Cytokine-induced Immune Deviation as a Therapy for Inflammatory Autoimmune Disease

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Summary

The properties and outcome of an immune response are best predicted by the lymphokine phenotype of the responding T cells. Cytokines produced by CD4$^+$ T helper type 1 (Th1) T cells mediate delayed type hypersensitivity (DTH) and inflammatory responses, whereas cytokines produced by Th2 T cells mediate helper T cell functions for antibody production. To determine whether induction of Th2-like cells would modulate an inflammatory response, interleukin 4 (IL-4) was administered to animals with experimental allergic encephalomyelitis (EAE), a prototypic autoimmune disease produced by Th1-like T cells specific for myelin basic protein (MBP). IL-4 treatment resulted in amelioration of clinical disease, the induction of MBP-specific Th2 cells, diminished demyelination, and inhibition of the synthesis of inflammatory cytokines in the central nervous system (CNS). Modulation of an immune response from one dominated by excessive activity of Th1-like T cells to one dominated by the protective cytokines produced by Th2-like T cells may have applicability to the therapy of certain human autoimmune diseases.

Immune deviation was originally defined as the induction of a T cell–dependent antibody response in the absence of delayed type hypersensitivity (DTH) (1, 2). More recent studies have demonstrated that in a normal immune response, CD4$^+$ T lymphocytes differentiate into effector populations that produce restricted sets of cytokines and perform specific functions (3–5). These populations are polarized into Th1 lymphocytes, which play a major role in DTH responses by secreting IL-2 and IFN-γ, and Th2 lymphocytes, which are involved in antibody-mediated responses by secreting IL-4, IL-5, and IL-10. Thus, immune deviation would be characteristic of a response where Th2 cells predominate. Autoimmune diseases can be similarly subdivided into those mediated by Th1-like cells with primarily inflammatory manifestations and those mediated by Th2-like cells whose manifestations are secondary to autoantibody containing immune complexes (6). As the Th1 and Th2 subpopulations interact and crossregulate each other (7–9), one approach to the immunotherapy of inflammatory autoimmune disease might involve the antigen-specific deviation of an immune response dominated by a Th1 type response to a Th2 type response.

The in vitro requirements for the induction of IL-4–pro-ducing Th2-like cells are now well established. It appears that IL-4 itself may play the most dominant role in the differentiation of naive T cells toward a Th2-like phenotype (10). Using superantigens as a model antigen, we have recently demonstrated that administration of IL-4 during the period of T cell activation primes T cells in vivo for IL-4 production in a strictly antigen-specific fashion (11). If this mechanism is also valid for T cells activated by autoantigens, administration of IL-4 during the course of an inflammatory autoimmune disease could prime developing autoreactive T cells for IL-4 production and may prevent the tissue damaging effects of autoreactive Th1 cells.

Experimental allergic encephalomyelitis (EAE) is an autoimmune disease characterized by relapsing paralysis and central nervous system (CNS) inflammation and demyelination, features reminiscent of the human disease, multiple sclerosis (12). In experimental animals, EAE can be induced in susceptible strains by the adoptive transfer of myelin basic protein (MBP)-specific CD4$^+$ Th1-like lines, but not Th2 lines (13, 14). Here we have studied the effects of IL-4 treatment in mice in which EAE was induced by the transfer of short-term cultured, highly pathogenic MBP-primed T cells. The treatment resulted in induction of MBP-reactive Th2-like

cells, considerable amelioration of EAE, reduced demyelination, and reduced inflammatory cytokine gene expression in the CNS.

Materials and Methods

Mice. Female SJL mice were purchased from The Jackson Laboratory (Bar Harbor, ME). All mice used were between 8 and 12 wk of age. Experiments were done under an approved protocol in accordance with the animal use guidelines of the National Institutes of Health.

Antibodies, Cytokines, and Reagents. MBP was purified from guinea pig spinal cords as previously described (15). The purified murine rIL-4 had a biologic activity of 2×10^6 U/μg. Purified rat anti–mouse IL-4 (mAb 11B11) was the generous gift of Dr. W. E. Paul (National Institute of Allergy and Infectious Diseases [NIAID], Bethesda, MD).

