The Journal of Immunology



# Protection Against Experimental Autoimmune Encephalomyelitis Generated by a Recombinant Adenovirus Vector Expressing the V β8.2 TCR Is Disrupted by Coadministration with Vectors Expressing Either IL-4 or -10

Todd A. Braciak, Brian Pedersen, Judy Chin, Clay Hsiao, E. Sally Ward, Igor Maricic, Alex Jahng, Frank L. Graham, Jack Gauldie, Eli E. Sercarz and Vipin Kumar

*J Immunol* 2003; 170:765-774; ;
doi: 10.4049/jimmunol.170.2.765
http://www.jimmunol.org/content/170/2/765



*The Journal of Immunology* is published twice each month by
The American Association of Immunologists, Inc.,
9650 Rockville Pike, Bethesda, MD 20814-3994.

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

# Protection Against Experimental Autoimmune Encephalomyelitis Generated by a Recombinant Adenovirus Vector Expressing the V$\beta$8.2 TCR Is Disrupted by Coadministration with Vectors Expressing Either IL-4 or -10[1]

**Todd A. Braciak,[2]* Brian Pedersen,* Judy Chin,‡ Clay Hsiao,‡ E. Sally Ward,† Igor Maricic,* Alex Jahng,* Frank L. Graham,§ Jack Gauldie,§ Eli E. Sercarz,* and Vipin Kumar***

**Adenovirus vectors are increasingly being used for genetic vaccination and may prove highly suitable for intervention in different pathological conditions due to their capacity to generate high level, transient gene expression. In this study, we report the use of a recombinant adenovirus vector to induce regulatory responses for the prevention of autoimmune diseases through transient expression of a TCR $\beta$-chain. Immunization of B10.PL mice with a recombinant adenovirus expressing the TCR V$\beta$8.2 chain (Ad5E1 mV$\beta$8.2), resulted in induction of regulatory type 1 CD4 T cells, directed against the framework region 3 determinant within the B5 peptide (aa 76–101) of the V$\beta$8.2 chain. This determinant is readily processed and displayed in an I-A$^u$ context, on ambient APC. Transient genetic delivery of the TCR V$\beta$8.2 chain protected mice from Ag-induced experimental autoimmune encephalomyelitis. However, when the Ad5E1 mV$\beta$8.2 vector was coadministered with either an IL-4- or IL-10-expressing vector, regulation was disrupted and disease was exacerbated. These results highlight the importance of the Th1-like cytokine requirement necessary for the generation and activity of effective regulatory T cells in this model of experimental autoimmune encephalomyelitis.** ***The Journal of Immunology*****, 2003, 170: 765–774.**

Adenovirus vectors have been successfully exploited in the treatment of tumors and to induce immune response to infectious agents as vaccines (1–14). In each case, a correlation between the induction of effector cells by vector transgene expression and their efficacy is seen. Because higher E:T ratios can be achieved as the expression of vector transgene increases, adenovirus vectors may also be suitable for inducing regulatory cells in T cell-mediated disease, if control of pathogenic cells also requires a high E:T ratio. Thus, it was of interest to test the efficacy of an adenovirus vector expressing a TCR from a pathogenic cell as an agent of gene delivery in the well-characterized prototype of a T cell-mediated disease, experimental autoimmune encephalomyelitis (EAE),[3] in B10.PL mice.

In this model, we have previously shown that both CD4 and CD8 regulatory T (Treg) cells play essential roles in the control of myelin basic protein (MBP)-specific CD4 effector T cells which mediate demyelination. In the initial phases of this experimental disease, most MBP-specific T cells in B10.PL mice react to the N-terminal determinant, MBPAc1–9, and predominantly use the V$\beta$8.2 TCR (15, 16). We have shown that Treg cells specific for two different determinants within the V$\beta$8.2 TCR chain appear during the recovery phase of the disease (17–20). One determinant is within the framework 3 (Fr3) region and is recognized by CD4 Treg cells, while another determinant is from the CDR1/2 region and is recognized by CD8 Treg cells. Induction of both of these regulatory cells is required for controlling disease (21, 22).

This oligoclonal use of TCR V genes in EAE provides an opportunity to test immunospecific intervention strategies. Early evidence for the efficacy of immunospecific therapy was provided when it was shown that in vivo administration of mAbs against the V$\beta$8.2 chain could prevent EAE in B10.PL mice as well as in other rodent models (15, 16). This Ab targeting of the TCR in the treatment of autoimmune disease was preceded by the experimental introduction of T cell clones as a vaccine. In this study, attenuated MBP-reactive CD4$^+$ T cells were used as a vaccine to protect rats from EAE (23). The protection afforded by such T cell vaccination was postulated to be dependent on the generation of Treg cells reactive to determinants on the TCR displayed by the encephalitogenic T cells (24). Our laboratory subsequently provided conclusive evidence that a regulatory CD4$^+$ T cell was involved in response to a Fr3 region determinant on the encephalitogenic V$\beta$8.2 TCR (18).

More recently, we have demonstrated that an additional requirement in the induction of Treg cells in this model system is that a Th1 cytokine milieu, predominantly rich in IFN-$\gamma$ must be intact to generate effective regulation (25, 26). The priming of Treg cells in a Th2-directed manner resulted in exacerbation of EAE and in-

*Division of Immune Regulation, La Jolla Institute for Allergy and Immunology, San Diego, CA 92121; †Department of Microbiology and Cancer Immunobiology Center, University of Texas Southwestern Medical Center, Dallas, TX 75235; ‡Department of Microbiology and Molecular Genetics, University of California, Los Angeles, CA 90024; and §Department of Pathology, McMaster University, Hamilton, Ontario, Canada

Received for publication August 23, 2002. Accepted for publication November 13, 2002.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This study was supported by grants from the National Multiple Sclerosis Society (RG-2765-B-3) and National Institutes of Health Grant CA 98958 (to V.K.) and in part from National Institutes of Health Grant AI 24819 (to E.E.S.). This is publication 367 from the La Jolla Institute for Allergy and Immunology.

[2] Address correspondence and reprint requests to Dr. Todd A. Braciak, Torrey Pines Institute for Molecular Studies, 3550 General Atomics Court, San Diego, CA 92121-1122. E-mail address: tbraciak@tpims.org

[3] Abbreviations used in this paper: EAE, experimental autoimmune encephalomyelitis; Treg, regulatory T; MBP, myelin basic protein; Fr3, framework 3 region; SI, stimulation index; mCMV, murine CMV; PTX, pertussis toxin.



0022-1767/03/$02.00

creased the frequency of Th1 MBP-specific encephalitogenic T cells. Although in general, MBP-restricted T cells producing a Th2 pattern of cytokines have been considered nonencephalitogenic and the presence of endogenous levels of IL-4 or IL-10 have correlated with recovery from EAE, the effect of cytokine on regulatory cells and the disease state has not fully been addressed.

In the studies presented here, we report that V$\beta$8.2 TCR expressed by a recombinant adenovirus vector can protect B10.PL mice against EAE by inducing potent type 1 regulatory $CD4^+$ T cells targeted to a Fr3 region determinant on the V$\beta$8.2 chain. We also show that this regulation can be disrupted by the simultaneous delivery of adenoviral vectors expressing cytokines IL-4 and IL-10. These data clearly establish an obligatory cytokine dependence for the generation of Treg cells. The development of TCR V$\beta$-expressing vectors along with the use of appropriate cytokine adenoviral vectors should prove useful for intervention in T cell-mediated pathologic conditions.

## Materials and Methods

### *Construction of recombinant plasmids and adenovirus containing a functional coding minigene for the murine V$\beta$8.2 chain of the TCR (Ad5E1 mV$\beta$8.2)*

A schematic diagram for the construction of the Ad5E1 mV$\beta$8.2 vector is shown in Fig. 1. To rescue V$\beta$8.2 TCR sequences into a translatable minigene cassette, an oligonucleotide was designed containing 5′ flanking restriction enzyme sites for *Bam*HI and *Hin*dIII, followed subsequently by a sequence coding for the consensus optimal ribosomal translation initiation site (Kozak sequence) (27), and bases incorporating the first 33 nucleotides of the coding sequence for the V$\beta$8.2 TCR. The sequence of the 5′ oligonucleotide is GCGGATCCAAGCTTGCCGCCGCCATGGAGGCTGCAGTCACCCAAAGCCCAAGAAAC. An additional oligonucleotide containing bases complementary to the 3′ end of the V$\beta$8.2 TCR, flanked by residues containing stop codons to provide a translational termination signal and a restriction site, *Xho*I, was created. The sequence of the 3′ oligonucleotide is ACATCAGTGTACTTCTGTGCCAGCGGTGATGCAGGGTGATAGCTCCAGCGG. PCR using the 5′- and 3′-designed oligonucleotides with plasmid

