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PREVENTION OF MURINE COLLAGEN-INDUCED ARTHRITIS IN THE KNEE AND IPSILATERAL PAW
BY LOCAL EXPRESSION OF HUMAN INTERLEUKIN-1 RECEPTOR ANTAGONIST PROTEIN IN THE KNEE

ANDREW C. BAKKER, LEO A. B. JOOSTEN, ONNO J. ARNTZ, MONIQUE M. A. HELEN, ALISON M. BENEDE, FONS A. J. VAN DE LOO, and WIM B. VAN DEN BERG

Objective. To determine the efficacy of local human interleukin-1 receptor antagonist (HuIL-1Ra) gene therapy in murine collagen-induced arthritis (CIA).

Methods. DBA/1 mice were immunized against bovine type II collagen. Before the onset of arthritis, NIH/3T3 fibroblasts transfected with pMFG-IRAP were transplanted into the knee cavity. Normal NIH/3T3 cells served as controls. Paws were evaluated macroscopically for redness, swelling, and deformities during the course of arthritis. Swelling of the knee joints was measured by external gamma counting of 99mTc accumulation in the joint. Paws and knee joints were dissected and processed for histologic studies to evaluate inflammation and cartilage destruction.

Results. The NIH/3T3 fibroblasts survived in the joint cavity of DBA mice for at least 7 days. The transduced cells expressed immunoreactive and bioactive HuIL-1Ra in the knee joint, and produced sufficient amounts to block the effect of 1 ng of recombinant murine IL-1α on chondrocyte proteoglycan synthesis. The onset of CIA was almost completely prevented in knee joints containing HuIL-1Ra-producing cells, whereas joints containing normal cells showed severe inflammation and destruction of cartilage. Moreover, onset of CIA in the draining joints (ipsilateral paws) of the HuIL-1Ra gene-bearing knees was also prevented.

Conclusion. Local production of HuIL-1Ra in the knee was able to ameliorate the effects of IL-1 on cartilage and could prevent the onset of CIA not only in that knee, but also in the “draining” paw. This indicates the feasibility of gene transfer as a therapeutic approach to modulating arthritis.

Rheumatoid arthritis (RA) is an autoimmune disease characterized by progressive joint destruction and immobility. Prevention of cartilage erosion and dysfunction would be of great therapeutic benefit to patients. There is convincing evidence that the cytokines tumor necrosis factor α (TNFα) and interleukin-1 (IL-1) play a decisive role in arthritic processes. This is based on neutralizing studies in various models of experimental arthritis (1–8) and recent observations from clinical trials in patients with RA (9–12). Prolonged treatment with antibodies, scavenging receptors, or receptor antagonists is probably needed to obtain sustained control, and in that respect, a gene therapy approach might offer a valuable alternative.

Gene therapy in experimental arthritis has been proposed by Bandara et al (13), and their first study in rabbits showed that local injection of synovial cells that had been transduced in vitro with human interleukin-1 receptor antagonist (HuIL-1Ra) complementary DNA could inhibit the effects of IL-1 injection into the knee joint (14,15). Recently, the efficacy of such an approach was also demonstrated in antigen-induced arthritis in the rabbit and bacterial cell wall–induced arthritis in the rat (16,17), providing further evidence of the role of IL-1.

Collagen-induced arthritis (CIA) is an autoimmune type of arthritis which shows many characteristics in common with human RA. Onset of arthritis in DBA/1 mice occurs 4–6 weeks after the first immunization with type II collagen and is dependent on TNFα and IL-1 (1–5,8). We have recently shown that IL-1 is important in both arthritis and cartilage destruction in
this model and that systemic treatment with IL-1Ra provides impressive protection (8).

