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Central Nervous System Expression of IL-10 Inhibits Autoimmune Encephalomyelitis[1]

Daniel J. Cua,* Beth Hutchins,† Drake M. LaFace,† Stephen A. Stohlman,‡§ and Robert L. Coffman[2]*

Multiple sclerosis, an inflammatory, demyelinating disease of the CNS currently lacks an effective therapy. We show here that CNS inflammation and clinical disease in experimental autoimmune encephalomyelitis, an experimental model of multiple sclerosis, could be prevented completely by a replication-defective adenovirus vector expressing the anti-inflammatory cytokine IL-10 (replication-deficient adenovirus expressing human IL-10), but only upon inoculation into the CNS where local infection and high IL-10 levels were achieved. High circulating levels of IL-10 produced by i.v. infection with replication-deficient adenovirus expressing human IL-10 was ineffective, although the immunological pathways for disease are initiated in the periphery in this disease model. In addition to this protective activity, intracranial injection of replication-deficient adenovirus expressing human IL-10 to mice with active disease blocked progression and accelerated disease remission. In a relapsing-remitting disease model, IL-10 gene transfer during remission prevented subsequent relapses. These data help explain the varying outcomes previously reported for systemic delivery of IL-10 in experimental autoimmune encephalomyelitis and show that, for optimum therapeutic activity, IL-10 must either access the CNS from the peripheral circulation or be delivered directly to it by strategies including the gene transfer described here. *The Journal of Immunology,* **2001, 166: 602–608.**

Interleukin-10 is a regulatory cytokine that plays a critical role in preventing uncontrolled T cell-mediated tissue destruction and is a potential therapeutic protein for the treatment of organ-specific autoimmune diseases. The anti-inflammatory role of IL-10 in the CNS has been extensively studied in experimental autoimmune encephalomyelitis (EAE),[3] an animal model of the human disease, multiple sclerosis. The increase of IL-10 mRNA expression in the CNS during the recovery phase of EAE and the inability of IL-10 gene-deficient mice to recover from EAE suggest that the presence of IL-10 in the CNS is one of the factors required for disease remission (1–3). The potential of IL-10 to prevent EAE is shown by the complete resistance of transgenic mice expressing IL-10 regulated either by a T cell- or an APC-specific promoter (2, 4). However, systemic administration of IL-10 for treatment of EAE has yielded contradictory results. Intravenous injection of rIL-10 exacerbated, rather than suppressed, an adoptive transfer model of disease (5), whereas s.c. and intranasal rIL-10 treatment partially inhibited disease in rat and mouse models of actively induced EAE (6–8). Studies of targeted delivery of IL-10 to the CNS have also yielded conflicting results. Adoptive transfer of a retrovirus transduced, myelin basic protein-specific, T cell hybridoma did not inhibit EAE; however, IL-10

produced by proteolipid protein (PLP)-specific T memory cells suppressed EAE when adoptively transferred to PLP peptide-immunized mice 1 day before expected disease onset (9, 10). These results suggest that, although IL-10 has the potential to prevent induction of CNS inflammation, the localization and timing of IL-10 production or administration may determine its effectiveness.

The complete protection of EAE observed in IL-10-transgenic mice (4) suggested that transfer of an expressible IL-10 gene might be similarly effective, yet would permit evaluation of such variables as the timing and anatomic localization of IL-10 expression. We have used a replication-deficient adenovirus (rAdV) vector for efficient transfer the human IL-10 gene to the brain with minimal inflammation caused by the virus itself. These data demonstrate the effectiveness of IL-10 in the treatment of this model of multiple sclerosis and suggests that gene-transfer provides a way of maintaining effective doses of IL-10 when frequent injection is not feasible.

Materials and Methods

Mice and immunization

SJL/J and CSJLF$_1$/J mice were obtained from The Jackson Laboratory (Bar Harbor, ME). BALB/cAnN were obtained from Taconic Farms (Germantown, NY). All mice were housed under specific pathogen-free conditions and were used between 7 and 12 wk of age. Mouse spinal cord homogenate (MSCH) was prepared from 8- to 12-wk-old BALB/cAnN mice as previously described (11). PLP$_{139-151}$ peptide was synthesized by Biosynthesis (Lewisville, TX). For active induction of EAE, mice were immunized intradermally over three right flank sites with a total of 150 μl of an PBS-oil emulsion containing 1.5 mg of MSCH and 75 μg of heat-killed *Mycobacterium tuberculosis* (strain H37RA; Difco, Detroit, MI). Immunized mice were challenged over three left flank sites with the same MSCH preparation at day 7 again. The frequency of disease is nearly 100%, and the day of disease onset is predictably between days 13 and 14 after the initial immunization. For induction of remitting-relapsing form of EAE, SJL/J mice were immunized intradermally over four dorsal flank sites with a total of 150 μl of PBS-oil emulsion containing 40 μg of PLP$_{139-151}$ peptide and 75 μg of H37RA stain of *M. tuberculosis*. The incidence of primary disease is >95%, and the subsequent disease relapse frequency is ~85% after a

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[3] Abbreviations used in this paper: EAE, experimental autoimmune encephalomyelitis; PLP, proteolipid protein; rAdV, replication-deficient adenovirus; MSCH, mouse spinal cord homogenate; hIL-10-rAdV, replication-deficient adenovirus expressing human IL-10; i.c., intracerebral.

single PLP peptide immunization. Clinical signs of EAE were scored as previously described (11). Significance was determined by the Mann-Whitney *U* nonparametric statistical analysis. Differences were considered significant if $p \leq 0.05$.