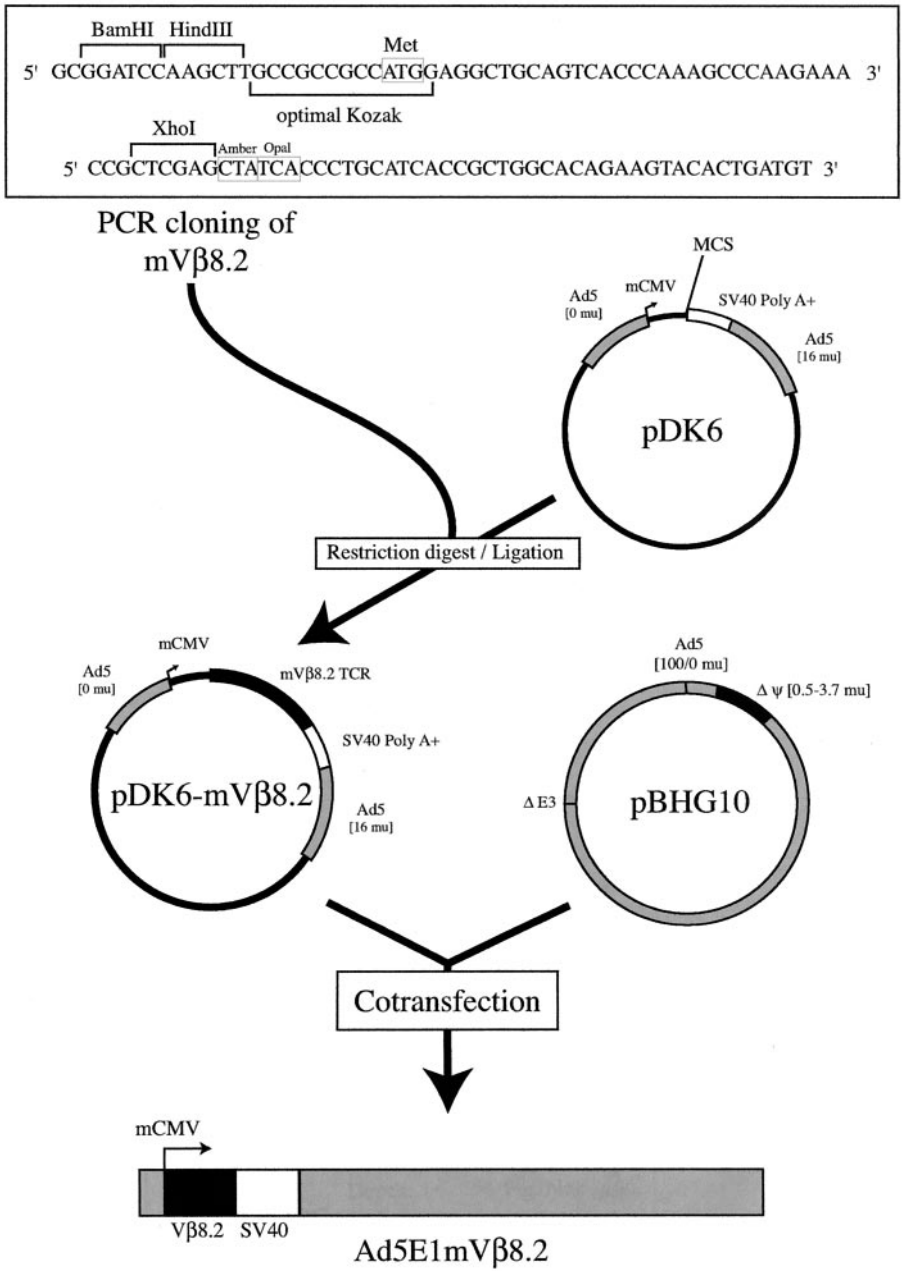


**FIGURE 1.** Schematic diagram showing the construction strategy and structure of the Ad5E1 mV$\beta$8.2 recombinant adenovirus vector. Plasmid pDK6-mV$\beta$8.2 was constructed by ligating the PCR fragment containing the murine V$\beta$8.2 TCR flanked by *Bam*HI and *Xho*I sites into the multicloning site of pDK6, placing the gene under the transcriptional control of the mCMV promoter and the SV40 polyadenylation signal. Cotransfection of pDK6-mV$\beta$8.2 with pBHG10 DNA in 293 cells generated the recombinant adenovirus vector Ad5E1 mV$\beta$8.2. Ad5 sequences with corresponding map units (mu) are indicated.

DNA encoding the single chain V$\beta$8.2 TCR (28) allowed rescue of a constructed mini-gene fragment of 342 bp containing the V$\beta$8.2 TCR. A *Bam*HI/*Xho*I fragment containing the mini-gene encoding the murine V$\beta$8.2 TCR was cloned into the polylinker site of pDK6, to generate pDK6.m$\beta$V8.2. This construct places the transgene under the control of the murine CMV (mCMV) promoter and provides a polyadenylation signal from SV40. To obtain the resultant adenovirus vector expressing the V$\beta$8.2 TCR, pDK6 mV$\beta$8.2, DNA was cotransfected with pBHG10 into 293 cells using standard adenovirus rescue protocols and produced the recombinant Ad5E1 mV$\beta$8.2 vector (29, 30).

### *Northern blot analysis for the detection of transcripts of the murine Vβ8.2 chain of the TCR after Ad5E1 mVβ8.2 vector treatment*

Transgenic expression of the V$\beta$8.2 transcripts was detected both in vitro and in vivo following Ad5E1 mV$\beta$8.2 vector treatment. To verify expression of the TCR V$\beta$8.2 transcripts, 293 cells were infected at a multiplicity of infection of 10 with the Ad5E1 mV$\beta$8.2 vector, or B10.PL mice were injected with a $2 \times 10^9$ PFU dose of Ad5E1 mV$\beta$8.2 vector in a 50-$\mu$l volume of PBS into the femoris muscle of the left hind leg. Twenty-four hours later, 20 $\mu$g of total cellular RNA was prepared and analyzed by Northern blot analysis as previously described (31). Strong mRNA expression of mV$\beta$8.2 was detected in cells and muscle tissue infected with Ad5E1 mV$\beta$8.2, but not DL70-3, vector-infected control cells (data not shown). This signal corresponded to an approximate size mRNA of 550 bp and is consistent with an expected length of 342 bp for the mV$\beta$8.2 mini-gene cassette with an ~200-bp polyadenylated tail.

### *Animals and immunization*

B10.PL mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and bred under specific pathogen-free conditions in the La Jolla Institute for Allergy and Immunology animal facility (San Diego, CA). Female mice were used at 8–14 wk of age. Mice were immunized i.p. with $2 \times 10^9$ PFU of the Ad5E1 mV$\beta$8.2 vector in 200 $\mu$l of PBS. For i.m. immunization, $2 \times 10^9$ PFU of Ad5E1 mV$\beta$8.2 vector were injected in 50 $\mu$l of PBS into the left hind leg. Disease induction was followed 10 days later using 100 $\mu$g of peptide Ac1–9 or whole MBP. All recombinant viruses were propagated and purified as described for the Ad5E1 mV$\beta$8.2 vector. For cytokine coexpression experiments, cytokine vectors were injected at $5 \times 10^8$ PFU dose admixed with $2 \times 10^9$ PFU of Ad5E1 mV$\beta$8.2 vector in 50 $\mu$l of PBS injected i.m. The adenoviruses expressing IL-4, IL-10, and IL-12 were all previously used to express biologically active cytokine upon in vivo administration in other model systems (2, 4, 32, 33). Control vector DL70-3 is an Ad5 variant deleted in the E1 region while the Ad5LacZ vector expresses a nonrelevant Ag, $\beta$-galactosidase (29). All work was performed in accordance with La Jolla Institute for Allergy and Immunology guidelines for animal use and care.

### *Polymerase chain reaction*

V$\beta$8.2 TCR mini-gene cDNA synthesis was performed with Vent DNA polymerase (New England Biolabs, Beverly, MA) using the Superscript preamplification system according to the manufacturer's instructions (Life Technologies, Grand Island, NY). PCR were performed on a Gene Amp PCR System 9700 (Applied Biosystems, Foster City, CA) using denaturation for 2 min at 94°C, and then 30 cycles of 94°C for 1 min, 55°C for 2 min, and 72°C for 2 min followed by a 10-min extension at 72°C.

### *TCR peptides*

TCR peptides were synthesized by S. Horvath (California Institute of Technology, Pasadena, CA) using a solid phase technique on a peptide synthesizer (430A; Applied Biosystems) and were purified on a reverse phase column by HPLC, as described earlier (34). TCR V$\beta$8.2 chain peptides correspond to the sequences predominantly used in the MBP-specific response in B10.PL mice (16) and are as follows (single-letter amino acid code): B1, aa 1–30 (L): EAAVTQSPRNKVAVTGGKVTLSCNQT NNHNL; B5, aa 76–101: LILELATPSQTSVYFCASGDAGGGYE; p41–50, aa 41–50: HGLRLIHYSY; p72–80, aa 72–80: ENFSLILEL.

### *Splenic proliferation assay*

Spleens of mice were removed 10 days after immunization with the Ad5E1 mV$\beta$8.2 vector to test initial responses to TCR determinants, or 10 days after disease induction with 100 $\mu$g of MBP to test the outcome of response after vector vaccination to MBP determinants. Splenocytes ($8 \times 10^5$ cells per well) were cultured in 96-well microtiter plates in 200 $\mu$l of serum-free medium (HL-1; Ventrex, Portland, ME) supplemented to 2 mM glutamine; peptides were added at concentrations ranging from 0.1–7 $\mu$M final concentration. Proliferation was assayed by the addition of 1 $\mu$Ci [$^3$H]thymidine (International Chemical and Nuclear, Irvine, CA) for the last 18 h of a 5-day culture, and incorporation of the label was measured by liquid scintillation counting.

### *Induction of EAE*

MBP was isolated from the brains of B10.PL mice as described (35). For induction of EAE, mice were immunized s.c. with either 100 $\mu$g of MBP or MBP peptide Ac1–9 in CFA, and 0.1 $\mu$g of pertussis toxin (PTX; List Biological Laboratories, Campbell, CA) was injected i.p. in 500 $\mu$l of saline, 24 and 72 h later. Mice were observed daily for signs of EAE and until >60 days after MBP immunization. The average disease score for each group was calculated by averaging the maximum severity at each time point of all of the affected animals in each group. Disease severity was scored on a five-point scale (36): 1, flaccid tail; 2, hind limb weakness; 3, hind limb paralysis; 4, whole body paralysis/moribund; 5, death. Onset of disease is defined as the first signs of loss of tail tone or hind limb weakness.

