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Adenovirus-Mediated Transfer of Viral IL-10 Gene Inhibits Murine Collagen-Induced Arthritis

Florence Apparailly, Claudie Verwaerde, Chantal Jacquet, Claude Auriault, Jacques Sany, and Christian Jorgensen

IL-10 is a potent anti-inflammatory cytokine that has received growing attention for its therapeutic potential. We examined the efficiency of adenoviral-mediated gene transfer of IL-10 on the incidence and severity of murine collagen-induced arthritis (CIA). Male DBA1 mice immunized with collagen II were treated by systemic administration of 10⁸ plaque-forming units of replication-defective adenoviral vector expressing viral IL-10 (vIL-10) on day 30, when clinical symptoms of arthritis start. The transgene was shown to inhibit the onset of CIA, to decrease severity, and profoundly suppress the overall joint histopathology of the experimental arthritis. Significant IL-10 concentrations were obtained in the serum of injected animals for 7 days. Inhibition of arthritis was enhanced by administration of increasing doses of adenovirus-vIL-10. In addition, the local immunosuppressive effect of gene-delivered vIL-10 could be neutralized by a monoclonal anti-vIL-10 Ab. The CIA symptoms in the group treated with the same construct expressing inactive vIL-10 (vIL-10 mut) were similar to those in untreated animals. Our data indicate that a single systemic administration of an adenoviral vector encoding vIL-10 may be a good candidate to suppress arthritis. The Journal of Immunology, 1998, 160: 5213–5220.

Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic inflammation of joints resulting in monocyte infiltration and progressive destruction of cartilage and bone. Male DBA1 collagen-induced arthritis model (CIA) is a widely accepted murine experimental model for RA, classified as a Th1-mediated disease. Previous studies demonstrated the conversion of the collagen-specific CD4⁺ T cells from an early dominant Th0/Th1 response (IL-2, TNF-α, IFN-γ) to a clear Th2 phenotype (IL-4, IL-5, IL-10) between days 15 and 30 of the development of CIA in DBA1 mice (1). Although the Th1/Th2 switch occurs, Th2-type cytokines remain insufficient, and proinflammatory cytokines derived from macrophages and fibroblasts such as TNF-α, IL-1, IL-6, and granulocyte-macrophage-CSF predominate in the synovial fluid, leading to CIA symptoms progression.

Numerous studies have indicated that cytokines of the Th2 phenotype or specific inhibitors of Th1 cytokines (IL-ra, anti-TNF-α) play an important immunoregulatory role in inhibiting the disease process (2–5). Among these factors, IL-10 has received growing attention for its therapeutic potential.

IL-10 is a cytokine synthesis-inhibitory factor produced by Th2 cells (6) that predominantly mediates immunosuppressive effects through the down-regulation of macrophage functions (7, 8) and the inhibition of proinflammatory cytokines produced by Th1 cells, such as IL-1β, IL-6, IL-8, TNF-α, IFN-γ, and granulocyte-macrophage-CSF (9–11). Endogenous IL-10 expression by T cell clones is enhanced in the synovium during RA (9, 12, 13), and previous data have suggested that IL-10 might be the major endogenous mediator of spontaneous immunosuppression that occurs to prevent disease expression and development (2, 3). Indeed, twice daily i.p. injections of murine rIL-10 (2000 U/day) suppressed established CIA in DBA1 mice and protected against cartilage destruction (2). In contrast, neutralization with anti-murine IL-10 (anti-mIL-10) Ab further accelerated the expression of CIA and increased its severity. Others showed that systemic mIL-10 treatment with miniosmotic pumps (100 µg/day) delayed the onset of arthritis and reduced the severity of CIA in rats when injected before the symptoms appeared (3). IL-10 activities have also been intensively investigated in a number of in vivo models including endotoxemia (14–18), transplantation (19–21), autoimmune diseases (22), diabetes in NOD mice (23), and allergen-induced lung inflammation (24).

