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The Roles of Fas, Fas Ligand and Bcl-2 in T cell Apoptosis in the Central Nervous System in Experimental Autoimmune Encephalomyelitis

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Abstract

The selective apoptotic elimination of autoreactive T cells in the central nervous system (CNS) contributes to the resolution of inflammation and the spontaneous clinical recovery from experimental autoimmune encephalomyelitis (EAE). To assess the molecular mechanisms involved in this process, we used three-colour flow cytometry to examine the expression of apoptosis-regulating proteins by inflammatory cells isolated from the spinal cords of Lewis rats immunized with myelin basic protein (MBP) and complete Freund's adjuvant. Throughout the course of the disease, which peaked 12–14 days after inoculation and was followed by clinical recovery, we analyzed the DNA content of the spinal cord inflammatory cells to assess apoptosis and, simultaneously, we measured the expression of five proteins (Fas, Fas ligand (Fas-L), Bcl-2, Bcl-x and Bax) which modulate the apoptotic process. Cells expressing the death effector molecules Fas and Fas-L were particularly prone to undergo apoptosis, and were over-represented in the apoptotic population. Of the cells expressing the cell death inhibitor Bcl-2, a low proportion were undergoing apoptosis compared to the proportion of the total inflammatory cell population undergoing apoptosis, indicating that expression of Bcl-2 protects against T cell apoptosis in this disease. There was no evidence, however, that the apoptotic regulators Bcl-x and Bax influenced the susceptibility to apoptosis. We also found that Vβ8.2$^+$ T cells, which constitute the predominant encephalitogenic MBP-reactive T cell population in the Lewis rat, have a high frequency of Fas and Fas-L expression compared to other inflammatory cells. This would account for the previously demonstrated susceptibility of Vβ8.2$^+$ T cells to apoptosis in the CNS in EAE. These findings support the hypothesis that autoreactive T cells are eliminated from the CNS during spontaneous recovery from EAE by activation-induced apoptosis involving the Fas pathway.

Author Keywords: T cell apoptosis; Fas (CD95); experimental autoimmune encephalomyelitis; Bcl-2; autoimmunity

1. Introduction

T cell apoptosis (programmed cell death) occurs in the central nervous system (CNS) in experimental autoimmune encephalomyelitis (EAE) (Pender et al., 1991 and Pender et al., 1992; Schmied et al., 1993). This T-cell-mediated demyelinating disease of the CNS is the best available animal model of multiple sclerosis, and can be induced in susceptible animals by immunization with myelin antigens, such as myelin basic protein (MBP), and adjuvants or by the passive transfer of T lymphocytes activated by these antigens (Pender, 1995). In acute EAE in the Lewis rat, encephalitogenic Vβ8.2$^+$ MBP-reactive T cells are selectively eliminated from the CNS by apoptosis (Tabi et al., 1994 and Tabi et al., 1995; McCombe et al., 1996a), with resultant resolution of inflammation and clinical recovery.

We have proposed that the mechanism responsible for the apoptosis of autoreactive T lymphocytes in the target organ of this autoimmune disease is activation-induced cell death due to the interaction of previously activated T cells with non-professional antigen-presenting cells (APCs) in the CNS, such as astrocytes or microglia, which fail to deliver the costimulatory signal needed for T cell survival(Pender et al., 1992; Tabi et al., 1994 and Tabi et al., 1995). This hypothesis is supported by our finding that the selective apoptosis of Vβ 8.2$^+$ T cells in the CNS in EAE is reduced by dexamethasone, which inhibits activation-induced cell death (McCombe et al., 1996b). It is also supported by in vitro experiments demonstrating that CD4$^+$ MBP-specific T cell lines undergo apoptosis after reactivation by activated microglia but proliferate after reactivation by non-microglial CNS macrophages (Ford et al., 1996). Furthermore, antigen presentation by astrocytes in vitro has been shown to increase the susceptibility of T lymphocytes to apoptosis (Gold et al., 1996).

Recent research has revealed important insights into the molecular biology of apoptosis. The interaction of Fas and Fas ligand (Fas-L) is required for the activation-induced death of previously stimulated T cells (Russell et al., 1993; Russell and Wang, 1993; Bossu et al., 1993; Alderson et al., 1995). Fas (APO-1 or CD95) is a cell surface protein that induces apoptosis via activation of cysteine proteases (caspases) (Fraser and Evan, 1996). A soluble secreted form of Fas has also been identified (Cheng et al., 1994). Its natural ligand, Fas-L, is primarily expressed on the cell surface, but overexpression causes shedding of the protein in a soluble form (Suda et al., 1993). Activation of T lymphocytes increases their expression of Fas and induces their expression of Fas-L within 24 h, and they become susceptible to Fas-mediated apoptosis 3–4 days after activation (Brunner et al., 1995; Ju et al., 1995; Dhein et al., 1995).

Other gene products which modulate lymphocyte apoptosis are those of the Bcl-2 family. Bcl-2, which is chiefly expressed on the mitochondrial outer membrane, is an effective inhibitor of apoptosis in many systems. Bcl-x is homologous to Bcl-2, and has long (Bcl-x$_L$), short (Bcl-x$_S$) and transmembrane region-negative forms (Bcl-x$_{\Delta TM}$), the latter two forms being the result of alternative splicing (Fang et al., 1994). The bulk of the Bcl-x$_L$ protein, like Bcl-2, is localized in the periphery of mitochondria, and it and Bcl-x$_{\Delta TM}$ have also been shown to prevent cell death. In contrast, Bcl-x$_S$ appears to facilitate apoptosis by inhibiting the death suppressor activity of Bcl-2 (Boise et al., 1993; Fang et al., 1995), as does Bax, an intracellular protein that forms heterodimers with Bcl-2 and Bcl-x (Oltvai et al., 1993; Vaux and Strasser, 1996). Thus, the response of a cell to death signals is modulated by the differential expression and interaction of inhibitors and facilitators of apoptosis (Núñez et al., 1994).