### *ELISA*

IFN-$\gamma$ and IL-4 levels were measured by a sandwich ELISA, using supernatants obtained from peptide-pulsed lymphocytes isolated from adenovirus vector- and control-immunized B10.PL mice. Briefly, splenocytes ($8 \times 10^6$ cells/ml) were cultured for 48 h in 24-well plates either with medium alone or together with the B1, B5, p41–50, or p72–80 TCR peptides. Nunc Immuno Plates MaxiSorp F96 (Roskilde, Denmark) were coated with anti-IFN-$\gamma$ or anti-IL-4 Abs. After blocking with PBS containing 10% FBS, supernatants were added overnight at 4°C. Plates were extensively washed with PBS-Tween and incubated with biotin-conjugated anti-IFN-$\gamma$ or anti-IL-4 Ab. Finally, plates were washed and developed using avidin-peroxidase and 2–2′-azino-bis(3-ethyl-benzthiazoline-6-sulfonic acid) substrate (Sigma-Aldrich, St. Louis, MO). $OD_{405}$ was measured, and the values were determined against a recombinant protein standard. All cytokine capture and Ab detecting pairs were obtained from BD PharMingen (San Diego, CA).

### *Cellular ELISA-spot*

IFN-$\gamma$- and IL-4-producing cells were enumerated from peptide-pulsed lymphocytes isolated from adenovirus vector and control immunized B10.PL mice by the cellular ELISA-spot assay as described (37). Briefly, splenocytes ($8 \times 10^6$ cells/ml) were cultured for 48 h in 24-well plates either with medium alone or the B1, B5, p41–50, p72–80, or Ac1–20 peptides. Millititer HA nitrocellulose plates (Millipore, Bedford, MA) were coated overnight at 4°C with anti-IFN-$\gamma$ (purified from R46A2 supernatants) or anti-IL-4 Ab. Plates were blocked as above, and Ag-stimulated cells were added at different concentrations for 24 h at 37°C. The wells were then incubated with biotin-conjugated anti-IFN-$\gamma$ (purified from XMG1.2 supernatants) or anti-IL-4 Ab followed by incubation with avidin-peroxidase (Vector Laboratories, Burlingame, CA). Spots were developed by the addition of 400 $\mu$g/ml 3-amino-9-ethylcarbazole substrate (Sigma-Aldrich) and enumerated by a computerized image analysis system (Lightools Research, Encinitas, CA) using the image analyzer program NIH Image 1.61 (National Institutes of Health, Bethesda, MD).

### *Statistical analysis*

Data are expressed as the mean ± SEM for each group. Statistical analysis were performed using Statview 4.5 programs from Abacus Concepts (Berkeley, CA). A Wilcoxon test was used for the final determination of significance testing the effects of the Ad5E1 mV$\beta$8.2 vector vs controls.

## Results

### *Ad5E1 mVβ8.2 vector immunization leads to priming/expansion of T cells reactive to the Fr3 region peptide (B5) of the Vβ8.2 chain of the TCR*

We have previously proposed a Treg cell circuit involving both TCR-peptide-reactive $CD4^+$ and $CD8^+$ cells which bring about the physiological regulation of EAE in B10.PL mice. Because the CD8 Treg population does not proliferate well in in vitro peptide recall assays, we tested whether CD4 Treg were primed following adenoviral V$\beta$8.2 vector administration. We examined the in vitro recall proliferative responses to the CD4 determinant B5 (aa 76–101) from the spleens of B10.PL mice that were injected i.p., 10

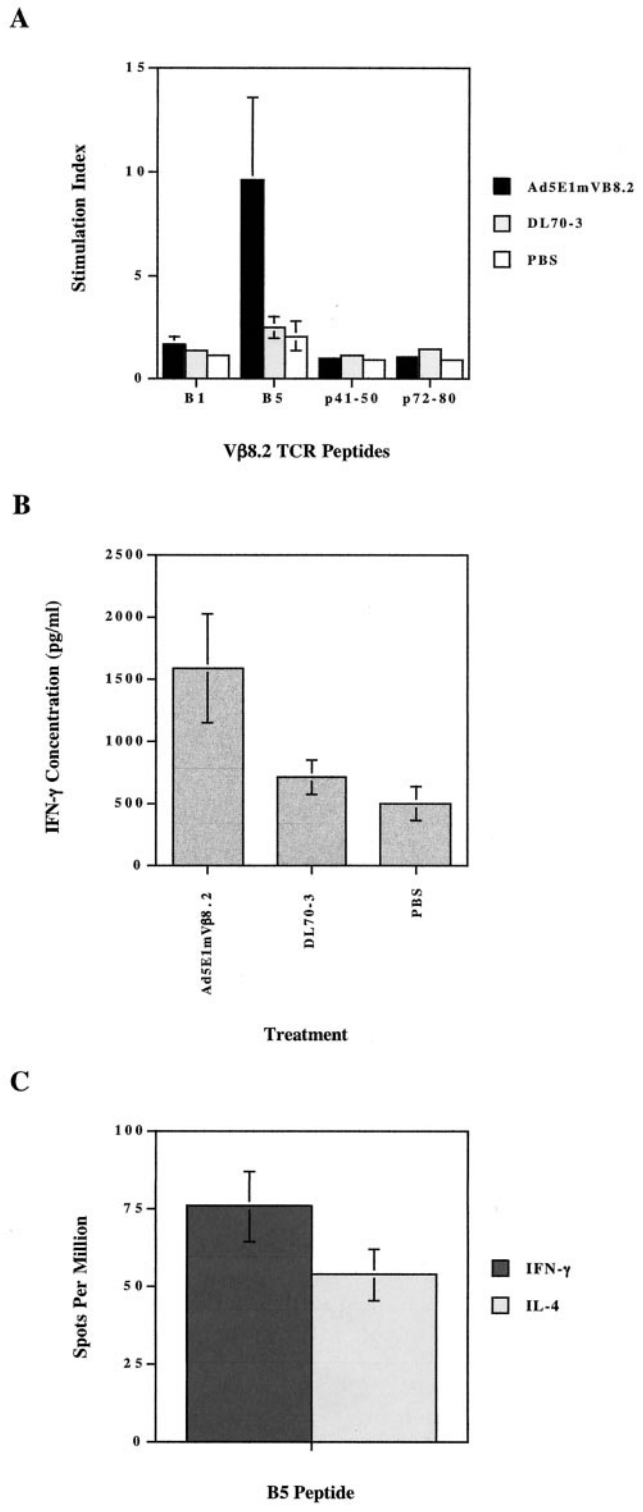


**FIGURE 2.** *A*, T cells isolated from B10. PL mice immunized with Ad5E1 mV$\beta$8.2 vector are reactive to the immunodominant Fr3 region determinant within TCR peptide B5. Proliferation to TCR peptides was tested in spleens from mice 10 days after vaccination with Ad5E1 mV$\beta$8.2 or vector controls. Responses to TCR peptides at an optimum concentration (3 $\mu$M) are shown. The data are expressed as arithmetic means $\pm$ SE of SIs that were derived from [$^3$H]thymidine incorporation. This experiment was repeated six times and the cumulative data are shown. Background cpm averaged 2493 $\pm$ 882. No detectable proliferative responses were seen to B1 control peptide in any treatment group. *B*, IFN-$\gamma$ production by V$\beta$8.2 TCR-reactive T cells following vaccination with Ad5E1 mV$\beta$8.2 or vector controls. Cells were harvested from spleens for each treatment group and incubated for 24 h in vitro at a density of $10^6$ cells/ml with 100 $\mu$g of B5 peptide. IFN-$\gamma$ production in the culture supernatants was then determined by ELISA as described in *Materials and Methods*. Data are expressed as the mean $\pm$ SD of four mice. No detectable IFN-$\gamma$ production was seen to B1 control peptide in any treatment group. *C*, ELISA-spot analysis of the B5 proliferative response in the Ad5E1 mV$\beta$8.2 vector-treated mice revealed a Th1 bias. IFN-$\gamma$- and IL-4-producing cells were enumerated as described in *Materials and Methods*. Splenocytes were cultured for 48 h with 100 $\mu$g of B5 peptide and plated on Millititer HA nitrocellulose plates coated with either anti-IFN-$\gamma$ or -IL-4 Abs. Spots were enumerated using image analyzer program NIH Image 1.61. Data are expressed as the mean $\pm$ SD of three mice. Responses to B5 in the PBS- or DL70-3 control vector-treated mice did not rise above background.

days earlier, either with a $2 \times 10^9$ PFU dose of Ad5E1 mV$\beta$8.2 or with vector controls.

As shown in Fig. 2*A*, T cells reactive to the TCR peptide B5 were primed after vaccination with the Ad5E1 mV$\beta$8.2 vector. This experiment was repeated six times and cumulative data are shown. Stimulation indices (SI) averaging 9.35 were achieved to the B5 peptide whereas responses to another control peptide, B1, averaged below 2 in V$\beta$8.2 vector-treated mice. In contrast, in all experiments performed, no significant responses to any other region of the TCR were detectable in the DL70-3 vector or PBS-treated controls. Our results show that recall responses to the TCR peptide B5 seen after vector treatment are similar to those that we have previously reported during spontaneous recovery from EAE (18) or induced by other TCR-based interventions in B10.PL mice (21).

### *Ad5E1 mV$\beta$8.2 vector immunization of B10.PL mice results in the expansion of Treg cells with a Th1 phenotype*

We have previously shown that the profile of cytokines produced by the regulatory CD4$^+$ T cells specific for the Fr3 region (B5) determinant of the V$\beta$8.2 TCR profoundly influences the resultant responses to the target autoantigen MBP and disease outcome (25). Protection was afforded only when the TCR peptide B5-reactive regulatory CD4$^+$ cells produce a Th1 cytokine profile. To determine whether a B5-specific Th1 response was obtained after adenovirus vector vaccination, the cytokine phenotype of the B5-reactive cells generated after Ad5E1V$\beta$8.2 immunization was investigated. Lymphocytes isolated from the vaccinated animals were tested for the secretion of IFN-$\gamma$ or IL-4 cytokine. IFN-$\gamma$ production against the B5 determinant was ~2- to 3-fold higher in Ad5E1V$\beta$8.2 vector-immunized mice vs the DL70-3 vector and PBS-treated controls (Fig. 2*B*). IFN-$\gamma$ concentrations averaged 1592 pg/ml in Ad5E1V$\beta$8.2 vector-treated mice compared with 720 and 505 pg/ml for DL70-3 and PBS controls, respectively. Although low but detectable levels of IL-4 were found in all treatment groups, no significant differences above baseline levels were detected for Ad5E1V$\beta$8.2 vector-immunized animals vs controls (data not shown).

