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Effective Treatment of Established Murine Collagen-Induced Arthritis by Systemic Administration of Dendritic Cells Genetically Modified to Express IL-4[1]

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Dendritic cells (DC) are APCs that are able to stimulate or inhibit immune responses, depending on levels of expression of MHC class I and II costimulatory molecules and cytokines. Our previous studies have suggested that the observed contralateral effect, where injection of a vector carrying certain immunomodulatory genes into one joint resulted in inhibition of arthritis in untreated joints, is mediated by in vivo modification of DC. Therefore, we have examined the ability of genetically modified DC to suppress established murine collagen-induced arthritis (CIA) after i.v. delivery. IL-4 has been shown to partially reduce the severity of CIA after repeated injection of recombinant protein or by injection of an adenoviral vector expressing IL-4. Here we demonstrate that i.v. injection of immature DC, infected with an adenoviral vector expressing IL-4, into mice with established CIA resulted in almost complete suppression of disease, with no recurrence for up to 4 wk posttreatment. Injection i.v. of fluorescently labeled DC demonstrated that the cells rapidly migrated to the liver and spleen after 6 h and to the lymph nodes by 24 h. In culture, spleen cells from DC/IL-4-treated mice produced less IFN-γ after stimulation by collagen than did control groups. In addition, DC/IL-4 administration decreased the level of specific Abs against type II collagen, in particular the IgG2 Th1 isotype 14 days posttreatment. These results demonstrate the ability to treat effectively established murine arthritis by systemic administration of DC expressing IL-4. ***The Journal of Immunology,*** **2001, 166: 3499–3505.**

Rheumatoid arthritis (RA)[4] is a chronic systemic autoimmune disease characterized by joint inflammation as well as progressive cartilage and bone erosion. The intraarticular cytokines in active disease are produced predominantly by macrophages and fibroblasts, although increased levels of T cell lymphokines expressed preferentially from Th1 cells such as IFN-γ and IL-2 are also present (1). These Th1 cytokines have been implicated in exacerbation of autoimmunity (2–5), whereas products of Th2 cells, such as IL-4, have been found to be protective (6).

IL-4, similar to TNF-α and IL-1 inhibitors, has a therapeutic effect in murine collagen-induced arthritis (CIA) when administered i.v. as a recombinant protein, either alone or in combination with IL-10. IL-4 can down-regulate the production of proinflammatory and Th1-type cytokines by inducing their mRNA degradation and can up-regulate the expression of inhibitors of proinflammatory cytokines such as IL-1 receptor antagonist (7, 8). IL-4 inhibits IL-2 and IFN-γ production by Th1 cells, resulting in suppression of both macrophage activation and production of the proinflammatory cytokines IL-1, IL-6, IL-8, and TNF-α (9–12). In addition, IL-4 inhibits growth factor-induced RA synoviocyte proliferation, expression of PGE, and matrix metalloproteinase-3 from RA synovial fibroblasts (13, 14). IL-4 also blocks bone resorption. Given these properties, IL-4 presents an attractive cytokine for use in treating arthritis. We have suggested previously that gene transfer is a promising strategy for the therapeutic delivery of antiarthritic cytokines and cytokine antagonists (15–17). We and others have shown recently that intraarticular and systemic delivery of IL-4 using an adenoviral vector can partially suppress established murine CIA (18–20).

T cells and dendritic cells (DC) interact to initiate immune responses against invading pathogens. The combination of the different Ag-presenting functions of the APCs can determine whether an immune response is immunogenic or tolerogenic (21). DC not only provide a common set of signals to initiate clonal expansion of T cells but also provide T cells with selective signals that lead to either Th1 or Th2 immunity. Because Th1 overactivity predominates in most animal models of autoimmune disease (22), skewing differentiation of T helper cells toward a Th2 phenotype should be therapeutic in treating Th1-driven autoimmune diseases. Previously several groups have shown that genetic modification of APCs to express Fas ligand (FasL) in conjunction with specific Ag(s) results in elimination of Ag-specific T cells (23, 24). In addition, expression of gene products able to block costimulation, such as CTLA4-Ig, appear able to induce T cell anergy to specific Ags (25–27). Therefore, it may be possible to drive differentiation of Th0 cells toward a Th2 response by expression of certain cytokines in DC such as IL-4.

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[4] Abbreviations used in this paper: RA, rheumatoid arthritis; CIA, collagen-induced arthritis; DC, dendritic cells; FasL, Fas ligand; vIL-10, viral IL-10; Ad-IL-4, adenoviral vector expressing IL-4; Ad-vIL-10, adenoviral vector expressing viral IL-10; BmDC, bone marrow-derived DC; CM, complete medium; MOI, multiplicity of infection; Ad-eGFP, adenoviral vector expressing enhanced green fluorescent protein.

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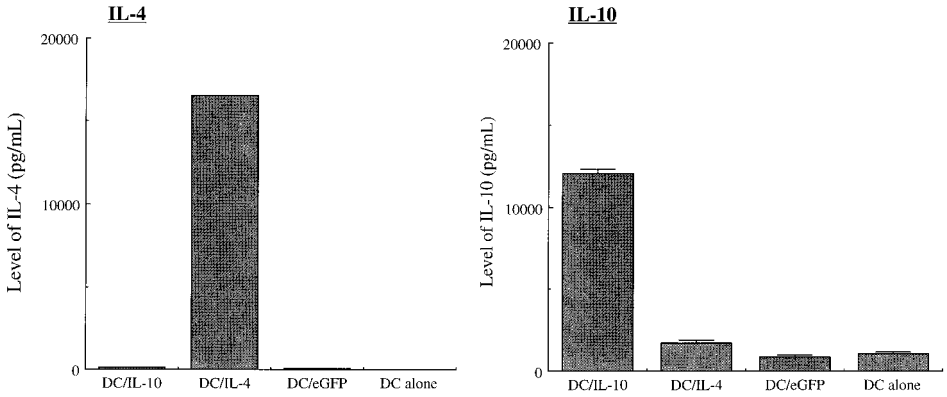

FIGURE 1. Production of IL-4 and IL-10 by dendritic cells after Ad-IL-4 transduction. BmDC were infected with 50 MOI adenoviral murine IL-4, Ad-IL-10, or Ad-eGFP on day 5 after DC generation for 24 h in serum-free medium. On day 6, the medium was replaced with fresh CM, and infected cells were incubated for 24 h. Cell-free media were collected, and the IL-4 and IL-10 concentrations were measured by ELISA.

