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Inhibition of Collagen-Induced Arthritis in Mice by Viral IL-10 Gene Transfer

Yuhe Ma,* Sherry Thornton,* Laura E. Duwel,* Gregory P. Boivin,† Edward H. Giannini,* Jeffrey M. Leiden,‡ Jeffrey A. Bluestone,§ and Raphael Hirsch2*

Autoimmune arthritides are characterized by an imbalance between pro- and anti-inflammatory cytokines. Viral IL-10 (vIL-10) shares many of the anti-inflammatory properties of mouse and human IL-10, but lacks their immunostimulatory properties and may therefore offer superior immunosuppression. Viral IL-10 has a short half-life; however, genetic modification of cells in vivo offers a potential means of achieving prolonged therapeutic titers. To determine the effects on collagen-induced arthritis of vIL-10 gene transfer, DBA/1 mice were administered i.v. or intra-articular injections of Av(vIL-10), a replication-deficient adenovirus encoding vIL-10. The i.v. injection of Av(vIL-10) before disease onset delayed the onset and reduced the severity of collagen-induced arthritis, but treatment of established disease was ineffective. The preventative effects were not due to decreased anti-type II collagen Ab production. Rather, T cells from mice treated with Av(vIL-10) demonstrated a decreased in vitro proliferative response to type II collagen, and a delay was observed in up-regulation of synovial mRNA for the proinflammatory cytokines IL-2 and IL-1β. Intra-articular injection of Av(vIL-10) into knee joints did not reduce arthritis in the knees, but inhibited the development of arthritis in the paws. Humoral and cellular immune responses against Av(vIL-10) were observed. These results demonstrate that vIL-10 can significantly alter the course of autoimmune arthritis and emphasize the complexities of using gene transfer as a method of drug delivery for arthritis. The Journal of Immunology, 1998, 161: 1516–1524.

Rheumatoid arthritis (RA) is an autoimmune disease characterized by both cellular and humoral responses. There exists a complex interaction among T cells, monocytes, and fibroblasts in the rheumatoid lesion, leading to perpetuation of the inflammatory process. Monocyte-derived cytokines, such as IL-6, TNF-α, and IL-1β, are found in the synovium and play a major role in the progression of joint destruction (1, 2). Th cells can be divided into Th1 cells, which mediate delayed-type hypersensitivity by secreting IL-2 and IFN-γ, and Th2 cells, which are involved in Ab-mediated responses by secreting IL-4 and IL-10 (3, 4). A growing body of evidence supports the hypothesis that Th2-derived cytokines can protect against arthritis (5–7), while Th1-derived cytokines exacerbate disease (8). Thus, one strategy for treating autoimmune arthritis is to deviate the immune response from a Th1 to a Th2 pattern.

One Th2 cytokine of potential interest as a therapeutic agent in arthritis is IL-10. IL-10 (9), originally termed cytokine inhibitory factor, is a 35-kDa homodimeric cytokine product of Th2 cells, B cells, and macrophages that has mixed immunosuppressive and immunostimulatory properties. IL-10 can inhibit CIA (10–12).

1 Division of Rheumatology, Children’s Hospital Medical Center; and 2 Division of Comparative Pathology, University of Cincinnati, Cincinnati, OH 45229; and Department of Medicine and 3 Ben May Institute for Cancer Research, University of Chicago, Chicago, IL 60637.

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Address correspondence and reprint requests to: Dr. Raphael Hirsch, Division of Rheumatology, Children’s Hospital Medical Center, PAV 2-129, 3333 Burnet Ave nue, Cincinnati, OH 45229.

Abbreviations used in this paper: RA, rheumatoid arthritis; CIA, collagen-induced arthritis; m, murine; h, human; BCRF1, BamHI C fragment rightward reading frame 1; v, viral; CII, type II collagen; RPA, ribonuclease protection assay.

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thus avoiding the necessity for repeated administrations and allowing sustained therapeutic levels. The present study was designed to determine the effects of gene transfer of vIL-10 on autoimmune arthritis in the murine CIA model, which shares many immunologic features with rheumatoid arthritis (27, 28).

Materials and Methods

Mice

Male DBA/1J mice, 6 to 10 wk of age, were purchased from The Jackson Laboratory (Bar Harbor, ME) and housed in the animal resource facility at Children’s Hospital Research Foundation (Cincinnati, OH).