Adoptive Transfer of EAE. Donor SJL/J mice were primed at the flanks and shoulders of 400 μg of guinea pig MBP in complete Freund's adjuvant as described (16). 10 d later, draining lymph node cells were harvested and 8×10^6 cells were stimulated in 2 ml with MBP (25 μg/ml) for 4 d. Cells (3×10^7 in 0.2 ml PBS) were immediately injected intravenously into syngeneic, naive recipients. Mice received an injection of encephalitogenic T cells on day 0, and either PBS or IL-4 (1 μg every 8 h) on days 0–11 or 6–11. Mice were monitored daily and a mean clinical score was assigned for each group using the following scale (17): 0, no abnormality; 1, a limp tail; 2, moderate hind limb weakness; 3, severe hind limb weakness; 4, complete hind limb paralysis; 5, quadriplegia or premoribund state.

In experiments modifying the encephalitogenicity of MBP-specific T cells in vitro, anti–IL-4 (mAb 11B11 [18, 19], 10 μg/ml) or IL-4 (1,000 U/ml) were present during antigenic stimulation. After two antigen stimulations, cells were resuspended in PBS (3×10^7 cells/0.2 ml) and injected into naive SJL recipients.

Histopathology. On day 25 post transfer, mice were perfused with PO_4 buffered 2.5% glutaraldehyde. CNS tissue was removed and thin slices were prepared of all levels of the neuraxis as described (16). 1-μm sections of tissue stained with toluidine blue were examined by light microscopy in a blinded fashion. Inflammation was graded as follows: –, no inflammatory cells; ±, a few positive cells; +, organization of inflammatory infiltrates around blood vessels; + +, extensive perivascular cuffing with extension

into the adjacent subarachnoid space; + + +, extensive perivascular cuffing with increasing subarachnoid inflammation. Demyelination was scored according to the following scale: –, no demyelination; ±, a few, scattered, naked axons; +, small groups of naked axons; + +, large groups of naked axons; + + +, confluent foci of demyelination.

Lymphokine Assays. 25 d (experiments 1 and 2) or 32 d (experiment 3) after cell transfer, the animals were killed and $CD4^+$ T cells were purified as described (20). $CD4^+$ T cells (3×10^5) were cultured in the presence of irradiated T cell–depleted spleen

Table 1. *Modification of Encephalitogenicity by In Vitro Stimulation of MBP-specific Lymph Node Cells in the Presence of IL-4 or Anti-IL-4*

In vitro treatment	Incidence	Mean onset	Mortality
		day	
None	5/5	7	0
Anti-IL-4	5/5	5	3
IL-4	0/5	–	–