Production of replication-deficient adenovirus expressing human IL-10 (hIL-10-rAdV)

The vectors were constructed using standard DNA manipulation techniques. The final stage of construction relies on in vivo recombination between the transfer plasmid and the large fragment of a *Cla*I-digested lacZ-containing derivative of Ad5/d1237 (A/C/β-galactosidase). The final vector is replication deficient, with the E1a, Elb, and the pIX region deleted. The vector backbone also has a partial deletion of the E3 region (12). The plasmid, pDSRG-IL10 containing the human IL-10 cDNA sequence was obtained from Kevin Moore (DNAX, Palo Alto, CA). The plasmid was digested with *Hin*dIII (5′ end) and *Eco*RI (3′ end) to produce a full-length fragment encoding human IL-10, which was ligated with the plasmid pBK-RSV (Stratagene, La Jolla, CA). The insert was excised with *Xba*I (5′ end) and *Bam*HI (3′ end), and then cloned into the *Xba*I *Bam*HI sites of the transfer plasmid, pACN (12) to generate pACNhIL-10. Linearized pAC-NhIL-10 was cotransfected with the large *Cla*I fragment of a plasmid encoding a derivative of Ad5 (13) into human embryonic kidney 293 cells to obtain recombinant adenovirus expressing hIL-10. The resulting hIL-10-rAdV was produced in 293 cells and column purified as previously described (14). The empty expression cassette control vector used a transfer plasmid, where a stop codon was placed after the CMV promoter portion; otherwise, the vector construction was identical with that of the hIL-10-rAdV.

IL-10 and hIL-10-rAdV treatment

For CNS delivery, the indicated number of viral particles were suspended in 10 μl of PBS and injected into the right lateral ventricle using a 28-gauge needle. For peripheral delivery, rAdV suspended in 200 μl of PBS was injected into the tail vein. Alternatively, rAdV was suspended in 50 μl of PBS and delivered intranasally into anesthetized mice.

For in vivo treatment with rhIL-10 (Schering-Plough, Madison, NJ), mice were injected either s.c. with 10 μg in 50 μl of PBS or intracerebrally (i.c.) with 10 μg in 10 μl of PBS using a 28-gauge needle. For daily treatment, rhIL-10 or OVA-protein control was injected into the right and left lateral ventricles on alternating days. CSJLF₁/J mice tolerated the daily i.c. IL-10 treatment regiment for 5 days with no apparent signs of stress or weight loss. However, daily OVA or PBS i.c. treatment was not well tolerated.

Histology and immunochemistry

Mice were killed by CO_2 asphyxiation, and spinal cords were removed, fixed in 10% formalin, and embedded in paraffin blocks. Sections were stained with hematoxylin and eosin for light microscopy (11). To determine localization of rAdV 2 days after i.c. injection with LacZ-rAdV, frozen tissue sections of the brain and spinal cord were prepared and stained with X-gal reagent and counterstained with hematoxylin and eosin. To determine the levels of hIL-10 after in vivo gene transfer, serum and CNS samples were taken at the days indicated. Brain and spinal cord samples were collected from mice killed by CO_2 inhalation and perfused via the heart with ice-cold glucose-containing PBS. The CNS samples were homogenized in glass Tenbrock tissue grinders with 4 volumes of PBS and centrifuged for 15 min at $400 \times g$. The concentration of hIL-10 in the serum and CNS extract was determined by a sandwich ELISA using JES3-9D7 and biotinylated JES3-12G8 Abs as described (4).