However, mIL-10 has some immunostimulatory activities, including enhanced expression of class II MHC molecules on B cells (25) and induction of cytotoxic T cell differentiation. Viral IL-10 (vIL-10), a product encoded by EBV, was found to be highly homologous to both murine and human IL-10. Viral IL-10 shares many biologic properties with murine and human IL-10, but it does not possess the T cell costimulatory activities (10, 19). Moreover, Qin et al. (20) showed that retroviral gene transfer of vIL-10 significantly prolonged murine cardiac allograft survival through induction of local immunosuppression. Suzuki et al. (19) also demonstrated that local vIL-10 but not mIL-10 production can suppress immune rejection of immunogenic tumor cells. Thus, all of these in vitro and in vivo data support viL-10 as a better candidate for...
reversing the immune response in the setting of autoimmune disease.

Most of the studies to date have used daily injections of rIL-10 because of its short half-life (26). Gene transfer technologies have developed efficient and prolonged expression of cytokines in vivo. Replication-defective adenoviral vectors are appealing candidates for in vivo gene therapy because of their capacity to transduce a wide variety of cell types, including nonreplicative cells. Recently, Xing et al. described miL-10 production in the sera of mice after intramuscular injection of miL-10 recombinant adenovirus (15).

The purpose of this study was to examine the efficiency of vIL-10 adenovirus-mediated (AdvIL-10) gene transfer on the incidence and severity of murine CIA. Our results show that systemic administration of adenovirus expressing vIL-10, but not the control recombinant adenovirus, can inhibit CIA onset and reduce clinical and histopathologic symptoms of the autoimmune animal model. This effect is dose dependent and is specifically neutralized by anti-vIL-10 Ab. Finally, these data suggest that adenovirus-mediated vIL-10 gene transfer may be a valuable therapeutic alternative to arthritis.

Materials and Methods

Animals

Male DBA/1 mice were bred in our facilities and immunized at the age of 8 to 10 wk.

Materials

Bovine type II collagen was purchased from Institute Jacques Boy (Reims, France). PBS was obtained from Eurobio (Les Ulis, France). LPS (Escherichia coli 0111:B4), BSA, Freund’s complete adjuvant, Tween 20, orthophenylene diamine, and all chemicals were purchased from Sigma (St. Louis, MO). Monoclonal anti-human and anti-viral IL-10 Abs for ELISA and in vivo neutralization were obtained from Pharamingen (San Diego, CA). The 96-well flat-bottom Maxisorb microtiter plates were obtained from Nunc (Life Technologies, Cergy Pontoise, France). Avidin-peroxidase was purchased from Immunotech, (Marseille, France).

Recombinant adenoviral vectors

Replication-defective type 5 adenovirus vector was deleted for sequences E1a and E1b, impairing the ability of the virus to replicate into cell or transform them. Construction of the recombinant AdvIL-10 and AdvIL-10 mut was described in detail elsewhere (C.V., manuscript in preparation). Briefly, the BCRF1-coding gene (–16, +625), flanked in 5’ of the promoter of the cytomegalovirus and in 3’ with a SV40 polyadenylation sequence, was inserted in place of E1 region into the adenoviral genome. In the AdvIL-10 mut control, a mutation was introduced by inserting a cytomegalo-20

Induction of arthritis

Bovine type II collagen was diluted at 2 mg/ml with 0.1 M acetic acid overnight at 4°C, then emulsified with an equal volume of CFA before use. CIA was induced by intradermal injection at the base of the tail with 100 μl of solution (100 μg collagen) at day 0. On day 21, the animals were boosted with an i.p. injection of 100 μg type II collagen at 1 mg/ml in PBS. On day 28, the onset of arthritis was initiated by a single i.p. injection of 40 μg of LPS (in 100 μl of PBS). This resulted in the onset of CIA within a week, as previously described (2, 27–29).

Treatment of CIA

Before the onset of arthritis, animals were divided into five groups. On day 30 after immunization, mice were either untreated or injected i.v. with 107 or 108 of adenoviral vector expressing the native or mutated vIL-10 gene. On day 29, one group received 0.2 mg of rat anti-human and vIL-10 mAb (clone JES3-19F, PharMingen). This group was then injected on day 30 with 109 pfu of adenoviral vector expressing the native or mutated vIL-10 gene. On day 29, one group received 0.2 mg of rat anti-human and vIL-10 mAb (clone JES3-19F, PharMingen). This group was then injected on day 30 with 109 pfu of adenoviral vector expressing the native or mutated vIL-10 gene.

