An Antibody to Lymphotoxin and Tumor Necrosis Factor Prevents Transfer of Experimental Allergic Encephalomyelitis

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Summary

Uncertainty regarding pathogenic mechanisms has been a major impediment to effective prevention and treatment for human neurologic diseases such as multiple sclerosis, tropical spastic paraparesis, and AIDS demyelinating disease. Here, we implicate lymphotoxin (LT) (tumor necrosis factor β [TNF-β]) and TNF-α in experimental allergic encephalomyelitis (EAE), a murine model of an autoimmune demyelinating disease. In this communication, we report that treatment of recipient mice with an antibody that neutralizes LT and TNF-α prevents transfer of clone-mediated EAE. LNC-8, a myelin basic protein–specific T cell line, produces high levels of LT and TNF-α after activation by concanavalin A, antibody to the CD-3ϵ component of the T cell receptor, or myelin basic protein presented in the context of syngeneic spleen cells. LNC-8 cells transfer clinical signs of EAE. When LNC-8 recipient mice were also treated with TN3.19.12, a monoclonal antibody that neutralizes LT and TNF-α, the severity of the transferred EAE was reduced, while control antibodies did not alter the disease. The effect of anti-LT/TNF-α treatment was long lived and has been sustained for 5 mo. These findings suggest that LT and TNF-α and the T cells that produce them play an important role in EAE.

It has been suggested that the cytokines lymphotoxin (LT)[1] (TNF-β) and TNF-α could contribute to pathogenesis in several human neurologic diseases, including multiple sclerosis (MS) (1) and AIDS dementia (2). If that were the case, inhibition of the induction or activity of such cytokines might alleviate the tissue damage and demyelination associated with these diseases. In this study, we test the hypothesis that LT and TNF-α are involved in experimental allergic encephalomyelitis (EAE), a murine model for MS, and demonstrate that inhibition of their activity prevents transfer of clinical signs of this paralytic disease.

LT and TNF-α are genetically related cytokines with several activities that could contribute to demyelinating diseases. TNF-α is produced by macrophages after stimulation with LPS, whereas both LT and TNF-α are released by T cells activated by antigen or infection with some viruses, including human T cell leukemia virus type I (HTLV-I) (3, 4). LT and TNF-α activities appear to be beneficial in defense against tumors and virus-infected cells (3), and detrimental in their association with cachexia (5) and (for TNF-α) in the pathogenesis of cerebral malaria (6). Evidence has accumulated that supports but does not yet prove a role for LT and TNF-α in certain neurologic diseases. One of the earliest descriptions of LT was derived from a study of lymphocytes of rats with EAE (7). TNF-α causes demyelination and death of oligodendrocytes in vitro (8). Further corroboration and a suggestion for the biologic relevance of these observations is indicated by the recent description of TNF-α in MS plaques (9). The high levels of LT produced by HTLV-I-infected T cell lines (10–12) is also consistent with an involvement of LT in tropical spastic paraparesis, a neurologic disease associated with infection with that human retrovirus.

In related studies (13, 14), we have presented circumstantial evidence for a role of LT in EAE. A series of myelin basic protein (MBP-reactive) PL/J T cell clones had identical antigen fine specificity and MHC restriction, used the same TCR Vβ gene (15), and produced IL-2 in response to the encephalitogenic peptide (amino acids 1–11) of MBP presented in the context of H-2^u. Despite these similarities, the clones varied in their ability to transfer EAE, and this was positively correlated with the amount of LT/TNF-α cytotoxic activity and

[1] *Abbreviations used in this paper:* EAE, experimental allergic encephalomyelitis; HTLV-I, human T cell leukemia virus type I; LT, lymphotoxin; MBP, myelin basic protein; MS, multiple sclerosis.

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the amount of LT mRNA that they produced. Clones that transferred EAE efficiently, as assessed by incidence of disease, severity of clinical signs, and day of onset, produced high levels of cytotoxic factors (256 U) and mRNA for LT. Those that did not transfer disease produced low or undetectable levels (0–16 U) of cytotoxic activity and LT mRNA. The correlation with TNF-α was not as clear. Most clones that made LT mRNA also made TNF-α mRNA. However, one clone made high levels of TNF-α mRNA, but did not secrete cytotoxic material, and did not transfer disease particularly well. No correlation was found between IFN-γ and IL-2 production and encephalitogenicity.