Adenovirus constructs

Two recombinant, Ela-E3-deleted replication-defective adenovirus type 5 vectors were used in this study. Av(vIL-10) encodes the BCRF1 cDNA driven by the elongation factor-1α promoter and contains the 4F2 heavy chain enhancer and the bovine growth hormone polyadenylation site. Av(BgII), which was used as a control, is identical to Av(vIL-10), except that it lacks the BCRF1 cDNA, the elongation factor-1α promoter, the 4F2 heavy chain enhancer, and the bovine growth hormone polyadenylation site. Recombinant adenoviruses were produced and propagated in 293 cells and were purified by cesium chloride density centrifugation, as previously described (29, 30). Viruses were plaque purified three times before the production of seed stocks, and their identities were confirmed by restriction endonuclease and DNA sequence analysis. Viral titers (particles per milliliter) were determined by OD_{530} \times 10^{12} following lysis of viral stocks in 0.1% SDS, 10 mM Tris-HCl (pH 7.4), and 1 mM EDTA at 56°C for 10 min.

Treatment protocols

Arthritis was induced with bovine type II collagen (CII; Elastin Products Co., Owensville, Missouri), as previously described (31). Mice were injected intradermally with 100 μg of CII in CFA at the base of the tail on day 0. A booster was administered on day 21. For i.v. injections, 10^{10} particles of adenovirus were administered via the lateral tail vein in 100 μl of buffer containing 10 mM Tris (pH 7.4), 1 mM MgCl₂, and 10% (v/v) glycercer. For intra-articular injections, 10^6 particles of virus in a volume of 5 μl of the above buffer were injected into the knee joints, as previously described (32). Mice were evaluated several times a week for arthritis using an established macroscopic scoring system (31) ranging from 0 to 4 (0 = no detectable arthritis, 1 = swelling and/or redness of paw or one digit, 2 = two joints involved, 3 = three or four joints involved, and 4 = severe arthritis of the entire paw and digits). The arthritic index for each mouse was scored using an adaptation of the scoring system of Ginsberg (34) as very severe with cartilage damage. The Mann-Whitney U test (two-tailed, independent) was used to test the statistical significance of intergroup differences in the arthritic indices. This non-parametric statistic was used because the scale of measurement was ordinal, and the distribution of values was typically non-normally distributed. Kaplan Meier (product limit) life table analysis was used to observe the time required for arthritis to develop. The generalised Wilcoxon test was used to compare the two survival curves. At various time points, serum samples were collected and were kept at −20°C for further analysis. Paws were frozen in liquid nitrogen immediately after harvesting and were kept at −80°C for mRNA analysis. Unless otherwise indicated, in vivo experiments were performed with 10 mice/group and repeated to ensure reproducibility.

Viral IL-10 serum titers

Titers of vIL-10 were determined by ELISA. Plates were coated overnight at 4°C with 1 μg/ml of the rat anti-vIL-10 mAb, JES3-9D7 (PharMingen, San Diego, CA), washed (PBS-Tween), and blocked by overnight incubation at 4°C with PBS containing 1% BSA. After washing, aliquots of the serum samples were added to duplicate wells and incubated at 4°C overnight. After washing, the sera were incubated with 125 μCi of mIgG labeled with 5% (v/v) goat anti-mouse IgG (Kirkegaard & Perry Laboratories), and incubated at room temperature for 1 h. Plates were washed and developed with Peroxidase Substrate System ABTS (Kirkegaard & Perry Laboratories). The plates were read at 410 nm on a kinetic microplate reader (Molecular Devices). OD readings for the duplicate wells were averaged and subtracted from background readings of normal mouse serum.

Anti-vIL-10 Ab titers

Titers of anti-vIL-10 Abs were determined by ELISA. ELISA plates were coated overnight at 4°C with 1 μg/ml of the rat anti-vIL-10 mAb, JES3-9D7 (PharMingen), washed (PBS-Tween), and blocked for 1 h at 4°C with PBS containing 1% BSA. After washing, vIL-10-containing supernatant from Av(vIL-10)-infected L293 cells (diluted 1/4 in PBS) was added to each well and incubated overnight at 4°C. After washing, aliquots of the serum samples (diluted in PBS) were added to duplicate wells and incubated at 4°C overnight. After washing, the sera were incubated with 125 μCi of mIgG labeled with 5% (v/v) goat ant-mouse Ig (Kirkegaard & Perry Laboratories), and incubated at room temperature for 1 h. Plates were washed and developed with Peroxidase Substrate System ABTS (Kirkegaard & Perry Laboratories). The plates were read at 410 nm on a kinetic microplate reader (Molecular Devices). OD readings for the duplicate wells were averaged and subtracted from background readings of normal mouse serum.