Results

CNS expression of IL-10 is necessary for inhibition of EAE

To test the ability of IL-10 produced within the CNS to influence the course of EAE, mice were injected with various numbers of hIL-10-rAdV particles 2–3 days before the predicated onset of disease. A dose of 3×10^9 viral particles delivered by the i.c. route fully protected mice from induction of EAE, whereas 3×10^8 viral particles provided partial protection and 3×10^7 viral particles showed marginal or no protection and a reduced mortality rate (Fig. 1A). Control mice not injected with rAdV showed a hyperacute course of disease, which resulted in an 80% mortality rate. Injection of 3×10^9 particles of an empty control rAdV not expressing hIL-10 had no measurable effect on the course of disease.
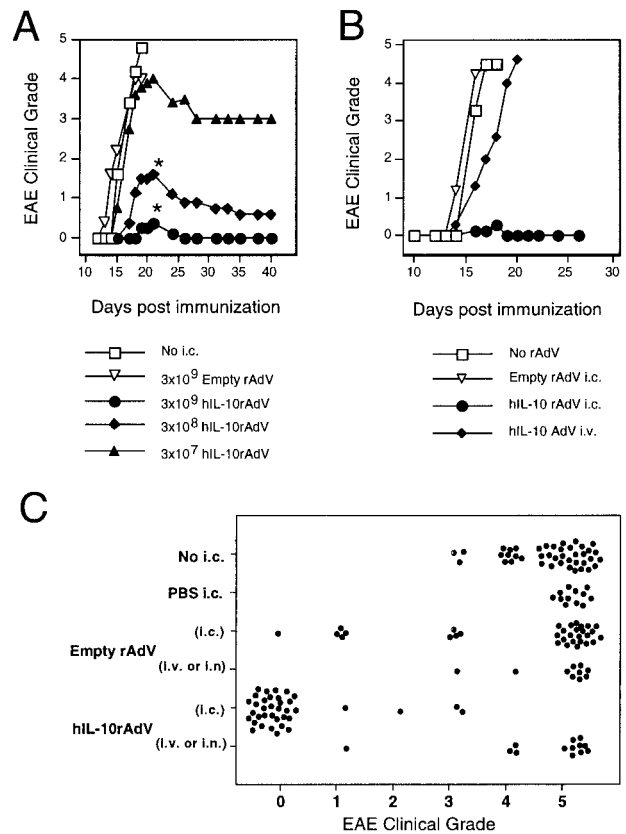

FIGURE 1. CNS localization of IL-10 is required for optimal inhibition of EAE. Mice were immunized with MSCH-CFA at days 0 and 7. At 10 days after initial priming, mice received empty control rAdV or hIL-10-rAdV by the i.c., i.v., or intranasal route. *A*, Average severity score of CSJLF₁/J mice receiving indicated number of hIL-10-rAdV and empty control rAdV particles by the i.c. route. Mortality rate of mice was as follows: no i.c. injection = 4/5; 3×10^9 empty control vector = 4/5; 3×10^7 hIL-10-rAdV = 5/8; 3×10^8 hIL-10-rAdV = 1/8; and 3×10^9 hIL-10-rAdV = 0/8. Asterisks indicate a significant protection from EAE ($p \leq 0.029$). Data represent one of two experiments. *B*, Average severity score of mice ($n = 3–7$/group) receiving 3×10^9 empty rAdV or hIL-10-rAdV by the i.c. or the i.v. route. Data represent one of at least five experiments. *C*, Circles represent peak severity of clinical disease for individual mice given 3×10^9 rAdV vectors by the indicated route of administration. Data complied from six separate experiments.

Therefore, 3×10^9 viral particles were used for all subsequent studies. Intravenous injection of 3×10^9 hIL-10-rAdV particles, in contrast, slightly delayed the progression but did not alter the severity of disease (Fig. 1, *B* and *C*). The inhibition of EAE was transgene specific because mice injected i.c. with PBS-vehicle control or the empty rAdV particles had similar kinetics of disease onset and severity, compared with untreated mice. Thus, neither the i.c. injection procedure nor CNS infection by the rAdV vector influenced the course of EAE.

Tissue expression of IL-10 induced by hIL-10-rAdV

In the present study, the human IL-10 gene was used to construct the rAdV vector. The activity of human IL-10 is comparable to endogenous murine IL-10 and has been shown to be active in suppressing EAE after both s.c. administration (8) and in a transgenic model of EAE (4). After i.c. injection of hIL-10-rAdV, frozen tissue sections of the brain were prepared for immunohistochemical staining with either a hIL-10-specific mAb or an isotype control mAb. An intense cytoplasmic staining of hIL-10 was found