The B5 proliferative response seen after Ad5E1V$\beta$8.2 vector immunization was further characterized by ELISA-spot analysis performed on isolated splenocytes (Fig. 2*C*). The ratio of IFN-$\gamma$-IL-4 spots (1.54) detected after immunization indicated that responses to B5 had a Th1 bias. Thus, Ad5E1V$\beta$8.2 vector vaccination enhanced the production of IFN-$\gamma$-secreting Th1 regulatory cells, specific to the Fr3 region determinant of the V$\beta$8.2 TCR.

### *Intraperitoneal or intramuscular immunization with the Ad5E1 mV$\beta$8.2 vector protects B10.PL mice against EAE induced with MBPAc1–9*

To determine whether Ad5E1 mV$\beta$8.2 vector immunization could influence the course of EAE, B10.PL mice were inoculated i.p. with PBS or a $2 \times 10^9$ PFU dose of the V$\beta$8.2 or DL70-3 vectors.

The $2 \times 10^9$ dose was chosen because this dose was shown to be the most effective for inducing response to self Ags in tumor models (38, 39). Ten days later, induction of EAE was attempted by s.c. administration of MBP Ac1–9 peptide as detailed in *Materials and Methods*. Mice were monitored daily for disease until day 30, and in one experiment up to day 60, to look for disease relapse. As shown in Fig. 3*A*, mice receiving i.p. immunization with the Ad5E1 mV$\beta$8.2 vector were significantly protected against induction of EAE. Table I shows the clear difference in EAE incidence following Ad5E1 mV$\beta$8.2 i.p. vector treatment. In this treatment group, a greater percentage of animals remained disease-free with no animal getting disease with severity greater than a score of 2, whereas mice in DL70-3- or PBS-immunized groups contracted a more severe EAE and had a higher incidence of disease. In addition, no disease relapse was evident in any Ad5E1 mV$\beta$8.2 vector-treated group when the animals were monitored until day 60 while some mild relapses were detected in the controls (data not shown).

Next, we wished to determine whether other routes of vector delivery could induce a protective response similar to that seen by IP immunization. Intramuscular immunization with the Ad5E1 mV$\beta$8.2 vector was chosen as an alternative route of administration to reduce concerns of immunotoxicity that have been reported with the i.v. and airway administration of adenovirus vectors (40–42). Injection of adenovirus into the muscle should offer no more, or similar, risk to that associated with standard vaccination procedures with adjuvant.

To test the efficacy of i.m. vaccination, B10.PL mice were immunized i.m. with a $2 \times 10^9$ PFU dose of the Ad5E1 mV$\beta$8.2 or vector controls and EAE was induced in these groups of mice as before. As seen in Fig. 3*A* and summarized in Table I, i.m. immunization with the Ad5E1 mV$\beta$8.2 vector provided B10.PL mice protection against EAE nearly equivalent to that seen for i.p. treatment. This approach of using i.m. injection with the Ad5E1 mV$\beta$8.2 vector to induce a regulatory response may be the most suitable use of adenovirus vectors in transient gene expression therapies (such as TCR-directed therapy) that have immunomodulation as a primary target. We have previously shown that i.m. vaccination generally leads to more localized transgene expression (32).

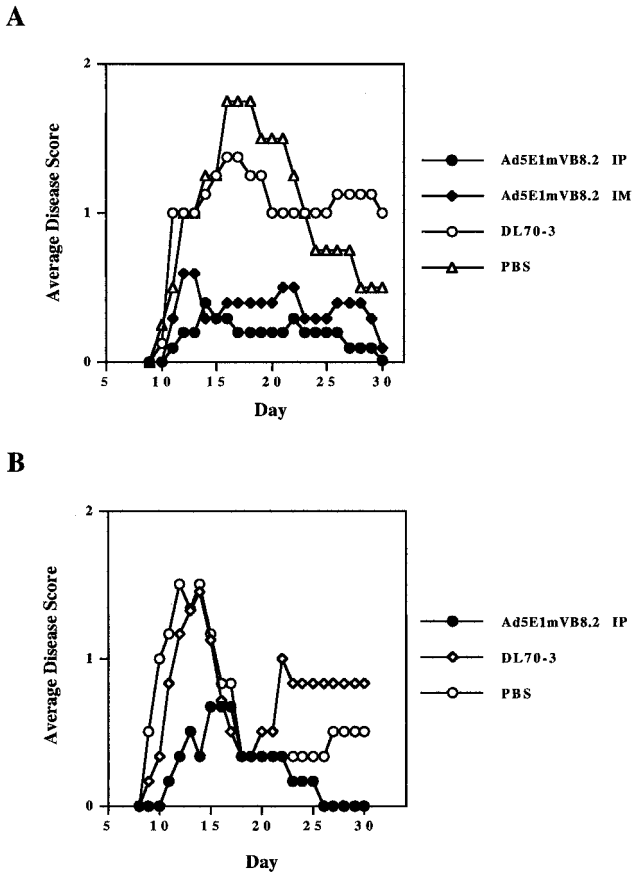


**FIGURE 3.** *A*, Intraperitoneal and i.m. vaccination with Ad5E1 mV$\beta$8.2 vector ameliorates EAE induced with the peptide Ac1–9 of MBP. Groups of B10.PL mice were immunized with a $2 \times 10^9$ PFU dose of Ad5E1 mV$\beta$8.2, DL70-3 control vector, or PBS in a 300-$\mu$l volume or were injected with a $2 \times 10^9$ PFU dose in a 50-$\mu$l volume into the femoral bicep muscle of the left hind limb. Ten days later, disease was induced in mice by challenge with MBP Ac1–9/CFA followed by PTX. Clinical symptoms of EAE were monitored until day 30 following antigenic challenge and were scored on the typical graded scale from 1 to 5. Mean values at each time point are shown for the treatment groups; $n = 10$ for each data point. Values of $p < 0.05$ for differential of peak disease score at day 15 for i.p. or i.m. treatments compared with control virus or saline. *B*, Intraperitoneal vaccination with the Ad5E1 mV$\beta$8.2 vector prevents EAE induced with whole MBP. Groups of B10.PL mice were immunized i.p. with a $2 \times 10^9$ PFU dose of Ad5E1 mV$\beta$8.2 vector or controls and 10 days later, disease was induced as described earlier, except with whole mouse MBP (100 $\mu$g/mouse), injected in CFA followed by PTX. Clinical symptoms of EAE were monitored until day 30 following antigenic challenge and were scored on a graded scale from 1 to 5. Mean values at each time point are shown for the treatment groups; $n = 6$ for each data point. Values of $p < 0.05$ for differential of peak disease score at day 15 for i.p. treatment compared with control virus or saline.

*Intraperitoneal immunization with the Ad5E1 mV$\beta$8.2 vector protects B10.PL mice from EAE induced with whole MBP*

Because T cells not displaying the V$\beta$8.2 TCR may arise with encephalitogenic capacity, owing to intramolecular or intermolecular spread of neuroantigen determinants, we tested the efficacy of Ad5E1 mV$\beta$8.2 vector vaccination against EAE induced with whole mouse MBP (Fig. 3*B*). EAE induced with whole MBP and additional adjuvant is more chronic than Ac1–9 monophasic disease. Treatment with the $2 \times 10^9$ PFU dose of Ad5E1 mV$\beta$8.2 vector provided protection against disease. In addition, mice in the PBS control group showed signs of mild relapses whereas no such relapses were seen in the V$\beta$8.2 vector-treated group. Thus, Ad5E1V$\beta$8.2 immunization was able to produce a degree of protection in B10.PL mice against MBP-induced disease despite the presence of potential additional T cell determinants capable of inducing EAE.

*Protective response generated by Ad5E1 mV$\beta$8.2 vector immunization is dose-dependent*

We have found that self-Ags, when compared with foreign Ags expressed from recombinant adenovirus vectors, generally require higher dosages to induce effective Ag-reactive immune response ($10^9$ vs $10^7$ PFU dosages). Because response to the conserved Fr3 region of the V$\beta$8.2 TCR appears to be hardwired into immunological memory, we wanted to determine how this response would be affected by vector-mediated expression of the V$\beta$8.2 transgene. Vector dose response analysis revealed that a relatively low immunizing dose of $5 \times 10^8$ PFU is capable of inducing a protective response while the greatest efficacy was seen at the highest dose tested of $4 \times 10^9$ PFU (Fig. 4).

*Ad5E1 mV$\beta$8.2 vector immunization attenuates proliferative and Th1 responses to the dominant determinant of MBP in B10.PL mice*

We asked whether Ad5E1V$\beta$8.2 vector immunization could modulate the dominant encephalitogenic T cell response to MBP.

Table I. *Treatment with Ad5E1mVβ8.2 decreases severity of EAE in B10.PL mice*[a]

| Treatment | EAE Incidence[b] | Mean Disease Onset (Days) | Mean Duration (Days) |
|---|---|---|---|
| Ad5E1mVβ8.2 i.p. | 4/10 (2, 1, 1, 1, 0, 0, 0, 0, 0, 0) | 14.7 ± 2.1 | 10.5 ± 2.3 |
| DL70–3 i.p. | 7/8 (5, 5, 2, 2, 2, 1, 1, 0) | 12.8 ± 1.2 | 12.7 ± 2.5 |
| PBS i.p. | 9/12 (5, 4, 3, 2, 2, 1, 1, 1, 1, 0, 0, 0) | 13.0 ± 1.1 | 12.0 ± 2.2 |
| Ad5E1mVβ8.2 i.m. | 5/10 (3, 3, 1, 1, 1, 0, 0, 0, 0, 0) | 15.2 ± 1.7 | 9.8 ± 2.0 |
| DL70–3 i.m. | 5/8 (5, 3, 3, 1, 1, 0, 0, 0) | 13.2 ± 1.0 | 15.2 ± 3.0 |
| PBS i.m. | 4/4 (3, 3, 2, 2) | 13.0 ± 1.1 | 15.5 ± 2.2 |

[a] B10.PL mice were injected with Ac1-9/CFA/PTX for the induction of EAE. Animals were injected once with recombinant adenovirus vectors or PBS 10 days prior to peptide challenge. All animals were monitored for 30 days.
[b] Diseased/total number of mice (individual maximum score).