Assessment of CIA

From day 21, the paw widths were measured with a micrometer. Swelling, redness, or the presence of erythema or wound were visually evaluated for macroscopic scoring of arthritis as previously described (30). The severity of arthritis was graded on a scale of 0 to 3 for each paw, where 0 = normal, 1 = swelling and moderate redness, 2 = marked edema, and 3 = severe edema with wound. The macroscopic score was expressed as a cumulative value for all paws, with a maximum possible score of 12. Scoring was done by two independent observers, without knowledge of the experimental groups. Mice were sacrificed at day 50, and the hind paws from five to nine animals were randomly collected from two independent experiments for radiography, then fixed in 4% paraformaldehyde, decalcified in 5% formic acid, embedded in paraffin, and 2-μm sections were stained with hematoxylin/eosin/safranin O. Sections and x-rays were examined by two independent, blind observers, who were different from the persons who had grouped and clinically scored the animals. X-rays performed using Kodak film were scored for the tibio-tarsal arthritis and tarsus joint from 0 to 5 according to the degree of destruction: 0, normal; 1, demineralization; 2, narrowing <50% of joint space; 3, narrowing <50% of joint space and joint erosion; 4, loss of joint space; 5, complete joint destruction. Two different areas of each paw (midfoot and hindfoot joints) were scored on four successive sections for overall histopathologic analysis as previously described (31): 0, normal; 1, inflammatory infiltrates and syncytial hyperplasia; 2, pannus formation and cartilage erosion; 3, important cartilage erosion and bone destruction; 4, loss of joint integrity.

Immunoaassay of serum vIL-10

Sera were collected from blood samples on days 0, 28, and 37, and stored at −20°C until tested. The circulating vIL-10 levels were measured by specific ELISA for each individual. Wells of flat-bottom Maxisorb microtiter plates were coated with 100 ng of monoclonal rat Ab specific for human and viral IL-10 (clone JES3-9D7, Pharamingen) in 50 μl of carbonate buffer, pH 8.2, at 4°C overnight. Following two washes with PBS-0.05% Tween 20, the plates were blocked with 200 μl PBS-3% BSA 2 h at room temperature (RT). Then, blocking buffer was eliminated, and 50 μl of IL-10 standard (2226-01, Genzyme, Cambridge, MA) or serum samples were added. After 2 h of incubation at RT, wells were washed four times with PBS-0.05% Tween 20 and then incubated 1 h at RT with 100 μl of biotinylated anti-human and anti-viral IL-10 Ab (18562D, Pharamingen) diluted at 1 μg/ml in PBS-3% BSA. After four washes, wells were incubated with 100 μl of a 1/10,000 dilution (in PBS-3% BSA) of avidinperoxidase for 30 min at RT. Finally, wells were washed six times, and visualization was achieved by adding 100 μl of 0.5 mg/ml orthophenylene diamine, 12.5 ml 0.1 M sodium acetate, pH 5.5, and 5 μl 30% hydrogen peroxide. The incubation was performed in the dark for −10 min and reaction stopped by the addition of 100 μl of 1N HCl. Absorbance was measured at 492 nm.

Anti-type II collagen (CH) ELISA

Sera were collected from blood samples on days 0, 28, and 37, and 45 were separately stored at −20°C. Individual sera were analyzed for anti-CII Abs using an isotype-specific ELISA as previously described (1). In brief, 50 μl of 5 μg/ml bovine type II collagen solution was coated at RT on 96-well microtiter plates. After overnight incubation at 4°C, plates were washed and blocked for 1 h at RT with 2% casein (100 μl/well). All samples were tested in duplicate at 1/1000 dilution (in PBS-3% BSA) of avidin-peroxidase for 30 min at RT. Finally, wells were washed six times, and visualization was achieved by adding 100 μl of solution containing 5 μg of mouse ACP, 0.5% Tween 20. The plates were blocked with 2% casein for 2 h at RT, and then incubated 1 h at RT with 100 μl of biotinylated anti-human and anti-viral IL-10 Ab and anti-type II collagen Ab (02003E or 02013E, Pharamingen) conjugated to alkaline phosphatase (50 μg/ml) for 1 h. After washing, 100 μl of 0.2% miscol solution was added to the wells, and the absorbance was measured at 405 nm.

Statistics

Statistical analysis was done using Fisher’s exact test for contingency group comparisons and the nonparametric Mann-Whitney test or Student t test, as appropriate according to data distribution. All data were analyzed by the Instat2.1 software program for Macintosh.

