibition of knee swelling which followed the swelling pattern in the control animals, albeit at a reduced level. The reduction was statistically significant on day 7 in the low-dose group, and on days 1, 2 and 7 in the high-dose group.

Figure 2 shows the effect of sIL-1RII on histological parameters in rabbit antigen-induced arthritis. The graph shows that there was marked and significant inhibition of soft-tissue swelling at both low and high doses of sIL-1RII. A significant effect was also seen on the reduction of joint erosions at the high dose, while no effects were observed on inflammatory cell infiltration or proteoglycan loss.

The effects of sIL-1RII on synovial fluid concentrations of IL-1α are shown in Fig. 3. In the control and low-dose sIL-1RII animals, the concentration of IL-1α was higher in the synovial fluid from the arthritic knee compared to the control knee. In contrast, the synovial fluid concentrations in the high-dose group were not significantly different in the control and arthritic knees, but the high-dose right knee IL-1α concentration was significantly reduced compared to the control right knee.

Figure 4 illustrates the effect of sIL-1RII on synovial fluid concentrations of IL-1β. In the control and low-dose sIL-1RII animals, the concentration of IL-1β was again higher in the synovial fluid from the arthritic knee compared to the control knee; however, the difference was much more marked in the saline-treated group. Similar to the results for IL-1α, the synovial fluid concentrations of IL-1β in the high-dose group were not significantly different in the control and arthritic knees. In contrast to the IL-1α results, the right knee concentrations of both the low- and high-dose sIL-1RII were significantly reduced compared to the saline-treated right knee.

The effects of sIL-1RII on synovial fluid counts are shown in Table 2. There was a marked increase in the number of cells in the arthritic right knee joints
and right knee synovial fluid PGE$_2$ concentrations. There was no significant difference in the mean PGE$_2$ concentrations determined in the synovial fluid from sIL-1RII-treated animals compared to saline-treated animals. In contrast, there was a marked and dose-related reduction in plasma PGE$_2$ concentrations, with the concentrations in the high-dose group reduced to below the detection limit of the assay. The concentrations of PGE$_2$ in the left knees of all groups were also below the detection limit of the assay (data not shown).

**Discussion**

This study clearly demonstrates that infusion of sIL-1RII is effective in a rabbit model of RA. The dose-related effect was not only apparent on clinical parameters such as knee swelling, but also on biochemical and histological aspects of the disease. In this study, we used antigen-induced arthritis, where monoarticular arthritis is induced by an intra-articular injection of mBSA into rabbits pre-sensitized intradermally to the same antigen. Our model is a modification of the system originally described by Dumonde and Glynne [8], and was chosen because, of all the available arthritis models, it reflects most accurately the chronicity of the disease [9], the pathological changes in synovium and the pattern of tissue damage observed in rheumatoid joints [10]. Antigen-induced arthritis, especially in the rabbit, also responds to many current drug treatments in a similar way to RA [12–15].

In recent years, there has been increasing interest in the use of anti-cytokine antibodies, receptor antagonists and soluble cytokine receptors for the treatment of chronic inflammation, with animal studies being performed [16, 17] as well as a number of clinical trials in RA to evaluate these agents [18, 19]. In the mouse antigen-induced arthritis model, re-injection of a small amount of mBSA can elicit a flare-up of arthritis with expression of IL-1 in the inflamed synovium. Administration of anti-IL-1ra and β antibodies can inhibit joint swelling and pathology in both the flare-up [20] and standard mouse antigen-induced arthritis model [21]. The effect of recombinant human IL-1ra has also been investigated in this mouse model and shown to reduce cartilage damage [22]. In contrast, neutralizing anti-TNF-α antibody was ineffective on this parameter. These results indicate that in this model, IL-1 but not TNF is responsible for cartilage damage.