In the present study we investigated the efficacy of local gene therapy in the murine knee joint using HuIL-1Ra-transduced NIH/3T3 cells. The class I major histocompatibility complex antigens on NIH/3T3 cells are identical to those in the DBA/1 inbred mouse strain, and this strain is therefore suitable as a host for transplantation of 3T3 cells (18). Prolonged expression of HuIL-1Ra sufficient to neutralize nanogram quantities of IL-1 was obtained in the synovial tissue. Onset of CIA could be fully prevented in the transplanted joint. Remarkably, the “draining” paw of the knee transplanted with HuIL-1Ra–producing cells was also protected from CIA, whereas the remote paws were unaffected and showed full-blown arthritis. This suggests either a linked onset of arthritis between knee and paw, probably through IL-1, or an overflow of HuIL-1Ra from the knee to the nearby joints.

MATERIALS AND METHODS

Production of HuIL-1Ra–producing 3T3 cells. The 3T3 cells were infected with the retroviral vector pMFG-IRAP (kindly provided by Dr. C. Evans, Pittsburgh, PA) as described elsewhere (15). Briefly, 3T3 cells were grown to ~75% confluence in a 25-cm² flask. Medium was removed and replaced with 1 ml of viral suspension in the presence of 8 μg/ml Polybrene. After 2 hours, 3 ml of medium was added and the cultures were incubated for 72 hours. Clones were obtained by limiting dilution and selected for HuIL-1Ra secretion, using a radiolmmunoassay (RIA). Two clones were selected for their high HuIL-Ra production (2.1 μg/10⁶ cells/24 hours and 5 μg/10⁶ cells/24 hours). Evidence that 3T3 cells are suitable vehicles for protective gene constructs is provided by the fact that production of proinflammatory cytokines such as IL-6, TNFα, or IL-1 could not be detected by bioassay or by reverse transcription–polymerase chain reaction. Furthermore, transplantation of 2 × 10⁵ normal 3T3 cells to knees of normal C57Bl/6 mice did not elicit an inflammatory reaction (follow-up of 1 week).

HuIL-1Ra radiolmmunoassay. HuIL-1Ra was measured by RIA according to the method of Poutsirka et al (19). Briefly, diluted polyclonal rabbit anti–HuIL-1Ra was added to the samples, and they were kept overnight at room temperature. Iodinated IL-1Ra was added to each sample, followed by incubation overnight at room temperature. Immunoprecipitates were formed by the addition of sheep anti-rabbit IgG and polyethylene glycol, at respective final concentrations of 1% and 3%. After 1 hour at room temperature, immunoprecipitates were recovered and counted.

HuIL-1Ra immunohistochemistry studies. Whole mouse knees were snap frozen in liquid nitrogen. Sections were attached to adhesive tape (20), fixed in 4% paraformaldehyde in phosphate buffered saline (PBS) for 10 minutes, and either used immediately or dried and stored at −70°C in an airtight container. Sections were rehydrated in PBS and incubated for 2 minutes in 0.2% Triton X-100 to permeabilize cell membranes. Endogenous peroxidase was blocked by incubation in 0.5% H₂O₂ in PBS. After a further wash in PBS, sections were incubated overnight with polyclonal rabbit anti–HuIL-1Ra antibodies (Genzyme, Cambridge, MA) diluted 1:500 in 4% nonfat dry milk in PBS. Sections were washed and incubated for 1 hour with goat anti-rabbit biotinylated antibodies (Vector, Burlingame, CA). Secondary antibodies were detected with an avidin–biotin–peroxidase complex (Vec­tauatin ABC Kit; Vector). After washing again in PBS, peroxi­dase activity was detected by 10-minute incubation in 0.5 mg/ml 3,3′-diaminobenzidine tetrahydrochloride (Sigma, St. Louis, MO) dissolved in 0.05M Tris HCl buffer, pH 7.6, containing 0.02% H₂O₂. Finally, sections were counterstained with Mayer’s hematoxylin and embedded in gelatin–glycerin.

Induction of zymosan-induced arthritis (ZIA) and treatment protocol. A homogeneous suspension of 30 mg zymosan A (Saccharomyces cerevisiae) dissolved in 1 ml endotoxin-free saline was obtained by boiling twice followed by sonic emulsification. Arthritis was induced by intraarticular injection of 180 μg zymosan into the knee joint. HuIL-1Ra–producing cells or 3T3 cells (2 × 10⁵) were injected intraarticularly into the knee joint 2 days before induction of ZIA. Two days after induction of ZIA, knee joint inflammation was assessed by ⁵⁹ᵐTechnetium pertechnetate uptake, and proteoglycan (PG) synthesis was determined by in vitro ³⁵S-sulfate incorporation in patellae.