FIGURE 2. Intracerebral gene transfer induced hIL-10 expression in both the ependymal cell layer and the parenchyma. Three days after injection of 3×10^9 hIL-10rAdV into the right lateral ventricle of mice, frozen cross-section of brain samples were prepared for hIL-10 immunohistochemical staining. Serial cross-sections were stained with (*A*) hematoxylin-eosin, (*B*) isotype control mAb, or (*C* and *D*) anti-hIL-10 mAb. Original magnification for *A–C* was ×100; *D* is a higher magnification (×400) of *C*. Data shown are one representative sample of five mice. *E* and *F*, A total of 3×10^9 β-galactosidase rAdV particles were injected into the right lateral ventricle and frozen sections of brain samples were prepared for X-gal staining 2 days after gene transfer. Shown are two of five representative brain samples. Original magnification was ×100.
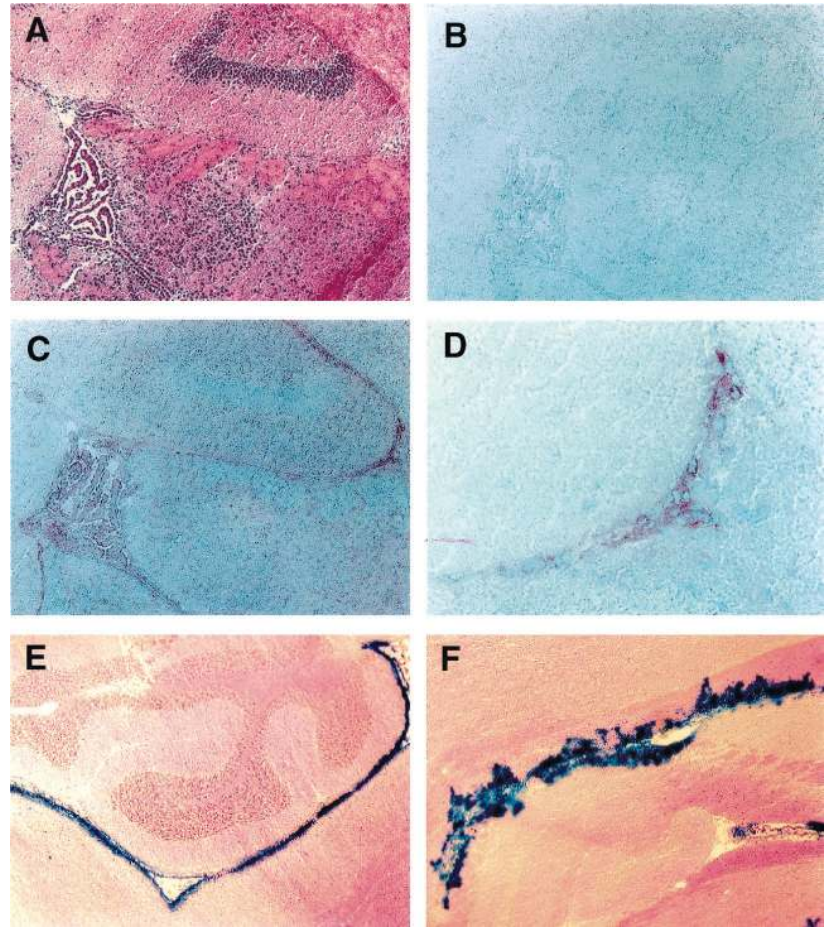
in the ependymal cells lining the ventricles (Fig. 2, *C* and *D*). This staining was hIL-10 specific because no hIL-10 immunostaining was observed after injection of empty rAdV control (data not shown). In addition, the pattern of staining is very similar to the pattern of X-gal staining after i.c. injection of LacZ-rAdV vector (Fig. 2, *E* and *F*). The brain tissue levels of hIL-10 at days 4 and 7 after i.c. injection of 3×10^9 hIL-10-rAdV particles ranged from 13 to 155 ng/g of tissue and spinal cord tissue level at day 7 were

between 16 and 100 ng/g of tissue, suggesting that hIL-10 secreted into the cerebral spinal fluid had diffused throughout the CNS (Table I). The CNS levels of hIL-10 began to decline at day 8 after i.c. injection, and hIL-10 was not detectable after day 12 (Table I). Human IL-10 also reached high concentrations in the serum after i.c. injection, although it was no longer detectable by day 7 (Table I). Intravenous injection of 3×10^9 hIL-10-rAdV particles produced comparable levels of serum hIL-10 to that achieved after i.c.

Table I. *hIL-10 expression following rAdV-mediated gene transfer*

Days Post-rAdV Injection	i.c. Injection[a]			i.v. Injection		
	Brain	Spinal cord serum	Serum	Brain	Spinal cord serum	Serum
4	22, 27, 120[b]					
7	155, 13, 45, 26, 71, 20, 8.5	16, 100, 75, 21, 63		<, <, <, <, <, <[c]	<, <, <, <	
8	8.2, 3.5, 1.8, 1.7, 1.9					
12	<, 1.0, <[c]					
16	<, <, <					
20	<, <, <					
2			31, 25, 35, 37			8.5, 12.5, 12.5, 0.4
5			22, 53, 42, 15, 35, 32, 70, 97			35, 6.4, 2.4, 12.8, 2.9, 10.5, 7.5, 44
7			<, <, <, <, <, <, <, <			<, <, <, <, <, <, <, <

[a] Mice were injected i.c. or i.v. with 3×10^9 rAdV particles. At the indicated days after gene transfer, serum and brains tissue were prepared for hIL-10 ELISA. Results are compiled from three separate experiments.

[b] Data expressed as ng hIL-10/g of brain tissue or ml of serum.

[c] <, ELISA detection limit was 0.2 ng/g tissue or ng/ml serum.

injection, but no hIL-10 was detectable in the CNS (Table 1). Thus, the distinction between the two routes of rAdV delivery was the presence vs the absence of IL-10 in the CNS, because the IL-10 levels in the serum were remarkably similar after either route of gene transfer.