Results

Reduced onset of CIA in AdvIL-10-treated DBA/1 mice

To test the effect of IL-10 gene therapy on the incidence of disease, mice were primed with intradermal CIA on day 0. Recombinant AdvIL-10 was given i.v. at a dose of 107 pfu/mouse on day 30. This treatment resulted in inhibition of arthritis onset, only 20 to
30% of the mice having developed arthritis by the end of the experiment (Fig. 1). The difference was statistically significant from the control group (p < 0.01 on day 44, and p < 0.04 on day 47). These results cannot be attributed to the vector, since mice that received the same inoculum of the non-vIL-10-expressing recombinant adenovirus (AdvIL-10 mut) developed an arthritic profile similar to that of untreated mice. In both groups, 100% of the animals progressively reached arthritis by day 50. Moreover, when anti-IL-10 Ab (0.2 mg/mouse) was administered i.v. 24 h before the injection of 10^9 pfu of AdvIL-10, the incidence of arthritis was enhanced, with 50% of the treated animals developing arthritic symptoms on day 30. On the contrary, arthritis onset was markedly delayed in the group injected with 10^10 pfu of AdvIL-10, suggesting a dose-dependent effect. On day 48, 22% of the mice presented clinical symptoms, as did mice treated with 10^9 pfu AdvIL-10. These results suggested that vIL-10 could prevent the onset of arthritis with a single injection of the adenoviral vector on day 30, when clinical symptoms of arthritis start.

Effects of AdvIL-10 on ankle thickness and macroscopic scoring

From day 24 to day 47 after induction of arthritis, thickness of the paws was measured and macroscopic arthritis score was determined in all groups as defined (see Materials and Methods). The injection of 10^9 pfu of AdvIL-10 on day 30 had a suppressive and significant (p < 0.03) effect on joint thickness (Fig. 2). Moreover, in the group treated with an increasing dose of adenoviral vector (10^10 pfu AdvIL-10), a marked reduction was observed when compared with the CIA-untreated group (p < 0.0007 on day 35). When a more complete picture of arthritis was considered, both AdvIL-10 treatments were beneficial, since macroscopic scoring of arthritis revealed significant improvement (Fig. 3; p < 0.001 and p < 0.008, respectively, on day 37).

Confirmation of AdvIL-10 infection and transduction was determined by the use of a viral IL-10 sandwich ELISA for the measurement of serum vIL-10. The Ab used to detect serum vIL-10 did not cross-react with mL-10 (not shown). Seven days after adenoviral vector i.v. injection, ELISA analysis of the sera showed that levels of vIL-10 circulating protein were 50 times higher in the group injected with 10^10 pfu AdvIL-10 compared with 10^9 pfu (31.2 ng/ml vs 0.60 ± 0.48 ng/ml, respectively; data not shown). The protein level in serum peaks between days 3 and 7 following i.v. vector injection, then decreased until day 14 (1.81 ± 0.95 ng/ml vs 0.58 ± 0.76 ng/ml, respectively, in groups injected with 10^10 AdvIL-10 and AdvIL-10 + Ab groups by nonparametric Mann-Whitney test.
with $10^{10}$ pfu and $10^9$ pfu AdvIL-10). However, we could not find a correlation between serum vIL-10 and arthritic scores.

The control group of DBA1 mice treated on day 30 with the control adenoviral vector (AdvIL-10 mut) developed full arthritis on days 35 through 37. This profile of CIA course was similar to that in untreated CIA mice: respectively, $1.80 \pm 0.09$ mm vs $1.81 \pm 0.05$ mm for paw thickness on day 37 (Fig. 2).

At the arthritis peak (day 37), changes measured in joint thickness and arthritic score were significantly different ($p < 0.0032$ and $p < 0.01$, respectively) between mice treated with $10^9$ pfu of AdvIL-10 or AdvIL-10 mut (Figs. 2 and 3).