Assessment of knee joint swelling. Joint swelling was quantified by ⁹⁹ᵐTc uptake as previously described (6). Briefly, 10 μCi ⁹⁹ᵐTc in 0.2 ml saline was injected subcutaneously in the neck region, and mice were sedated by intraperitoneal injection of 4.5% chloral hydrate (0.1 ml/10 mg body weight). Accumulation of the isotope in the knee was determined after 30 minutes by external gamma counting.

Induction of CIA and treatment protocol. DBA/1 mice, age 9–10 weeks at the start of experiments, were immunized intradermally at the base of the tail with bovine type II collagen (100 μg) emulsified in Freund’s complete adjuvant (Difco, Detroit, MI). On day 21, the animals were boosted with an intraperitoneal injection of 100 μg type II collagen. Gradual onset of arthritis normally starts approximately 4 weeks after immunization. In the first experiment, 10 mice that did not yet have any macroscopic signs of arthritis were selected at 32 days after the initial immunization. These mice received 2 × 10⁵ HuIL-1Ra–producing cells in the right knee and 2 × 10⁵ normal NIH/3T3 cells in the left knee. In the second experiment, we took mice before arthritis onset, 23 days after the initial immunization, and divided them into 3 groups receiving either 2 × 10⁵ normal 3T3 cells (n = 10), HuIL-1Ra cells (5 μg/10⁶ cells/24 hours; n = 10), or a second HuIL-1Ra–producing cell-line (2.1 μg/10⁶ cells/24 hours; n = 9) in the right knee joint. Saline was injected into the left knee joint. CIA development in the first experiment was evaluated by assessment of knee swelling on day 4 and macroscopic scoring of the draining paws and histologic analysis on day 5. CIA development in the second experiment was assessed by macroscopic scoring of the draining paws and histologic analysis of the knees on day 5.

Macroscopic scoring of CIA. Erythema and swelling of the paws were scored on a 0–2 scale, with a maximum score of 2 for each paw. Scoring was done by 2 independent observers,
and the mean scores from the 2 observers were used for analyses.

Assessment of PG synthesis. Patellae were dissected so as to minimize the amount of adjoining synovium, tendon, and muscle, and placed in 200 μl RPMI-HEPES medium (Life Technologies, Breda, The Netherlands) supplemented with gentamicin (50 mg/liter), L-glutamine (2 mM), and 40 μCi 35S-sulfate/2 ml. After a 3-hour incubation, patellae were fixed in 10% formalin, decalcified in formic acid (4%), punched out, and dissolved in 0.5 ml Lumasolve (Omnilab, Breda, The Netherlands). The 35S content of each patella was measured by liquid scintillation counting and expressed as counts per minute.

To determine the in vitro potency of recombinant HuIL-1Ra (rHuIL-1Ra), patellae from normal C57Bl/6 mice were isolated. PG synthesis was measured after incubation of the patellae with or without 1 ng/ml or 10 ng/ml recombinant murine IL-1α (rMuIL-1α) for 48 hours in medium supplemented with 0.5 μg/ml insulin-like growth factor in the presence of various amounts of rHuIL-1Ra (generously provided by Amgen, Boulder, CO). After 48 hours, the 35S-sulfate content of the patellae was measured as described above. PG breakdown was measured by first prelabeling the patellae as described above, followed by a 48-hour incubation in medium in the presence of 10 ng/ml rMuIL-1α and various concentrations of rHuIL-1Ra. The 35S-sulfate content of the patellae was again measured as described above. Data are shown as the percentage of 35S-sulfate incorporation in the right (experimental) knee compared with the left (control) knee.