The rational for using genetically modified DC for treatment of arthritis is supported further by our previous studies suggesting that DCs are important for mediating the observed contralateral effect. Intraarticular injection of adenoviral vectors expressing IL-4, viral IL-10 (vIL-10), and a combination of IL-1- and TNF-soluble receptors resulted in a therapeutic effect in the injected joint as well as untreated distal or contralateral joints in rabbit and murine models of inflammatory disease (28–30). Furthermore, adoptive transfer of DC from animals treated with Ad-vIL-10 to naive animals resulted in a therapeutic effect.[5] Therefore, we have examined the ability of genetically modified DC to suppress established murine CIA. Here we demonstrate that i.v. injection of immature DC, infected with Ad-IL-4, into mice with established CIA resulted in almost complete suppression of disease with no recurrence of disease for up to 4 wk posttreatment.

Materials and Methods

Mice

Male DBA1/lacJ (H-2^q) mice, 7–8 wk of age, were purchased from The Jackson Laboratory (Bar Harbor, ME). They were maintained in a pathogen-free animal facility at the University of Pittsburgh Biotechnology Center (Pittsburgh, PA).

Vector construction and adenovirus generation

Ad-IL-4, Ad-IL-10, and adenoviral vector expressing enhanced green fluorescent protein (Ad-eGFP) were constructed, propagated, and titered according to standard protocols as previously described (31). Briefly, the recombinant adenoviruses were generated by homologous recombination in 293 cells expressing Cre recombinase (CRE8 cells), after cotransfection of DNA, an adenovirus 5-derived, E1- and E3-deleted adenoviral backbone (psi 5), and pAd-lox, the adenoviral shuttle vector. The inserted cDNA sequences are expressed under the transcriptional control of the human CMV promoter. The recombinant adenoviral vectors were purified over three consecutive CsCl gradient ultracentrifugations, dialyzed at 4°C against sterile virus buffer, aliquoted, and stored at −80°C until use. Recombinant adenoviruses were titrated on CRE8 cells for PFUs. The CRE8 cell line was grown in DMEM (Life Technologies, Gaithersburg, MD) supplemented with 10% FBS.

Generation and culture of DC

Bone marrow-derived DC (BmDC) were generated as described previously (32). Briefly, bone marrow was collected from tibias and femurs of female DBA1/lacJ mice and passed through a nylon mesh to remove small pieces of bone and debris. Contaminating erythrocytes were lysed with 0.83 M NH_4Cl buffer, and lymphocytes were depleted with a mixture of Abs (RA3-3A1/6.1, anti-B220; 2.43, anti-Lyt2; GK1.5, anti-L3T4; all from American Type Culture Collection, Manassas, VA) and rabbit complement (Accurate Chemical and Scientific, Westbury, NY) on day 0. The cells then were cultured for 24 h in complete medium (CM; RPMI 1640 containing 10% FBS, 50 μM 2-ME, 2 mM glutamine, 0.1 mM nonessential amino acids, 100 μg/ml streptomycin, and 100 IU/ml penicillin) to remove the adherent macrophages. The nonadherent cells were then placed in fresh CM containing recombinant murine GM-CSF (1000 U/ml) and recombinant murine IL-4 (1000 U/ml) on day 1. DCs were defined by phenotypic analysis for expression of CD11b, CD11c, CD80, CD86, and MHC class I and class II in the majority of the cultured cells (60–95%) by FACScan (Becton Dickinson, Sunnyvale, CA). Cells were cultured for 4 days and harvested for adenoviral transduction on day 5.

For adenoviral infection, 1×10^6 DC/well were plated on 24-well plates, and 5×10^7 PFU viruses were added in a total volume of 1 ml serum-free medium. After incubation for 24 h at 37°C, 1 ml CM was added into each well and incubated for a further 24 h. On day 6, infected DC was recovered and injected into animals.

Induction of arthritis

Bovine type II collagen (Chondrex LLC, Redmond, WA) was dissolved in 0.05 M acetic acid at a concentration of 2 mg/ml by stirring overnight at 4°C and was emulsified in an equal volume of CFA. The mice were immunized intradermally at the base of the tail with 100 μg collagen. On day 21 after priming, the mice received an intradermal booster injection with 100 μg collagen in IFA. For the synchronous onset of arthritis, 40 μg LPS (Sigma, St. Louis, MO) were injected i.p. on day 28.

Disease evaluation

Mice were monitored every other day by an established macroscopic system ranging from 0 to 4 : 0 = normal; 1 = detectable arthritis with erythma; 2 = significant swelling and redness; 3 = severe swelling and redness from joint to digit; and 4 = maximal swelling and deformity with ankylosis. The average of macroscopic score was expressed as a cumulative value for all paws, with a maximum possible score of 16 per mouse. The thickness of each paw was also measured with a spring-loaded caliper. The paw swelling for each mouse was calculated by adding the thicknesses of all four paws. In addition, the numbers of arthritic paws per mouse were added to represent the number of arthritic paws in a group, with a maximum possible score of 80 per group of 20 mice. The in vivo experiments were performed with 10 mice/group and repeated twice to ensure reproducibility.

Histologic examination

Joint tissue from freshly dissected mice was immersion-fixed in 10% neutral buffered formalin and decalcified in 15% EDTA, 30% glycerol. Tissues were then dehydrated in a gradient of alcohols, paraffin embedded, sectioned at 5 μm, mounted on glass slides, and stained with hematoxylin and eosin.

For frozen sections, tissues were fixed in 2% paraformaldehyde solution overnight and snap frozen in embedding medium (10.24% polyvinyl alcohol, 4.26% polyethylene glycol). The specimens were sectioned at 5 μm using a cryostat and were observed using a BH2-RFCA fluorescence microscope (Olympus, Tokyo, Japan).

Flow cytometry

For phenotypic analysis, DC were stained with PE- or FITC-conjugated mAbs against murine surface molecules (CD11b, CD11c, CD80, CD86, Gr-1, H-2Kb, I-Ab, and appropriate isotype controls; all Abs were obtained from PharMingen, San Diego, CA) and examined by FACScan (Becton Dickinson).

[5] J. D. Whalen, A. W. Thomson, L. Lu, P. D. Robbins, and C. H. Evans. Short-term transfer and expression of the vIL-10 gene in vivo induces a suppressor population of antigen presenting cells which can adoptively transfer suppression to soluble antigens. *Submitted for publication.*

Luciferase assays

To determine the migration of DC transduced with adenoviral vector expressing-Luciferase, tissues were dissected after sacrifice of the animals and stored at −80°C. Approximately 0.1 g of each tissue was mixed with 0.2 ml 0.25 M Tris-HCl, pH 7.5, and the mixture was homogenized by hand with a tightly fitting Dounce homogenizer. The homogenate was collected, subjected to three freeze-thaw cycles, and centrifuged for15 min at low speed in a tabletop clinical centrifuge. Luciferase activity in 50 μl supernatant was measured in a luminometer using a luciferase assay kit purchased from Roche (Somerville, NJ). Luciferase activity was standardized per milligram of tissue after quantitative assay of protein.