The AdV vector is not a cofactor for inhibition of EAE

The complete prevention of disease with hIL-10-rAdV contrasted with the partial protection or, in some cases, exacerbation previously reported with peripherally injected rIL-10 (5, 7, 8). The ability of hIL-10-rAdV to cause sustained IL-10 production within the CNS seemed the likely explanation for this difference, but a role for the rAdV vector itself, or the immune response to it, as a cofactor was also possible. To test this, immunized $CSJLF_1/J$ mice treated with hIL-10-rAdV were compared with mice given daily injections of rhIL-10, either by i.c. or s.c. injection. Mice receiving 5 daily i.c. injections of rhIL-10 (day 10–14 after priming) were completely protected from EAE during the course of the IL-10 treatment, although 4 of 10 mice began to show signs of disease within 3 days of the last injection (Fig. 3). Mice receiving s.c. injections of the same dose of rhIL-10 were not different from untreated controls or controls injected with OVA (Fig. 3). Thus, the host response to the rAdV vector is not required for protection and rIL-10 can be effective if given repeatedly by the i.c. route.

IL-10 inhibits the effector phase of EAE

After immunization with myelin Ag, substantial development of autoreactive T cells occurs within the first 10 days (15), suggesting that hIL-10-rAdV injection at day 10 exerted its action by preventing the pathological effector functions of primed T cells. To determine whether IL-10 treatment could be equally effective at preventing priming of autoreactive T cells, mice were injected with hIL-10-rAdV either 2 days before or 10 days after the initiation of priming. Because hIL-10-rAdV injection resulted in a transient expression of IL-10 (Table I), these time points should preferentially influence the priming and effector phases, respectively. Mice injected i.c. with hIL-10-rAdV 2 days before immunization had reduced EAE severity but were not fully protected, as were mice that received hIL-10-rAdV 10 days after immunization (Fig. 4). Injection of hIL-10-rAdV at day 10, however, seems to prevent disease for at least 60 days (Fig. 4B) despite the fact that little, if any, hIL-10 remained within the CNSs after 10 days (Table I).
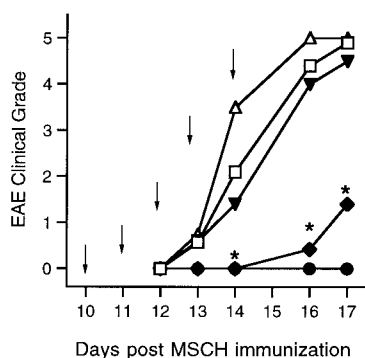

FIGURE 3. CNS administration of soluble rhIL-10 inhibited induction of EAE. Average severity score of mice ($n = 6–12$/group) receiving soluble rhIL-10 by either the i.c. ($\blacklozenge$) or the s.c. ($\blacktriangledown$) route from 10 to 14 days after initial immunization. Control mice received no i.c. injection ($\square$), 10 μg of OVA ($\triangle$), or 3×10^9 hIL-10-rAdV ($\bullet$) particles by the i.c. route at day 10 of immunization. Asterisks indicate a significant inhibition of EAE in i.c. hIL-10-treated mice, compared with OVA-treated mice ($p \leq 0.029$). Data shown were compiled from two experiments.
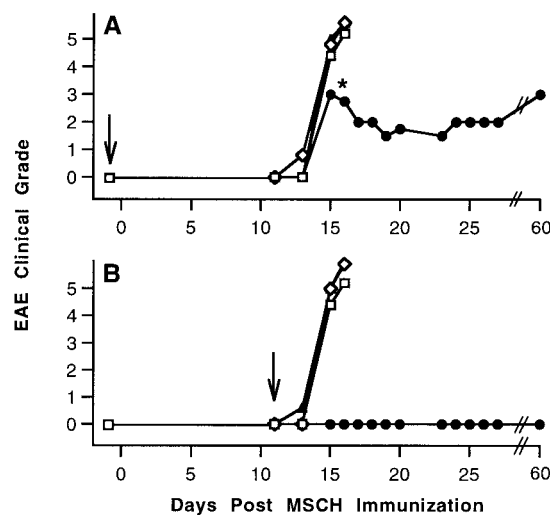

FIGURE 4. IL-10 gene transfer inhibited the effector phase of EAE. Mice ($n = 5–6$/group) were immunized with MSCH-CFA at day 0 and 7. *A*, Two days before initial Ag priming, mice received i.c. injection of hIL-10-rAdV ($\bullet$), empty control-rAdV ($\square$), PBS vehicle control ($\diamond$), or no i.c. injection ($\triangle$). The asterisk indicates a significant protection from EAE ($p \leq 0.0159$). *B*, Ten days after initial priming, mice received i.c. injection of hIL-10-rAdV and the same controls as in *A*. Average severity score of mice were shown and data represent one of three experiments.

This suggests that IL-10 may have long lasting effects on self-reactive T cells.