Histologic processing and analysis of knee joints. Knee joints were dissected, fixed, decalcified, dehydrated, and embedded in paraffin. Standard frontal sections of 7 μm were prepared, stained with Safranin O, and counterstained with fast green. Cartilage depletion was visualized by diminished staining of the matrix and scored arbitrarily as 0 when normal or 1-3 according to the degree of depletion (loss of staining). Exudate was scored arbitrarily as 0 when no cells were present in the joint space or 1-3 according to the cell content of the joint space (predominantly polymorphonuclear cells).

For autoradiography, 35S-sulfate (50 μCi per animal) was administered to the animals shortly (2 hours) before killing and knee and ankle joints prepared for histologic study. Sections were mounted on gelatin-coated slides, dipped in K5 emulsion (Ilford, Basildon, UK) and developed after 2-4 weeks (21).

Statistical analysis. Data are expressed as the mean ± SD. Statistical analyses were performed using the 2-sample t-test for parametric data, and the Wilcoxon signed rank test for nonparametric data.

RESULTS

Potency of HuIL-1Ra in blocking effects of IL-1 on patellae in vitro. To determine the amount of rHuIL-1Ra needed to completely block rMuIL-1α, patellae from normal C57Bl/6 mice were isolated and cultured with saline, 1 ng/ml rMuIL-1α, or 10 ng/ml rMuIL-1α, either alone or together with various concentrations of rHuIL-1Ra (Table 1). From these experiments, it was evident that in vitro, at least a 1,000-fold excess of rHuIL-1Ra over rMuIL-1α is needed to completely block the effects of rMuIL-1α on PG synthesis and breakdown in patellar cartilage.

In vivo application of HuIL-1Ra by transplantation of HuIL-1Ra-producing 3T3 cells to knee joints. We examined, by immunohistochemistry, local production of HuIL-1Ra after transplantation of HuIL-1Ra-producing 3T3 cells to C57Bl/6 and DBA/1 mice. In C57Bl/6 mice, HuIL-1Ra-containing cells were retained in the knees for at least 7 days (Figure 1). No signs of inflammation (granulocyte influx) were observed, but a slight thickening of the synovial lining was seen. Knee swelling as measured by 99Tc uptake was negligible, and cartilage damage or PG depletion was not observed on histologic sections. Since the CIA experiments had to be performed in susceptible DBA/1 mice, we also injected 3T3 cells or HuIL-1Ra–producing cells into the knee joints of mice of this strain. In none of the animals was significant joint pathology noted on day 5, and local HuIL-1Ra production was similar compared with the immunohistologic pattern seen in C57Bl/6 mice.

To investigate whether the immunoreactive HuIL-1Ra was bioactive, HuIL-1Ra–producing cells were injected into knee joints 1 day before the intra-articular injection of 1 ng or 10 ng rMuIL-1. This treatment blocked the inhibition of PG synthesis in cartilage induced by 1 ng rMuIL-1, whereas the effects of 10 ng rMuIL-1 could not be blocked (Figure 2). Since the amount of IL-1 produced in arthritic joints does not exceed the nanogram level (22), these cells were subsequently used in arthritis models.

Table 1. Potency of HuIL-1Ra in blocking the effects of IL-1 on mouse patellae in vitro

<table>
<thead>
<tr>
<th>Concentration of rHuIL-1Ra, ng/ml</th>
<th>% inhibition of PG synthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>rMuIL-1α 1 ng/ml</td>
<td>53</td>
</tr>
<tr>
<td>rMuIL-1α 10 ng/ml</td>
<td>53</td>
</tr>
<tr>
<td>rHuIL-1Ra 0</td>
<td>10</td>
</tr>
<tr>
<td>rHuIL-1Ra 100</td>
<td>94</td>
</tr>
<tr>
<td>rHuIL-1Ra 300</td>
<td>93</td>
</tr>
<tr>
<td>rHuIL-1Ra 1,000</td>
<td>90</td>
</tr>
<tr>
<td>rHuIL-1Ra 10,000</td>
<td>86</td>
</tr>
</tbody>
</table>

