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Suppression of Autoimmune Diabetes by Viral IL-10 Gene Transfer

Zandong Yang,* Meng Chen,* Runpei Wu,* Lawrence B. Fialkow,* Jonathan S. Bromberg,‡ Marcia McDuffie,*† Ali Naji,*§ and Jerry L. Nadler*‡

Th1 cell activation and cytokine production shift the balance between Th1 and Th2, favoring the up-regulation of proinflammatory activity that leads to destruction of insulin-producing pancreatic β cells in type 1 diabetes. Th2-type cytokines, such as IL-10, have immune regulatory function. Administration of IL-10, or IL-10 gene transfer, prevents autoimmune diabetes in nonobese diabetic (NOD) mice. However, constant administration of purified rIL-10 is not practical for long-term therapy to prevent diabetes. In this study, we transferred the BCRF-1 gene, an open reading frame in the Epstein-Barr viral genome with remarkable homology to mouse IL-10 (viral IL-10 or vIL-10), by an adeno-associated viral (AAV) vector to NOD mice to attain sustained vIL-10 gene expression. Like endogenous mouse IL-10, vIL-10 has potent immunoregulatory and immunosuppressive functions, but can be specifically distinguished from endogenous mouse IL-10 for monitoring of the transgene expression. A single systemic administration of AAV vIL-10 significantly reduced insulitis and prevented diabetes development in NOD mice. This protective effect correlated with sustained transgene expression and protein production. Moreover, splenocytes from the treated mice blocked diabetes transfer to NOD recipients, suggesting that vIL-10 induces an active suppression of autoimmunity. This study provides evidence to support the possibility of using vIL-10 gene therapy to prevent type 1 diabetes. The Journal of Immunology, 2002, 168: 6479–6485.

Abbreviations used in this paper: NOD, nonobese diabetic; AAV, adeno-associated virus; AF, aldehyde fuchsin; mIL, murine IL; Tg, transgenic; vIL-10, viral IL-10.

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Harbor, ME) and housed in a pathogen-free colony in the Center for Comparative Medicine at the University of Virginia. The experimental protocols were approved by the Institutional Animal Care and Use Committee. In our colony, 75% of female NOD mice become diabetic by 20 wk of age.

**rAAV vectors**

Full-length CDNA of a vIL-10 plasmid, pAC-EBNA, was digested with EcoRI and Kpnl, then ligated with an AAV cloning plasmid, pZAC2.1, with EcoRI and Kpnl arms by T4 DNA ligase (Life Technologies, Grand Island, NY). The recombinant plasmid, pAAV/vIL-10, was confirmed by DNA sequence. AAV vIL-10 virus was produced, and the titer of viral stocks was determined by Southern hybridization, as previously described (20, 21). A rAAV virus encoding β-galactosidase gene (AAV lacZ) was also produced for viral control use.

**Reagents and Abs**

vIL-10-specific oligonucleotide primers were synthesized for vIL-10 mRNA detection: GGAGGGAAGGTTAGTGG and GACAATTAAAGC CAGG. All mAbs used in this study were purchased from BD Pharmingen (San Diego, CA): anti-vIL-10 mAb (JES3-9D7 and JES3-6B11), anti-mouse IgG1 mAb (A85-3), and anti-mouse IgG2a mAb (R11-89). Polyclonal anti-insulin Ab (H-86; Santa Cruz Biotechnology, Santa Cruz, CA) was used to detect insulin in immunohistochemical staining of islet structure.

**Animal treatment**

Female NOD mice received a single i.m. injection at upper hind limbs. Particles (10^5) of a rAAV vector in a volume of 100 μl normal saline, containing either AAV vIL-10 or AAV lacZ, were used for each mouse. Age-matched mice were injected with 100 μl normal saline as controls. Levels of nonfasting blood glucose were monitored one to two times/week using tail vein whole blood by an Accu-Chek Glucose Monitor (Roche, Indianapolis, IN). Hyperglycemia was defined by blood glucose higher than 250 mg/dl for 3 consecutive days. Body weight was monitored weekly. To demonstrate the linkage of protection to vIL-10 protein function, a neutralizing Ab was injected i.v. to AAV vIL-10-treated mice at 50 mg/mouse twice within 5 days. As a control, mouse IgG (Sigma-Aldrich, St. Louis, MO) was given by the same protocol to AAV vIL-10-treated NOD mice.