In this communication, we present additional data that support the hypothesis that LT and/or TNF-α are involved in the pathogenesis of EAE. These results obtained with the SJL/J murine model of EAE indicate that inhibition of LT and TNF-α reduces the severity of disease symptoms transferred by a T cell line. One implication of these results is that inhibition of these cytokines might alleviate clinical signs in certain immunologically mediated human neurologic diseases.

Materials and Methods

LNC 8 T Cell Line. LNC-8 was derived from the popliteal lymph nodes of an SJL mouse immunized with porcine MBP. LNC-8 was maintained with biweekly addition of human rIL-2 (Amgen Biologicals, Thousand Oaks, CA) and stimulated with porcine MBP (10 μg/ml; Calbiochem-Behring Corp., San Diego, CA) and irradiated SJL splenocytes every other week.

Activation of LT and TNF-α Production. LNC-8 cells (2×10^6/ml) were incubated in medium that contained 10% FCS alone, or were supplemented with Con A (5 μg/ml), or 25 or 100 μg/ml porcine MBP and irradiated syngeneic SJL spleen cells. For stimulation with anti-CD3, LNC-8 cells were added as above to tissue culture flasks that had been incubated overnight with anti-CD3 ϵ antibody YCD3-1 (16), then rinsed twice with PBS. Culture supernatants were collected from activated cells at various times between 8 and 48 h and tested for cytotoxic activity against WEHI 164 cells.

Biologic Assay for LT and TNF-α. For determination of LT/TNF units, a sensitive WEHI 164 subline obtained from Edward Lattime (Memorial Sloan-Kettering, New York) was used as a target. WEHI 164 cells (5×10^3/well) were set up in 96-well tissue culture plates in RPMI 1640 with 10% FCS, 1% nonessential amino acids, 1% essential amino acids, 1% sodium pyruvate in dilutions of test and control samples in a volume of 100 μl. After 44 h, WEHI 164 survival was evaluated by the cells' 4-h uptake of MTT (17), which was then acidified with 150 μl 0.04M HCL in isopropenol. The plate was read on a Vmax plate reader at 470 nm with a 650-nm reference standard. Units were calculated as the highest dilution causing 50% cytotoxicity. Percent cytotoxicity was calculated as: 100× (1.00 − OD of sample wells)/OD of control wells.

Neutralization of LT/TNF-α Biologic Activity. Supernatants containing WEHI 164 cytotoxic activity were diluted to 64 U and incubated with TN3.19.12 obtained from Dr. Robert Schreiber (Washington University, St. Louis, MO). This hamster mAb has been previously demonstrated to neutralize both LT and TNF-α (18). After incubation for 1 h at 37°C, the supernatants were added to WEHI 164 cells and assayed as above.

Northern Blot Analysis. Total RNA was obtained by the guanidinium thiocyanate cesium chloride method (19) from unstimulated murine L cells, and from SJL splenocytes and LNC-8 cells after activation. RNA (20 μg) was subjected to electrophoresis in a 0.66 M formaldehyde/MOPS 1% agarose gel, transferred to Gene Screen Plus (New England Nuclear, Boston, MA), and hybridized with TNF-α cDNA (20) (a generous gift from Dr. Bruce Beutler, University of Texas Southwestern Medical School), LT cDNA (21), or β-actin cDNA (22) (a generous gift from Dr. D. Cleveland, Johns Hopkins University Medical School) labeled by the random primer method (23). The filters were washed twice for 15 min at 55°C with 0.2× SSC and 0.1% SDS, and exposed to XAR film with intensifying screens at −70°C.

Transfer and Evaluation of EAE. LNC-8 cells were stimulated with MBP (10 μg/ml) and spleen cells for 3 d. The cells were then exposed to 2 U/ml IL-2 for 1 d. After that time, $7.5–12 \times 10^6$ LNC-8 cells were injected intraperitoneally or intravenously as indicated in individual experiments. The mice were divided into groups and, 48 h later, injected with PBS, hamster control antibody L2D39, rat anti-IL-4 antibody 11B11 (24), or hamster anti-LT/TNF-α antibody TN3.19.12 (18). Mice were evaluated for clinical signs of EAE daily for at least 21 d after injection of cells. The study was done in a blinded fashion. That is, the observer was unaware of the protocol. The clinical scale was as follows: 0 = normal, 1 = tail limpness, 2 = paraparesis with a clumsy gait, 3 = hind limb paralysis, 4 = hind and fore limb paralysis, 5 = death.