Anti-CII Ab titers

The titers of anti-CII Abs in the serum samples were determined by ELISA, as previously described (31). All samples were measured in duplicate. Peroxidase-labeled goat-antimouse IgG and IgM (NA26-5, Kirkegaard & Perry Laboratories) were used to measure CII-specific total IgG1 and IgG2a were measured using biotinylated rat anti-mouse IgG1 or IgG2a (PharMingen), followed by streptavidin-peroxidase. Plates were developed with Peroxidase Substrate System ABTS (Kirkegaard & Perry Laboratories). The plates were read at 410 nm, as described above. OD readings for the duplicate wells were averaged. A serum sample from one of the control mice was tested at various dilutions and used as a standard to generate a curve from which relative titers of the other serum samples were calculated.

Proliferation assays

Mice were sacrificed on day 28. Spleens were removed, and single cell suspensions were prepared. Cells (5 \times 10^6/well) were plated in triplicate in 96-well flat-bottom microtiter plates in DMEM containing 0.5% normal DBA/1 mouse serum in the presence of varying concentrations of heat-denatured (56°C for 10 min) CII or the anti-CII Ab, 2C11 (33). Cells were incubated at 37°C for 48 h, followed by addition of 1 μCi of [3H]TdR for an additional 18 h. Cells were harvested and [3H]TdR incorporation per well was measured and averaged for each triplicate. Background counts from unstimulated cells were subtracted from each group.

mRNA analysis

Joints were homogenized with a Tissue Tearor (Biospec Products, Bartville, OK), and RNA was extracted with RNASTAT 60 (TelTest, Friendswood TX). RNase protection assays (RPA) were performed on 2.5 to 5 μg of poly(A)⁺ RNA using the Riboquant MultiProbe RNase Protection Assay System (PharMingen) following the manufacturer’s instructions. Each commercial kit contained a set of cytokine/chemokine templates as well as a template for the housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase. [α-32P]UTP-labeled antisense RNA probes were synthesized by in vitro transcription from these cDNA templates. Antisense RNA probes were purified by phenol/chloroform extraction and ethanol precipitation and were hybridized with the mRNA samples at 56°C overnight. Unhybridized single-stranded RNA was digested by RNase treatment. Double-stranded RNA was purified by phenol/chloroform extraction and ethanol precipitation. The samples were electrophoresed in a 12% denaturing polyacrylamide gel. The gel was then dried and exposed to a PhosphorImager screen. Radioactivity of the samples was measured by scanning on a PhosphorImager (Molecular Dynamics, Sunnyvale, CA) and analyzed by ImageQuant software (Molecular Dynamics). The mRNA level of each cytokine was expressed as the ratio of the PhosphorImager units of the cytokine to those of glyceraldehyde-3-phosphate dehydrogenase from the same RNA sample.

Histology

The knees and paws were fixed in 10% neutral buffered formalin and dehydrated in a gradient of alcohols, paraffin embedded, sectioned at 5 μm, mounted on glass slides, and stained with hematoxylin and eosin. Histopathologic analysis was performed by a blinded observer. Inflammation was scored using an adaptation of the scoring system of Ginsberg (34) as follows: 0 = no inflammation, 1 = mild; 2 = moderate, 3 = severe, and 4 = very severe with cartilage damage. The Mann-Whitney U test (two-tailed, independent) was used to test the statistical significance of intergroup differences in the histopathologic scores.
Results

Viral IL-10 expression following in vivo administration of Av(vIL-10)

To determine whether administration of Av(vIL-10) resulted in detectable levels of vIL-10 in vivo, DBA/1 mice were injected i.v. with $10^7$, $10^8$, $10^9$, or $10^{10}$ particles of Av(vIL-10). Mice were bled 1, 3, 7, 10, 14, 21, and 28 days following injection (two mice per dose per time point), and serum samples were pooled and analyzed for vIL-10 by ELISA. Administration of $10^{10}$ particles of Av(vIL-10) resulted in detectable serum levels of vIL-10 as early as 1 day after administration and peaking at 3 days (Fig. 1A). This was followed by a drop in titer such that by 10 days postinjection vIL-10 was undetectable by ELISA. The drop in vIL-10 titer was associated with development of circulating Abs to vIL-10 (Fig. 1B). Administration of $10^9$ particles resulted in lower, but still detectable, vIL-10, whereas vIL-10 was not detected in the serum of mice administered $10^8$ or $10^7$ particles (data not shown). Doses $>10^{10}$ particles induced morbidity.