**Pancreatic islet isolation and insulin secretion assay**

Pancreatic islets were isolated from 3-wk-old male NOD mice using the method described previously (22). Freshly isolated islets were cultured with either AAV vIL-10 or AAV lacZ at 1:1000 (islet:viral particle) ratios for 4 days at 22°C, 5% CO2. Control islets were cultured without addition of virus. One hundred islets from each group were set in duplicate and treated with 3 mM glucose-supplemented Krebs-Ringer bicarbonate HEPES buffer solution (115 mM NaCl, 24 mM NaHCO3, 5 mM KCl, 2.5 mM CaCl2, 1 mM MgCl2, and 7.5 mM HEPES, pH 7.4) for 30 min at 37°C. Medium was then replaced, and samples from each group were cultured in either 3 or 28 mM glucose-Krebs Ringer bicarbonate HEPES buffer solution for 2 h at 37°C. Insulin released into supernatants was measured by RIA (23). The concentration was defined by comparison with a standard curve for murine insulin (Linco Research, St. Charles, MO).

**Transgene detection**

Total RNA was purified from AAV vIL-10-treated NOD tissue and from in vitro transfected islets by RNAgent Total RNA Isolation System (Promega, Madison, WI). RT-PCR for vIL-10 mRNA was performed with vIL-10 primers and a GeneAmp E Z TTh RNA PCR kit (PerkinElmer, Branchburg, NJ). Protein samples were purified and loaded in equal amounts (50 μg each) on a 10% polyacrylamide gel, separated by electrophoresis, then transferred to Hybond ECL membrane (Amersham Pharmacia Biotech, Piscataway, NJ). An anti-human/VIL-10 mAb was used to detect vIL-10 proteins. ECL Plus kit (Amersham Pharmacia Biotech) was used for signal development. Protein levels of vIL-10, IgG1, and IgG2a were measured in mouse sera by ELISA methods according to BD Pharmingen protocols for specific mAbs.

**Cytokine assay**

Serum levels of mouse cytokines (IL-2, IL-4, IL-10, and IFN-γ) were measured by ELISA kits (BioSource International, Camarillo, CA) at 1, 3, and 5 wk after treatments.

**Pathology and serology assessments**

Skeletal muscle samples of the injection sites and livers from both groups of mice were fixed in 10% Formalin, then stained with H&E. Pancreatic tissues were fixed in Bouin’s solution. A pair of adjacent sections was separately stained with H&E or aldehyde fuchsins (AF) for scoring cellular infiltration and β cell granulation in a blinded fashion (24). Immunohistochemical stain of insulin was performed in 4% parafomaldehyde-fixed paraffin sections of pancreata using a polyclonal anti-insulin Ab (H-86; Santa Cruz Biotechnology) with a Vectastain ABC peroxidase standard kit and a diaminobenzidine peroxidase substrate kit (Vector Laboratories, Burlingame, CA). Serum samples from both groups of mice were tested for alanine aminotransaminase by the university’s clinical laboratory to assess liver damage.

**Adaptive transfer**

Splenocytes were isolated from AAV vIL-10-treated, AAV lacZ-treated, and saline-treated NOD mice using the method described previously (25). Freshly isolated splenocytes at dose of 40 × 10^7 or 80 × 10^7/mouse were i.v. injected into 3-wk-old NOD.scid mice or naïve wild-type female NOD mice at 3 or 9 wk old. Measurements of vIL-10 mRNA and protein in splenocytes used for transfer were performed by either RT-PCR or immunoblotting. Serum levels of vIL-10 were measured in splenocyte recipients by ELISA method at 4 wk after transfer.

**Statistical analysis**

Data are presented as the mean ± SEM. Either Student’s t test or ANOVA testing was used to compare the differences among groups. Differences were considered statistically significant if p < 0.05.

**Results**

**Efficient transgene expression after a single systemic administration of rAAV virus**

NOD mice were treated with a single i.m. injection of AAV vIL-10 at a dose of 10^11 particles/mouse at the age of 6 wk. On day 21 after virus administration, vIL-10 mRNA was detected by RT-PCR in skeletal muscle, liver, and splenocytes from AAV vIL-10-treated mice using vIL-10-specific oligonucleotide primers (Fig. 1). vIL-10 mRNA was also detectable in isolated NOD islets that had been cultured with AAV vIL-10 for 4 days in vitro (Fig. 1). In addition, vIL-10 protein was confirmed in these tissues by Western blotting (Fig. 2). Similarly, the β-galactosidase gene was expressed in skeletal muscle of AAV lacZ-treated mice by X-gal staining (data not shown). Mice that received i.m. injection of up to 10^11 particles of recombinant virus per mouse appeared healthy and gained body weight at a rate similar to saline-treated mice. There was no evidence of local inflammation in the injection areas.
checked at 10 days after injection (data not shown). These studies demonstrated that administration through a single i.m. injection was sufficient to achieve systemic transgene expression.