FACS Analysis. LNC-8 cells were analyzed for Vβ gene usage 12 d after being stimulated with irradiated splenocytes and MBP. Cells were incubated with supernatant from KJ23a, a generous gift of Drs. John Kappler and Phillippa Marrack (National Jewish Center, Denver, CO), or a control supernatant and stained with fluorescein-conjugated goat anti-mouse antibody. Binding was evaluated with a FACS analyzer (Becton Dickinson & Co., Mountain View, CA).

Results

Characteristics of LNC-8 Cells. The IL-2-dependent T cell line LNC-8 used in the present studies to transfer EAE proliferates in response to MBP in the context of H-2^s. It proliferates to all three major pepsin-digested fractions of MBP (data not shown). One of these fractions includes the peptide, which is encephalitogenic for SJL/J mice, that lies in the COOH-terminal end of the MBP molecule (25). We have found by FACS analysis that ~50% of LNC-8 T cells stain with KJ23a, an antibody that reacts with the Vβ17a gene product of the TCR (Fig. 1). This TCR gene usage is consistent with the observation that Vβ17a and at least one other as yet undefined Vβ gene are utilized by encephalitogenic SJL clones (26).

LT and TNF-α Production by LNC-8 Cells. When LNC-8 cells are stimulated by Con A, they produce mRNA for several cytokines, including IFN-γ and GM-CSF (data not shown) and LT and TNF-α (Fig. 2). Of particular interest is the fact that in the RNA samples from stimulated LNC-8 cells LT mRNA appeared to be at higher abundance than TNF-α mRNA, and that the T cell line made considerably more LT and TNF mRNA than do Con A–activated spleen cells. There are actually several molecular species of LNC-8 RNA that hybridize with both the LT and TNF-α cDNA probes. One high molecular weight species (~3 kb) hybridizes with both probes and could represent an LT promoter–regulated transcript driven through the entire 6-kb

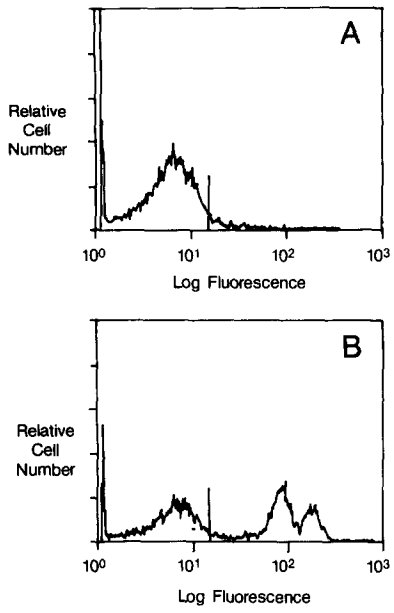

Figure 1. The LNC-8 cell line includes a Vβ17a$^+$ population. LNC-8 cells were analyzed for Vβ17a usage 12 d after last being stimulated with irradiated SJL splenocytes and MBP. Cells were incubated with supernatant KJ23a (anti-Vβ17a) or with control supernatant, washed, and stained with fluorescein-conjugated goat anti-mouse antibody. Binding was analyzed on a FACS analyzer (Becton Dickinson & Co.). (*A*) Control supernatant; (*B*) anti-Vβ17a.

TNF-α/β gene complex within the MHC on mouse chromosome 17 (27, 28).

Culture supernatants from LNC-8 cells activated by several different methods contained cytotoxic activity against WEHI 164 cells, a target for both LT and TNF-α (Table 1). Because the bioassay does not distinguish between the two cytokines, the results are expressed as LT/TNF-α activity. LNC-8 cells did not secrete LT/TNF unless activated. They secreted high levels of LT/TNF after stimulation with nonspecific mitogens, stimulation through the TCR by an anti-CD3 antibody YCD3-1 (16), or by MBP presented by syngeneic spleen cells. The amount of cytotoxic activity in the culture supernatants increased over time of stimulation, particularly when LNC-8 cells were activated with MBP and spleen cells. In experiment 1 (Table 1), the amount of cytotoxic activity in supernatants after antigen stimulation was 256 U at 8 h, 1,024 U at 24 h, and 2,048 U at 48 h. Northern blots of RNA prepared from LNC 8 cells activated under all conditions of Table 1 were positive for both LT and TNF-α mRNA, with usually higher accumulations of LT mRNA, as in Fig. 2. Though it is not possible to distinguish between LT and TNF-α in the biologic assay, it is likely that the WEHI 164 killing in the experiments reported here is due to both cytokines since the activated LNC-8 cells made both mRNAs. In other studies, we have observed that the kinetics of LT and TNF-α mRNA production can vary in individual T cell clones, and the cytotoxic activity attributable to the individual cytokines varies with activation time.