To assess specificity of observed effects in AdvIL-10-treated groups, we then neutralized the expression of vIL-10 with i.v. injection of a rat anti-vIL-10 Ab 1 day before AdvIL-10 injection. Figure 2 shows an earlier onset and enhanced magnitude of joint swelling in the group (AdvIL-10 + anti-IL-10), even though differences were never significant when compared with control groups (CIA ± AdvIL-10 mut). However, inhibition of exogenous vIL-10 significantly enhanced the expression of CIA when compared with the AdvIL-10-treated group ($p < 0.002$). The same results could be observed with macroscopic scores (Fig. 3) ($p < 0.001$). Figures 1 through 3 represent pooled data of two independent experiments, as can be seen in detail Table I.

**Effects of vIL-10 on joint histology**

Histologic analysis of the ankle joint was performed at day 50. Figure 4 shows representative pictures of untreated and AdvIL-10-treated CIA animals, and Table II summarizes the respective scores. The effects observed in untreated and AdvIL-10 mut-treated mice were infiltration of mononuclear cells into the joint cavity, synovial hyperplasia, pannus formation, and cartilage erosion (Fig. 4, A and B). Only minor thickening of the synovium can be seen in the AdvIL-10-treated group (Fig. 4C). As shown previously, treatment with anti-IL-10 Ab reversed the beneficial effects of AdvIL-10 injection, and joint destruction was dramatically increased (Fig. 4D). The histopathology results for the AdvIL-10-treated groups were comparable to those in age-matched animals that had not been immunized (data not shown).

Analysis of the histologic scoring results indicates that groups treated with $10^9$ or $10^{10}$ pfu of AdvIL-10 have significantly lower overall histopathology compared with the CIA control group (Table II). Thus, AdvIL-10-treated groups were significantly protected against synovial hyperplasia, cartilage erosion, bone destruction, and the overall histopathology as observed in the control groups.

**Radiography analysis**

The scoring of x-rayed paws on day 50 was correlated significantly to histology. When compared, CIA and AdvIL-10 mut radiologic scoring were not significantly different (Table II). However, AdvIL-10 treatment significantly decreased x-ray scoring ($p < 0.024$), and this effect was significantly reversed by anti-IL-10 treatment ($p < 0.023$).

**AdvIL-10 modifies IgG isotype autoantibody levels**

To determine whether the AdvIL-10 treatment of CIA was associated with a modified humoral response to CIA, levels of anti-CIA isotypes produced during the course of arthritis were measured by ELISA in serum samples at different times after immunization. Both IgG2a and IgG1 anti-CII Abs were present in the sera of the prearthritic (day 28) and arthritic (day 37) DBA1 mice (Table III). The relative isotype abundance was IgG1 > IgG2a in all groups. However, the ratio of IgG1:IgG2a increased in AdvIL-10-treated groups. The differences between day 27 and 28 of the isotype IgG1:IgG2a ratio (Th2:Th1) showed that AdvIL-10-treated groups presented a more pronounced Th2 phenotype on day 37 than on day 28, compared with the AdvIL-10 mut or untreated groups.

**Discussion**

The present results show that adenovirus-mediated gene transfer and expression of vIL-10 significantly decrease the frequency of arthritis, delaying the onset and reducing the severity of arthritic symptoms in the mouse DBA1 CIA model. The effect is dose dependent and specifically related to vIL-10 gene expression, since the vector containing mutated vIL-10 is unable to modify the arthritis course. Furthermore, the administration of neutralizing Ab anti-IL-10 is able to abolish the effect of vIL-10 gene transfer on the development of CIA.

These results were obtained not only at the level of macroscopic analysis, but were also confirmed at radiologic and histologic levels. Higher doses of AdvIL-10 ($10^{10}$ pfu/mice) totally prevent the onset of arthritis for 15 days, with a significant protective effect found on joint histopathology ($p < 0.0001$). The majority of mice treated with $10^9$ pfu AdvIL-10 did not show any clinical and histologic evidence of CIA, while the remaining 22% (animals developing arthritic symptoms) presented macroscopic and x-ray scores that were significantly reduced ($p < 0.001$ and $p < 0.024$), respectively; and significant protection of cartilage damage ($p < 0.0001$). We showed that the beneficial action of AdvIL-10 injection could be abolished by pretreating the mice with an anti-vIL-10 Ab. Although differences were never statistically significant, we observed that anti-IL-10-treated mice presented earlier and more severe clinical symptoms than untreated or AdvIL-10 mut-treated mice. This result was related to the cross-reactivity of the neutralizing vIL-10 Ab for mIL-10, due to high sequence homology between murine and viral cytokines. Accelerated expression of arthritis resulting from neutralization of both exogenous and