Viral IL-10 gene transfer delays the onset and reduces the incidence and severity of CIA

Having determined that administration of Av(vIL-10) results in circulating vIL-10, its effects on CIA were next investigated. To maximize circulating levels of vIL-10, a dose of $10^{10}$ particles was used for these experiments. DBA/1 mice were immunized on days 0 and 21 with CII (10 mice/group). Adenovirus was administered on day 20 to ensure that vIL-10 would be present during the early stages of disease. A delay in onset of CIA was observed in mice receiving Av(vIL-10). By day 33, 90% of Av(BglII)-treated mice, but only 20% of Av(vIL-10)-treated mice had CIA (Fig. 2). Life-table analysis showed that the time to develop arthritis was significantly longer in the Av(vIL-10)-treated group than in the Av(BglII)-treated controls ($p = 0.002$, by generalized Wilcoxon test). The incidence of CIA in the Av(vIL-10)-treated group gradually increased to 60% by day 40. The severity of CIA in mice treated with Av(vIL-10) was also significantly lower than that in the control group at early time points (days 28–33; Table I). There was an increase in the severity of CIA in Av(vIL-10)-treated mice at later time points; however, arthritic indexes remained significantly lower than those in the controls. Disease onset and severity were similar in mice receiving the Av(BglII) control virus and mice receiving buffer alone, suggesting that adenovirus by itself did not affect the disease.

vIL-10 gene transfer inhibits the proliferative response to CII

The mechanisms responsible for the observed decrease in incidence and severity of CIA following treatment with Av(vIL-10) were next investigated. Viral IL-10 has a number of potential immunoregulatory effects, including inhibition of proliferation and IL-2 production by T cells (22), and inhibition of proinflammatory cytokine production by monocytes (20, 21, 23). To determine the effects of vIL-10 on T cell responses to CII, mice were immunized with CII on days 0 and 21. On day 20, mice were treated with buffer, Av(vIL-10), or Av(BglII). Four mice per group were sacrificed on day 28, and spleen cells were analyzed in vitro for proliferation in response to CII or to the anti-CD3 mAb, 2C11. While spleen cells from control mice proliferated in response to CII, spleen cells from mice receiving Av(vIL-10) responded minimally to CII, comparable to the response observed in unimmunized DBA/1 mice (Fig. 3A). The proliferative response to the pan-T cell mAb, 2C11 was unaffected (Fig. 3B), consistent with the inhibitory effects of vIL-10.

Table I. Severity of CIA following i.v. administration of adenovirus

<table>
<thead>
<tr>
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<th>Day 28</th>
<th>Day 33</th>
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<td>Buffer</td>
<td>0</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Av(BglII)</td>
<td>1.5</td>
<td>7.5</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Av(vIL-10)</td>
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<td>0</td>
<td>3.5</td>
<td>4.5</td>
</tr>
<tr>
<td>p-value</td>
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<td>0.001</td>
<td>0.04</td>
<td>0.03</td>
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* Mice were immunized with CII as described in Materials and Methods. On day 20, mice received i.v. injections of buffer or $10^{10}$ particles of either Av(vIL-10) or Av(BglII). The data represent the median arthritic index of 10 mice/group. The p-values are between the Av(BglII) and Av(vIL-10) groups. Differences between buffer and Av(BglII) were not significant.
on Ag presentation by down-regulation of MHC class II expression on monocytes (22).