Ad5E1Vβ8.2 or control vector-vaccinated mice were challenged with murine MBPAc1–20 containing the dominant N-terminal determinant Ac1–9 and proliferative responses were determined 10 days later in isolated splenocytes (Fig. 5*A*). There were significant differences in the proliferative responses of Ad5E1Vβ8.2 vector-immunized mice compared with vector controls. SIs to Ac1–20 were ~3-fold lower in Ad5E1Vβ8.2-treated mice compared with DL70-3 and PBS controls, respectively. The decrease in responsiveness to murine MBP Ac1–20 following Ad5E1Vβ8.2 vector was specific because responses to purified protein derivative of the mycobacterium (a component in the CFA) were similar to those found after treatment with the vector controls. Proliferative responses to purified protein derivative ranged between 69,000 and 82,000 cpm in all groups.

In addition to the diminished proliferative recall response, IFN-γ production by Ac1–20-specific T cells was reduced >2-fold in Ad5E1Vβ8.2 vector-treated mice compared with the PBS-treated control (Fig. 5*B*). There was some background effect of the adenovirus vector alone on IFN-γ production as Ac1–20-specific lymphocytes isolated from DL70-3 controls showed a 27% reduction in IFN-γ levels compared with the PBS treatment group. The most significant difference was evident between the treatment groups when the frequency of IFN-γ-secreting cells in response to MBP Ac1–20 was examined by cellular ELISA-spot analysis (Fig. 5*C*). Approximately 22-fold fewer Ac1–20-specific IFN-γ-producing cells/$10^6$ were detected after Ad5E1Vβ8.2 vector immunization compared with PBS control animals (20 vs 439 IFN-γ spots) while the response was 6.6-fold less than the number detected following DL70-3 treatment (20 vs 133 IFN-γ spots). No significant differences in the frequency of IL-4-secreting cells were detected. Nevertheless, the resultant effect of Ad5E1Vβ8.2 vector immunization was to reduce the IFN-γ-IL-4 ratio of T cells (Th1/Th2) in the MBP Ac1–20-reactive population.

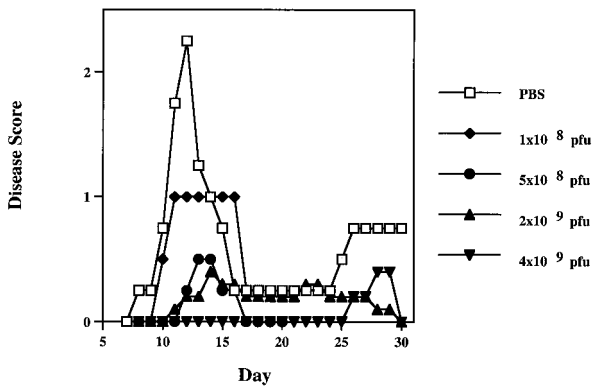


**FIGURE 4.** The therapeutic efficacy of Ad5E1 mVβ8.2 vector vaccination is dose-dependent. B10.PL mice were immunized i.p. with the various gradations in dose of the Ad5E1 mVβ8.2 vector. Disease induction was initiated as described before 10 days after immunization and the clinical symptoms of EAE were monitored until day 30 and scored as described above. Mean values at each time point are shown for the treatment groups; $n = 4$ for each data point.

### *Intramuscular coimmunization with adenoviruses expressing either IL-4 or IL-10 cytokine disrupts the protective response generated by the Ad5E1 mVβ8.2 vector alone*

We have previously reported that the generation of effective regulatory CD4 T cells was cytokine-dependent (25). To generate effective regulatory CD4 T cells, we had shown that a Th1 cytokine milieu during their priming was required. To test the stringency of this requirement, we coadministered with the Ad5E1Vβ8.2 vector, the recombinant adenovirus vectors expressing either IL-4 or IL-10 by i.m. injection. Each of these vectors had been previously demonstrated in other mouse model systems to alter immune responses. The IL-4 vector was used to skew immune responses in a Th2 direction in B10.D2 mice to render them susceptible to leishmaniasis (32). The IL-10 vector has been previously used to induce a transient immunosuppression that controlled inflammation induced by bacteria (33). We reasoned that each vector could disrupt the generation of regulatory cells by altering the cytokine microenvironment. As shown in Fig. 6, coadministration of either an IL-4 or IL-10 cytokine vector with the Ad5E1Vβ8.2 vector resulted in exacerbation of EAE, while administration of the Ad5E1 mVβ8.2 vector alone or together with the control vector DL70-3 significantly protects mice from EAE.

### *Cointramuscular immunization with adenoviruses expressing either IL-4 or IL-10 cytokine with the Ad5E1 mVβ8.2 vector changes both the proliferative and cytokine response generated by CD4 Treg cells reactive to B5*

As shown in Fig. 7, the proliferative response to the B5 determinant of the Vβ8.2 TCR was significantly reduced when the Ad5E1 mVβ8.2 vector was coadministered with either the IL-4 or IL-10 vector. Proliferative responses to B5 in both spleen and draining lymph nodes were present (SI ~3) when the Ad5E1 mVβ8.2 vector was administered with the control vector DL70-3. No significant proliferative response was detected to B1, the control peptide representing the region aa 1–30 of the Vβ8.2 TCR, in any treatment group.

Major differences in the Ad5E1 mVβ8.2 vector response were detected in animals after the IL-4 or IL-10 cytokine treatments. As shown in Table II, ELISA-spot analysis revealed a Th1 bias in response to B5 peptide in both spleen and draining lymph node cells isolated 10 days after i.m. immunization with the Ad5E1 mVβ8.2/DL70-3 mixture. The IFN-γ-IL-4 ratio was 1.42 vs 2.08 in spleen and lymph node in Ad5E1 mVβ8.2/DL70–3 treated

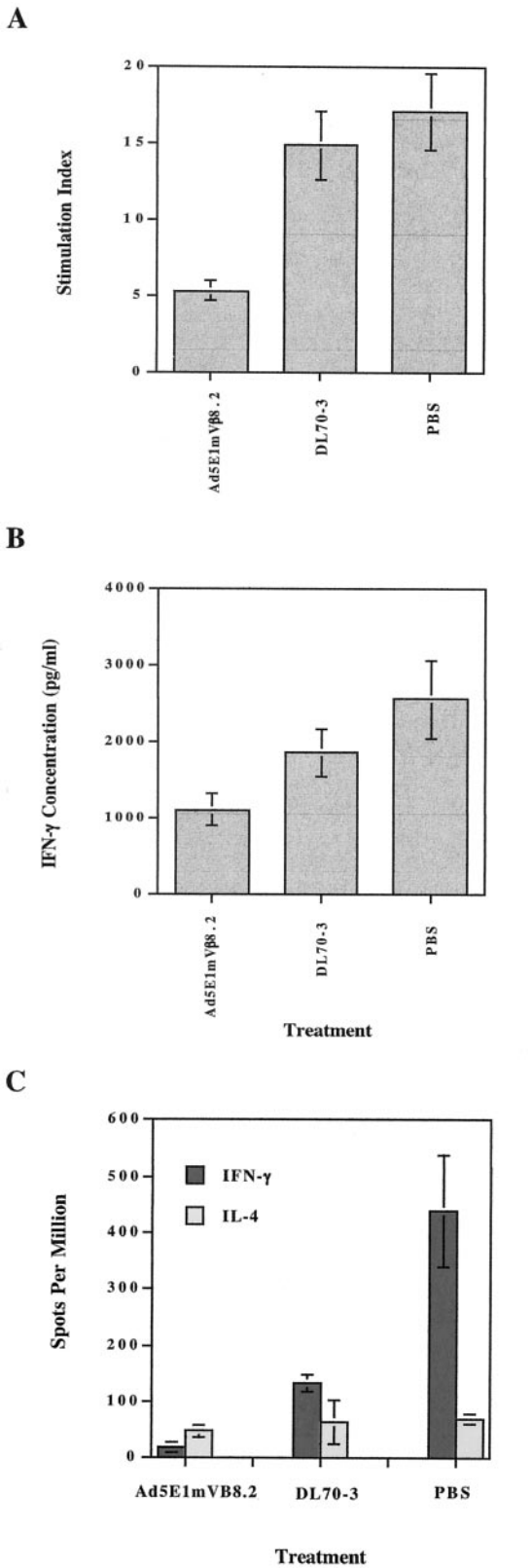


**FIGURE 5.** *A,* Ad5E1 mV$\beta$8.2 vector i.p. immunization down-regulates proliferative responses to MBP Ac1–20 in B10.PL mice. Groups of mice were immunized i.p. with a $2 \times 10^9$ PFU dose of Ad5E1 mV$\beta$8.2 or vector controls. Ten days later, disease was induced using MBP peptide Ac1–9/CFA as described in *Materials and Methods*. Splenocytes were harvested from Ag-challenged mice 10 days later and proliferative responses to peptide Ac1–20 of MBP were determined. The data are expressed as the arithmetic means of [$^3$H]thymidine incorporation in triplicate cultures. Background cpm averaged 3113 ± 385. *B,* IFN-$\gamma$ production by MBP Ac1–20-reactive T cells following vaccination with Ad5E1 mV$\beta$8.2 or vector controls. Cells were harvested from spleens for each treatment group and incubated for 24 h in vitro at a density of $10^6$ cells/ml with 100 $\mu$g of MBP Ac1–20 peptide. IFN-$\gamma$ production in the culture supernatants was then determined by ELISA as described in *Materials and Methods.* Data are expressed as the mean ± SD of four mice. *C,* ELISA-spot analysis of the MBP Ac1–20 proliferative response in the Ad5E1 mV$\beta$8.2 vector-treated mice revealed a Th2 bias. IFN-$\gamma$- and IL-4-producing cells were enumerated as described in *Materials and Methods.* Splenocytes were cultured for 48 h with 100 $\mu$g of MBP Ac1–20 peptide and plated on Millititer HA nitrocellulose plates coated with either anti-IFN-$\gamma$ or -IL-4 Abs. Spots were enumerated using image analyzer program NIH Image 1.61. Data are expressed as the mean ± SD of four mice.