Histopathological examination of the spinal cords from mice injected i.c. with hIL-10-rAdV at day 10 of priming showed a complete absence of inflammatory infiltrate, whereas mice injected with the control rAdV showed extensive inflammation consistent with the observed clinical severity (Fig. 5). Extensive inflammation was also observed in EAE-susceptible mice that were given hIL-10-rAdV i.v. (data not shown). Together, these results suggest that one of the mechanisms by which IL-10 suppresses EAE is inhibition of cellular invasion to the CNS parenchyma.

CNS IL-10 gene transfer modulates active disease

The complete protection achieved by injection of hIL-10-rAdV just 2–3 days before the onset of disease led us to test its effect on active disease. At day 15 after immunization, mice were divided into two groups according to disease severity, one with an average EAE grade of 1 (Fig. 6A) and the second with an average grade of 3 (Fig. 6B). When hIL-10-rAdV treatment was initiated in mice exhibiting an EAE grade of 1, clinical signs did not progress and mice recovered rapidly. In mice receiving the control rAdV vector, severe disease progressed unabated with an eventual mortality rate of >60% (Fig. 6A). In the group of mice tested with an initial EAE grade of 3, all the mice that received hIL-10-rAdV showed clinical improvement after progressing to EAE grade 4, whereas the control rAdV-treated mice showed no clinical improvement and had an eventual mortality rate of 78% (Fig. 6B). However, hIL-10-rAdV treatment was not effective for mice that had reached an EAE grade of 5 at the time treatment was initiated (data not shown). Thus, IL-10 gene transfer during active disease could protect mice from further disease exacerbation and promote recovery even in this hyperacute disease model.

CNS IL-10 gene transfer during disease remission inhibits EAE relapse

In the majority of patients with multiple sclerosis, clinical disease follows a remitting-relapsing course. Therefore, practical therapies

Empty Adenovirus **HuIL-10 Adenovirus**

FIGURE 5. CNS administration of hIL-10-rAdV during the effector phase of EAE inhibited cellular infiltration into the spinal cord. Mice receiving hIL-10-rAdV by the i.c. route 10 days after initial MSCH priming were EAE resistant, and spinal cords showed no inflammatory infiltration in the white matter. Spinal cord samples were taken at day 17 of immunization. Mice receiving empty control rAdV at day 10 of immunization by the i.c. route were EAE susceptible and showed intense inflammatory infiltrates in the white matter. Result is representative of at least 16 mice from four experiments. Original magnification was ×100.
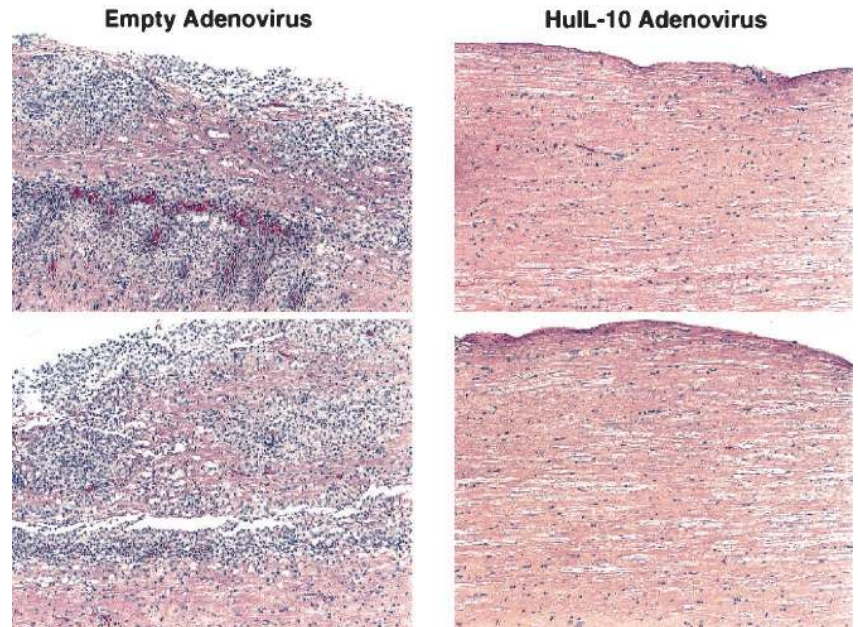
must be able to delay or prevent relapses. To address this issue, expression of IL-10 in the SJL mouse model of remitting-relapsing EAE was examined. SJL mice were immunized either with MSCH (Fig. 7A) or PLP$_{139-151}$ peptide (Fig. 7B). After initial remission, groups of mice were given a single injection of either hIL-10rAdV or control rAdV vector. Mice receiving hIL-10rAdV were significantly protected from disease relapse, whereas all mice receiving the control rAdV vector suffered multiple clinical relapses. This result clearly demonstrates that CNS IL-10 gene transfer provides significant benefit in remitting-relapsing CNS inflammation.