Lymph node cells from MBP-primed mice were cultured in vitro in the presence of MBP and media, anti-IL-4, or IL-4 for two cycles of antigen stimulation (total 18 d of culture). Recovered cells were then injected into naive SJL recipients and mice were monitored daily for clinical signs of EAE (17).
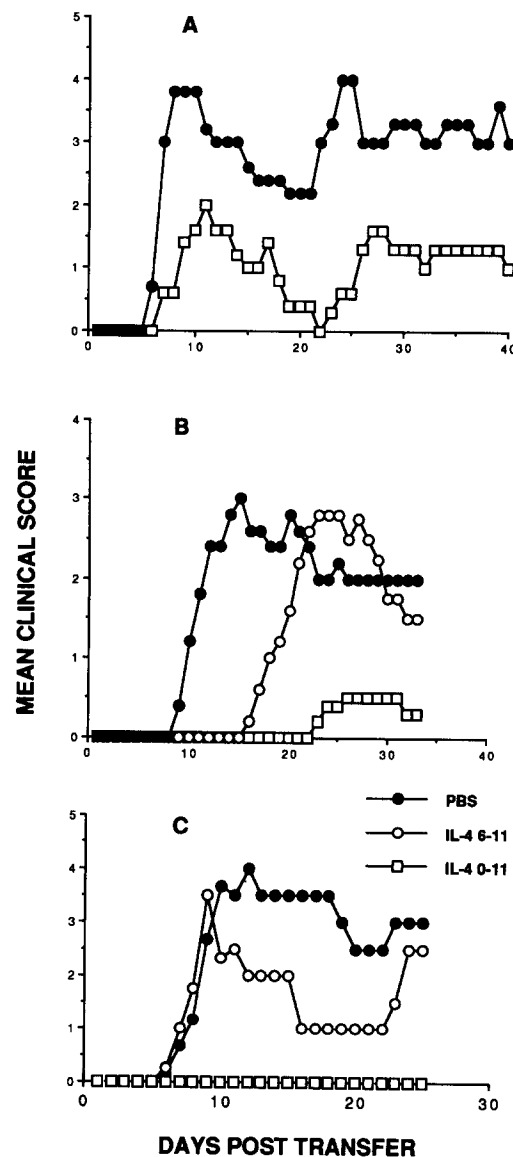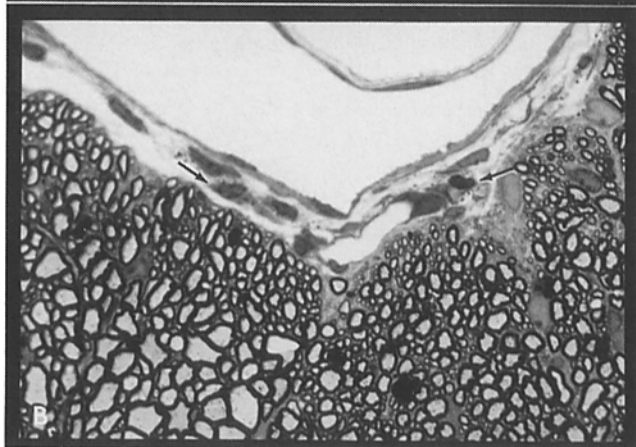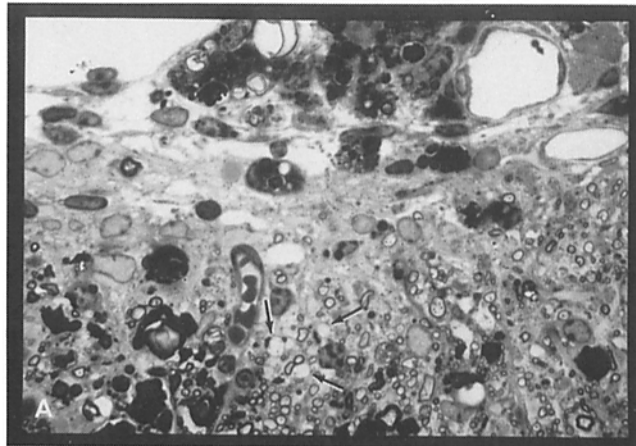
DAYS POST TRANSFER

Figure 1. Amelioration of EAE by IL-4 treatment. MBP-specific LNC were transferred into naive SJL/J mice which subsequently received either IL-4 (1 μg every 8 h) from days 0–11 or 6–11 or PBS intraperitoneally. Mean clinical scores represent groups of five mice from days 0–22 and three mice thereafter (*A*), groups of five mice from days 0–25 and four mice thereafter (*B*), or groups of six mice from days 0–10 and two mice thereafter (*C*). The decrease in number of mice was due to sampling for histology, CNS gene expression, and peripheral lymphocyte cytokine production.