In a rabbit antigen-induced arthritis study using an antibody against the inflammatory cytokine TNF-α [16], a dose of 0.6 mg/kg anti-TNF-α antibody given daily for 10 days, starting 1 day after the induction of the arthritis, significantly reduced knee swelling, but had no effect on leucocyte influx, nor on proteoglycan loss from cartilage. In contrast, our present rabbit study with soluble IL-1RII at a dose of 4 μg/h/animal demonstrated inhibitory effects not only on swelling, but also histologically on joint damage/erosions. In addition, a recent study in rat antigen-induced arthritis using the same soluble IL-1RII (paper submitted) demonstrated

**Table 2.** Total synovial fluid cell counts (× 10$^6$/ml) obtained from left (normal) and right (arthritic) knee joints in rabbit antigen-induced arthritis. Each value represents the mean ± s.e.m. of n = 5 animals. **P < 0.01 significant vs saline-treated group, one-way ANOVA followed by Dunnett’s test for multiple comparisons post hoc. NS, not significant.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Left knee</th>
<th>Right knee</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline 0.9% w/v</td>
<td>0.51 ± 0.13</td>
<td>19.45 ± 8.42</td>
</tr>
<tr>
<td>(500 μl + 5 μl/h)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low-dose sIL-1RII</td>
<td>0.94 ± 0.23$^*$</td>
<td>20.82 ± 5.48$^*$</td>
</tr>
<tr>
<td>(13.4 μg + 1.33 μg/h)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High-dose sIL-1RII</td>
<td>0.29 ± 0.06$^*$</td>
<td>17.03 ± 7.14$^*$</td>
</tr>
<tr>
<td>(40.2 μg + 4.05 μg/h)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ANOVA + post hoc Dunnett’s test for multiple comparisons. NS, not significantly different from the saline control group.

**Table 3.** Concentrations of PGE$_2$ (pg/ml) in synovial fluid from right knee joints (arthritic) and plasma in rabbit antigen-induced arthritis. Each value represents the mean ± s.e.m. of n = 5 animals.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Synovial fluid</th>
<th>Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline 0.9% w/v</td>
<td>1076 ± 764</td>
<td>180 ± 40</td>
</tr>
<tr>
<td>(500 μl + 5 μl/h)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low-dose sIL-1RII</td>
<td>1066 ± 338$^*$</td>
<td>54 ± 16$^*$</td>
</tr>
<tr>
<td>(13.4 μg + 1.33 μg/h)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High-dose sIL-1RII</td>
<td>1016 ± 576$^*$</td>
<td>&lt; dl$^*$</td>
</tr>
<tr>
<td>(40.2 μg + 4.05 μg/h)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^*$P < 0.05 and $^{**}$P < 0.01 ANOVA + post hoc Dunnett’s test for multiple comparisons. NS, not significantly different from the saline control group. < dl, below the detection limit of the assay (2.5 pg/ml).

**Fig. 4.** The effect of sIL-1RII on synovial fluid IL-1β concentrations in the rabbit antigen-induced arthritis model. Treatment was administered as an i.v. bolus (loading dose), followed by s.c. infusion via minipump. Each point represents the mean ± s.e.m. of n = 5 animals. **P < 0.01 significant vs saline-treated group, one-way ANOVA followed by Dunnett’s test for multiple comparisons post hoc. NS, not significant.
significant dose-related inhibition of knee swelling, as well as inhibition of both inflammatory cell influx and joint damage on histological examination after administration of a dose of 2 μg/h/animal. Recently, an alternative approach to cytokine inhibition has been described in rabbit antigen-induced arthritis using adenovirus gene transfer of IL-1 and TNF-α soluble type I receptors locally into knee joints [23]. These workers found that, individually, sIL-1 receptor reduced cell influx and cartilage degradation, while sTNF-α receptor was only moderately effective on cell influx. The best effects were seen when both agents were transfected together, and some beneficial effects were additionally seen in the contralateral arthritic knee which was not treated directly. These results, taken together, seem to confirm in this model that IL-1 is the major cytokine responsible for the hard-tissue destruction, while TNF-α has little or no direct effect on this aspect of the arthritis pathology. These results also agree with previously reported findings using exogenous administration of cytokines [24–27]. However, there seems to be a growing consensus that inhibition of both of these cytokines is likely to be required to show real benefit in RA.