* Proteoglycan (PG) synthesis was determined in normal patellae (n = 7) cultured for 48 hours in the presence of 1 ng/ml or 10 ng/ml recombinant mouse interleukin-1α (rMuIL-1α) with various concentrations of recombinant human IL-1 receptor antagonist (rHuIL-1Ra), followed by a 3-hour incubation with 35SO4. Breakdown of PG was measured in 35SO4-labeled patellae, by 48-hour incubation with 10 ng/ml rMuIL-1α and various concentrations of HuIL-1Ra. Inhibition of PG synthesis was measured as the percent reduction in 35SO4 incorporation compared with patellae incubated without rMuIL-1α or HuIL-1Ra. ND = not done.
Figure 1. Immunohistochemistry of human interleukin-1 receptor antagonist (HuIL-1Ra)–producing NIH/3T3 cells 7 days after transplantation of 2 × 10^5 cells into a murine knee joint. A, Normal 3T3 cells. B, HuIL-1Ra–producing cells. Cells adjacent to the lining are positive for HuIL-1Ra. Arrowheads indicate the position of the synovial lining. F = femur.

Effect of HuIL-1Ra–producing cells on ZIA-induced PG synthesis inhibition. HuIL-1Ra–producing cells (2 × 10^5) were injected intraarticularly into the mouse knees, followed 2 days later by 180 μg zymosan. PG synthesis by patellar chondrocytes was determined on day 4. Intraarticular injection of normal 3T3 cells did not have an effect on the inhibition of cartilage PG synthesis in ZIA. The HuIL-1Ra–producing cells significantly ameliorated PG synthesis, from 52% to 73% of normal (Figure 3). Histologic analysis revealed a similar infiltrate in the knees treated with normal 3T3 and those treated with HuIL-1Ra–producing cells (results not shown).

Figure 2. Expression of human interleukin-1 receptor antagonist (HuIL-1Ra) in the knee counteracts the effect of intraarticular injection of recombinant murine IL-1α (rMuIL-1α) on proteoglycan synthesis. Before injection of rMuIL-1α into the right knee and saline into the left knee, 2 × 10^5 normal 3T3 or hIL-1Ra–producing cells were injected intraarticularly into the right knee. Two days after the injection, patellae were isolated and incubated for 3 hours with 35SO_4. The 35SO_4 content in the patellae was measured (counts per minute) (n = 5). Values are the mean and SD percentage of 35SO_4 content in the right patellae compared with the left patellae. * = P < 0.01 compared with the 3T3 control, as determined by 2-sample t-test.

Figure 3. Effect of local human interleukin-1 receptor antagonist (HuIL-1Ra) production on inhibition of proteoglycan production in patellae with zymosan-induced arthritis (ZIA). Human IL-1Ra–producing cells (n = 22) or normal 3T3 cells (n = 20) (each at 2 × 10^5) were injected into the right knee joint. Control right knees (n = 15) received no injection. Two days later, 180 μg zymosan was injected into the right knee joint and saline was injected into the left knee joint. After 2 days, proteoglycan synthesis in the patellae was assessed by ex vivo measurement of 35SO_4 incorporation (counts per minute). Values are the mean and SD percentage of 35SO_4 content in the right patellae compared with the left patellae. * = P < 0.01 compared with the 3T3 control, as determined by 2-sample t-test.
Table 2. Histologic evaluation of collagen-induced arthritis in mouse knees transplanted with $2 \times 10^5$ 3T3 cells or 1 of 2 different HuIL-1Ra-producing 3T3 cell lines*

<table>
<thead>
<tr>
<th>Transplanted cell line</th>
<th>Histologic score</th>
<th>Arthritis incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infiltrate</td>
<td>Exudate</td>
</tr>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3T3</td>
<td>1.9 ± 1.5</td>
<td>1.9 ± 1.5</td>
</tr>
<tr>
<td>HuIL-1Ra (1)</td>
<td>0.4 ± 1.0</td>
<td>0.3 ± 1.0</td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>1.4 ± 1.4</td>
<td>1.6 ± 1.3</td>
</tr>
<tr>
<td>3T3</td>
<td>2.7 ± 1.0</td>
<td>2.8 ± 0.8</td>
</tr>
<tr>
<td>HuIL-1Ra (1)</td>
<td>0.7 ± 0.9§</td>
<td>0.8 ± 0.8§</td>
</tr>
<tr>
<td>HuIL-1Ra (2)</td>
<td>1.1 ± 1.1§</td>
<td>0.7 ± 0.9§</td>
</tr>
</tbody>
</table>