A

B

Figure 2. Pathologic analysis of the CNS of control and IL-4–treated mice. (*A*) PBS administration days 0–11, 32 d post transfer; L6 spinal cord; dorsal columns. A diffusely demyelinated lesion (demyelinated axons seen at *arrows*) is overlaid by infiltrates of lymphocytes and macrophages in the subarachnoid space. Toluidine blue stained, 1 micrometer epoxy section; original magnification ×875. (*B*) IL-4 administration days 0–11, 32 d post transfer; L6 spinal cord, dorsal columns. A few infiltrating hematogenous cells (*arrows*) can be seen in the subarachnoid space whereas the underlying white matter has a normal appearance; original magnification ×875.

cells (2 × 10⁵) from normal SJL/J mice in the presence or absence of MBP (50 μg/ml). After 48 h, supernatants were removed and assayed by bioassay for IL-2 and IL-4 and by ELISA for IFN-γ (18, 19). Results are expressed as units per milliliter of each cytokine.

CNS Gene Expression. The CNS was removed and RNA extracted using RNAzol (Cinna/Biotecs, Friendswood, TX). RNA was purified and the coupled reverse transcriptase (RT)–PCR performed as described (21). Briefly, RNA samples (3.0 μl of 0.5 μg/ml) were reverse transcribed with Superscript RT (BRL, Rockville, MD) and cytokine-specific primers were used to amplify selected cytokines. For each gene product, the optimum number of cycles (achieving a detectable concentration well below saturating conditions) was determined experimentally. To verify that equal amounts of RNA were added in each RT-PCR reaction, primers for HPRT were used in each experiment. Values for specific cytokines were normalized to hypoxanthine phosphoribosyl transferase (HPRT) values. Amplified PCR products were detected by Southern blot analysis and the resulting signal quantitated with a Phosphorimager (Molecular Dynamics, Sunnyvale, CA).

Results and Discussion

Since is has been previously shown that IL-4 itself is a critical factor required for the generation of Th2-like cells in vitro and in vivo (11, 18, 19), T cells from MBP-sensitized mice were activated in vitro with MBP in the presence or absence of IL-4. Very aggressive encephalitogenic T cells were produced when T cells were cultured with MBP in the presence of anti-IL-4, whereas T cells activated with MBP in the presence of IL-4 had markedly reduced encephalitogenicity (Table 1).

As MBP-reactive T cells lost their encephalitogenicity after in vitro priming with IL-4 and MBP (Table 1), we examined the ability of IL-4 to modify disease and lymphokine phenotype of MBP-reactive CD4⁺ T cells in vivo. SJL/J mice were treated with either PBS or IL-4 (1 μg every 8 h for 11 d) immediately after the adoptive transfer of encephalitogenic T cells. Administration of IL-4 from days 0–11 post transfer resulted in a dramatic reduction of clinical disease; after cessation of IL-4 therapy, the treated animals appeared to be protected from severe disease for up to 35 d following cell transfer (Fig. 1). Since IL-4 has been well characterized as a cytokine with marked inhibitory effects on macrophage activation and cytokine production (22, 23), it is possible that its therapeutic effects were secondary to the downregulation of cytokine production by resident or recruited inflammatory cells in the CNS. However, administration of IL-4 during the period of establishment of CNS inflammation (days 6–11 after transfer) in one experiment only delayed disease onset (Fig. 1 *B*), while in another had modest effects on disease severity (Fig. 1 *C*). Thus, direct suppression of the production of inflammatory cytokines such as IL-1 and TNF-α by exogenous IL-4 during establishment of CNS inflammation appears to be a minor component of its therapeutic action.

Histopathologic evaluation of the CNS of IL-4–treated mice vs. PBS-treated controls demonstrated a marked reduction of demyelination throughout the CNS in the IL-4–treated animals, yet only modest differences in levels of inflammation throughout the neuraxis (Fig. 2 and Table 2). This suggested that inflammatory cells were able to gain access to the CNS parenchyma, but that the factors necessary for the development of demyelination and paralysis were reduced. The interaction of the integrin, $\alpha 4 \beta 1$ (very late antigen type 4 [VLA-4]), with its counter-receptor vascular cell adhesion molecule type 1 (VCAM-1) on endothelial cells appears to be necessary for the entry of the MBP-specific T cells into the CNS as the encephalitogenicity of T cell clones correlates with $\alpha 4 \beta 1$ expression and mAbs to $\alpha 4 \beta 1$ can inhibit the induction of EAE (14, 24, 25). It is unlikely that IL-4 inhibits the entry of MBP-specific T cells into the CNS as IL-4 has been shown to augment the expression of VCAM-1 on endothelial cells (26, 27) and therefore might be expected to facilitate the entry of T cells to the CNS.

