Biochemical and Biophysical Research Communications **275,** 164–168 (2000)
doi:10.1006/bbrc.2000.3279, available online at http://www.idealibrary.com on IDEAL®

Intrathecal Administration of Neutralizing Antibody against Fas Ligand Suppresses the Progression of Experimental Autoimmune Encephalomyelitis

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Received July 7, 2000

A therapy aimed at blocking the Fas/Fas ligand (FasL) system was investigated using a relapsing form of experimental autoimmune encephalomyelitis (EAE) in mice, an animal model of multiple sclerosis (MS). Intracisternal administration of neutralizing antibody against FasL during the progression phase of EAE significantly reduced the severity of the disease with milder inflammation and myelin breakdown in the central nervous system (CNS). These results raised the possibility that the Fas/FasL system might contribute to tissue destruction in the CNS in the acute phase of EAE and that the intrathecal administration of neutralizing antibody against FasL may be beneficial for suppression of the acute phase of MS.

Fas ligand (FasL) is known to induce apoptotic cell death through its cell-surface receptor, Fas. The Fas/FasL system is an important mechanism for controlling the development and/or turnover of several organs, including the immune system and the impairment of this system can lead to the autoimmune disease (1). On the other hand, the Fas-mediated cytotoxic activity itself is considered to participate in some diseases such as fulminant hepatitis (2), insulin-dependent diabetes mellitus (3, 4), and multiple sclerosis (MS) (5–7).

MS is an inflammatory disease of the central nervous system (CNS) characterized by localized areas of demyelination (8). Although the etiology and pathogenesis of MS are not yet well understood, several studies have suggested that the Fas/FasL system may contribute to its pathogenesis. Indeed an increase of Fas antigen in the cerebrospinal fluid or blood of patients with MS has been observed (9–11), and histological studies have shown that Fas and FasL were up-regulated in MS lesions (5–7). Furthermore, an *in vitro* study showed that FasL could induce cell death in Fas-bearing oligodendrocytes (6).

The role of the Fas/FasL system has also been investigated in experimentally-induced inflammatory demyelinating diseases such as experimental autoimmune encephalomyelitis (EAE), an animal model of MS. We and others recently reported that Fas- and FasL-deficient mice were resistant to EAE, even though infiltration of the inflammatory cells into the CNS was present (12–15). These results suggested that the Fas/FasL system might play a crucial role in the expansion of inflammation and/or myelin destruction in the CNS of mice with EAE, and that the blockade of Fas/FasL-mediated tissue injury might suppress the progression of EAE. To further assess this possibility, we tested the potential inhibitory effect of a neutralizing antibody against FasL on the acute phase of EAE in mice.

MATERIALS AND METHODS

Induction and clinical evaluation of EAE. Female (SJL/J × PL/J) F1 mice were obtained from Jackson Laboratory (Bar Harbor, ME), and used between 8 and 10 weeks. The method for EAE induction has previously been reported (16). Briefly, each mouse was injected subcutaneously in the femoral region on both sides with an emulsion containing 500 μg of myelin basic protein (MBP) (Sigma, St. Louis, MO) mixed with complete Freund's adjuvant. Twenty-four hours after inoculation, 400 ng of *Bordetella pertussis* toxin was given intraperitoneally.

Mice were weighed and examined for clinical score daily for 55 days after immunization. The clinical score was graded as 0; no clinical sign, 0.5; tail weakness and overnight weight loss over 1.5 g, l; limp tail, 2; limp tail and impaired righting reflex, 3; apparent hind limb paresis, 4; complete hind limb paralysis, 5; moribund or death. Intermediate scores were assigned if neurologic signs were milder than typically observed. The onset was defined as the day a mouse developed a clinical sign (more than clinical score 0.5). In this paper, we designated the day of onset as "day 0." The relapse was defined