Table 1. *LNC-8 Cells Produce LT/TNF After Activation*

Exp.	Stimulating agent*	LT/TNF‡
1	–	0
	MBP/SC	256
	Con A	32,000
2	–	2
	MBP/SC	512
	Anti-CD3	25,600

* LNC-8 cells were incubatd for 8 h in growth media plus 10% FCS. Conditions of stimulation were as indicated in Materials and Methods. Exp. 1, MBP concentration was 25 μg/ml; Exp. 2, MBP was 100 μg/ml.
‡ LT/TNF units were determined by cytotoxic effect against WEHI 164 cells.

Neutralization of LNC-8 Cytotoxic Activity by Anti-LT/TNF-α Antibody. In a previous publication (18), we described a hamster mAb, TN3.19.12, that reacts both with LT and TNF-α derived from supernatants of T cell clones and with TNF-α derived from macrophage culture supernatants. This antibody also neutralized the cytotoxic activity secreted by LNC-8 cells, indicating that the WEHI 164 killing activity is due to LT and/or TNF-α. TN3.19.12 anti-LT/TNF-α antibody completely neutralized WEHI 164 cytotoxic activity of supernatants obtained after all methods of activation of LNC-8 cells, including antigen plus spleen cells (Fig. 3), Con A, and anti-CD3 antibody.

Inhibition of Transfer of EAE by LNC-8 by Treatment of Recipient Mice with Anti-LT/TNF-α Antibody. LNC-8 cells are potent mediators of EAE. When 1.5 $\times$ 10^7 cells are injected

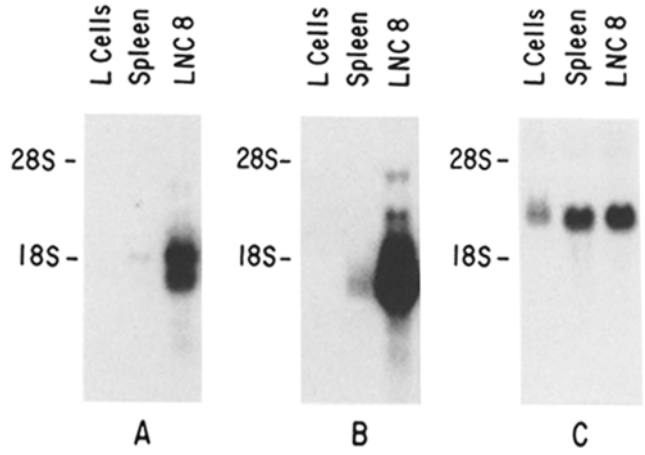

Figure 2. LNC-8 cells produce TNF-α and LT (TNF-β) mRNA. Northern blot analysis of total RNA (20 μg) obtained from unstimulated murine L cells and from SJL splenocytes and LNC-8 cells after 8-h exposure to 5 μg/ml Con A, hybridized with TNF-α cDNA (*A*), LT cDNA (*B*), or β-actin cDNA (*C*), and exposed with intensifying screens for 15 h (*A*), 2.5 h (*B*), or 30 min (*C*) to XAR-5 film.