Viral IL-10 gene transfer is associated with delayed expression of proinflammatory cytokine mRNA in response to CII immunization

As vIL-10 is known to inhibit secretion of the proinflammatory cytokine IL-2 from T cells (22) and of IL-1β from monocytes (20, 21, 23), paws were analyzed by RPA for expression of these products. Comparison was made between day 34, when the incidence of arthritis was significantly reduced in Av(vIL-10)-treated mice, and day 48, when the incidence was comparable to that of controls. Total RNA was extracted from four paws per group and subjected to RPA analysis. As a group, Av(vIL-10)-treated mice had decreased mean levels of mRNA for IL-2 and IL-1β on day 34 compared with those in the control mice (Fig. 4). On day 48, mRNA for these cytokines were higher in the Av(vIL-10) group, consistent with their delayed onset of arthritis. Individual paws with similar paw scores had similar cytokine mRNA levels regardless of which virus vector they received (data not shown). Thus, the early decrease in mean cytokine mRNA levels correlated with the decrease in mean paw scores of the Av(vIL-10)-treated mice. As was observed with disease incidence and severity, the late increase in cytokine mRNA expression in the Av(vIL-10)-treated mice correlated with the drop in circulating vIL-10 titers and the increase in the incidence and severity of disease.

Viral IL-10 gene transfer does not affect the humoral response to CII

CIA is mediated by both T cells and anti-CII Abs. To determine the possible effects of vIL-10 gene transfer on the humoral immune response to CII, mice were bled at various times following treatment (10 mice/group), and sera were analyzed for Abs to CII. Ab levels varied greatly between mice, but no significant differences were observed between the groups of mice in circulating titers of total Ig, IgG1, or IgG2a Abs against CII (Fig. 5). Thus, the inhibition of CIA by vIL-10 was unlikely to be due to inhibition of autoantibody production.

Viral IL-10 gene transfer does not inhibit established CIA

The late increase in the incidence and severity of arthritis in the Av(vIL-10)-treated group correlated with the observed drop in vIL-10 titers following the administration of Av(vIL-10). These findings suggested that circulating vIL-10 was necessary to maintain protection from disease. The rapid loss of circulating vIL-10 was most likely due to an immune response to vIL-10 as well as to adenoviral proteins. In numerous model systems, it has not been possible to readminister adenovirus unless concomitant immunosuppression is used due to the development of neutralizing Abs (35–40). Thus, it was unlikely that a second administration would prolong the effects of vIL-10. To determine whether gene therapy with vIL-10 could protect mice following disease onset, virus was

FIGURE 3. Viral IL-10 gene transfer inhibits the proliferative response to CII. Mice were immunized with CII as described in Materials and Methods. On day 20, mice received i.v. injections of buffer or 10^{10} particles of either Av(vIL-10) or Av(BglII). Mice were sacrificed on day 28, and spleen cells were stimulated with 100 μg of heat-denatured CII (A) or 25% anti-CD3 (2C11)-containing culture supernatant (B). A pool of three unimmunized DBA/1 spleens was used for assessment of the basal response. Proliferation was measured at 48 h. Data represent the mean ± SEM of four mice per group.

FIGURE 4. Viral IL-10 gene transfer inhibits transcription of proinflammatory cytokines by synovial cells. Mice were immunized with CII as described in Materials and Methods. On day 20, mice received i.v. injections of buffer or 10^{10} particles of Av(vIL-10) or Av(BglII). Mice were sacrificed on days 34 and 48. Hind paws were removed, and mRNA analysis was performed by RPA. Data represent the mean of four mice per group. A paw from an unimmunized mouse (dashed lines) was analyzed simultaneously for assessment of the basal response.
administered to 10 mice/group on day 32, when 75% of the animals had developed arthritis. No significant differences were observed in the severity of disease between mice receiving Av(vIL-10) and those receiving Av(BgIII) (Fig. 6), suggesting that vIL-10 was ineffective in established disease.

**Intra-articular gene transfer of vIL-10 does not protect the injected joint, but inhibits the development of CIA in distal joints**