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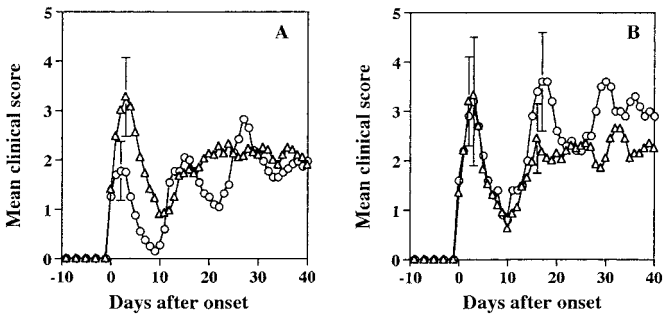

FIG. 1. The effect of an anti-FasL antibody on the progression phase of EAE. Mean clinical score of EAE-affected mice treated with 20 μg/day of the anti-FasL antibody by i.c. injection (circle) (n = 11) and normal hamster IgG by i.c. injection as controls (triangle) (n = 10). Mean clinical score (B) of EAE-affected mice treated with 200 μg/day of the anti-FasL antibody by i.p. injection (circle) (n = 5) and normal hamster IgG by i.p. injection as controls (triangle) (n = 5). Vertical bars indicate SD.

when a mouse developed an increase of the clinical score accompanied by weight loss.

Administration of anti-FasL antibody after onset of the disease. Mice with EAE were treated with hamster anti-mouse FasL monoclonal antibody (17, 18) for 3 days starting on the day of onset (day 0), by intraperitoneal (i.p.) or intracisternal (i.c.) administration as shown in Table 1. The mice subjected to daily i.p. injection received 100 μl of the antibody solution (200 μg/day) dissolved in pyrogen-free PBS. Control mice were treated with 100 μl of normal hamster IgG solution (200 μg/day) in the same way. The mice subjected to daily i.c. injection received 10 μl of the antibody solution (20 μg/day) dissolved in pyrogen-free PBS. Intracisternal injection was performed as previously reported (16). Briefly, each mouse was anesthetized with inhalation of diethyl ether, and the cisterna magna was punctured percutaneously by a 29 gauge needle and the antibody solution was administered.

Histological and histochemical evaluation. To document the suppressive effect of the i.c. administration of the anti-FasL antibody, spinal cords from mice with EAE receiving i.c. injection of the anti-FasL antibody or normal hamster IgG (control) for 3 days (from day