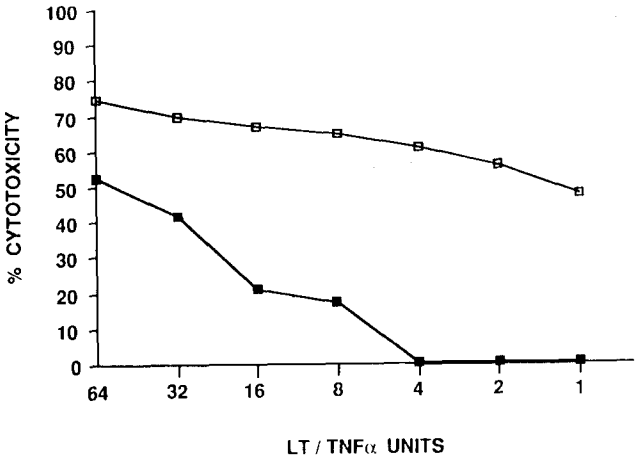

Figure 3. Hamster anti-LT/TNF-α mAb TN3.19.12 neutralizes cytotoxic activity in supernatants obtained from LNC-8 cells. Supernatant obtained from LNC-8 cells after incubation for 24 h with MBP and irradiated SJL spleen cells contained 1,024 U LT/TNF-α. The supernatant was diluted to 64 U and incubated in complete medium (□) or 850 ng TN3.19.12 (■) for 1 h at 37°C, and then added to WEHI 164 cells. Cytotoxicity was evaluated at 44 h.

intraperitoneally or intravenously, ~90% of the recipients develop clinical signs of severe EAE, usually within 7 d. Approximately 75% of the mice die. When lower numbers of LNC-8 cells are injected, the disease is not as debilitating, resulting in somewhat less severe clinical signs, fewer deaths, and delayed onset.

When mice that received LNC-8 cells were treated with the anti-LT/TNF-α mAb TN3.19.12, symptoms and severity of EAE were alleviated. In five different experiments (Table 2, protocol A), groups of four or five mice were injected with 7.5–9 × 10^6 LNC-8 cells intraperitoneally, and then treated 48 h later with a single intraperitoneal injection of 300 μg TN3.19.12, PBS, or a control hamster mAb, L2D39. Mice were evaluated daily for clinical signs of EAE for 3 wk. Treatment with anti-LT/TNF-α resulted in a dramatic reduction in the severity of clinical symptoms (Table 2). The effect of anti-LT/TNF-α treatment on the course of the disease is most apparent when the average disease scores of TN3.19.12-treated and control groups are compared. The average highest group score of mice that received 7.5–9 × 10^6 LNC-8 cells and PBS or the control hamster monoclonal L2D39 was 2.6 or 2.2, respectively, while the average highest group score of mice that received TN3.19.12 was 0.9, which is actually below clinically detectable disease (Table 2). These results are highly significant ($p < 0.001$) when TN3.19.12-treated mice were compared with PBS-treated mice by student's t test. L2D39 control (hamster antibody) treatment did not significantly affect the progression of the disease ($p < 0.252$). Those mice that did develop disease in the group receiving cells and TN3.19.12 developed clinical signs slightly later than did the control mice, and this disease was less severe. Those TN3.19.12-treated mice that did develop clinical signs had an average maximum disease score of 2, compared with 3.7 for mice that were treated with control antibody and developed clinical signs. In an additional experiment (Table 2, protocol B), 1.2 × 10^7 cells were injected intravenously in groups of 9 or 10 mice. In this experiment, a higher dose (1 mg) of anti-LT/TNF-α and a different control antibody (11B11, anti-IL-4) were used. The more severe disease that was induced with

Table 2. *Treatment of Mice with Anti-LT/TNF-α Antibody Reduces EAE Severity*

Protocol	Antibody treatment			Antibody specificity	Day of onset*	Average disease‡
	Amount	Antibody	n			
	mg					
A (7–9 × 10^6 LNC-8 cells i.p.)	–	PBS	10	–	6.2 ± 0.3	2.6 ± 0.4
	0.3	L2D39	15	–	7.8 ± 1.0	2.2 ± 0.7
	0.3	TN3.19.12	21	Anti-LT/TNF-α	8.6 ± 0.7	0.9 ± 0.3
B (1.2 × 10^7 LNC-8 cells i.v.)	–	PBS	9	–	7.1 ± 0.9	3.9 ± 0.6
	1	11B11	4	Anti-IL-4	7.5 ± 0.5	3.3 ± 1.6
	1	TN3.19.12	10	Anti-LT/TNF-α	7.0 ± 0.0	0.2 ± 0.1

$p < 0.001$ when 0.3 mg TN3.19.12 mice are compared with PBS-injected mice. $p < 0.2521$ when L2D39 mice are compared with PBS-injected mice. $p < 0.00035$ when 1 mg TN3.19.12-treated mice are compared with PBS mice. Protocol A was a summary of five separate experiments with four to five mice per group. Protocol B was one experiment.