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**Table I. Effect of AdvIL-10 treatment on CIA**

<table>
<thead>
<tr>
<th>Expt. Group</th>
<th>Incidence (%)</th>
<th>Paw width (mm)</th>
<th>Clinical score</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Control</td>
<td>60</td>
<td>1.81 ± 0.28</td>
<td>3.0 ± 2.0</td>
</tr>
<tr>
<td>AdvIL-10</td>
<td>33.3</td>
<td>1.62 ± 0.11</td>
<td>0.83 ± 1.86</td>
</tr>
<tr>
<td>II Control</td>
<td>66.6</td>
<td>1.68 ± 0.11</td>
<td>1.33 ± 0.95</td>
</tr>
<tr>
<td>AdvIL-10</td>
<td>0</td>
<td>1.61 ± 0.06</td>
<td>0.66 ± 0.94</td>
</tr>
</tbody>
</table>

- At day 30, mice were injected i.v. with or without $10^9$ pfu AdvIL-10.
- Days after immunization with bovine CII.
- Incidence is expressed as percentage of animals per group having at least one affected paw.
- Paw width was measured with a micrometer.
- Clinical score is expressed as mean ± SD, with a maximum cumulative value of 12 for all paws.
endogenous IL-10 emphasizes the important role of IL-10 in preventing CIA progression and has already been noted by other groups as occurring after mIL-10 neutralization (2).

Earlier studies had already suggested that IL-10 was one candidate for experimental CIA treatment (2, 3). Twice a day i.p. injection of murine rIL-10 suppressed established CIA and decreased levels of TNF-α and IL-1 mRNA in synovial tissue and articular cartilage, while levels of IL-1ra mRNA remained elevated. In contrast, neutralization of mIL-10 further accelerated the expression of CIA (2).

IL-10 is abundantly expressed in the joints of RA patients (9, 12, 13), and there is evidence that IL-10 is an important down-regulator of Th1 cells (11) and a deactivator of macrophage functions. In vitro studies have suggested that IL-10 could mediate immunosuppressive effects by: 1) inhibiting proinflammatory cytokines produced by Th1 cells and macrophages such as TNF-α, IL-1β, and IL-8 (9–11) implicated in lymphocyte migration into the synovium; 2) up-regulating soluble TNF-α receptor and IL-1ra monocyte production (32); 3) down-regulating metalloproteases and preferentially stimulating TIMP-1.
production by macrophages, which participate in the degradation and remodeling of extracellular matrix in acute and chronic inflammation situations (7, 8); and 4) inhibiting costimulatory molecule expression on synovial cells (33). Thus, the effects observed with AdvIL-10 treatment could be explained by a powerful anti-inflammatory action of vIL-10 that reduces lymphocyte infiltrates and local immune response within the joint, doubled by a chondroprotective action of the joint via the alteration of macrophage-degradative proteinase/antiproteinase balance.

As cellular IL-10, vIL-10 possesses the anti-inflammatory functions listed above; however, the T cell costimulatory activities were not demonstrated. First, vIL-10 does not costimulate the proliferation of murine thymocytes (34) and mast cell lines (10). Second, vIL-10 reduces the Ag-specific human T cell proliferative response by down-regulating APC class II MHC expression (35). Third, vIL-10 converts activated APCs into resting APCs by down-regulating the expression of costimulatory molecules (36). Fourth, vIL-10 doubles by a chondroprotective action of the joint via the alterations of macrophage-degradative proteinase/antiproteinase balance.

Table II. Clinical, radiologic, and histologic scores of arthritis for AdvIL-10 gene transfer in CIA mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Clinical Score* (day 38)</th>
<th>Radiologic Score*</th>
<th>Histologic Score*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT</td>
<td>3.16 ± 0.59</td>
<td>13.38 ± 8.31</td>
<td>1.80 ± 1.66</td>
</tr>
<tr>
<td>AdvIL-10 (10⁹ pfu)</td>
<td>0.25 ± 0.23**</td>
<td>9.00 ± 6.24*</td>
<td>0.25 ± 0.43*</td>
</tr>
<tr>
<td>AdvIL-10 (10¹⁰ pfu)</td>
<td>0.40 ± 0.35*</td>
<td>8.40 ± 7.02</td>
<td>0.40 ± 0.48**</td>
</tr>
<tr>
<td>AdvIL-10 mut</td>
<td>1.37 ± 0.39</td>
<td>16.75 ± 6.54</td>
<td>1.40 ± 1.80</td>
</tr>
<tr>
<td>AdvIL-10 + anti-IL10</td>
<td>3.16 ± 0.72</td>
<td>15.67 ± 11.4*</td>
<td>2.90 ± 1.37</td>
</tr>
</tbody>
</table>