In human clinical trials, the major therapeutic efforts have so far evaluated the effects of systemic or local application of soluble IL-1ra and sIL-1RII (to investigate IL-1 inhibition) and anti-TNF-α antibody and soluble TNF-α receptor fusion proteins (to examine the effects of TNF-α inhibition) [18]. The results obtained so far with the anti-TNF-α therapies seem to have shown good efficacy [28]; however, whether these therapies can demonstrate longer term relief with acceptable side-effect profiles remains to be seen. The results obtained with the anti-IL-1-directed therapies, while showing early promise in open trials, have been somewhat less efficacious in subsequent randomized placebo-controlled ones [28]. The binding properties of IL-1α and IL-1β vs IL-1ra to the two IL-1 receptors may provide explanations for this. Firstly, a very large excess of IL-1ra will be required in vivo to compete out the binding of IL-1β to the type I receptor and prevent cell activation since only a very low receptor occupancy (<1%) is required for this biological activity [29]. This means that very large amounts will be required to be present at all times for good pharmacological antagonism. Secondly, the higher affinity of the sIL-1 type I receptor for IL-1ra, rather than for IL-1β, means that the effects of this therapy may be at best equivocal, binding some excess IL-1β, but at worst counterproductive, since it may preferentially remove the endogenous antagonist of IL-1, i.e. IL-1ra, from the system, reducing the amount of IL-1β required to produce pathological effects. The use of sIL-1RII as a therapeutic should avoid all of the above problems since it has the highest affinity for IL-1β, and will not need to be present in vast excess to reduce significantly the pathological effects of IL-1β in RA. The synergistic anti-inflammatory effects of a combination of IL-1ra with sIL-1RII, and the antagonistic effects of combining IL-1ra and sIL-1RI to inhibit IL-1-stimulated synovial cell responses have been elegantly demonstrated in vitro [30], strengthening the above hypothesis further.

In our current study, the concentrations of IL-1α and, to a greater extent, IL-1β in synovial fluid were also significantly reduced in a dose-related manner. The observed reduction of IL-1α vs IL-1β in synovial fluid is consistent with the relative binding affinities of each of these molecules for IL-1RII, and additionally suggests that the soluble receptor molecule enters the inflamed joint after systemic administration. Indeed, it has been reported that soluble IL-1 receptors can reduce the concentrations of IL-1β and IL-1ra which can be detected in synovial fluid by ELISA [31]. Our study indicates that the same is true when RIAs are used to detect cytokine concentrations in rabbit synovial fluid.

Treatment with sIL-1RII had no effect on the concentration of PGE₂ which could be detected in synovial fluids, but did give a significant, dose-related reduction in the concentration of PGE₂ which could be measured in plasma from the same animals. PGE₂ production can be induced from both synoviocytes [32] and articular chondrocytes [33] in vitro by IL-1β, suggesting that either or both of these sites are probably responsible for the production of PGE₂ detected in rabbit synovial fluid. Whether the plasma concentrations of PGE₂ represent an excessive local production in the joints, which then leaks into the circulation, or a separate systemic induction of PGE₂, is open to question, and would require further investigation. However, since synovial fluid PGE₂ production was not affected by sIL-1RII, this strongly suggests that mediators other than IL-1 may be major inducers of PGE₂ synthesis in inflamed joints.

In conclusion, sIL-1RII has been used to test the concept that this rabbit model of RA arthritis is IL-1 dependent, and the results obtained indicate that this is indeed the case. Results from similar experiments using anti-TNF-α antibodies and gene therapy serve to validate this model as the best system for testing therapies which are ultimately destined for the treatment of RA.

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