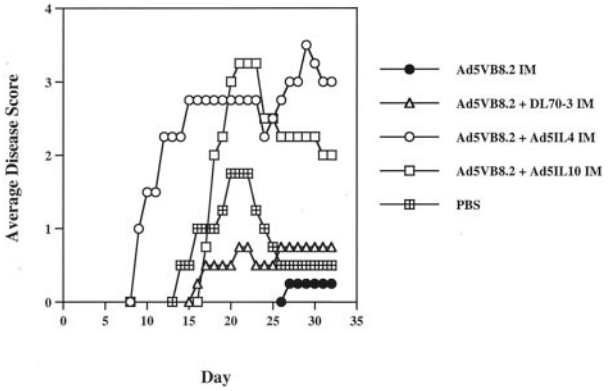


**FIGURE 6.** Intramuscular covaccination of Ad5E1 mV$\beta$8.2 vector with IL-4 or IL-10 adenovirus expression vectors exacerbates EAE induced with the peptide Ac1–9 of MBP. Groups of B10.PL mice were immunized with a $2 \times 10^9$ PFU dose of Ad5E1 mV$\beta$8.2, DL70-3 control vector or PBS, in a 300-$\mu$l volume or were injected with a $2 \times 10^9$ PFU dose in a 50-$\mu$l volume into the femoral bicep muscle of the left hind limb. Ten days later, disease was induced in mice by challenge with MBP Ac1–9/CFA followed by PTX. Clinical symptoms of EAE were monitored until day 30 following antigenic challenge and were scored on the typical graded scale from 1 to 5. Mean values at each time point are shown for the treatment groups; $n = 4$ for each data point. Values of $p < 0.05$ for differential of peak disease score at day 15 for IL-4 or IL-10 vector treatment compared with control virus or saline.

mice, respectively. This was in contrast to the ratios found in treatment groups that received IL-4 or IL-10 vector in addition to the Ad5E1 mV$\beta$8.2 vector. In splenocytes recovered from the IL-4- and IL-10-treated mice, a Th2 bias was evident and IFN-$\gamma$-IL-4 ratios were 0.27 and 0.44, respectively. The Th2 bias was also noticed in draining lymph node cells after IL-4 treatment; however, in IL-10-treated mice very few spots were detected. This possibly could reflect the immunosuppressive effect of IL-10 on the local tissue response.

## Discussion

In this study, we have used a recombinant adenovirus vector (Ad5E1V$\beta$8.2) expressing the murine TCR V$\beta$8.2 chain to prevent EAE in B10.PL mice induced either with whole murine MBP or its dominant N-terminal encephalitogenic determinant. We have previously shown that T cell clones raised against the dominant MBP determinant in B10.PL mice were highly restricted in their V$\beta$ gene usage with ~80% of the T cells displaying the V$\beta$8.2 TCR (16). Thus, in the B10.PL model of EAE, encephalitogenic T cells with such limited V gene usage provide an excellent model target for intervention. We and others have previously used adenovirus

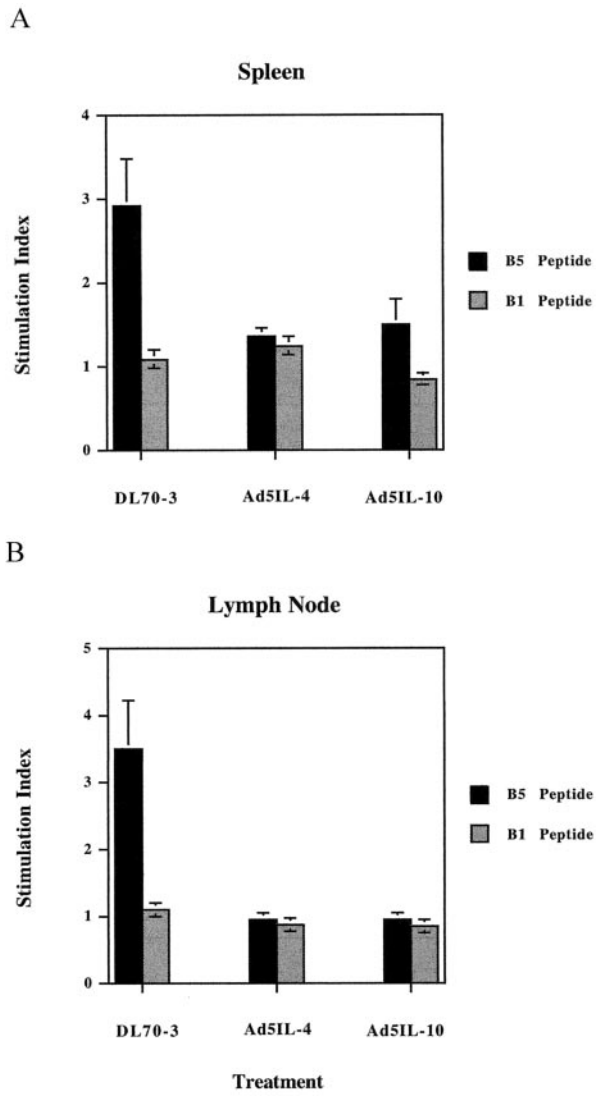


**FIGURE 7.** Coadministration of adenovirus vectors expressing IL-4 or IL-10 with Ad5E1 mV$\beta$8.2 vector disrupts the proliferative response to the immunodominant Fr3 region determinant within TCR peptide B5. Proliferation to TCR peptides was tested in spleens (*A*) or lymph nodes (*B*) from mice 10 days after i.m. vaccination with Ad5E1 mV$\beta$8.2 or vector controls. Responses to TCR peptides at an optimum concentration (3 $\mu$M) are shown. The data are expressed as arithmetic means $\pm$ SE of stimulation indices which were derived from [$^3$H]thymidine incorporation. Mean values are shown for the treatment groups; $n = 4$ for each data point. Background cpm averaged 15,339 $\pm$ 857 and 26,680 $\pm$ 1494 for spleen and lymph node assay, respectively.

vectors expressing transgenes as powerful immunogens to generate protective immunity to infectious diseases and in cancer therapy (2, 4–7, 9, 10, 13, 14, 43). Adenovirus vectors provide the ability to induce strong immune responses owing to their transient high expression relative to retrovirus vectors or naked DNA. It was predictable that an adenovirus vector directly expressing the TCR from encephalitogenic T cells would induce prolific cognate T cell responses with regulatory function.

In an attempt to gain some insight into the mechanism of V$\beta$8.2 vector-induced protection, we have examined the induction of a CD4 Treg population that has been extensively characterized in our laboratory. We were able to demonstrate a significant recall response to the B5 peptide that contains the Fr3 region of the V$\beta$8.2 TCR. This region is recognized by naturally primed Treg cells generated during the recovery phase of MBP-induced EAE in B10.PL mice (18).

Table II. *Th1-like response disruption*[a]

| Treatment | IL-4 Spots | IFN-$\gamma$ Spots | IFN-$\gamma$-IL-4 Ratio | Trend |
|---|---|---|---|---|
| Spleen | | | | |
| DL70–3 | 84 | 120 | 1.42 | Th1 |
| Ad5mIL-4 | 48 | 13 | 0.27 | Th2 |
| Ad5mIL-10 | 54 | 24 | 0.44 | Th2 |
| Lymph node | | | | |
| DL70–3 | 120 | 250 | 2.08 | Th1 |
| Ad5mIL-4 | 130 | 62 | 0.476 | Th2 |
| Ad5mIL-10 | 0 | 3 | NA | Unknown |

[a] The Th1-like response generated by i.m. immunization with the Ad5E1mV$\beta$8.2 vector to the Fr3 region determinant of the V$\beta$8.2 chain can be disrupted following coimmunization with recombinant adenovirus cytokine vectors expressing either IL-4 or IL-10.

Adenovirus vector expression of the V$\beta$8.2 TCR induced CD4 regulatory responses to the same region of the TCR as that previously witnessed after natural recovery from EAE or that seen after immunization with peptide, recombinant TCR, and DNA vaccination (18, 21, 44, 53). It will be important to directly compare the efficacy and efficiency of the adenovirus vector approach to that of other reported TCR-based modalities. It appears that the adenoviral vector-mediated delivery of TCR is quite efficient in the control of EAE. For example, a single i.m. immunization with V$\beta$8.2 DNA does not significantly protect mice from EAE (three weekly injections are required) (45, 53). However in this regard, the protection against EAE induced with whole MBP afforded by $2 \times 10^9$ PFU of the Ad5E1 mV$\beta$8.2 vector demonstrated that protection was significant especially at the later time points with all mice remaining disease-free. No relapses were evident in Ad5E1 mV$\beta$8.2 vector-treated mice while some animals in the control group relapsed. This effective protection may provide an overriding advantage in the use of adenovirus vector for treatment of autoimmune conditions in that establishment of a potentially lifelong Treg cell memory response may be generated which can be evoked by any later activation of encephalitogenic T cells.