Discussion

In this study, we have shown that IL-10 gene transfer is an effective way to provide continuous doses of IL-10 within the brain and spinal cord and that IL-10 expression within the CNS is required for the inhibition of EAE. The finding that IL-10 expression 10 days after the initial immunization with autoantigen, but just before the onset of symptoms, was fully protective suggests that IL-10 must be present in the CNS during the effector phase of EAE
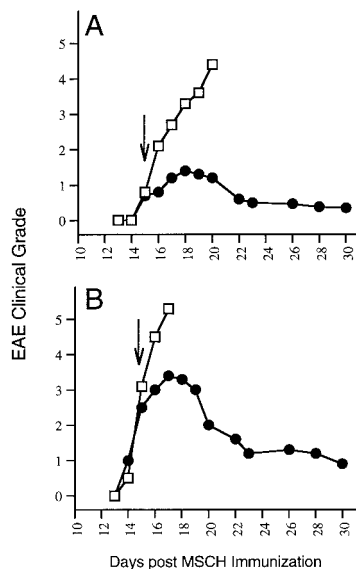

FIGURE 6. IL-10 gene transfer after the onset of EAE reduced clinical severity and mortality rate. At 15 days after initial priming, mice were divided into two groups according to the clinical severity and i.c. injected with gene transfer vectors. *A*, Mice had an average clinical grade of 1 at the time of i.c. IL-10 gene transfer. The mortality rate of the empty rAdV- (□) treated mice in this group was 6/9 and the hIL-10-rAdV- (●) treated mice was 0/13. *B*, Mice had an average disease severity of 3 at the time of gene transfer. The mortality rate of the empty rAdV-treated mice in group *B* was 5/6 and the hIL-10-rAdV-treated mice was 0/11. Result shown was compiled from three separate experiments.
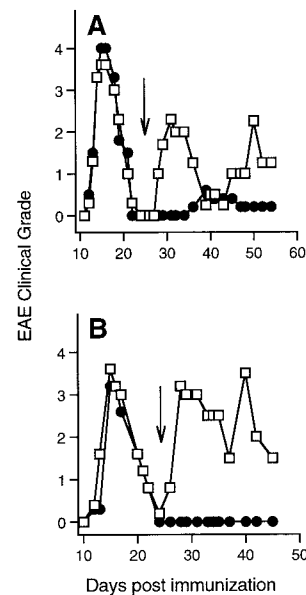

FIGURE 7. IL-10 gene transfer during disease remission inhibits disease relapse. A remitting–relapsing disease was induced in SJL mice after immunization with (*A*) MSCH or (*B*) PLP$_{139-151}$ peptide. During remission from the first EAE episode, mice were randomly divided into two groups and were injected with either hIL-10rAdV (●) or empty control rAdV (□). *A*, The rate of disease relapse after hIL-10rAdV treatment was 1/5 and empty rAdV treatment was 4/5. *B*, All seven7 mice did not have disease relapse after hIL-10rAdV treatment, whereas 4/5 mice had multiple relapses after empty control rAdV treatment. Result shown was representative of four experiments.

to prevent disease. The adenovirus vector itself was not required, because daily i.c. injection of rhIL-10 was also effective at inhibiting EAE.

The efficacy of hIL-10rAdV given i.c. vs i.v. could not be explained by large differences in the overall production of IL-10 in the brain vs the liver and spleen. Comparable serum IL-10 levels were produced with either route of injection, and the levels in either case were at least 10 times the serum levels measured in fully protected hIL-10-transgenic mice (D. J. Cua and R. L. Coffman, unpublished observations). However, the difference in hIL-10 concentration in the CNS was dramatic between the two routes of injection. Although hIL-10 produced by the ventricular ependymal cells rapidly diffused to the spinal cord and to the serum, hIL-10 produced in the liver and spleen did not reach detectable concentrations in the CNS (the limit of sensitivity of the ELISA was 0.2 ng/g tissue). The presence of hIL-10 in the serum after i.c. injection is primarily due to an unidirectional exit of fluids into the systemic circulation via the arachnoid villi (16). The daily turnover rate of cerebral spinal fluid is nearly 95%, which would result in rapid equilibration of IL-10 from the CNS to the systemic circulation. This is consistent with previous reports demonstrating efficient gene transfer into the ependymal cells lining the brain ventricles and secretion of transduced factors into the cerebral spinal fluid by replication-deficient viral vectors (17–20). Thus, CNS expression of IL-10 was necessary for inhibition of EAE, but it was not possible to determine whether IL-10 was also required in the periphery for this inhibition. These results may explain the inability of systemic IL-10 treatment to inhibit EAE induced by the transfer of activated T cells (5). Once the T cells are activated and enter the CNS, the impact of IL-10 in the systemic circulation may be limited. Two important features of IL-10 gene transfer contrast it to daily IL-10 injection; steady-state levels of IL-10, rather than wide daily fluctuations in IL-10 concentration, and the mild inflammatory response caused by the virus infection itself. The effectiveness of daily i.c. injections of hIL-10 demonstrates that neither of these parameters is critical for the prevention of EAE by hIL-10rAdV. Thus, the key parameter determining the efficacy of hIL-10 in EAE is the maintenance of effective levels of the cytokine within the CNS.