* Average day of onset ± SEM of clinical signs of EAE.

‡ Average disease per group ± SEM. Mice were graded on a scale of 0–5, as indicated in Materials and Methods.

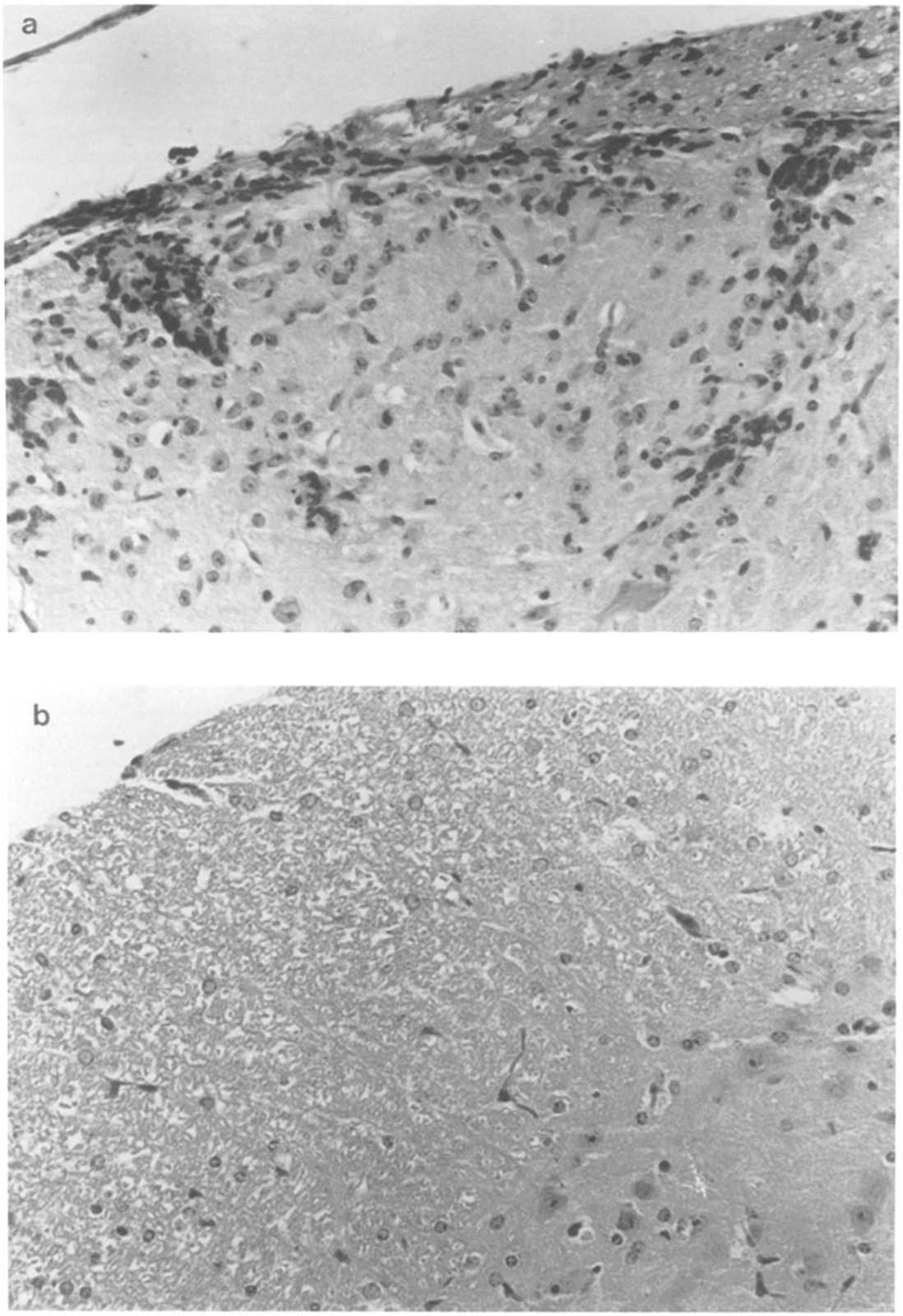

Figure 4. Anti-LT/TNF-α antibody treatment alleviates histologic signs of EAE. Analysis of spinal cord stained with hematoxylin and eosin. (*a*) Tissue section 9 d after injection of 9 × 10^6 LNC-8 cells and 7 d after PBS. Note perivascular infiltrate. (*b*) Tissue section 9 d after injection of 9 × 10^6 LNC-8 cells and 7 d after injection of 300 μg TN3.19.12. Note absence of infiltrate.