* Swelling, redness, and presence of arthryema or wound were evaluated visually for clinical scoring of arthritis. The severity of arthritis was graded 0 through 3 as described in Materials and Methods, * p < 0.008 and ** p < 0.001 vs ACI controls, by Mann-Whitney test.

IL-10 has a short circulating half-life (26, 38). Administration of IL-10 produced potent, albeit short, therapeutic effects in murine CIA models, requiring sustained injections (2, 3). We choose gene transfer technology because it developed efficient and prolonged expression of cytokines in vivo. With a single i.v. administration of 10¹⁰ pfu AdvIL-10, we could reach significant levels of circulating protein after 1 wk (~30 ng/ml) and maintain detectable levels for 2 wk (~1 ng/ml). We also demonstrated the absence of nonspecific effect due to the virus, since animals receiving the same dose of adenovirus expressing no vIL-10 (AdvIL-10 mut) developed an arthritic profile similar to that of untreated CIA controls. The use of systemic vs local delivery was intended to target all of the inflamed joints. Although it was debatable whether to express a cytokine systemically, transgene expression was demonstrated to be transient, with 90% of the Ad genome lost from the liver in the first 24 h (39). Thus, although the activity of vIL-10 is compromised by its immunogenicity in the mouse, we can expect the production of neutralizing Abs to be short, since the vector is rapidly eliminated from the blood, and to be weak, because of the high sequence homology between vIL-10 and cIL-10.

Direct injection of vectors into the joints of DBA1 mice was not possible; for technical reasons, injection could only be periarticular. That being the case, injection of first-generation AdvIL-10 into the paws provoked local inflammation (data not shown). Recent work from Whalen et al. showed that with the same model of CIA DBA1 mice only 28.5% of those injected in the rear footpads with 10¹⁰ pfu AdvIL-10 developed arthritis (40). Only the noninjected front paws were arthritic, whereas the AdvIL-10-injected rear paws were protected from CIA for up to 10 wk postimmunization. A critical role of TNF-α and IL-1β in CIA in the DBA1 mouse model has also been shown by several groups using anti-cytokine therapy (5) and IL-1ra (4–5, 41), but the reduction is transient and

Table III. Evolution of Th2/Th1 balance during CIA

<table>
<thead>
<tr>
<th>Days Postpriming</th>
<th>CT</th>
<th>AdvIL-10 (10⁹ pfu)</th>
<th>AdvIL-10 (10¹⁰ pfu)</th>
<th>AdvIL-10 + anti-IL10</th>
</tr>
</thead>
<tbody>
<tr>
<td>28</td>
<td>1.7 ± 0.8</td>
<td>2.1 ± 0.9</td>
<td>1.4 ± 0.4</td>
<td>2.0 ± 1.1</td>
</tr>
<tr>
<td>37</td>
<td>1.3 ± 0.3</td>
<td>1.8 ± 0.7</td>
<td>1.6 ± 0.5</td>
<td>3.3 ± 1.2</td>
</tr>
<tr>
<td>J37–J28</td>
<td>−0.38</td>
<td>−0.22</td>
<td>+0.26</td>
<td>+1.25</td>
</tr>
</tbody>
</table>