Cytokine production

Cytokine production in serum or by cultured spleen or lymph node cells was assessed by ELISA (R&D Systems, Minneapolis, MN). To test cytokine production, 1×10^6 cells from lymph nodes and spleen were isolated and stimulated with 100 μg/ml bovine type II collagen, or 50 μg/ml Con A in 1 ml medium in 24-well plates. Culture supernatant was collected 48 h after stimulation.

Type II collagen Ab titration

Serum level of Ab against type II collagen was measured by a standard ELISA. Briefly, a 96-well Immuno-Maxisorp Plate (Nunc, Roskilde, Denmark) was coated with murine type II collagen (10 μg/ml) overnight at 4°C and blocked with 10% FBS in PBS. Sample sera were diluted at 1/100,000 and incubated for 2 h at 37°C. After washing, bound Ab isotypes were detected with biotin-conjugated anti-rat whole IgG (heavy and light chain) Ab (PharMingen, San Diego, CA). Thereafter, plates were washed, incubated with 100 μl 2,2-azinodi(3-ethylbenzthiazoline sulfonate) substrate (ABTS; Boehringer Mannheim, Indianapolis, IN) at 1 mg/ml and read at 405 nm.

Statistical analysis

Results were compared using the Student's *t* test and by ANOVA. $p < 0.05$ was considered statistically significant.

Results

Ad-IL-4 transduction of BmDC

We have shown previously that injection of an adenovirus expressing murine IL-4 or vIL-10 into the ankle joints of mice with CIA was able to provide a therapeutic effect in the injected ankle joint as well as in untreated paws (20, 29). Analysis of the mechanism of this "contralateral effect" has suggested that in vivo modification of DC may be important).[5] Therefore, we examined the therapeutic effects of injecting DC, genetically engineered to express IL-4 or IL-10, into mice with established CIA. Initially, BmDC were infected for 24 h in serum-free medium with Ad-mIL-4, Ad-IL-10, or Ad-eGFP at a multiplicity of infection (MOI) of 50 on day 5 after DC generation. On day 6, media were changed with fresh CM, and infected cells were incubated for a further 24 h. Cell-free media were collected, and IL-4 production was measured by ELISA. DCs (10^6), infected with Ad-IL-4 or Ad-IL-10, secreted 16.7 and 12.1 ng/ml murine IL-4 and IL-10, respectively, during a 24-h period (Fig. 1). The phenotype of DC/IL-4 was determined by Ab staining and flow cytometry 2 days post-adenoviral infection (data not shown). Although 94.5% of DC/IL-4 showed MHC II expression, 92.1% showed B7.1 expression and 89.7% showed B7.2 expression surface molecules; the levels of these surface markers were only slightly higher than those of nontransduced DC and similar to those of DC/eGFP and DC/IL-10. Thus, IL-4 expression did not significantly effect the expression of MHC molecules and costimulatory surface molecules on the transduced DC.

Antiarthritic effect of systemic delivery of DC/IL-4 in CIA model

To determine whether genetically modified DC would have a therapeutic effect in treating established CIA, the cells were injected i.v. into mice with CIA. Cultures of DC were infected with either 50 MOI Ad-IL-4, Ad-IL-10, or Ad-eGFP and systemically injected

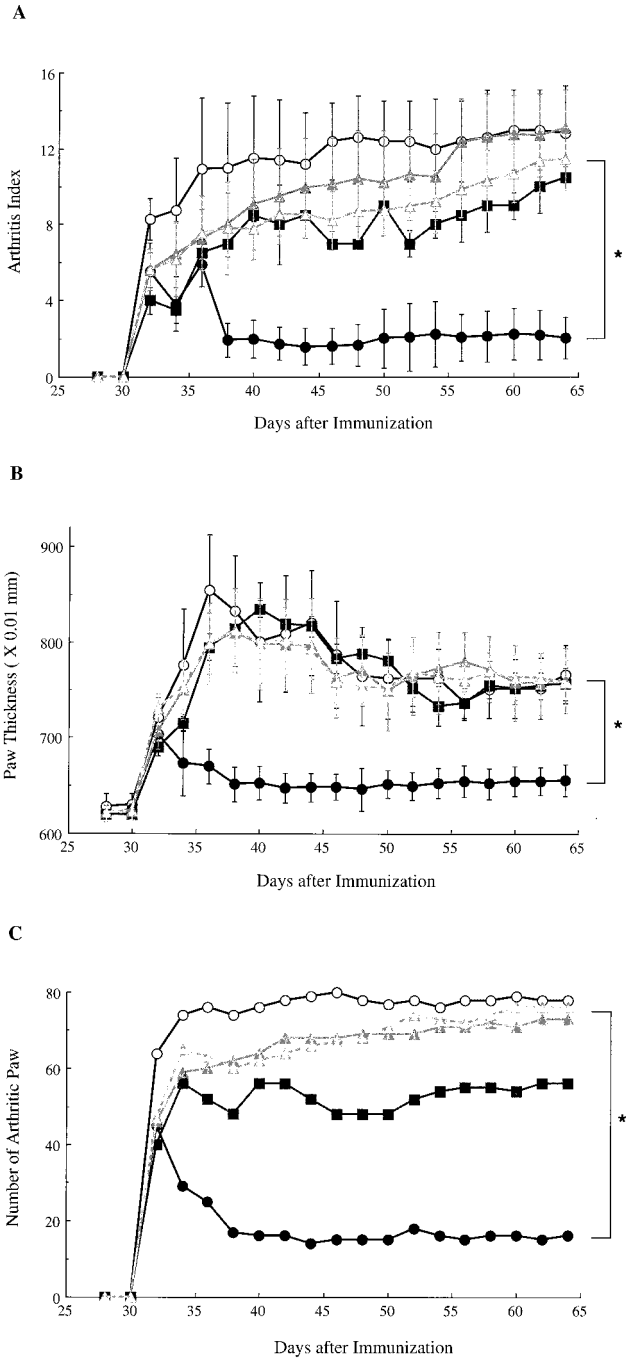

FIGURE 2. Antiarthritic effect of systemically delivered DC/IL-4 in CIA model. Immature DC were infected with 50 MOI Ad-IL-4, Ad-IL-10, or Ad-eGFP after 5 days in culture. One million genetically modified DCs were injected i.v. into DBA1 mice with CIA 4 days after LPS injection. ○, Saline; ●, DC/IL-4; ■, DC/IL-10; ▲, DC/eGFP; △, DC alone. *A*, Arthritis index. Mice were monitored every other day by established macroscopic scoring system on a 0 to 4 scale: 0 = normal, 1 = detectable arthritis with erythma, 2 = significant swelling and redness, 3 = severe swelling and redness from joint to digit, 4 = maximal swelling and deformity with ankylosis. The macroscopic score (mean ± SD) was expressed as a cumulative value for all paws, with a maximum possible score of 16 ($n = 20$). *, $p < 0.0001$. *B*, Paw swelling. The thickness of each paw was also evaluated with spring-loaded caliper. The paw swelling for each mouse was calculated by adding the thicknesses of the individual paws. *, $p < 0.0001$. *C*, Number of arthritic paws. At the same time, the arthritic paws of individual mouse were counted and added to represent the number of arthritic paws in each experimental group (maximum, 80). *, $p < 0.0001$.