*Five days after transplantation, histologic samples were obtained and sections were stained with Safranin O. Histologic data are expressed as the mean ± SD. 3T3 = normal 3T3 cells; HuIL-1Ra (1) = 3T3 cells producing 5 µg HuIL-1Ra/10^6 cells/24 hours; saline = contralateral knees from all 3 groups; HuIL-1Ra (2) = 3T3 cells producing 2.1 µg HuIL-1Ra/10^6 cells/24 hours. See Table 1 for other definitions.

† Exudate was scored arbitrarily as 0 when no cells were present in the joint space or 1–3 according to the cell content of the joint space (predominantly polymorphonuclear cells).

§ PG loss of femur and patellar cartilage. Cartilage PG depletion was visualized by diminished staining of the matrix and scored arbitrarily as 0 when normal or 1–3 according to the degree of loss of Safranin O staining.

§§ P < 0.05 versus 3T3 group, by Wilcoxon signed rank test.

shown), consistent with earlier observations that PG synthesis inhibition, but not synovial infiltration, is IL-1 dependent in this model (7).

**Effect of the transplanted HuIL-1Ra–producing cells on CIA in the knee joint.** Onset of murine CIA generally occurs approximately 4–6 weeks after the first immunization and has been shown to be IL-1 dependent (4,5,8). In the first experiment, 10 type II collagen–immunized mice without signs of arthritis on day 32 were selected. Cells producing HuIL-1Ra were injected into the right knee joints, whereas 3T3 cells injected into the left knee joints served as controls. Knee joint swelling was measured by 99mTc uptake 4 days after transplantation of the cells. The mean ± SD left/right ratio for the 10 mice was 1.72 ± 0.37. In only 1 mouse did both knees appear equally swollen; but in all other mice the left knee was swollen and the right knee appeared macroscopically normal. Thus, in 9 of 10 mice, the HuIL-1Ra cells completely blocked swelling of the knees. On day 5 after transplantation of the cells, knee joint sections were prepared for histologic study and autoradiography was performed to measure cartilage chondrocyte function (Table 2 and Figure 4). Autoradiography revealed that inflamed knees had fully suppressed 35SO_4 incorporation in cartilage, whereas noninflamed knees had normal 35SO_4 incorporation. Occurrence of marked cell influx and major tissue destruction was seen in 7 of 10 left knee joints transplanted with normal 3T3 cells, versus only 1 of 10 of the right knee joints transplanted with HuIL-1Ra–producing cells. Affected knees had severe histologic scores (>2) for infiltrate, exudate, and PG loss, resembling classic CIA.

In a second experiment, mice were selected just before the onset of CIA, on day 23 after the initial immunization. In this experiment, 3T3 cells or 2 different HuIL-1Ra–producing clones were transplanted into the right knee joints and saline was injected into the left knee joints. Five days later, knee joints were scored macroscopically. In the untreated group (no intraarticular injection), 50% of the right and left knee joints