* Levels of anti-CII IgG1 and IgG2a in individual sera collected on days 28 and 37 postimmunization with bovine CII were compared according to treatments: untreated CIA group (CT) or groups receiving a single injection of 10⁹ or 10¹⁰ pfu AdvIL-10 on day 30, control group injected with 10¹⁰ pfu of adenoviral vector expressing inactive vIL-10 (AdvIL-10 mut), and group injected with 0.2 mg of monoclonal rat anti-vIL-10 Ab 1 day before (AdvIL-10 + anti-IL10). Results are presented as mean ± SD (n = 5–9).
dependent on continued suppression. Anti-TNF-α treatment appeared to be efficient shortly after the onset of CIA and less so at later stages, while anti-IL-1 ameliorated both early and full-blown CIA (5). Fibroblasts transfected in vitro with the retroviral vector expressing human IL-1ra and then injected intra-articularly into the DBA1 mouse knee joint prevent the onset of CIA (4), attenuating but not abolishing the erosion of cartilage (42). These data suggest that the use of ex vivo gene transfer to obtain local and prolonged expression may lead to improved results in arthritis treatment. However, this approach seems to be utopian in a human RA application perspective because of the risk, the cost, and the time-consuming aspects. Moreover, it is debatable whether using retroviral vectors would achieve gene delivery directly into the resident synovial cells. Its low titer and weak frequency of transfection limits its application in vivo (42). In contrast, the recombinant adenovirus vector is characterized by high titers, lack of potential risk of insertional mutagenesis, and efficient gene transfer in a wide variety of cell types in vivo, including non-replicative cells such as synovial fibroblasts (43). Thus, replication-defective adenoviruses appear more suitable than retrovirus vectors for efficient gene therapy in RA. Particularly in that type of autoimmune disease characterized by chronic phases of joint inflammation, transient expression of the transgene is an ideal method.

Nonetheless, two major issues remain to be solved to optimize the use of adenovirus vectors in gene therapy: 1) stimulation of the host immune response to the vector with a second adenovirus infection, and 2) transgene expression at low levels, in only a small percentage of target cells, without ways to regulate promoter transcription. Better vectors will be available in the near future to optimize expression, targeting, and tolerance.

The AdvIL-10 treatment of CIA was associated with a modified humoral response to CII during the course of arthritis. Although the relative isotype abundance was IgG1 > IgG2a in all groups, the ratio of IgG1/IgG2a increased in AdvIL-10-treated groups between day 28 and 37. The Thy1/Th2 switch observed between days 15 and 30 of CIA development in DBA1 mice occurred (1), but was more marked on day 37 in AdvIL-10 groups than in control or AdvIL-10 mut groups. Thus, AdvIL-10 affects Th2/Th1 balance by orienting the CIA immune response toward a stronger Th2-type cytokine profile, since it has been shown that IgG2a production is associated with a Th1 response, while IgG1 production is associated with a Th2 response. This stronger switch might be due to different regulation of pro- vs anti-inflammatory cytokine production. However, because of the physical limitation of using a CIA mice model, we could not get access to the locally secreted cytokines within the joints. Moreover, ELISA analysis of blood samples from different groups after treatment showed no significant levels for murine IL-1, IL-4, IL-10, TNF-α, or IFN-γ (data not shown).

The LPS acceleration of CIA used in our protocol may also participate in the shift in Th2 cytokine balance observed in all groups by day 21 postimmunization. One explanation for the enhanced type 2 response in CIA groups treated with AdvIL-10 vector could be an IL-10 selective blockage of IL-12 synthesis by APCs (44).

Thus, we have demonstrated that replication-defective adenoviral vector delivery of vIL-10 transgene achieves therapeutic effects in murine CIA. Several mechanisms may contribute to this effect: vIL-10 may inhibit cytokines important for lymphocyte migration toward the joint by inhibiting proinflammatory synthesis of cytokines (9–11) and up-regulating production of their endogenous inhibitors (soluble TNFR and IL-1ra) (32). vIL-10 could also inhibit the specific proliferative response of T cells to CII Ag by down-regulating APC class II MHC molecule expression (33). Moreover, vIL-10 might convert activated into nonreactive APCs by down-regulating the expression of costimulatory molecules (CD86) implicated in the interaction with CTLA-4 and CD28 on synovial cells (33, 36). Finally, protection against cartilage destruction might be due to inhibition of metalloproteinases coupled to stimulation of TIMP-1 production induced by vIL-10 (7).

Cytokines other than IL-10 have natural anti-cytokine or anti-inflammatory activity and are in clinical development. Each of these agents has to be evaluated separately, even if optimal treatment of RA may require a combination of treatments because of the complex, intertwined nature of the cytokine network. However, vIL-10 gene therapy may represent one of the more efficient therapeutic treatments because of its combined anti-inflammatory and chondroprotective actions.

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