in to mice with established CIA at day 32, 4 days post-LPS injection. The severity of arthritis gradually deceased with DC/IL-4 injection, and disease was completely ameliorated within 1 wk in at least one-half of the mice. Furthermore, the animals remained disease free for at least 4 wk. In contrast, there were no substantial differences among the DC/IL-10, DC/eGFP, or nontransduced DC groups (Fig. 2*A*). Similar to the arthritic score, the swelling of arthritic paws also decreased after DC/IL-4 administration compared with control groups (Fig. 2*B*). The total number of arthritic paws in DC/IL-4 also was significantly less than those of saline-, nontransduced DC/, or DC/eGFP-treated groups (Fig. 2*C*). Mice injected with DC/IL-10 showed continuous disease progression even though the incidence of arthritis in DC/IL-10-delivered mice was slightly less than that in mice receiving DC/eGFP- or nontransduced DC. Analysis of the bones in the ankle joints of control and DC- and DC/eGFP-treated mice showed evidence of erosion with an associated monocytic infiltrate around the joint space compared with the nonarthritic control joint (Fig. 3). In contrast, the joints from DC/IL-4-treated mice showed less inflammatory joint tissue, a reduction in bone erosion, and a reduction in the number of inflammatory cells in histologic analysis. These results suggest that a single systemic administration of DC/IL-4 was able to reduce or ameliorate established CIA whereas DC/IL-10 had little effect.

Trafficking of DC after i.v. delivery

To test the migration of DC after systemic administration, DCs were either labeled with PKH26, a red fluorescence cell linker, or infected with an adenoviral vector carrying the luciferase marker gene before injection. Fluorescent DCs were observed in liver and spleen of recipient mice within 6 h of DC injection and in lymph nodes 24 h postinjection (data not shown). Similarly, analysis of luciferase expression 24 h postinjection of DC/luciferase showed luciferase expression in liver, spleen, and lymph nodes but not in other tissues (Fig. 4). Thus, i.v.-injected DC rapidly moved to lym-

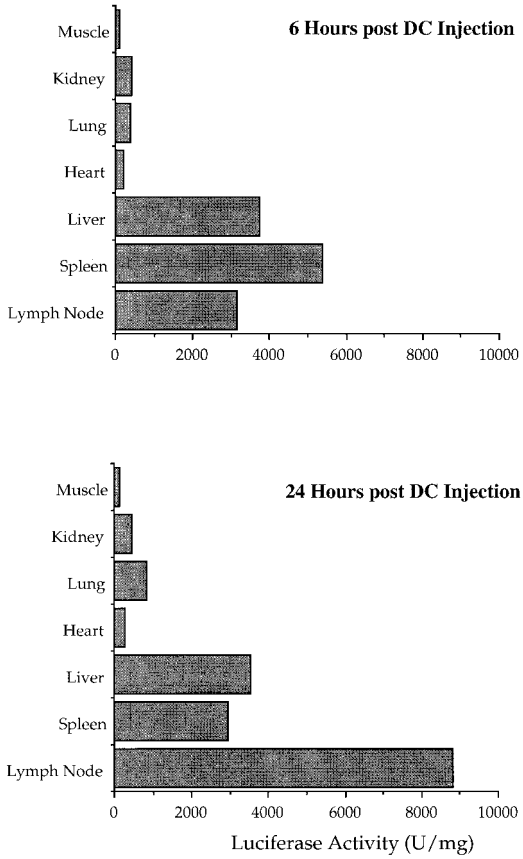

FIGURE 4. Tissue distribution of DC after i.v. delivery. After i.v. injection of DC transduced with adenoviral vector expressing-Luciferase, tissues were dissected and stored at −80°C. Each tissue was homogenized in lysis buffer. Luciferase activity in 50 μl lysate was measured in a luminometer and standardized per milligram of tissue after quantitative assay of protein.

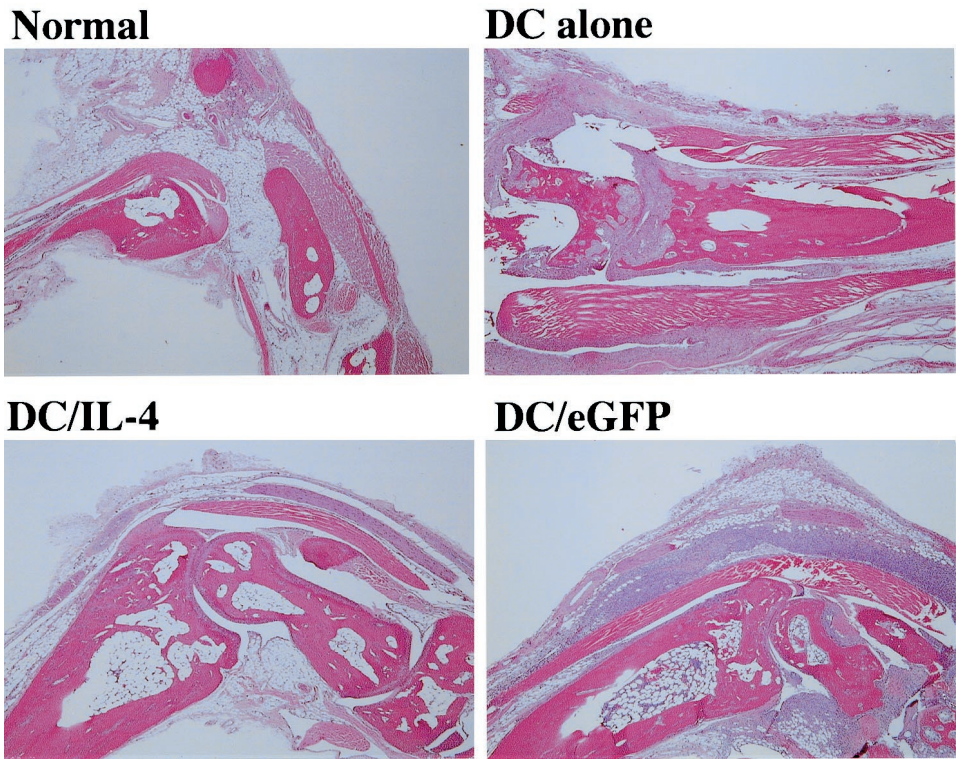

FIGURE 3. Histologic analysis. Ankle joints of mice were isolated from CIA and age-matched normal DBA mice 28 days after the injection of 1 million DC. Joint tissue freshly dissected from mice was immersion-fixed in 10% neutral buffered formalin and decalcified in 15% EDTA, 30% glycerol. Tissues were then dehydrated in a gradient of alcohols, paraffin embedded, sectioned at 5 μm, and mounted on glass slides. Tissues were stained with hematoxylin and eosin and are shown at ×200 magnification.

phoid organs, such as the spleen and lymph nodes, within 24 h where they are positioned to modulate T cell immune responses.