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**Figure 4.** Autoradiography of the right (human interleukin-1Ra–producing cell transplanted) (A) and left (normal 3T3 cell–transplanted) (B) knee joints of the same collagen-induced arthritis–prone mouse, 5 days after transplantation of $2 \times 10^5$ cells. Black spots represent $^{35}$SO_4 incorporation. Arrowheads show femoral and patellar cartilage. P = patella; F = femur; GP = growth plate; JS = joint space. Note the absence of grains in cartilage of the arthritic left knee, showing strong inhibition of proteoglycan synthesis by chondrocytes. The growth plates are evenly stained.
developed arthritis (Figure 5). Occurrence of arthritis was enhanced in the 3T3-transplanted knees (90%). In the HuIL-1Ra–transplanted knees, occurrence of arthritis was markedly decreased, to 10% (5 μg HuIL-1Ra/10^6 cells/24 hours) and 22% (2.1 μg HuIL-1Ra/10^6 cells/24 hours) (Figure 5). The left, saline-injected knees from all 3 treatment groups were comparable with those from the untreated group, suggesting that HuIL-1Ra expression in the right knee had no effect on the contralateral saline-injected knee. Histologic examination showed marked protection of the knee joints transplanted with either of the HuIL-1Ra–producing cell lines compared with the knee joints transplanted with normal 3T3 cells, with respect to exudate, infiltrate, and PG loss (Table 2). The contralateral saline-injected knees from all 3 groups all developed CIA and had similar scores for infiltrate, exudate, and PG depletion of cartilage.

**Effect of HuIL-1Ra expression on draining and remote paws.** Human IL-1Ra expression in the knee seemed to also have a positive effect on the draining paws. To further assess this phenomenon, paws were macroscopically scored at the same time points as histologic specimens were obtained from the knees. In the second experiment, 50% of the untreated group developed arthritis in the right and left paws (Figure 6). Expression was slightly enhanced in the draining paws from the HuIL-1Ra–transplanted knees, the occurrence of arthritis was markedly decreased, to 10% (5 μg HuIL-1Ra/10^6 cells/24 hours) and 11% (2.1 μg HuIL-1Ra/10^6 cells/24 hours) (Figure 6). The draining paws of the contralateral legs (knees injected with saline) from all 3 groups showed an arthritis incidence comparable with that found in the untreated group, demonstrating that HuIL-1Ra expression in the right knee had no effect on the contralateral paws. Occurrence of linkage between arthritis in the knee and paw of one leg was further analyzed. Either preferential expression in both (3T3 group) or dominant absence at both sites (the 2 HuIL-

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Knee and paw</th>
<th>Only knee</th>
<th>Only paw</th>
<th>Neither</th>
</tr>
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<tbody>
<tr>
<td>3T3</td>
<td>20</td>
<td>70</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>HuIL-1Ra (1)</td>
<td>20</td>
<td>15</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HuIL-1Ra (2)</td>
<td>9</td>
<td>11</td>
<td>11</td>
<td>0</td>
</tr>
</tbody>
</table>

* Data from experiments 1 and 2 are combined. 3T3 = normal 3T3 cells; HuIL-1Ra (1) = 3T3 cells producing 5 μg human interleukin-1 receptor antagonist (HuIL-1Ra)/10^6 cells/24 hours, transplanted into the knee; HuIL-1Ra (2) = 3T3 cells producing 2.1 μg HuIL-1Ra/10^6 cells/24 hours, transplanted into the knee.

† Number of legs evaluated, experiments 1 and 2 combined.
1Ra groups) was noted (Table 3), whereas selective expression either in knee or in paw was rarely seen.

DISCUSSION

We used the ex vivo method of gene transfer as described by Bandara et al (15) to obtain local and sustained expression of HuIL-1Ra in the knee. It was found that this approach prevented the onset of CIA not only in that knee, but also in the nearby, draining paw. Contralateral knee and paw joints were not protected. Control NIH/3T3 cells not expressing HuIL-1Ra enhanced, rather than suppressed, the onset of arthritis.

Using immunohistochemistry methods, we have shown that the transduced NIH/3T3 cells transplanted into the knee cavity continue producing the HuIL-1Ra protein for at least 7 days. Furthermore, transplantation of $2 \times 10^5$ HuIL-1Ra-producing cells into the knee joint could completely block the effects of an intraarticular injection of 1 ng rMuIL-1α on patellar PG synthesis. Since it is known from previous studies that the peak levels of IL-1 produced by the inflamed synovial tissue after onset of arthritis do not exceed the nanogram level, the selected HuIL-1Ra-producing cells are sufficiently potent.