Intravenous injection of adenovirus results in transgene expression mostly in the liver (41–43). To determine the effects on CIA of local synovial expression of vIL-10, Av(vIL-10) or buffer was administered on day 20 into the knee joints of CII-immunized mice (10 mice/group). On days 28 and 34, mice were sacrificed, and knee joints were evaluated by histopathology. Knee joints were not protected by intra-articular administration of Av(vIL-10). Both Av(vIL-10)-treated and buffer-treated knees demonstrated severe inflammation, characterized by inflammatory cells in the synovium, joint space, and fat pad with moderate fibrosis of the fat pad and severe synovial hyperplasia, necrosis and sloughing (Fig. 7, A and B). Median histopathology scores were 4 and 3.5, respectively (difference not significant), at both time points. This lack of protection was not due to failure of adenovirus to infect the synovium, as vIL-10 mRNA in synovial tissues could be detected by PCR for up to 8 wk (data not shown). To determine whether this lack of apparent protection by vIL-10 might be due to an anti-adenovirus inflammatory response, unimmunized mice were injected intra-articularly with buffer, Av(vIL-10), or Av(BgIII). Mice were sacrificed 7 and 14 days later, and knees were evaluated histologically (six knees per group per time point). Surprisingly, the Av(vIL-10) vector was significantly more inflammatory at both time points than Av(BgIII), with increased inflammation in the synovium, fat pad, and joint space (Fig. 7, C–E). High power views demonstrated that this inflammatory response was mostly neutrophilic (not shown), consistent with our earlier description of synovial inflammation following intra-articular administration of adenovirus (32). The median histopathologic score following administration of Av(vIL-10) was 3, compared with 2 following administration of Av(BgIII) (two-tailed \( p = 0.004 \)). Thus, vIL-10 expression in the synovium did not inhibit adenovirus-induced inflammation, and actually promoted inflammation.

Intra-articular expression of IL-1ra in the knees of mice has been shown to inhibit CIA in the hind paws, and it has been suggested that this may be due to a specific effect on the “draining joints” (44). To determine whether expression of vIL-10 in the knees would affect distal paws, virus was administered into knee joints, and paws were scored for arthritis (10 mice/group). Intra-articular injection into the knees resulted in effects on the paws similar to those observed following i.v. injection. Administration of \( 10^9 \) particles of Av(vIL-10) into each knee significantly delayed the onset of arthritis in the paws (Fig. 8). By day 31, 100% of Av(BgIII)-treated mice, but only 25% of Av(vIL-10)-treated mice had detectable CIA, as defined by arthritis in the paws. Life-table analysis showed that the time to develop arthritis was significantly longer in the Av(vIL-10)-treated group than that in the Av(BgIII)-treated controls (\( p < 0.001 \), by generalized Wilcoxon test). The incidence of CIA in the Av(vIL-10)-treated group gradually increased to 80% by day 36. The severity of CIA in mice treated with Av(vIL-10) was also significantly lower than that in the control groups at early time points (days 28–34; Table II). As was observed following i.v. administration, there was an increase in the severity of CIA in Av(vIL-10)-treated mice at later time points; however, arthritic indexes remained significantly lower than those in the controls. Fore as well as hind paws were protected, suggesting that the effects were global, rather than limited to draining joints.

**Discussion**

The present study demonstrates for the first time that vIL-10 can inhibit the development of joint inflammation in an experimental model of autoimmune arthritis. Since vIL-10 appears to lack the potential adverse effects of cellular IL-10 on autoantibody production, it has been suggested that it might be preferable to cellular
The ability of vIL-10 to inhibit T cell proliferation in response to CII is consistent with the findings of earlier studies on the effects of vIL-10 on mononuclear cells (20, 21, 23) and is likely to result from down-regulation of MHC class II expression on APCs (22), which can be bypassed with anti-CD3 mAb. IL-1β has been shown to exacerbate CIA (45–47), and it is likely that the anti-arthritisogenic effects of vIL-10 are due at least in part to its suppression.

The inability of vIL-10 to inhibit ongoing disease is interesting in light of recent studies with murine IL-10 that showed a mild therapeutic effect (10, 12). However, another study showed no effect of murine IL-10 on ongoing disease (48). These apparently discrepant findings might be a result of dose and timing of administration. In the case of vIL-10, it is also possible that certain biologic properties distinct from murine IL-10 are responsible for the lack of effect. We were unable to increase the dose of adenovirus administered due to toxicity. Recent promising clinical studies using soluble TNF receptor (49), Abs to TNF (50), and IL-1R antagonist (51, 52) suggest that such proteins may have improved efficacy over conventional drug therapy in the treatment of arthritis. However, they can be associated with wide swings in peak and trough levels and require frequent repeated administration because of short half-lives. Gene transfer is attractive as a potential means to deliver consistent, prolonged therapeutic titers of anti-inflammatory proteins with fewer side effects and without the need for repeated administrations.

Two general gene transfer strategies have been proposed for RA. The first is systemic delivery, based on the hypothesis that RA is a systemic disease and that the drug should therefore reach regional lymph nodes and lymphoid organs for maximal effect. The observed T cell proliferative response to CII in spleen cells of CII-immunized mice demonstrates that the autoimmune response is not limited to the synovium and suggests that the systemic approach used in the present study may be advantageous. Similar
or 10^9 particles of either Av(vIL-10) or Av(BglII). The data represent 10 mice/group.