Systemic administration of DC/IL-4 moderates the inflammatory response

To determine the mechanism(s) through which DC/IL-4 delivery ameliorated established disease, the levels of Th1 and Th2 cytokines produced by lymphocytes from spleen and lymph nodes of treated animals were examined. Lymphocytes were isolated from spleen or lymph nodes (data not shown) 2 wk post DC injection and cultured with type II non-Ag or in the presence of collagen or ConA for 2 days. A significant reduction in the production of IFN-γ, a Th1 cytokine, was observed in the untreated (Fig. 5*A*) and collagen-stimulated (Fig. 5*B*) cells derived from the DC/IL-4-treated animal. In contrast, non-Ag-specific stimulation by Con A resulted in similar levels of IFN-γ production in all groups, including the DC/IL-4 group (Fig. 5*C*). In contrast to the effect on IFN-γ production, the levels of expression of IL-4 (Fig. 5, *D–F*) and IL-10 (not shown) were not significantly different among groups after collagen stimulation (Fig. 5*D*), Con A treatment (Fig. 5*F*), or with no Ag (Fig. 5*D*). Similar effects on IFN-γ and IL-4 expression were observed on days 7 and 28 posttreatment (data not shown). Taken together, these results suggest that DC/IL-4 treatment was able to inhibit the collagen-specific Th1 response.

To test whether DC/IL-4 were able to inhibit the generation of anti-collagen type II Abs, the levels of Ab isotypes were titrated from immunized mouse sera 14 days after DC injection. Treatment of mice with DC/IL-4 resulted in moderately reduced levels of all the Ig Ab isotypes tested against murine collagen type II (Fig. 6). All of the values were standardized against the level of Abs in the serum of saline-treated CIA mouse. In particular, the levels of the Th1 isotypes IgG2a and IgG2b in DC/IL-4 were significantly reduced compared with Th2-specific isotypes, IgG1 and IgG3 (Fig. 6). No effect of DC/IL-4 on the levels of IgE was observed. Taken together, these results suggest that DC/IL-4 treatment was able to inhibit Th1 IFN-γ production after collagen stimulation as well as production of the Th2 IgG Ab isotypes.

Discussion

We have previously shown that local intraarticular injection of adenoviral vectors expressing IL-4-, vIL-10-, and IL-1-soluble receptors results in a therapeutic effect not only in the injected joint but also in other untreated joints (20, 28–30). The therapeutic effects in the contralateral joint have been observed in Ag-induced arthritis in the rabbit and in CIA in the mouse. Several sets of experiments suggest that modification of the activity of dendritic cells is important for conferring the contralateral effect. In particular, the adoptive transfer of DC isolated from mice treated with an adenoviral vector carrying a therapeutic gene resulted in suppression of disease.[5] These results suggest that DC, genetically modified in vivo or in vitro to express the appropriate cytokine, might be used as a potential therapy for RA.

In this report, we have demonstrated that systemic delivery of DC, infected with an adenoviral vector carrying the gene for murine IL-4, was effective in treating established murine CIA. Injection of genetically modified DC 4 days after the onset of disease resulted in amelioration of disease in >50% of the treated animals with a significant reduction in all parameters of disease activity including paw swelling and clinical score as well as at the histologic level. Moreover, the antiarthritic effect was far greater after systemic administration of DC expressing IL-4 than observed previously after i.v. injection of Ad-IL-4 (20). However, serum levels