We have previously reported that another critical feature of TCR-centered regulation of EAE in B10.PL mice is the requirement that TCR peptide-reactive regulatory CD4 T cells secrete a Th1 cytokine profile (25). Ad5E1 mV$\beta$8.2 vector administration resulted in priming of B5-reactive Treg cells predominantly secreting IFN-$\gamma$, which is consistent with the findings from our previous report (25) as well as one describing vaccination with a vaccinia virus vector expressing the V$\beta$8.2 TCR (46). The resultant response to the dominant determinant of murine MBP, Ac1–20, following treatment with the V$\beta$8.2 TCR adenovirus vector revealed a skewing toward Th2 with a dramatically reduced level of IFN-$\gamma$ production. We are also in the process of using IFN-$\gamma$ knockout mice to fully investigate the requirement for this cytokine in generation of a regulatory response.

The results we have generated using adenovirus vectors expressing IL-4 or IL-10 to disrupt the protective response generated by Ad5E1 mV$\beta$8.2 vector immunization alone add further support for the Th1 requirement in regulatory cell induction. ELISA-spot analysis suggested two possible different mechanisms for interference with the protective response generated by Ad5E1 mV$\beta$8.2 vector in response to cytokine. IL-4 vector resulted in skewing of the B5 response in the draining node toward a Th2 profile. This could effectively prevent the development of Th1 regulatory cells and loss of normal physiologic regulation leading to exacerbation of

disease. In IL-10 vector-treated mice, few spots of either IFN-$\gamma$ or IL-4 were detected suggesting a direct immunosuppression of B5-reactive Treg cells. However, it is possible that lymphocytes isolated from this site could produce a unique phenotype (expressing TGF-$\beta$ for example), although we have not yet fully explored this possibility. The mechanism of action of IL-10 and its impact on EAE is still unclear, but it has been shown to exert either no effect or efficacy in the prevention of disease (47, 48). In this study, we propose that the tissue-localized expression of IL-10 in the context of the microenvironment prevents the development of a Th1-directed B5 response.

The importance of the Th1 requirement may be reflected in the following considerations: we have proposed that IFN-$\gamma$ production by CD4 Treg may be required to up-regulate costimulatory molecules on APC for the appropriate induction of a CD8 Treg population. These CD8 T cells then become able to induce apoptosis of V$\beta$8.2$^+$ Th1 pathogenic cells,[4] leaving behind Th2 cells which are less susceptible to apoptosis. This results in deviation of the global response to MBP in a Th2 direction. IL-4 production by MBP-specific T cells was also a characteristic of the protection reported following vaccination with DNA encoding V$\beta$8.2 TCR (22, 45, 53). Given the importance of inducing Treg for disease recovery, the ability to modify effector phenotypes of Treg cells by cytokine modulation may be decisive in optimizing regulatory control. We plan to include different cytokines in future studies as adjuvants to enhance protection induced by the Ad5E1V$\beta$8.2 vector, as well as to enhance response using other methods such as multimerization (49).

In this study, we have not yet documented directly whether protection is dependent upon a CD8 regulatory population. In our earlier work using mutants of recombinant single chain TCR (21) or DNA constructs (53), we were able to demonstrate that the region from 41–50 of the V$\beta$8.2 TCR is essential in the induction of the protective regulatory CD8 response. In addition, we have been able to generate short-term CD8 Treg cell lines reactive to this region which are able to adoptively transfer protection from EAE (N. Purohit and V. Kumar, unpublished observations). However in this study, we were unable to detect strong proliferative responses to this CDR1/CDR2 region of the TCR after Ad5E1 mV$\beta$8.2 vector immunization. This finding is consistent with our previous experience and the inability of CD8 cells to readily proliferate in vitro in B10.PL mice. In an attempt to further address this issue, we are currently exploring CD8 function with suitable CTL target cell lines and CD8 knockout mice.

Because EAE serves as a prototype for T cell-mediated autoimmune diseases, the understanding obtained in animal models of this disease may provide some insights for the development of preventive/therapeutic approaches for autoimmune conditions in humans. In this study, we have reported the successful use of a recombinant adenovirus vector expressing the murine V$\beta$8.2 TCR to induce an efficient regulatory response and protection from EAE. The potential advantages of this vector lie in the creation of generic therapies for autoimmune conditions by targeting the TCR V regions of pathogenic T cells. The ability of the Ad5 vectors to deliver whole TCR genes in a highly efficient manner allows processing of determinants into both class I and II pathways using the host's endogenous proteolytic processing enzymes. Furthermore, additional processing motifs could be inserted at crucial sites to improve accessibility for known MHC binding domains (50). Providing the whole TCR gene in the adenovirus eliminates the necessity of matching MHC binding domains to each of the different TCR V regions.

If a given pathogenic condition involves induction of more than a single TCR-specific repertoire via inter or intramolecular spread, it may be necessary for an additional regulatory repertoire to expand. In a recent report, DNA vaccination using two diverse V$\beta$ TCR chains of cardiac myosin-restricted T cells regulated autoimmune myocarditis, demonstrating that T cell-centered regulation can be achieved when more than a single V$\beta$ repertoire is involved in pathogenesis (51). Furthermore, it may not be necessary to induce regulation to all potential T cells reactive to an autoantigen(s), but targeting of an appropriate dominant clone or "driver" clone (52), accompanied by bystander suppression of other aggressive clones, could be successful in the induction of regulation and protection.

The experiments reported here provide a first step proof-of-principle for the development of a transiently expressed vector approach for a regulatory intervention in autoimmune conditions. The rationale for its further development as a practical therapeutic device depends on the generation of vectors with reduced immunotoxicity and the proven capacity to maintain a memory population of Treg cells.

[4] L. T. Madakamutil, I. Maricic, E. Sercarz, and V. Kumar. Regulatory T cells control autoimmunity in vivo by inducing apoptotic depletion of activated pathogenic lymphocytes. *Submitted for publication.*

## Acknowledgments

We thank Dr. Steve Wilson for help in the production of figures, Dr. David Stevens for supplying mouse MBP, and Duncan Chong for rescue of the Ad5E1 mV$\beta$8.2 recombinant adenovirus vector.

## References

1. Braciak, T. A., W. S. Gallichan, F. L. Graham, C. D. Richards, A. J. Ramsay, K. L. Rosenthal, and J. Gauldie. 2000. Recombinant adenovirus vectors expressing interleukin-5 and -6 specifically enhance mucosal immunoglobulin A responses in the lung. *Immunology 101:388.*
2. Addison, C. L., T. Braciak, R. Ralston, W. J. Muller, J. Gauldie, and F. L. Graham. 1995. Intratumoral injection of an adenovirus expressing interleukin 2 induces regression and immunity in a murine breast cancer model. *Proc. Natl. Acad. Sci. USA 92:8522.*
3. Addison, C. L., J. Gauldie, W. J. Muller, and F. L. Graham. 1995. An adenoviral vector expressing interleukin-4 modulates tumorigenicity and induces regression in a murine breast cancer model. *Int. J. Oncol. 7:1253.*
4. Bramson, J. L., F. L. Graham, and J. Gauldie. 1995. The use of adenoviral vectors for gene therapy and gene transfer in vivo. *Curr. Opin. Biotechnol. 6:590.*
5. Graham, F. L. 1990. Adenoviruses as expression vectors and recombinant vaccines. *Trends Biotechnol. 8:85.*
6. Graham, F. L., and L. Prevec. 1992. Adenovirus-based expression vectors and recombinant vaccines. *Biotechnology 20:363.*
7. Haddada, H., L. Cordier, and M. Perricaudet. 1995. Gene therapy using adenovirus vectors. *Curr. Top. Microbiol. Immunol. 199:297.*
8. Prevec, L., J. B. Campbell, B. S. Christie, L. Belbeck, and F. L. Graham. 1990. A recombinant human adenovirus vaccine against rabies. *J. Infect. Dis. 161:27.*
9. Prevec, L., B. S. Christie, K. E. Laurie, M. M. Bailey, F. L. Graham, and K. L. Rosenthal. 1991. Immune response to HIV-1 *gag* antigens induced by recombinant adenovirus vectors in mice and rhesus macaque monkeys. *J. Acquired Immune Defic. Syndr. 4:568.*
10. Randrianarison-Jewtoukoff, V., and M. Perricaudet. 1995. Recombinant adenoviruses as vaccines. *Biologicals 23:145.*
11. Hitt, M. M., and J. Gauldie. 2000. Gene vectors for cytokine expression in vivo. *Curr. Pharm. Des. 6:613.*
12. Emtage, P. C., Y. Wan, W. Muller, F. L. Graham, and J. Gauldie. 1998. Enhanced interleukin-2 gene transfer immunotherapy of breast cancer by coexpression of B7-1 and B7-2. *J. Interferon Cytokine Res. 18:927.*
13. Emtage, P. C., Y. Wan, M. Hitt, F. L. Graham, W. J. Muller, A. Zlotnik, and J. Gauldie. 1999. Adenoviral vectors expressing lymphotactin and interleukin 2 or lymphotactin and interleukin 12 synergize to facilitate tumor regression in murine breast cancer models. *Hum. Gene Ther. 10:697.*
14. Yarosh, O. K., A. I. Wandeler, F. L. Graham, J. B. Campbell, and L. Prevec. 1996. Human adenovirus type 5 vectors expressing rabies glycoprotein. *Vaccine 14:1257.*
15. Acha-Orbea, H., D. J. Mitchell, L. Timmermann, D. C. Wraith, G. S. Tausch, M. K. Waldor, S. S. Zamvil, H. O. McDevitt, and L. Steinman. 1988. Limited heterogeneity of T cell receptors from lymphocytes mediating autoimmune encephalomyelitis allows specific immune intervention. *Cell 54:263.*
16. Urban, J. L., V. Kumar, D. H. Kono, C. Gomez, S. J. Horvath, J. Clayton, D. G. Ando, E. E. Sercarz, and L. Hood. 1988. Restricted use of T cell receptor V genes in murine autoimmune encephalomyelitis raises possibilities for antibody therapy. *Cell 54:577.*