A second gene transfer strategy for RA is intra-articular administration of the transfer vector or of transduced cells to obtain local gene expression in the synovium. This strategy is based on the hypothesis that local gene expression will control disease with fewer systemic side effects. Studies in a number of animal systems, including rabbits, rats, and mice, demonstrate that local expression of IL-1 RA can inhibit the inflammatory process within a particular joint (54–57), as can expression of Fas ligand (58). Our observations that transgene expression in the knee joints can inhibit arthritis in the paws has also been reported following intra-articular injection of cells transduced with the IL-1R antagonist gene (44). However, in contrast to that study, in which only the draining paws were protected, in our study all paws were protected by treatment. Our findings are unlikely to be due to leakage of adenovirus particles to the paws, since infection remains localized to the synovial lining following intra-articular injection of adenovirus (32). Given the similar response observed following i.v. or intra-articular administration of Av(vIL-10) it is probable that the protective effect on distant joints is a result of leakage of sufficient cytokine protein into the circulation. However, these findings do not rule out the possibility that different sites of disease are interdependent. Further studies will be necessary to address these issues and to determine the optimal route of administration.

We and others have previously demonstrated that injection of adenovirus into mouse joints induces a viral synovitis (32, 59). This may exacerbate an already inflamed joint. In the present study, despite successful gene transfer and expression of the vIL-10 transgene, knee joints developed increased inflammation, most likely secondary to viral synovitis, indicating that vIL-10 could not overcome the immune response to adenovirus. Similar findings were observed following intra-articular injection of an adenovirus encoding TNF receptor (59). At the present time, adenovirus is the most efficient gene transfer vector due to its ability to infect a wide variety of cell types at a high frequency. It can be made replication incompetent, is incorporated episomally, and does not integrate into the host chromosome. It is thus relatively safe. The liver is the main target of adenovirus following i.v. administration (41–43), and the transient expression observed in the present study is a well-recognized limitation of adenoviral vectors and is considered to be due to cell-mediated and humoral immune responses resulting in rapid elimination of transduced cells (34–40, 42, 43).

The data presented here indicate that the humoral response to the transgene protein, in this case vIL-10, probably also contributes to the loss of effect. The loss of circulating vIL-10 was probably responsible for the inability of vIL-10 to completely prevent disease. If gene therapy is to become a practical treatment, methods to further prolong gene expression will be necessary. Retreatment with the identical adenovirus vector has not proven effective in most studies unless the mice are immunosuppressed, due to the development of an immune response to both the xenogeneic protein and the adenovirus particles (35–40). It was hoped that the immunosuppressive properties of vIL-10 might give long lasting transgene expression; however, this was not the case. In fact, the vIL-10-encoding vector was more inflammatory than the control vector, suggesting that the vIL-10 protein was itself immunogenic in this setting. This is supported by the rapid rise in anti-vIL-10 Abs observed in the present study. This finding is of interest in view of the recent report that vIL-10 was able to inhibit the immune response to adenovirus administered into cardiac allografts (60).

RA is a leading cause of long term disability in the United States. Present therapies, while partially effective at controlling symptoms, have shown minimal efficacy at controlling disease progression. Gene therapy for arthritis is still in its infancy, and the optimal gene products, transfer vectors, and route of administration have yet to be determined. As demonstrated in the present study, the clinical use of presently available adenoviral vectors for treatment of arthritis appears premature. It is evident that if gene transfer is to be used to treat arthritis, it will be necessary to develop better gene delivery vectors that will allow stable gene expression without inducing an immune response against either the vector or the transgene product. These are significant obstacles. Nonetheless, these and other studies demonstrate the feasibility of this approach and suggest that gene transfer holds promise as a therapeutic modality for arthritis.

Table II. Severity of CIA following intra-articular administration of adenovirus*

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<tr>
<th></th>
<th>Day 28</th>
<th>Day 34</th>
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<tr>
<td></td>
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</tr>
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<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Av(BglII)</td>
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<td>0</td>
<td>1</td>
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<tr>
<td>Av(vIL-10)</td>
<td>0</td>
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*p-values are between the Av(BglII) and Av(vIL-10) groups. Differences between buffer and Av(BglII) were not significant.*
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References