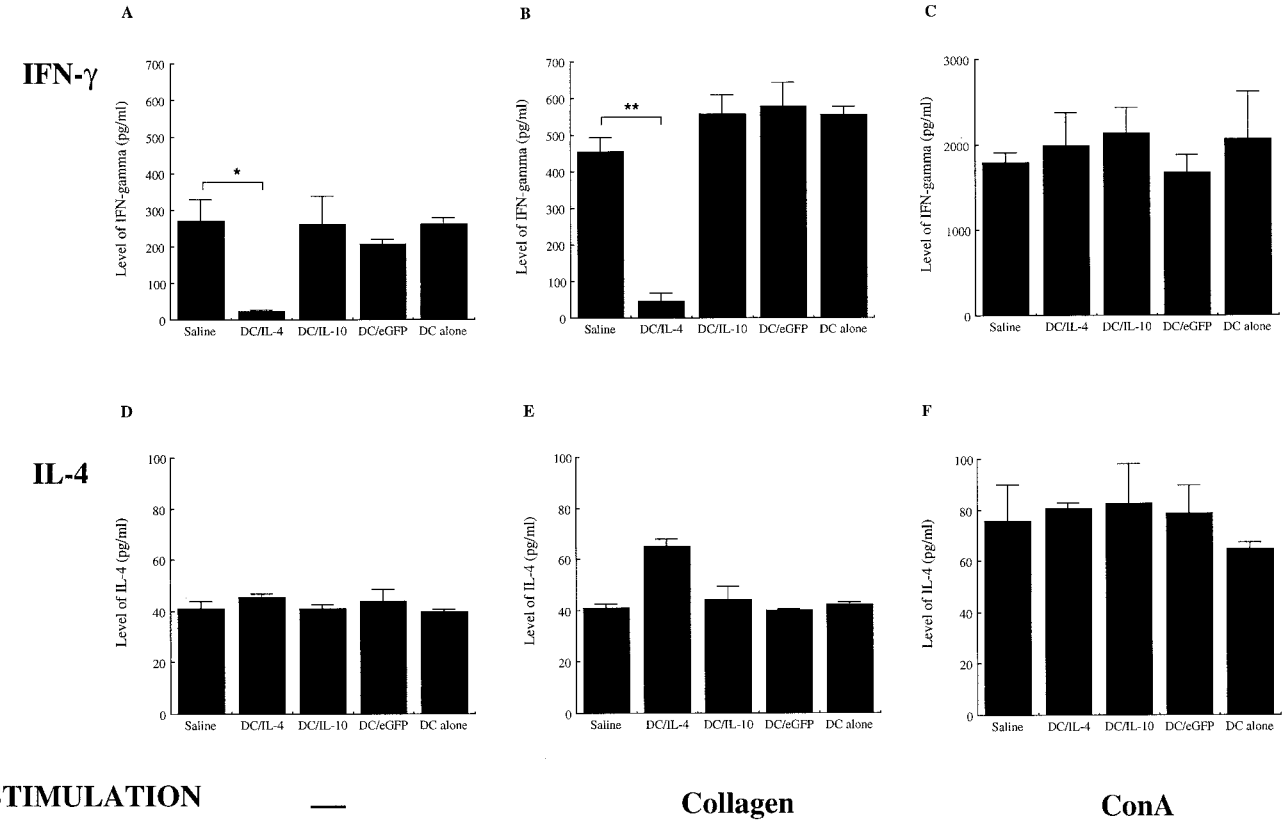

FIGURE 5. Cytokine production by lymphocytes after collagen stimulation. Lymphocytes were isolated from spleen 2 wk post-DC injection, and 10^6 cells/well were cultured in 1 ml medium with no Ag (*A* and *D*), bovine type II collagen (*B* and *E*), or Con A (*C* and *F*) for 2 days. Culture supernatants were collected and measured for cytokine production by ELISA for IFN-γ (*A–C*) and IL-4 (*D–E*). $n = 4$; *, $p < 0.05$; ** $p < 0.005$.

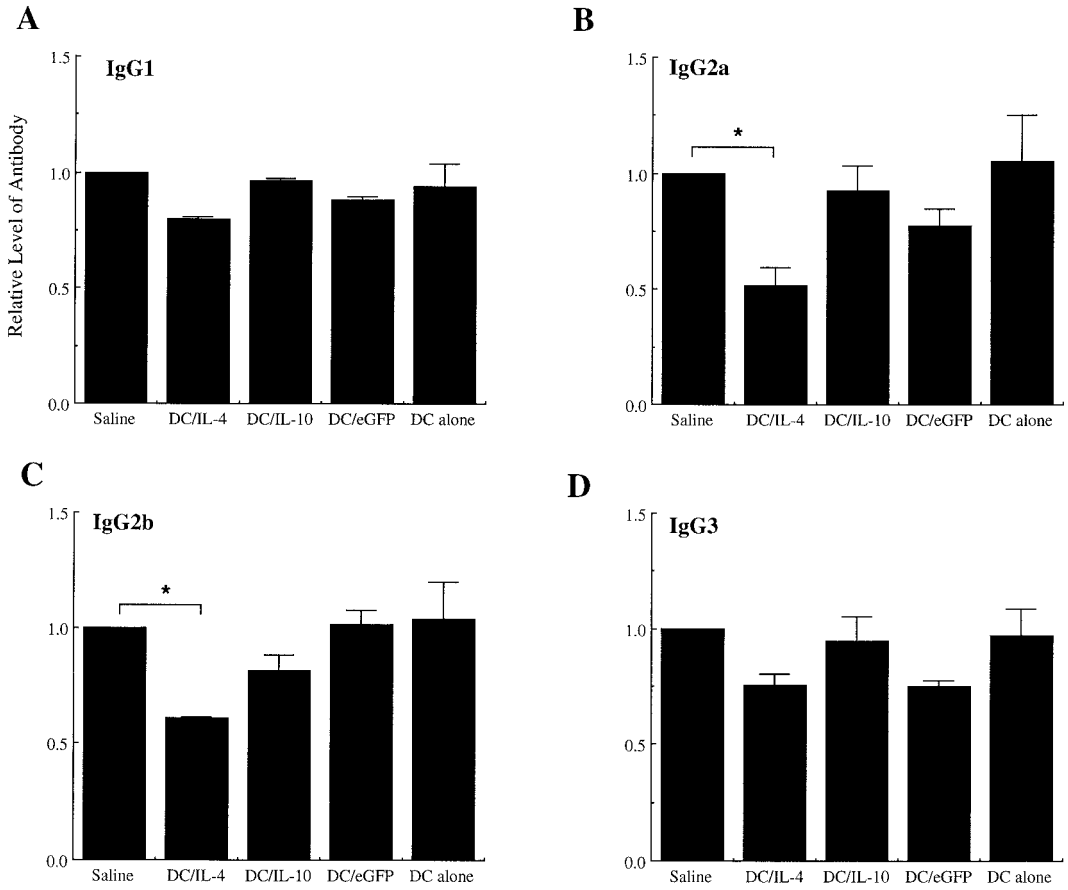

FIGURE 6. Titer of Ab isotypes against murine collagen type II. Serum was isolated from the mouse days 14 after injection of DC/IL-4. Sample sera were diluted 1/100,000 and incubated in murine collagen type II-coated wells. After binding mouse Ab isotopes, the titers of each Ab isotype were measured with biotin-conjugated anti-rat whole IgG Ab. *A,* IgG1; *B,* IgG2a; *C,* IgG2b; *D,* IgG3. $n = 6$ in each groups; $*$, $p < 0.05$.

of IL-4 after DC/IL-4 administration were not significantly above background (unpublished data), whereas the level of IL-4 after Ad-IL-4 injection was significantly higher (20). Thus, the therapeutic effect conferred by DC/IL-4 is not due simply to higher systemic levels of IL-4 but instead is potentially due to local effects of DC/IL-4 upon immune function. Analysis of trafficking of the DC following tail vein injection demonstrated that the cells went to the spleen, liver, and lymph nodes within 6 h, with a greater concentration of cells in the lymph nodes at 24 h. It is possible that the high concentration of DC/IL-4 in the lymph node is able to modulate the immune response to collagen. The therapeutic efficacy of the DC/IL-4 treatment was more effective than administration of a collagen-specific T cell clone genetically modified to express IL-4 (Q. Yao, T. Oligino, and P. D. Robbins, unpublished observations).

Analysis of the effects of DC/IL-4 treatment on immune responses demonstrated a reduction in IFN-γ production after collagen stimulation from lymphocytes isolated from lymph nodes at 7 and 14 days posttreatment. However, the lymphocytes were able to produce IFN-γ in response to Con A stimulation, demonstrating that the effects of DC/IL-4 were at least partially Ag specific. In addition, there was a significant reduction in both the levels of IgG2a and IgG2b anti-collagen Ab isotypes after DC/IL-4 treatment specifically. Taken together, these results suggest that DC/IL-4 administration is able to suppress the overactivity of Th1 response and to skew the immune response from a Th1 to a Th 2 response. This result is in contrast to the effects of Ad-IL-4 systemic administration where there was no statistically significant reduction in IFN-γ production or in the level of anti-collagen Ab isotypes. Treatment of DC/IL-4 also did not stimulate an IgE response (data not shown) that could lead to allergic or asthmatic side effects.