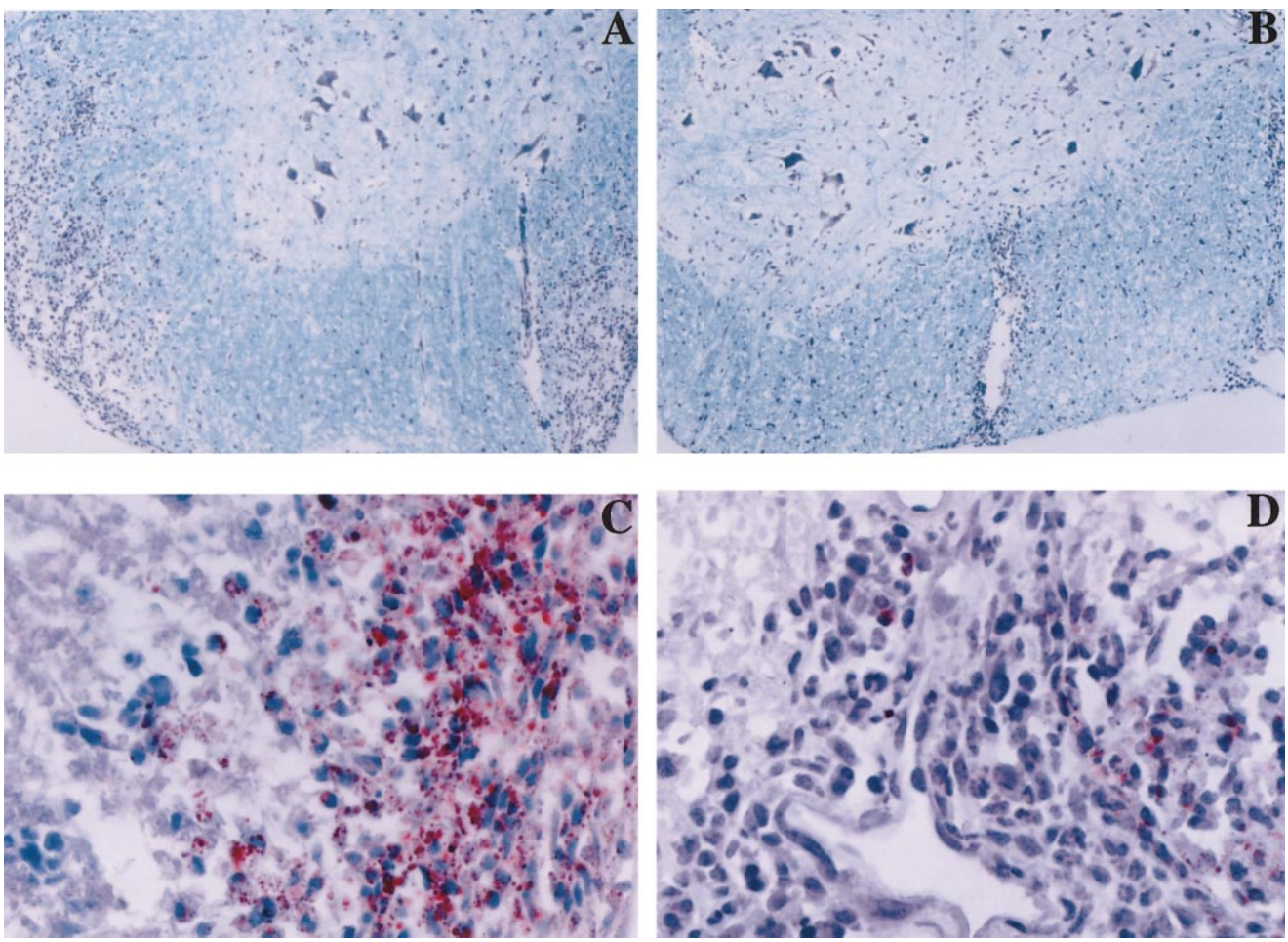

FIG. 2. Representative microscopic photographs of the spinal cord. Each spinal cord from EAE-affected mice (control), sacrificed at day 2 after onset, was stained with KB (A), and ORO (C). Each spinal cord from EAE-affected mice treated with i.c. injection of 20 μg/day of an anti-FasL antibody, sacrificed at day 2 after onset was stained with KB (B), and ORO (D). (A) and (B), lower magnification (×40), showing the cellular infiltrations in the spinal cord. (C) and (D), higher magnification (×400), showing myelin breakdown products as visualized with red dots by the ORO staining.

TABLE 1

The Effect of Anti-FasL Antibody on MBP-Induced EAE

	i.c. injection control (n = 12)	20 μg/day (n = 15)	i.p. injection control (n = 6)	200 μg/day (n = 6)
First attack				
Day of onset (mean ± SD)	11.0 ± 0.8	11.3 ± 0.8	11.3 ± 1.1	10.7 ± 0.8
Maximal score (mean ± SD)	4.0 ± 0.7	3.1 ± 1.1*	3.9 ± 0.8	3.6 ± 1.1
Mortality (death/total)	2/12	4/15	1/6	1/6
Second attack				
Frequency (sick/total)	10/10	11/11	5/5	5/5
Day of onset (mean ± SD)	29.2 ± 3.6	25.2 ± 3.3*	29.0 ± 2.4	24.6 ± 0.9*
Maximal score (mean ± SD)	3.9 ± 0.5	3.5 ± 0.7	3.7 ± 0.7	3.9 ± 0.6

* $P < 0.05$ versus control with Student's t test.