One concern about application of local gene therapy is a potential disturbing effect of the inflammatory process on protein production and, for the ex vivo method in particular, the nonspecific effect of the transplanted cells on the inflammation process. To evaluate this we used the model of ZIA, in which we demonstrated previously that the inhibition of chondrocyte PG synthesis could be completely prevented, but that the joint swelling was only marginally suppressed, with neutralizing anti-IL-1 antibodies (7). In the present study we noted that local expression of HuIL-1Ra in the knee during ZIA barely affected joint swelling ($^{99m}$Tc uptake), consistent with IL-1 independence, but inhibition of cartilage PG synthesis was clearly reduced. Furthermore, transplantation of NIH/3T3 cells did not influence ZIA-related joint swelling or cartilage PG synthesis inhibition. These observations demonstrate that the cells do not influence ZIA and that HuIL-1Ra is being produced during the inflammation. In comparison with our in vitro blocking studies (Table 1), we can extrapolate that we must have achieved at least a 300-fold excess of HuIL-1Ra above local murine IL-1 levels to explain the positive effect on PG synthesis. This indirectly proves that the transplanted cells still produce considerable amounts of HuIL-1Ra under conditions of inflammation.

In the model of murine collagen arthritis, both joint inflammation and cartilage destruction are IL-1 dependent (4,5,8). Consistent with this, the local expression of HuIL-1Ra prevented both events. Recent studies with IL-1β-converting enzyme inhibitors (23) as well as observations in IL-1β knockout mice confirm this strong IL-1 dependence. Bandera et al (15) have shown, in studies of rabbits, that autografted synoviocytes exhibit a much longer intraarticular transgene expression as compared with allografted cells. Although we used a cell line compatible with the DBA/1 mice (18), the followup of effects of local HuIL-1Ra transgene expression was limited to 5 days, which excludes the possibility that the effects were flawed by graft reactions. We have not yet performed prolonged treatment studies or therapeutic protocols, but we are currently producing a DBA/1 autologous synovial fibroblast cell line for this purpose. The fact that we found similar effects with 2 different HuIL-1Ra-producing cell lines of comparable potency provides further proof that the observed suppression of arthritis is probably not due to an aberrant character of the cell lines used.

Two earlier publications, by Makarov et al (17) and Otani et al (16), have shown the potential of the ex vivo method in, respectively, antigen-induced arthritis in rabbits and bacterial cell wall–induced arthritis in rats. Due to the monarthritic nature of these experimental arthritis models, those investigators could not determine the effect of HuIL-1Ra production in the knee on nearby and more remote joints. The polyarthritic nature of the CIA model allowed us to obtain such information. We observed that local HuIL-1Ra production in the knee not only prevented onset of CIA in that knee, but also prevented onset of CIA in the draining paw, whereas the contralateral leg was not affected. This seems to link with previous work (24), in which we showed that expression of collagen arthritis in a particular joint can be accelerated by local injection of cytokine-generating stimuli such as zymosan, and that with high doses, concomitant onset occurred in the draining paw but not in the remote joints. Furthermore, Staite et al (11) have shown that IL-1 administered in the knee augments a local inflammatory response of methylated bovine serum albumin at that site, but also affects the ipsilateral paw. These data suggesting coupling of arthritic processes in nearby joints may indicate that gene therapy applied to a larger joint could exert protective effects in smaller joints of hands or feet.

It is debatable whether the ex vivo method is the ideal gene therapy technique. In comparison with local transduction at the site, using adenoviral vectors or liposomes, it is labor intensive, and the cells could change during the transfer from the knee to culture
conditions and back. However, cell targeting is possible in vitro, and some of the adenoviral vectors show inflammatory properties and need further constructive cleaning. The present data provide good evidence for the possibilities of local expression of therapeutic proteins for treatment of arthritis. We have shown that transplantation of cells producing HuIL-1Ra to the knee cavity can protect articular cartilage against IL-1-induced inhibition of PG synthesis and can prevent onset of CIA not only in the transplanted knees, but also in the draining paws. It is expected that better vectors will become available in the near future to optimize expression and targeting to relevant cells and tissues of the joint.

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