Preservation of insulin-secretory function after AAV viral transfection

One of important biological functions of β cells in pancreatic islets is insulin secretion in response to glucose. Therefore, it is crucial if this function is altered by AAV transfection. We had previously shown no dysfunction of insulin secretion in isolated islets after culture with rAAV vectors (13). In this study, we cultured freshly isolated NOD islets with an AAV vector at a 1:1000 (islet:viral particle) ratio for 4 days, then tested their insulin-secretory function upon glucose stimulation. No reduction of insulin secretion was found in either AAV vIL-10- or AAV lacZ-treated NOD islets compared with those in saline-treated islets in response to basal (3 mM) or stimulatory (28 mM) glucose conditions (Fig. 3).

Prevention of autoimmune diabetes in NOD mice by AAV-mediated vIL-10 gene therapy

A majority (70–80%) of female NOD mice spontaneously develop insulitis, leading to overt diabetes by 20 wk of age. Insulitis can be detected by histology at 8 wk of age, and overt diabetes is seen from 12 to 15 wk of age in female NOD mice. Therefore, we chose young (3-wk-old), prediabetic (6-wk-old), and close-to-diabetic (17-wk-old) female NOD mice for studies in vivo. Female NOD mice received a single i.m. injection of 1 × 10¹⁰ particles/mouse of either AAV vIL-10 or AAV lacZ. Age-matched female NOD mice received 100 μl normal saline as controls. AAV vIL-10 treatment delayed and inhibited the onset of diabetes in the groups treated at 3 and 6 wk of age (Fig. 4, A and B). The onset of hyperglycemia was delayed 5–6 wk in both groups, and the cumulative incidence of diabetes was significantly reduced (18 and 33%, respectively), compared with the incidence in age-matched saline-treated mice (75%). AAV lacZ treatment did not alter the incidence of diabetes as compared with saline treatment (69% vs 75% in saline group). No significant protection from diabetes was seen in older mice that received therapy at 17 wk of age (Fig. 4C).
Serum levels of vIL-10 were detected beginning at 2 wk postinjection, and peaked at 4 wk in treated mice. Gene-transferred cytokine (vIL-10) was specifically quantitated with vIL-10 mAbs that have no cross-reactivity to endogenous mIL-10 (Fig. 5). Consistent with the minor effects reported for vIL-10 on B lymphocytes, no difference was found in IgG1 and IgG2a levels between AAV vIL-10-treated and saline-treated mice (data not shown).

To demonstrate a requirement for functional vIL-10 in diabetes protection, a neutralizing Ab to vIL-10, which does not cross-react with mIL-10, was injected (i.v.) into euglycemic NOD mice that had received AAV vIL-10 6–8 wk ago. Three of four mice became hyperglycemic 2 wk after the neutralizing Ab injection. No diabetes developed in control mice treated with mouse IgG (n = 3). Although we were unable to detect serum levels of vIL-10 at 8 wk after AAV vIL-10 injection, the effect of the neutralizing Ab suggested that function of vIL-10 protein might be responsible for the protective effect.

Histologic assessment was performed on pancreatic sections obtained from all study groups. In the NOD mice that received AAV vIL-10 treatment at either 3 or 6 wk of age, no cellular infiltration was observed in most islets at 5 and 8 wk after treatment (infiltration free in 89% of 131 islets from seven mice). In both AAV lacZ- and saline-treated mice, massive cellular infiltration (intraislet insulitis) or complete destruction of islets was found (Fig. 6A). The results from insulin immunohistochemical stains were consistent with the data from H&E and AF staining (Fig. 6B), indicating loss of insulin-containing β cells in AAV lacZ- and saline-treated mice.