0 to day 2) after the onset were randomly taken at day 2 for histological examination. Each mouse was anesthetized with pentobarbital and the spinal cord was fixed with transcardiac perfusion with 4% paraformaldehyde in 0.1 M phosphate buffer. Each spinal cord was removed and immersed in the same fixative for 24 h. The cervical, thoracic, and lumbar segments of each spinal cord were frozen after washing through graded sucrose solutions. Consecutive 5 μm thick sections were prepared with a cryostat and stored at −80°C until use. Serial sections from those three segments from each mouse were used for hematoxylin-eosin (HE), Klüver-Barrera (KB), and Oil Red O (ORO) staining for detection of morphological changes and myelin breakdown, and for *in situ* terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) for detection of DNA fragmentation. The TUNEL procedure was performed using an *in situ* cell death detection kit (Boehringer Mannheim, Germany) according to the instruction from the manufacturer.

Semi-quantitative histological evaluation was performed by two examiners in a blind fashion using the following score based on the severity of inflammation: 0, no inflammation; 1, cellular infiltrate only in the perivascular areas and meninges; 2, mild cellular infiltrate in parenchyma; 3, moderate cellular infiltrate in parenchyma; 4, marked cellular infiltrate in parenchyma.

RESULTS

Progression of EAE Was Blocked by Intracisternal Administration but Not by Systemic Administration of Anti-FasL Antibody after Onset

The effect of i.c. and i.p. administration of anti-FasL antibody on the progression phase of EAE is shown in Fig. 1. It can be seen that the intracisternal administration of the anti-FasL antibody at a dose of 20 μg/day for 3 days, starting on the day of disease onset, significantly suppressed the severity of EAE. However, as indicated in Table 1, these treated animals displayed an earlier onset of the second attack. Intraperitoneal administration of the anti-FasL antibody in the dose of 200 μg/day during the same period showed no suppressive effect on the severity of EAE, but hastened the onset of the second attack significantly (Table 1), and tended to worsen the clinical course of the second attack (Fig. 1B).

Intracisternal Administration of Anti-FasL Antibody during Progression of EAE Suppressed Inflammation and Myelin Breakdown

Histological findings are summarized in Table 2 and representative microscopic photographs are shown in Fig. 2. Intracisternal administration of the anti-FasL antibody, which reduced the clinical score, significantly suppressed the infiltration of inflammatory cells into parenchyma (Table 2), though many inflammatory cells were present in the blood vessels and meninges in the spinal cord of EAE-affected mice treated with the anti-FasL antibody and control mice (Figs. 2A and 2B). Myelin breakdown products, visualized by the ORO staining, were clearly visible in many lesions of mice with acute EAE, but they were observed only occasionally in mice treated with the anti-FasL antibody (Figs. 2C and 2D). When the number of ORO-positive lesions

TABLE 2

Histological Findings of Spinal Cords from EAE-Affected Mice Treated with i.c. Injection of an Anti-FasL Antibody

	No. of mice	No. of sections	Clinical score (mean ± SD)	Histological score (mean ± SD)	ORO positive lesions per total lesions	Percent of TUNEL positive cells[a] (mean ± SD)
Control	4	12	3.3 ± 0.8	3.0 ± 0.7	47/90	25.3 ± 12.8
Anti-FasL antibody	6	18	2.3 ± 0.8	2.2 ± 0.7*	38/105**	11.8 ± 6.6*

[a] The number of TUNEL positive nuclei per total number of nuclei in randomly selected lesions (n = 50) from each group were counted by two examiners in a blind fashion.

* $P < 0.05$ versus control with Student's t test.

** $P < 0.05$ versus control with χ^2 test.

were counted regardless of their sizes and expressed as percent of the total number of lesions, they were significantly reduced by i.c. administration of the anti-FasL antibody (Table 2). The ratio of TUNEL-positive nuclei, suggestive of the presence of apoptosis, was significantly decreased by administration of the anti-FasL antibody as shown in Table 2.

DISCUSSION

The present study demonstrated that i.c. but not i.p. treatment with a neutralizing antibody against FasL could suppress the progression of EAE, possibly through protection of the CNS tissue from FasL-mediated injury.