To determine if IL-4 treatment enhanced the development of MBP-specific Th2-like cells in vivo, freshly isolated, peripheral CD4⁺ T cells were stimulated in vitro with MBP and the levels of production of various cytokines examined. T cells from paralyzed animals that had received either PBS

Table 2. *Pathologic Analysis of Mice Receiving IL-4 or PBS*

		Treatment			
		PBS	IL-4	PBS	IL-4
	Location	Inflammation		Demyelination	
Experiment 1	Cerebrum	+ + / +	+ + / + + +	+ / −	+
	Cervical cord	+ +	+	+ +	−
	Thoracic cord	+	−	+ +	−
	Upper lumbar cord	+	−	+ + / +	−
Experiment 2	Cerebrum	+ / −	+ + +	−	+
	Cervical cord	+	−	+ + / + + +	−
	Thoracic cord	+	+	−	−
	Upper lumbar cord	+ / +	+ / +	−	−

Summary of pathologic analysis of CNS of mice receiving IL-4 or PBS (Experiment 1, 32 d post transfer; Experiment 2, 25 d post transfer).
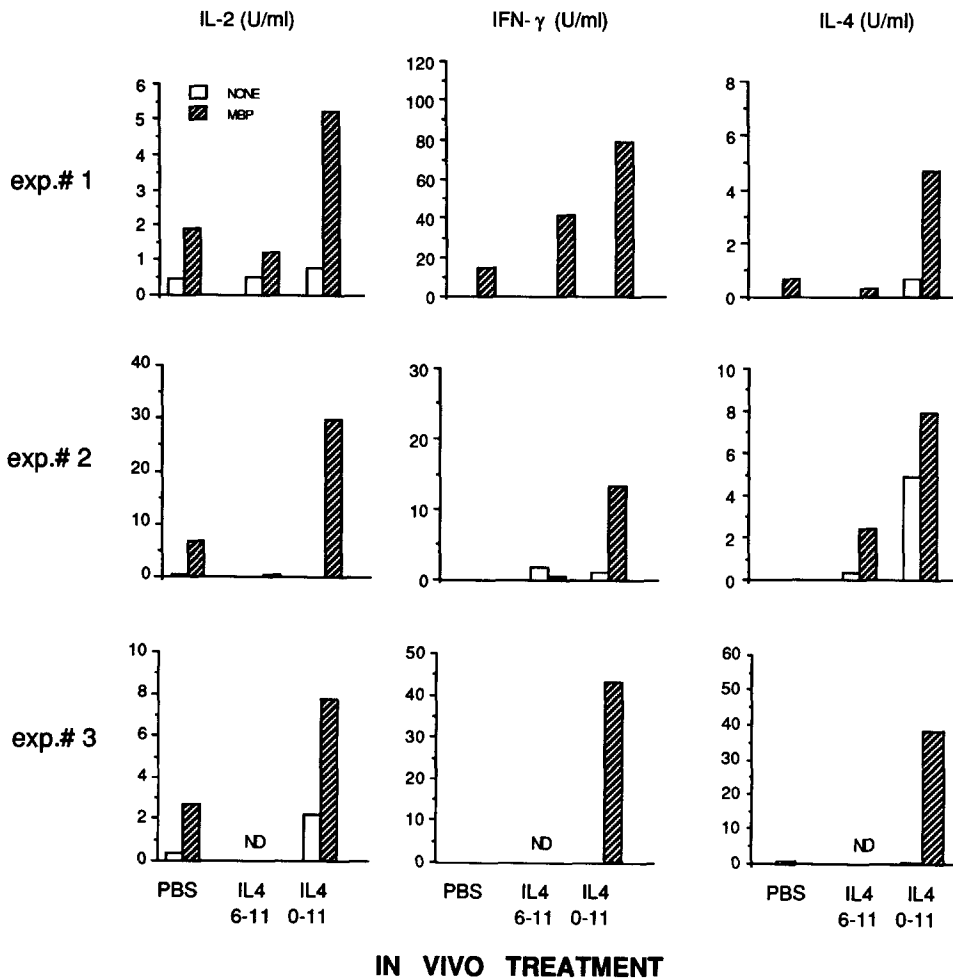