17. Kumar, V., and E. E. Sercarz. 1996. Dysregulation of potentially pathogenic self reactivity is crucial for the manifestation of clinical autoimmunity. *J. Neurosci. Res. 45:334.*
18. Kumar, V., and E. E. Sercarz. 1993. The involvement of T cell receptor peptide-specific regulatory $CD4^+$ T cells in recovery from antigen-induced autoimmune disease. *J. Exp. Med. 178:909.*
19. Kumar, V., and E. Sercarz. 1993. T cell regulatory circuitry: antigen-specific and TCR-idiopeptide-specific T cell interactions in EAE. *Int. Rev. Immunol. 9:287.*
20. Kumar, V., K. Stellrecht, and E. Sercarz. 1996. Inactivation of T cell receptor peptide-specific CD4 regulatory T cells induces chronic experimental autoimmune encephalomyelitis (EAE). *J. Exp. Med. 184:1609.*
21. Kumar, V., E. Coulsell, B. Ober, G. Hubbard, E. Sercarz, and E. S. Ward. 1997. Recombinant T cell receptor molecules can prevent and reverse experimental autoimmune encephalomyelitis: dose effects and involvement of both CD4 and CD8 T cells. *J. Immunol. 159:5150.*
22. Kumar, V., and E. Sercarz. 2001. An integrative model of regulation centered on recognition of TCR peptide/MHC complexes. *Immunol. Rev. 182:113.*
23. Ben-Nun, A., H. Wekerle, and I. R. Cohen. 1981. Vaccination against autoimmune encephalomyelitis with T-lymphocyte line cells reactive against myelin basic protein. *Nature 292:60.*
24. Vandenbark, A. A., G. Hashim, and H. Offner. 1989. Immunization with a synthetic T-cell receptor V-region peptide protects against experimental autoimmune encephalomyelitis. *Nature 341:541.*
25. Kumar, V., and E. Sercarz. 1998. Induction or protection from experimental autoimmune encephalomyelitis depends on the cytokine secretion profile of TCR peptide-specific regulatory CD4 T cells. *J. Immunol. 161:6585.*
26. Kumar, V., and E. Sercarz. 1999. Distinct levels of regulation in organ-specific autoimmune diseases. *Life Sci. 65:1523.*
27. Kozak, M. 1987. At least six nucleotides preceding the AUG initiator codon enhance translation in mammalian cells. *J. Mol. Biol. 196:947.*
28. Ward, E. S. 1992. Secretion of T cell receptor fragments from recombinant *Escherichia coli* cells. *J. Mol. Biol. 224:885.*
29. Bett, A. J., W. Haddara, L. Prevec, and F. L. Graham. 1994. An efficient and flexible system for construction of adenovirus vectors with insertions or deletions in early regions 1 and 3. *Proc. Natl. Acad. Sci. USA 91:8802.*
30. Graham, F. L., and L. Prevec. 1995. Methods for construction of adenovirus vectors. *Mol. Biotechnol. 3:207.*
31. Virca, G. D., W. Northemann, B. R. Shiels, G. Widera, and S. Broome. 1990. Simplified Northern blot hybridization using 5% sodium dodecyl sulfate. *BioTechniques 8:370.*
32. Gabaglia, C. R., B. Pedersen, M. Hitt, N. Burdin, E. E. Sercarz, F. L. Graham, J. Gauldie, and T. A. Braciak. 1999. A single intramuscular injection with an adenovirus-expressing IL-12 protects BALB/c mice against *Leishmania major* infection, while treatment with an IL-4-expressing vector increases disease susceptibility in B10.D2 mice. *J. Immunol. 162:753.*
33. Xing, Z., Y. Ohkawara, M. Jordana, F. L. Graham, and J. Gauldie. 1997. Adenoviral vector-mediated interleukin-10 expression in vivo: intramuscular gene transfer inhibits cytokine responses in endotoxemia. *Gene Ther. 4:140.*
34. Clark-Lewis, I., R. Aebersold, H. Ziltener, J. W. Schrader, L. E. Hood, and S. B. Kent. 1986. Automated chemical synthesis of a protein growth factor for hemopoietic cells, interleukin-3. *Science 231:134.*
35. Swanborg, R. H. 1988. Experimental allergic encephalomyelitis. *Methods Enzymol. 162:413.*
36. Zamvil, S., P. Nelson, J. Trotter, D. Mitchell, R. Knobler, R. Fritz, and L. Steinman. 1985. T-cell clones specific for myelin basic protein induce chronic relapsing paralysis and demyelination. *Nature 317:355.*
37. Kumar, V., V. Bhardwaj, L. Soares, J. Alexander, A. Sette, and E. Sercarz. 1995. Major histocompatibility complex binding affinity of an antigenic determinant is crucial for the differential secretion of interleukin 4/5 or interferon $\gamma$ by T cells. *Proc. Natl. Acad. Sci. USA 92:9510.*
38. Wan, Y., P. Emtage, Q. Zhu, R. Foley, A. Pilon, B. Roberts, and J. Gauldie. 1999. Enhanced immune response to the melanoma antigen gp100 using recombinant adenovirus-transduced dendritic cells. *Cell. Immunol. 198:131.*
39. Wan, Y., J. Bramson, R. Carter, F. Graham, and J. Gauldie. 1997. Dendritic cells transduced with an adenoviral vector encoding a model tumor-associated antigen for tumor vaccination. *Hum. Gene Ther. 8:1355.*
40. Simon, R. H., J. F. Engelhardt, Y. Yang, M. Zepeda, S. Weber-Pendleton, M. Grossman, and J. M. Wilson. 1993. Adenovirus-mediated transfer of the CFTR gene to lung of nonhuman primates: toxicity study. *Hum. Gene Ther. 4:771.*
41. Crystal, R. G., A. Jaffe, S. Brody, A. Mastrangeli, N. G. McElvaney, M. Rosenfeld, C. S. Chu, C. Danel, J. Hay, and T. Eissa. 1995. A phase 1 study, in cystic fibrosis patients, of the safety, toxicity, and biological efficacy of a single administration of a replication deficient, recombinant adenovirus carrying the cDNA of the normal cystic fibrosis transmembrane conductance regulator gene in the lung. *Hum. Gene Ther. 6:643.*
42. Harvey, B. G., P. L. Leopold, N. R. Hackett, T. M. Grasso, P. M. Williams, A. L. Tucker, R. J. Kaner, B. Ferris, I. Gonda, T. D. Sweeney, et al. 1999. Airway epithelial CFTR mRNA expression in cystic fibrosis patients after repetitive administration of a recombinant adenovirus. *J. Clin. Invest. 104:1245.*
43. McDermott, M. R., F. L. Graham, T. Hanke, and D. C. Johnson. 1989. Protection of mice against lethal challenge with herpes simplex virus by vaccination with an adenovirus vector expressing HSV glycoprotein B. *Virology 169:244.*
44. Kumar, V., and E. Sercarz. 1996. Genetic vaccination: the advantages of going naked. *Nat. Med. 2:857.*
45. Waisman, A., P. J. Ruiz, D. L. Hirschberg, A. Gelman, J. R. Oksenberg, S. Brocke, F. Mor, I. R. Cohen, and L. Steinman. 1996. Suppressive vaccination with DNA encoding a variable region gene of the T-cell receptor prevents autoimmune encephalomyelitis and activates Th2 immunity. *Nat. Med. 2:899.*
46. Chunduru, S. K., R. M. Sutherland, G. A. Stewart, R. W. Doms, and Y. Paterson. 1996. Exploitation of the V$\beta$8.2 T cell receptor in protection against experimental autoimmune encephalomyelitis using a live vaccinia virus vector. *J. Immunol. 156:4940.*
47. Croxford, J. L., M. Feldmann, Y. Chernajovsky, and D. Baker. 2001. Different therapeutic outcomes in experimental allergic encephalomyelitis dependent upon the mode of delivery of IL-10: a comparison of the effects of protein, adenoviral or retroviral IL-10 delivery into the central nervous system. *J. Immunol. 166: 4124.*
48. Cua, D. J., B. Hutchins, D. M. LaFace, S. A. Stohlman, and R. L. Coffman. 2001. Central nervous system expression of IL-10 inhibits autoimmune encephalomyelitis. *J. Immunol. 166:602.*
49. Toes, R. E., R. C. Hoeben, E. I. van der Voort, M. E. Ressing, A. J. van der Eb, C. J. Melief, and R. Offringa. 1997. Protective anti-tumor immunity induced by vaccination with recombinant adenoviruses encoding multiple tumor-associated cytotoxic T lymphocyte epitopes in a string-of-beads fashion. *Proc. Natl. Acad. Sci. USA 94:14660.*
50. Schneider, S. C., J. Ohmen, L. Fosdick, B. Gladstone, J. Guo, A. Ametani, E. E. Sercarz, and H. Deng. 2000. Cutting edge: introduction of an endopeptidase cleavage motif into a determinant flanking region of hen egg lysozyme results in enhanced T cell determinant display. *J. Immunol. 165:20.*
51. Matsumoto, Y., Y. Jee, and M. Sugisaki. 2000. Successful TCR-based immunotherapy for autoimmune myocarditis with DNA vaccines after rapid identification of pathogenic TCR. *J. Immunol. 164:2248.*
52. Wilson, S. S., P. van den Elzen, E. Maverakis, J. T. Beech, T. A. Braciak, V. Kumar, and E. E. Sercarz. 2000. Residual public repertoires to self. *J. Neuroimmunol. 107:233.*
53. Kumar, V., J. Maglione, J. Thatte, B. Pederson, E. Sercarz, and E. S. Ward. 2001. Induction of a type 1 regulatory CD4 T cell response following V$\beta$8.2 DNA vaccination results in immune deviation and protection from experimental autoimmune encephalomyelitis. *Int. Immunol. 13:835.*