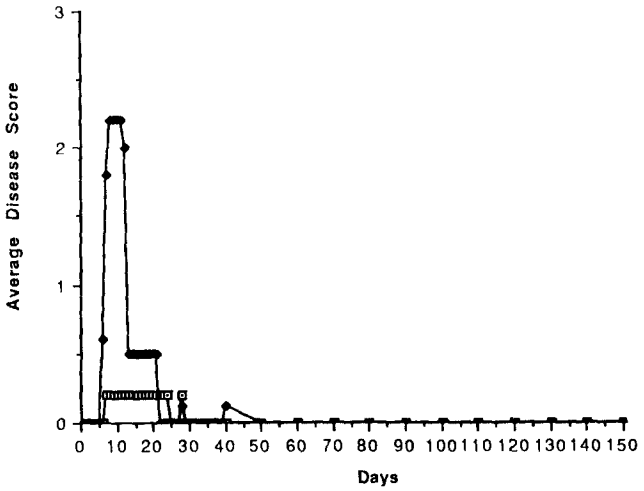

Figure 5. The alleviation of clinical signs of EAE induced by treatment with anti-LT/TNF-α antibody is persistent. Mice were injected with 7.5 × 10^6 LNC-8 cells intraperitoneally. 2 d later, one group of mice received PBS (◆), and one group received 300 μg anti-LT/TNF-α antibody TN3.19.12 (⊡). Mice were evaluated for clinical signs of EAE daily for 3 wk, and then three times a week for an additional 4 mo.

this dose of cells was dramatically inhibited with one injection of anti-LT/TNF-α. As indicated in Table 2, the average disease score was 3.9 for the PBS-treated group; 3.3 for the anti-IL-4-treated group, and 0.2 for the anti-LT-TNF-α treated group (the latter score again below clinically detectable disease). The incidence of disease was also reduced from 89% in the PBS treated group to 20% in the anti-LT/TNF-α group. The highest score of disease was 1 in this group.

Histological evaluation of mice that received LNC-8 cells and TN3.19.12 that remained clinically normal revealed that some of these mice showed no evidence of cellular infiltration or abnormality, while others showed meningeal infiltration and mild perivascular cuffing. All mice from the group that received LNC-8 cells and control antibodies or PBS that were examined had histologic signs of cuffing and infiltration consistent with their clinical signs (Fig. 4).

The inhibition of transfer of EAE by treatment with anti-LT/TNF-α antibody was long lived. Several groups of mice were observed for 2–3 mo. None developed disease if it had not occurred by day 12. In the representative experiment depicted in Fig. 5, mice were observed for 5 mo after receipt of 7.5 × 10^6 LNC-8 cells and antibody or PBS. The only mouse in the group of five that received both cells and TN3.19.12 to develop clinical signs had a limp tail (grade 1) from day 7 through day 26 and then recovered. Neither it nor any of the other mice in the antibody-treated group developed any further symptoms. In the control group (five mice) that received cells and PBS, four developed clinical signs, one of these died on day 12, and the others eventually recovered.

Discussion

The data presented here implicate LT and TNF-α in the passively transferred autoimmune neurological disease EAE. We demonstrate that a MBP-specific T cell line produces LT and TNF-α cytotoxic activity after activation with any of several different agents. The biologic activity is neutralized by an anti-LT/TNF-α antibody. The ability of LNC-8 cells to transfer EAE into naive mice is prevented if those mice are treated with the anti-LT/TNF-α antibody, but not with control antibodies. The use of an antibody that completely neutralizes both LT and TNF-α may be important since both cytokines are made by LNC-8 cells after activation with MBP, the relevant neuroantigen in this model of EAE. Absolute certainty that only one or the other cytokine is involved will only come with mAbs that distinguish between the murine forms. These are not yet available, and the polyclonal antibodies that we have tested in vitro are variable and inconsistent. It is particularly relevant to the human situation that we were able to inhibit the transfer of EAE by a cell line that contains at least two T cell populations that utilize different TCR-β genes. This suggests that these experiments may be broadly relevant to the human situation where, even though particular TCR-β genes may be preferentially utilized by siblings with MS (29), the actual gene(s) have not yet been identified. It is likely that the number of TCR-β genes utilized in this disease by the highly polymorphic human population is limited, but it is certainly greater than one.