No evidence for liver damage in AAV vIL-10-treated mice
Liver histology was assessed in both AAV vIL-10- and saline-treated NOD mice, as well as in the recipient mice that received splenocytes from the AAV IL-10-treated donors. Both diabetic and euglycemic NOD mice that had received AAV vIL-10 treatment >20 wk ago were used for liver histology, and compared with the age-matched diabetic saline-treated mice. A single focal mononuclear infiltration was found in one of five samples from five individual AAV vIL-10-treated mice and in two of five mice that had been treated with saline alone. There was no cellular infiltration in any mouse that received splenocytes from AAV vIL-10-treated donors (n = 6). No elevation of serum levels of alanine aminotransaminase was found in any sample (four samples for each group). These results demonstrate no evidence for liver toxicity related to this viral-mediated therapy and splenocyte transfer.

Modulation of cytokine profiles after AAV vIL-10 therapy
vIL-10 may prevent diabetes by altering the balance of Th1 and Th2 cytokines. We measured the serum levels of both Th1 (IL-2 and IFN-γ) and Th2 (IL-4 and IL-10)-type cytokines. Serum samples were obtained from both AAV vIL-10- and saline-treated mice for cytokine ELISA. We observed a significant decrease in IL-2 and IFN-γ at both 3- and 5-wk posttherapy in AAV vIL-10-treated mice. However, no difference was detected at the 1-wk point following treatment, and no difference was found in mIL-4 and mIL-10 levels between the two groups at any time point (Table I).

Blocking diabetes induction by adoptive splenocyte transfer
Adoptive transfer of splenocytes from diabetic NOD mice induces hyperglycemia in NOD.scid recipients. Splenocytes were isolated from AAV vIL-10-treated NOD donors that had been euglycemic for 6–8 wk after initial therapy, as well as from either AAV lacZ- or saline-treated donors that were hyperglycemic. Freshly isolated/washed splenocytes (40 × 10⁶/mouse) were injected (i.v.) into 3-wk-old NOD.scid mice. One hundred percent of the mice (20/20) that received splenocytes from AAV lacZ-treated donors became diabetic within 3 wk after transfer. Similarly, 90% of the mice (18/20) that had received splenocytes from saline-treated donors had become diabetic by 7 wk after transfer. In contrast, only 25% (5/20) recipients that received splenocytes from the euglycemic AAV vIL-10-treated donors had become hyperglycemic by the end of a 9-wk observation period (Fig. 7A). mRNA for vIL-10 was detected in the transferred splenocytes obtained from all AAV vIL-10-treated donors by RT-PCR. Serum levels of vIL-10 were detectable in the recipients that received splenocytes from AAV vIL-10-treated donors 4 wk after transfer.

We compared the ability of splenocytes from either nondiabetic or diabetic donors that were previously treated with AAV vIL-10 to induce diabetes in NOD.scid recipients. One hundred percent of recipients that received splenocytes from diabetic donors became hyperglycemic within 6 wk after transfer, compared with a 25% incidence of diabetes in mice that received nondiabetic AAV vIL-10 splenocytes (Fig. 7B). Cotransfer using mixed splenocytes (1:1 for total 40 × 10⁶ cells/mouse) from both diabetic and nondiabetic donors resulted in similar incidence (90%) of hyperglycemia to the use of splenocytes from diabetic donors alone in NOD.scid recipients.

We also compared the dose effect for splenocyte adoptive transfer. Splenocytes were isolated from euglycemic AAV vIL-10-treated donors 6–8 wk after gene therapy. We i.v. injected the splenocytes into 3-wk-old NOD.scid recipients at doses of either 40 × 10⁶ or 80 × 10⁶ cells per mouse, and found that larger...
amount of splenocytes (80 × 10^6 cells/mouse) completely blocked diabetes transfer (0% in 80 × 10^6/mouse vs 25% in 40 × 10^6/mouse groups) (Fig. 7C).

We further tested the ability of AAV vIL-10-treated splenocytes to prevent spontaneous autoimmune diabetes in wild-type NOD mice. Freshly isolated/washed splenocytes from euglycemic AAV vIL-10-treated donors were injected into either 3-wk-old or 9-wk-old NOD.scid mice. A value of 5% vs saline controls at the same time points.

Discussion

Both T cell activation and cytokine action play central roles in the initiation and progression of autoimmune diabetes in NOD mice (26, 27). IL-10 inhibits cytokine synthesis and other functions of macrophages (28–30). IL-10 also suppresses T cell and NK cell activation/proliferation by both direct and indirect effects (31, 32). These activities of IL-10 render this cytokine a potent suppressor of the effector functions of macrophages, T cells, and NK cells. In addition, IL-10 most likely has stimulatory and regulatory actions on proliferation and differentiation of B lymphocytes, mast cells, and thymocytes (33).