We also have examined the effects of DC genetically engineered to express other potentially therapeutic gene products including FasL (data not shown) and IL-10. As shown in this study, DC/IL-10 was not effective in treating established disease after systemic administration but did have a marginal effect when injected intraarticularly. In fact, DC/IL-10 was more effective than DC/IL-4 after intraarticular injection (data not shown). Similar to several previous reports using APCs genetically engineered to express FasL to suppress Ag-specific immune responses (23, 24, 33, 34), DC/FasL was partially effective in treating established arthritis in the mouse. However, DC/FasL treatment was not as effective as DC/IL-4 treatment, and the antiarthritic effects were only transient. The ability of DC/IL-4 to ameliorate established disease in 50% of the treated animals is unique among the many gene therapy approaches tested to date. Intraarticular or intravenous injection of Ad-vIL-10 was able to block the progression of only early stage disease, although the combination of systemic Ad-vIL-10 and Ad-soluble TNFR was partially effective in transiently reversing established disease (35).

We also have demonstrated that treatment of DC with a NF-κB decoy is able to block maturation of DC, rendering the cells more tolerogenic. Administration of donor-derived DC, treated with NF-κB decoy, facilitated long term engraftment of allogeneic cardiac allografts (36). This observation is significant in that it has

been reported that intraarticular injection of NF-κB decoys into the ankle joints of rats with streptococcal cell wall-induced arthritis resulted in a therapeutic effect in the contralateral joint (37, 38). It is possible that inhibition of DC maturation by NF-κB decoys in vivo is important for the observed therapeutic effect in untreated joints. We currently are examining the efficacy of NF-κB decoy-treated DC in reversing established arthritis in the mouse. Taken together, these results suggest that administration of genetic modification of DC to express either a Th 2 cytokine (IL-4), an apoptotic-inducing ligand, or possibly a NF-κB decoy that is able to block DC maturation is able to confer a therapeutic effect in treating animal models of arthritis. Furthermore, the ability of DC/IL-4 to ameliorate disease without recurrence suggests that the use of genetically modified DC could be used not only as a treatment but also as a cure for certain chronic autoimmune diseases.