IL-10 gene transfer just before disease onset was able to completely or significantly prevent disease in several models of EAE. These include immunization of CSJLF$_1$/J, SJL, and BALB/c mice with MSCH or PLP$_{139-151}$ peptide and C57BL/6 mice with myelin oligodendrocyte glycoprotein 35–55 peptides (BALB/c and C57BL/6, data not shown). Intracerebral injection of hIL-10rAdV worked most effectively when given at day 10, after priming twice with autoantigen, and a few days before the onset of neurological symptoms. The reduced effectiveness of IL-10 given at day −2 was likely due to the transient expression of this vector rather than a counterprotective effect of early IL-10 expression. Because virally transduced IL-10 production lasted only ∼8 days in the CNS, it would have ceased in this group well before the onset of CNS inflammation. Expression of IL-10 during autoantigen priming only partially suppressed subsequent disease. Both i.c. (Fig. 4) and i.v. (data not shown) injections of hIL-10-rAdV 2 days before initial priming, which provided comparable serum levels of IL-10, only partially inhibited EAE. This is consistent with a number of studies showing partial, but not complete, reduction of disease severity after daily systemic administration of soluble IL-10 starting from the day of immunization (6–8). These experiments, taken together, show that hIL-10 delivered by gene transduction inhibits EAE optimally at the end effector stage within the CNS. Previous EAE susceptibility studies with mice transgenic for hIL-10

reached the same conclusion regarding the principal site of action of IL-10 (4).

These results encouraged us to attempt treatment with hIL-10rAdV after the onset of disease. In mice with mild or moderate disease, i.c. injection of hIL-10rAdV halted disease progression and induced rapid remission, whereas control mice continued to progress and often required termination. In addition, IL-10 gene transfer after remission from acute disease also prevented disease relapses for at least 55 days, whereas control mice had two subsequent disease relapses. These results suggest that the presence of IL-10 for 8 days in the CNS during events leading to disease onset has significant long-term therapeutic benefits for CNS inflammatory disorders. It should be noted that CNS administration of a single 20-μg dose of mouse IL-10 protein after clinical disease onset had no inhibitory effect on severity of EAE (21). These results illustrate the importance of continuous expression of IL-10 within the CNS during the effector phase of disease for inhibition autoimmune encephalomyelitis. Many recent studies have begun to address the need for local expression of therapeutic agents in this disease model. These include local CNS gene transfer of IL-4, TGF-β, CTLA-4-Ig, platelet-derived growth factor-A, and dimeric p75 TNFR using replication-deficient viral vectors or genetically engineered cells (17, 22–25). It should be mentioned that many different types of immune modulations including blockade of IL-1β-converting enzyme and OX40L/OX40R complex and administration of 1,25-dihydroxyvitamin D$_3$ as well as inducible NO synthase inhibitors have been shown to reduce EAE severity (26–29); however, relatively few were able to significantly prevent or reverse disease when given to mice just before or after the onset of disease symptoms (30, 31).

IL-10 could potentially affect EAE pathogenesis at several points distal to T cell priming, including recruitment of inflammatory cells to the CNS and CNS tissue destruction. The absence of visible cellular infiltrates in mice given hIL-10-rAdV suggests inhibition of macrophage and granulocyte recruitment and extravasation. IL-10 is known, for example, to inhibit TNF-α induction of VCAM-1 expression in the CNS and VLA-4 expression on T cells (32–35). However, the lack of effectiveness of systemic IL-10 as well as the ability of CNS IL-10 to rapidly modulate active disease suggests that IL-10 acts principally on steps during and after entry into the CNS. During the acute phase of EAE, as demyelination and axonal degeneration take place, IL-10 could inhibit both the production and the actions of proinflammatory cytokines and chemokines, including IL-1, IL-6, IL-12, GM-CSF, M-CSF, IFN-γ TNF-α, macrophage inflammatory protein (MIP)-1α, MIP-1β, and MIP-2, by both resident microglia and infiltrating mononuclear cells (36–39). We are currently measuring gene expression patterns in hIL-10-rAdV and control mice to define more precisely the mechanism of action of IL-10 in this disease model.

The experiments described here demonstrate the therapeutic potential IL-10 in both the prevention and treatment of a Th1-mediated autoimmune disease, provided it can be delivered to the target organ for an appropriate period of time. Although systemic expression or injection of IL-10 allows penetration into many organs, the blood–brain barrier effectively limits the entry of IL-10 to the CNS, despite disruptions to the barrier that might result from CNS inflammation. Gene transfer provides an attractive alternative to recombinant cytokine therapy, especially for prolonged treatment of anatomical sites that are difficult to access. Replication-deficient adenovirus vectors have proven to be convenient experimental tools for cytokine delivery studies in animal models, but their immunogenicity and limited duration of expression make them less than ideal for treatment of chronic conditions, such as multiple sclerosis. These experiments do show, however, that therapy with

a vector conferring extended, and perhaps regulated, expression of IL-10 would have promise for the treatment of multiple sclerosis.

Acknowledgments

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