Study of IL-10 in autoimmune diabetes in NOD mice has been conducted for years, but the results of these studies are somewhat confusing. Various methods for IL-10 supplementation prevent autoimmune diabetes in NOD mice (5–9). Transgenic (Tg) BALB/c mice expressing mIL-10 restricted to the pancreas exhibited significant perin-bulinsits, although they did not progress to frank diabetes. Backcrossing of these mice to the NOD strain resulted in acceleration of the onset of diabetes in the Tg offspring (34). Islet-specific expression of mIL-10 promoted diabetes that appeared to be independent of Fas, perforin, TNR-1, and TNR-2 molecules in the Tg mice (35, 36). However, expression of vIL-10 in pancreata of Tg NOD mice resulted in suppression of Th1 cell activation and prevention of autoimmune diabetes (37). These results suggest a functional variance between mIL-10 and vIL-10. In addition, local expression of high levels of mIL-10 in nonlymphoid tissue may have proinflammatory effects. In contrast, systemic expression is likely to favor anti-inflammatory or immunoregulatory effects (38–41). It is clear that IL-10 has immunostimulatory properties, not only leading to B lymphocyte proliferation and increased Ab production, but also augmenting the T cell proliferative response in the presence of IL-2/IL-4 and increasing expression of CTL after Con A stimulation in mouse T cells in vitro (see review

FIGURE 7. Cumulative incidence of hyperglycemia (%) in NOD recipients after adoptive splenocyte transfer. A. Adoptive transfer splenocytes from either AAV vIL-10-, or AAV lacZ-, or saline-treated donors. Donors were hyperglycemic in AAV lacZ and saline groups, but euglycemic in AAV vIL-10 mice at time of splenocytes isolation. A total of 40 × 10^6 splenocytes/mouse was injected i.v. into 3-wk-old NOD.scid mice. A value of p < 0.05 (AAV vIL-10 cells vs saline or AAV lacZ cells). B. Adoptive transfer splenocytes from either diabetic or euglycemic AAV vIL-10-treated donors. A total of 40 × 10^6 cells/mouse was injected i.v. into 3-wk-old NOD.scid mice. A value of p < 0.05. C. 40 × 10^6 or 80 × 10^6 splenocytes/mouse from AAV vIL-10-treated euglycemic donors were injected i.v. into 3-wk-old NOD.scid mice. A value of p < 0.05. D. Either 3- or 9-wk-old wild-type NOD females received i.v. 40 × 10^6 splenocytes/mouse from AAV vIL-10-treated euglycemic donors. Controls were age-matched mice that received splenocytes from diabetic AAV lacZ-treated donors. p < 0.05 in each pair of age group at the end of the observation.
in Ref. 40). The therapeutic effect of IL-10 in vivo depends upon the balance of all properties of the cytokine to both local and systemic immune responses.

The discovery of vIL-10 from the Epstein-Barr viral genome, BCRF1, provides a new biological agent that shares many of mammalian IL-10’s activities, but differs in its reduced ability to stimulate class II MHC expression on mouse B cells (41) and proliferation of mouse mast cells (15). By using vIL-10 as a therapeutic agent, we can monitor the kinetics of the transgene expression without confusion of endogenous IL-10 in mouse studies.

AAV viral delivery has the advantage of providing sustained transgene expression when compared with adenoviral vectors. The reason may be partly due to reduced viral genome in rAAV vectors and the ability of AAV to integrate into genomic DNA. Both characteristics of rAAV reduce the antiviral immune response in host. Although additional studies need to be performed to evaluate long-term effects of rAAV vectors in vivo (42), their use provides a promising approach for many gene therapy applications, especially after the development of a helper-virus-free system for rAAV production (43).