Acknowledgments

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References

1. Firestein, G. S., and N. J. Zvaifler. 1990. How important are T cells in chronic rheumatoid synovitis? *Arthritis Rheum. 33:1437.*
2. Rosenberg, Y., A. Steinberg, and, T. Santoro.1984. T cells from autoimmune "IL 2-defective" MRL-*lpr/lpr* mice continue to grow in vitro and produce IL 2 constitutively. *Immunology 133:2545.*
3. Jacob, C. O., P. H. van der Meide, and H. O. McDevitt. 1987. In vivo treatment of (NZB × NZW)F_1 lupus-like nephritis with monoclonal antibody to γ interferon. *J. Exp. Med. 166:798.*
4. Kroemer, G., and G. Wick. 1989. The role of interleukin 2 in autoimmunity. *Immunol. Today 10:246.*
5. Mauritz, N. J., R. Holmdahl, R. Jonsson, P. H. Van der Meide, A. Scheynius, and L. Klareskog. 1988. Treatment with γ-interferon triggers the onset of collagen arthritis in mice. *Arthritis Rheum. 10:1297.*
6. Marcelletti, J. F., J. Ohara, and D. H. Katz. 1991. Collagen-induced arthritis in mice: relationship of collagen-specific and total IgE synthesis to disease. *J. Immunol. 147:4185.*
7. Moore, K. W., A. O'Garra, R. De Waal-Malefyt, P. Vieira, and T. R. Mosmann. Interleukin-10. *1993. Annu. Rev. Immunol. 11:165.*
8. Dechanet, J., M. C. Rissoan, J. Banchereau, and P. Miossec. 1995. Interleukin 4, but not interleukin 10, regulates the production of inflammation mediators by rheumatoid synoviocytes. *Cytokine 7:176.*
9. Miossec, P., P. Chomarat, J. Dechanet, J. F. Moreau, J. P. Roux, P. Delmas, and J. Banchereau. 1994. Interleukin-4 inhibits bone resorption through an effect on osteoclasts and proinflammatory cytokines in an ex vivo model of bone resorption in rheumatoid arthritis. *Arthritis Rheum. 37:1715.*
10. Allen, J. B., H. L. Wong, G. L. Costa, M. J. Bienkowski, and S. M. Wahl. 1993. Suppression of monocyte function and differential regulation of IL-1 and IL-1Ra by IL-4 contribute to resolution of experimental arthritis. *J. Immunol. 151:4344.*
11. Taylor, D. J. 1994. Interleukin-4 (IL-4) induces down-modulation and shedding of the p55 tumor necrosis factor receptor and inhibits TNF α's effect on rheumatoid synovial fibroblasts. *Rheumatol. Int. 14:21.*
12. Joosten, L. A. B., E. Lubberts, P. Durez, M. M. Helsen, M. J. Jacobs, M. Goldman, and, W. B. van den Berg. 1997. Role of interleukin-4 and interleukin-10 in murine collagen-induced arthritis. *Arthritis Rheum. 40:249.*
13. Wang, P., P. Wu, M. I. Siegel, R. W. Egan., and M. M. Billah. 1995. Interleukin (IL)-10 inhibits nuclear factor κB (NF κB) activation in human monocytes: IL-10 and IL-4 suppress cytokine synthesis by different mechanisms. *J. Biol. Chem. 270:9558.*
14. Gerard, C., C. Bruyns, A. Marchant, D. Abramowicz, P. Vandenabeele, A. Delvaux, W. Fiers, M. Goldman, and T. Velu. 1993. Interleukin-10 reduces the release of tumor necrosis factor and prevents lethality in experimental endotoxemia. *J. Exp. Med. 177:547.*
15. Robbins, P. D., and C. H. Evans. 1996. Prospects for treating autoimmune and inflammatory diseases by gene therapy. *Gene Ther. 3:187.*
16. Evans, C. H., S. C. Ghivizzani, and P. D. Robbins. 1998. Blocking cytokines with genes. *J. Leukocyte Biol. 64:55.*
17. Evans, C. H., and P. D. Robbins. 1999. Gene therapy of arthritis. *Intern. Med. 38:233.*
18. Dechanet, J., J. L. Taupin, P. Chomarat, M. C. Rissoan, J. F. Moreau, J. Banchereau, and P. Miossec. 1994. Interleukin-4 but not interleukin-10 inhibits the production of leukemia inhibitory factor by rheumatoid synovium and synoviocytes. *Eur. J. Immunol. 24:3222.*
19. Lubberts, E., L. A. B. Joosten, L. van Den Bersselaar, M. M. Helsen, A. C. Bakker, J. B. van Meurs, F. L. Graham, C. D. Richards, and W. B. van Den Berg. 1999. Adenoviral vector-mediated overexpression of IL-4 in the knee joint of mice with collagen-induced arthritis prevents cartilage destruction. *J. Immunol. 163:4546.*
20. Kim, S. H., C. H. Evans, S. Kim, T. Oligino, S. C. Ghivizzani, and P. D. Robbins. 2000. Gene therapy of established murine collagen-induced arthritis by local and systemic adenoviral mediated delivery of IL-4. *Arthritis Res. 2:293.*
21. Finkelman, F. D., A. Lees, R. Birnbaum, W. C. Gause, and S. C. Morris. 1996. Dendritic cells can present antigen in vivo in a tolerogenic or immunogenic fashion. *J. Immunol. 157:1406.*
22. Liblau, R. S., S. M. Singer, and H. O. McDevitt. 1995. Th1 and Th2 $CD4^+$ T cells in the pathogenesis of organ-specific autoimmune diseases. *Immunol. Today 16: 34.*
23. Zhang, H. G., X. Su, D. Liu, W. Liu, P. Yang, Z. Wang, C. K. Edwards, H. Bluethmann, J. D. Mountz, and T. Zhou. 1999. Induction of specific T cell tolerance by Fas ligand-expressing antigen-presenting cells. *J. Immunol. 162: 1423.*
24. Matsue, H., K. Matsue, M. Walters, K. Okumura, H. Yagita, and A. Takashima. 1999. Induction of antigen-specific immunosuppression by CD95L cDNA-transfected "killer" dendritic cells. *Nat. Med. 5:930.*
25. Lu, L., D. McCaslin, T. E. Starzl, and A. W. Thomson. 1995. Bone marrow-derived dendritic cell progenitors (NLDC 145^+, MHC class II^+, B7-1^{dim}, B7-2^-) induce alloantigen-specific hyporesponsiveness in murine T lymphocytes. *Transplantation 60:1539.*
26. Fu, F., Y. Li, S. Qian, L. Lu, F. Chambers, T. E. Starzl, J. J. Fung, and A. W. Thomson. 1996. Costimulatory molecule-deficient dendritic cell progenitors (MHC class II^+, $CD80^{dim}$, $CD86^-$) prolong cardiac allograft survival in nonimmunosuppressed recipients. *Transplantation 62:659.*
27. Khoury, S. J., L. Gallon, R. R. Verburg, A. Chandraker, R. Peach, P. S. Linsley, L. A. Turka, W. W. Hancock, and M. H. Sayegh. 1996. Ex vivo treatment of antigen-presenting cells with CTLA4Ig and encephalitogenic peptide prevents experimental autoimmune encephalomyelitis in the Lewis rat. *J. Immunol. 157: 3700.*
28. Ghivizzani, S. C., E. R. Lechman, R. Kang, C. Tio, J. Kolls, C. H. Evans, and P. D. Robbins. 1998. Direct adenovirus-mediated gene transfer of interleukin 1 and tumor necrosis factor α soluble receptors to rabbit knees with experimental arthritis has local and distal anti-arthritic effects. *Proc. Natl. Acad. Sci. USA 95:4613.*
29. Whalen, J. D., E. L. Lechman, C. A. Carlos, K. Weiss, I. Kovesdi, J. C. Glorioso, P. D. Robbins, and C. H. Evans. 1999. Adenoviral transfer of the viral IL-10 gene periarticularly to mouse paws suppresses development of collagen-induced arthritis in both injected and uninjected paws. *J. Immunol. 162:3625.*
30. Lechman, E. R., D. Jaffurs, S. C. Ghivizzani, A. Gambotto, I. Kovesdi, Z. Mi, C. H. Evans, and P. D. Robbins. 1999. Direct adenoviral gene transfer of viral IL-10 to rabbit knees with experimental arthritis ameliorates disease in both injected and contralateral control knees. *J. Immunol. 163:2202.*
31. Hardy, S., M. Kitamura, T. Harris-Stansil, Y. Dai, and M. L. Pipps. 1997. Construction of adenovirus vectors through Cre-lox recombination. *J. Virol. 71:1842.*
32. Inaba, K., M. Inaba, N. Romani, H. Aya, M. Deguchi, S. Ikehara, S. Muramatsu, and R. M. Steinman. 1992. Generation of large numbers of dendritic cells from mouse bone marrow cultures supplemented with granulocyte/macrophage colony-stimulating factor. *J. Exp. Med. 176:1693.*
33. Min, W. P., R. Gorczynski, X. Y. Huang, M. Kushida, P. Kim, M. Obataki, J. Lei, R. M. Suri, and M. S. Cattral. 2000. Dendritic cells genetically engineered to express Fas ligand induce donor-specific hyporesponsiveness and prolong allograft survival. *J. Immunol. 164:161.*
34. Kezuka, T., and J. W. Streilein. 2000. Evidence for multiple CD95-CD95 ligand interactions in anterior chamber-associated immune deviation induced by soluble protein antigen. *Immunology 99:451.*
35. Kim, K. N., S. Watanabe, Y. Ma, S. Thornton, E. H. Giannini, and R. Hirsch. 2000. Viral IL-10 and soluble TNF-receptor act synergistically to inhibit collagen-induced arthritis following adenovirus-mediated gene transfer. *J. Immunol. 164:1576.*
36. Giannukakis, N., C. A. Bonham, S. Qian, Z. Zhou, L. Peng, J. W. Harnaha, L. Li, A. W. Thomson, J. J. Fung, P. D. Robbins, and L. Lu. 2000. Prolongation of cardiac allograft survival using dendritic cells treated with NF-kB decoy oligodeoxyribonucleotides. *Mol. Ther. 1:430.*
37. Miagkov, A. V., D. V. Kovalenko, C. E. Brown, J. R. Didsbury, J. P. Cogswell, S. A. Stimpson, A. S. Baldwin, and S. S. Makarov. 1998. NF-κB activation provides the potential link between inflammation and hyperplasia in the arthritic joint. *Proc. Natl. Acad. Sci. USA 95:13859.*
38. Tomita, T., E. Takeuchi, N. Tomita, R. Morishita, M. Kaneko, K. Yamamoto, T. Nakase, H. Seki, K. Kato, Y. Kaneda, and T. Ochi. 1999. Suppressed severity of collagen-induced arthritis by in vivo transfection of nuclear factor κB decoy oligodeoxynucleotides as a gene therapy. *Arthritis Rheum. 42:2532.*