Figure 3. MBP-specific IL-2, IFN-γ, and IL-4 production by peripheral CD4^{+} T cells from mice receiving either PBS (days 0–11), IL-4 (days 6–11), or IL-4 (days 0–11).
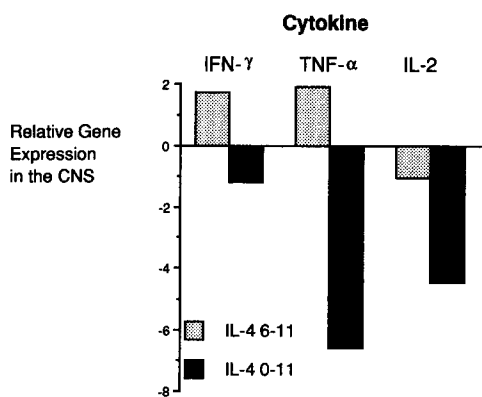
Cytokine

Figure 4. Cytokine gene expression in the CNS. Relative levels of cytokine gene expression in the CNS were determined at day 25 post transfer. Cytokine gene expression was normalized to HPRT and then compared with the gene expression in the PBS-treated animals.

or IL-4 (days 6–11) produced IL-2 and IFN-γ, but little or no IL-4, whereas T cells from IL-4–treated, healthy animals showed a marked increase in IL-4 production upon antigenic stimulation (Fig. 3). It is interesting to note that T cells from IL-4–treated animals produced amounts of IL-2 and IFN-γ equivalent to or greater than those produced by T cells from the PBS-treated controls suggesting that the induction of MBP-specific Th2 cytokines was not accompanied by a down-regulation of MBP-specific Th1 cells.

Gene expression of TNF-α and IL-2 in the target organ, the CNS, was decreased severalfold only in animals treated with IL-4 from days 0–11 (Fig. 4), but levels of IFN-γ were similar to those seen in controls. The levels of IL-4 mRNA

in CNS tissues were variable and unrelated to the experimental protocol or clinical course. However, one should be cautious in the interpretation of these data because of differences in the magnitude and cell types of the inflammatory infiltrates.

Collectively these data indicate that the therapeutic effects of IL-4 on EAE (Fig. 1) strongly correlated with the induction or enhancement of MBP-specific Th2-like cells in the treated animals (Fig. 3). Previous approaches to the therapy of EAE have shown that disease can be prevented by inhibition of T cell receptor–target cell interactions (28), by induction of oral tolerance (29), by inhibition of entry of cells into the CNS (14, 24, 25), or by neutralization of inflammatory cytokines (16, 30–33). Our data are consistent with a model where production of IL-4 or other Th2 cytokines (e.g., IL-10) modulates disease activity by antagonizing the effects of pathogenic Th1 cytokines, such as IFN-γ, thereby inhibiting the production of inflammatory mediators such as TNF-α by CNS macrophages or glial cells (Fig. 4). Very similar mechanisms may be operative in "infectious" transplantation tolerance (34), in the suppression of Th1 cells in chronic infectious diseases such as leprosy (35), and in the maintenance of peripheral tolerance in a transgenic model of autoimmune diabetes (36).

It is likely that autoantigen-specific T cells which are not yet committed to the Th1 and Th2 pathway are constantly being exported from the thymus during the course of a chronic autoimmune disease. The ability to generate Th2 populations that can ameliorate disease even in the presence of pathogenic Th1 cells raises the possibility that strategies designed to augment Th2 activity may have therapeutic efficacy in many autoimmune diseases mediated by Th1 cells, including multiple sclerosis.

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