In our study, a single i.m. injection of rAAV vectors led to systemic transgene expression and therapeutic function for >5 mo. The therapeutic effect correlated with serum levels of transgene protein (vIL-10) and with the reduction of IL-2 and IFN-γ serum levels. No difference in serum IgG1 and IgG2a levels among rAAV- and saline-treated mice suggested that B cell stimulation was unlikely after AAV vIL-10 therapy. AAV vIL-10 gene therapy was safe. AAV vIL-10-treated mice were healthy and gained body weight similar to the mice treated with saline. No difference was found in liver serology and histology in the mice treated with AAV vIL-10 or saline. High dose transfection with rAAV in vitro did not cause dysfunction of insulin secretion to glucose stimulation in isolated islets. We also showed prevention or delay of the onset of diabetes in both NOD.scid and wild-type NOD mice using splenocytes from AAV vIL-10-treated donors. This result suggests that introducing splenocytes bearing the vIL-10 gene and/or altering T cell function may interrupt the autoimmune process. Therefore, the results from this study support the potential of AAV-mediated gene therapy, particularly the use of AAV vIL-10 for type 1 diabetes.

The precise mechanism of vIL-10-induced protection in NOD mice needs to be further defined. vIL-10, like mammalian IL-10, strongly reduces Ag-specific T cell proliferation by diminishing the Ag-presenting capacity of macrophages/monocytes via down-regulation of class II MHC expression (41). vIL-10 acts directly on T cells to inhibit costimulatory signals (44) and suppresses Th1 cell activation (37). vIL-10 decreases inflammation and cellular adhesion molecular expression (45), and regulates dendritic cell migratory responses via modulation of chemokine receptor expression (46), indicating that vIL-10 has the ability to impair functions of APCs (47). The result of reduction of IL-2 and IFN-γ in vivo after AAV vIL-10 treatment in this study should contribute to the mechanism.

In adoptive transfer studies, both CD4+ and CD8+ T cells in NOD splenocytes are required for diabetes induction (48, 49). There are at least two possible explanations for diabetes protection provided by splenocytes from AAV vIL-10-treated donors. First, transferred splenocytes from treated mice continue to express the vIL-10 gene and produce vIL-10 protein after entering the recipient circulation. Our RT-PCR and immunoblotting data of splenocytes from treated mice support this possibility. This is further supported by detection of serum levels of vIL-10 in NOD recipients after adoptive transfer with splenocytes from AAV vIL-10-treated donors. Second, regulatory T cells may be developed in treated donors and transferred into recipients to prevent autoimmunity. Although we have no direct evidence to confirm this possibility, studies from other laboratories suggest that endogenous or supplemented rmIL-10 can induce regulatory T cell subsets (50–52). It is likely that systemic vIL-10 gene therapy may be able to induce general immunoregulation in vivo through an inhibition of Th1 type cytokine production, and alteration of the functions of APCs and chemokine receptor expression (41, 45–47). Further characterization of AAV vIL-10-treated T cell function and phenotype will provide more information to explain the mechanism.

The protective effect of AAV vIL-10 therapy was age sensitive in NOD mice. NOD mice develop spontaneous diabetes from age of 12–15 wk as the result of the development of invasive insulitis. The inability of vIL-10 to prevent diabetes in older NOD mice suggests that the cytokine cannot reverse established diabetes once β cell destruction has occurred. It is also possible that immune deviation by vIL-10 may not regulate functions of effector cells once they become fully activated in vivo. This may also explain the result from our cotransfer study. Therefore, early treatment with vIL-10 certainly may provide better protection in NOD mice.

Atkinson and his coworkers (53) recently reported a study using AAV vectors to deliver mIL-10 for preventing diabetes in NOD mice. Although there is a biological difference between mIL-10 and vIL-10, several observations were similar between their study and ours. As expected, AAV mIL-10 prevented spontaneous diabetes in NOD mice. However, because they were unable to distinguish transferred mIL-10 from endogenous cytokine, the persistence of transgene expression could not be monitored and specific protective effects could not be confirmed by blocking the cytokine. In addition, the AAV mIL-10 treatment did not produce a transferrable protective effect by adoptive transfer, suggesting that expression of this cytokine might have been more short-lived than that demonstrated for AAV vIL-10 in this study. The diminished levels of both Th1 and Th2 cytokines (IL-2, IL-4, IL-10, and IFN-γ) following AAV mIL-10 treatment suggested that high levels of mIL-10, but not vIL-10, might produce strong feedback to inhibit both proinflammatory and regulatory cytokines, potentially limiting its long-term therapeutic effectiveness.

Our study provides the first evidence that AAV-mediated vIL-10 gene transfer can prevent autoimmune diabetes in the NOD model. The result suggests that vIL-10 is a potent immunoregulatory factor that may be useful for autoimmune diabetes prevention. vIL-10 may also have potential for treatment of other autoimmune disorders and for tolerance induction in transplantation.

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