Several *in vitro* studies suggested that the Fas/FasL system (6), tumor necrosis factor (TNF) (19), interferon (IFN)-γ (20), and nitric oxide (NO) (21) might be responsible for myelin and oligodendrocytic injury in inflammatory demyelinating diseases. However, TNF-α-deficient mice (22, 23), IFN-γ-deficient mice (24), IFN-γ receptor-deficient mice (25), and inducible NO synthase-deficient mice (26, 27) actually developed more severe EAE with impaired recovery. Insofar as the Fas/FasL system is concerned, several studies using Fas- or FasL-deficient mice have demonstrated that the Fas/FasL system played a crucial role in the progression phase of EAE through the destruction of the CNS tissues (12–15, 28). The Fas/FasL system is considered to participate in the myelin damage in a cytolytic manner (6, 7) and/or in the expansion of inflammation through astrocytic injury (15). The present study, showing a reduction of inflammation and myelin breakdown as well as the suppression of EAE progression by i.c. administration of a neutralizing antibody against FasL after disease onset, may be consistent with these findings. Taken together, these results imply that the Fas/FasL system by itself plays an important role in myelin and/or other tissue destruction in the CNS of mice with EAE.

However, FasL may not only be involved in the initiation of EAE but also in the recovery from EAE (29). Indeed, as we and others have reported, apoptosis is observed in infiltrated cells such as T cells (30–33) and macrophages (32–34) in the CNS lesions during the course of EAE, but to a lesser extent observed in oligodendrocytes (33). Furthermore, the administration of apoptosis inhibitors to mice with acute EAE, resulted in the impaired recovery or earlier relapse (35), suggesting that the apoptotic deletion of encephalitogenic cells contribute to the spontaneous recovery from EAE. On the basis of these findings, it is likely that the Fas/FasL system participates in the regulation of EAE through apoptotic deletions of immune cells (36, 37). This notion is supported by the present study, showing that the anti-FasL antibody treatment of mice with EAE decreased the number of apoptotic cells in the CNS and produced an earlier onset of relapse. However, given the fact that many apoptotic infiltrated cells are observed in the CNS and that an almost complete recovery from the disease is observed in Fas- and FasL-deficient mice with EAE (12–15), it is likely that the Fas/FasL system is not solely involved in the recovery of disease or in the apoptotic elimination of inflammatory cells in EAE.

Thus, the Fas/FasL system may play a dual role in EAE. One major function of this system would be the destruction of oligodendrocytes (6) and/or astrocytes (15). The other, perhaps less important, would be implicated in the apoptotic deletion of infiltrated cells and/or pathogenic cells. Therefore, the beneficial effect of i.c. injection of anti-FasL antibody that we observed in the acute phase of EAE could be mediated by the protection of cytolytic oligodendrocytic death rather than the inhibition of apoptotic cell death in the CNS.

Our results also suggested that the CNS delivery of agents blocking the Fas/FasL system might be necessary for an effective treatment of the inflammatory demyelinating disease, particularly in the acute phase. Indeed, the systemic administration of a neutralizing antibody against FasL might have a limited access to the lesions in the CNS as well as inhibit to some extent, apoptosis of encephalitogenic cells. As a result, the antibody may fail to suppress EAE and produce an earlier onset of relapse. On the other hand, the intrathecal administration of a neutralizing antibody against FasL may effectively prevent myelin or other tissue damages in the CNS and as a result, suppress the progression of EAE. Since the Fas/FasL system may have different functions, its blockade may have both beneficial as well as harmful effects. Therefore, the route of administration of any agents acting on the Fas/FasL system may have a major impact on successful treatment. Based on the present study, intrathecal administration of a neutralizing antibody against FasL may be a new and promising approach for treatment of MS in the progression phase.

ACKNOWLEDGMENTS

This study was supported by Grant-in-Aid for Scientific Research (B) from the Ministry of Education, Science, and Culture, Japan and by the Multiple Sclerosis Society of Australia. Y. Okuda is the recipient of a Fellowship from Toyobo Biotechnology Foundation.

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