The studies reported here are the first antibody neutralization experiments of EAE that provide insight into the identity of the mediators of the disease. The extent of prevention of transfer of EAE was dependent on the dose of anti-LT/TNF-α antibody used, and these doses (0.3 or 1 mg) were comparable with or less than those used in experiments with other antibodies to inhibit transfer or development of EAE. Sriram et al. (30) used three injections of 3 mg of anti-I-A antibodies on successive days before and after injection of encephalitogenic T cell clones to inhibit transfer of EAE. Urban et al. (31) injected 500 μg anti-Vβ8 antibody to prevent development of EAE, and Acha Orbea et al. (15) used two 100-μg injections of anti-Vβ8 antibody to inhibit T cell transfer of disease. In the previous experiments, the design was based on preventing T cell activation (30) or a major component of the T cell repertoire (15, 31). The experiments reported here represent a different approach to EAE; that is, TN3.19.12 inhibits the activity of mediators that are produced in vivo after stimulation of an antigen-specific population of effector T cells.

The mechanism by which the anti-LT/TNF-α antibody prevents the transfer of EAE is under investigation. It is possible that the antibody inhibits the disease in part by binding to and eliminating TNF-bearing T cells. This possibility is raised by the identification of membrane-bound TNF on a murine CTL clone with a polyclonal rabbit anti-TNF antibody (32), and the demonstration of TNF/cachectin on activated normal human T cells with an anti-TNF mAb (33). We do not believe that this is the most likely explanation for our results, because we have not been able to detect membrane TNF from macrophage lysates with TN3.19.12 (18), though we were able to detect a high molecular weight species from membranes of PU.5.1.8 with a polyclonal antibody (T. James and N. Ruddle, unpublished results). Moreover,

we have not been able to demonstrate surface staining by FACS with TN3.19.12 of LNC-8 cells treated identically to those used in the transfer studies. Maximum staining of 10^6 cells treated with 1, 10, or 100 μg/ml TN3.19.12 and a rabbit anti-hamster fluoresceinated antibody was 3.1%; under the same conditions, 94% of the cells were positive by anti-CD3. Because the concentration of TN3.19.12 antibody used in FACS experiments was comparable with that used in the in vivo experiments (i.e., 300 μg/7 $\times$ 10^6 cells), we believe it is unlikely that elimination of TNF-bearing cells is the mechanism. Nevertheless, this possibility exists and is under investigation in in vivo experiments with labeled cells. Histological analysis of tissue from mice that have received LNC-8 cells and TN3.19.12 has revealed that some treated, clinically normal mice had minimal evidence of cellular infiltrates, while others did not. Thus, the antibody may inhibit transfer at various stages by inhibiting several different activities of the LT and TNF-α secreted by LNC-8 cells. These activities involve a number of effects relevant to the pathogenesis of EAE, including an increase in MHC determinants on endothelial cells (34), an alteration of central nervous system permeability, and an influx of inflammatory cells through an increase in cell adhesion molecules on the endothelium (34) or astrocytes (35). Cytokines produced by these inflammatory cells may also directly affect the myelin sheath and influence viability of oligodendrocytes (8). TN3.19.12 antibody could inhibit any of these activities by directly neutralizing secreted LT and TNF-α. Further insight into these questions will derive from experiments underway designed to reverse established disease. Whatever the mechanism(s) by which this antibody inhibits transfer of EAE, this study demonstrates that inhibition of LT and TNF-α biologic activity prevents transfer of the symptoms of a severe an often fatal neurologic disease. These studies suggest a role for these cytokines or cells that express them in some neurologic diseases. If the transfer of disease in the EAE model is inhibited through inhibiting LT or TNF-α activity, the studies point the way for the development of TNF antagonists in such disease. The recent cloning of a receptor for LT and TNF-α (36, 37) elicits cautious optimism regarding feasibility of therapy for cytokine-mediated neurologic disease.

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