The present study was performed to assess the roles of five apoptosis-regulating proteins (Fas, Fas-L, Bcl-2, Bcl-x and Bax) in the apoptosis of T lymphocytes in the spinal cords of Lewis rats with acute EAE induced by immunization with MBP and complete Freund's adjuvant.

Using three-colour flow cytometry, we demonstrate that Fas^+ cells and $Fas\text{-}L^+$ cells are particularly prone to apoptosis, whereas $Bcl\text{-}2^+$ cells are protected against apoptosis in the CNS. Furthermore, $V\beta8.2^+$ T cells, which constitute the predominant encephalitogenic MBP-reactive T cell population in the Lewis rat (Chluba et al., 1989; Burns et al., 1989; Imrich et al., 1995), have a high frequency of Fas and Fas-L expression, which increases their susceptibility to apoptosis. These findings support the hypothesis that autoreactive T cells are eliminated from the CNS during spontaneous recovery from EAE by activation-induced apoptosis involving the Fas pathway, and may have implications for the design of therapeutic strategies to facilitate this process.

2. Materials and methods

2.1. Animals

Female Lewis rats (JC strain), 7–10 weeks old, were obtained from the Central Animal Breeding House of the University of Queensland.

2.2. Preparation of inoculum and induction of EAE

MBP was prepared from guinea pig brains by the method of Deibler et al. (1972). MBP in 0.9% saline was emulsified in an equal volume of incomplete Freund's adjuvant containing 4 mg/ml *Mycobacterium butyricum*. Under anaesthesia, rats were inoculated in one hind footpad with 0.1 ml emulsion. The total dose of MBP was 50 μg per rat.

2.3. Clinical assessment

Tail, hindlimb and forelimb weakness were each graded on a scale of 0 (no weakness) to 4 (total paralysis) as previously described (Pender, 1986). The total clinical score was obtained by adding these three scores (maximum=12).

2.4. Extraction of cells from the spinal cord

Using our previously described methods (Tabi et al., 1994), cells were isolated from the spinal cords of rats perfused with ice-cold saline. The entire spinal cord was removed by insufflation, weighed, and a single cell suspension in ice-cold RPMI containing 1% foetal calf serum (FCS) was prepared by passage of the spinal cord through a 200-mesh stainless steel sieve. The cell suspension was mixed with isotonic Percoll (Percoll:HBSS 9:1) in a 3:2 ratio (density 1.052) in 50 ml centrifuge tubes and spun for 25 min at 640×*g* at 4°C. The cell pellet and the last 9 ml supernatant were resuspended, transferred to a conical centrifuge tube, underlaid with 1 ml Ficoll and spun for 20 min at 600×*g* at 4°C. The cells from the interface above the Ficoll were collected, washed and counted. To enrich for T lymphocytes, the cells were then passed through a nylon wool column, as previously described (Tabi et al., 1994).

2.5. Antibodies

Mouse monoclonal antibody to Vβ8.2 (R78) was kindly provided by Dr. T. Hünig (Torres-Nagel et al., 1993). Rabbit polyclonal antibodies specific for rat Fas (M-20), Fas-L (W-20), Bcl-2 (N19), Bcl-x$_{L/S}$ (L-19) and Bax (P19) were purchased from Santa Cruz Biotechnology. Western blot analysis of the CNS inflammatory cell protein extract confirmed the specificity of antibody binding and indicated that Bcl-x$_L$ was the predominant form of

Bcl-x expressed (not shown). The secondary antibodies employed were fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit IgG $F(ab')_2$ (Rockland) and phycoerythrin (PE)-conjugated goat anti-mouse Ig (Dako). Mouse IgG_1 (Dako) and rabbit IgG (Rockland) were used as control antibodies. The primary and secondary antibodies were diluted in phosphate-buffered saline (PBS)/azide (1% FCS/1% sodium azide in PBS) plus 10% autologous rat serum.

2.6. Labelling of cells and flow cytometric analysis

Three-colour analysis was used for the simultaneous detection of surface and intracellular antigens and for analysis of DNA content for assessment of apoptosis. Each sample was stained with the following: propidium iodide (PI) which stains DNA; the anti-Vβ8.2 antibody followed by PE-conjugated secondary antibody; and an antibody against one of the five apoptosis-regulating proteins followed by an FITC-conjugated secondary antibody. Briefly, 10^5–10^6 cells were stained for expression of the cell-surface antigens Vβ8.2, Fas and Fas-L as previously described (McCombe et al., 1996a). After washing to remove unbound secondary antibody, cells were fixed with 1 ml ice-cold 0.25% paraformaldehyde in PBS (pH 7.2) overnight at 4°C. They were then washed in PBS and permeabilized by being gently resuspended in 1 ml 0.2% Tween 20 in PBS and incubated at 37°C for 15 min. Samples were washed, and the intracellular antigens Bcl-2, Bcl-x$_{L/S}$ and Bax were labelled in the same manner as for the surface antigens.

The washed samples were then resuspended in an appropriate volume (100–300 μl) of PI-staining solution which was freshly prepared by diluting stock solution (RNase (5 mg/ml) and PI (250 μg/ml) in 0.1 M PBS containing 0.1 mM EDTA, pH 7.4) with PBS/azide. Samples were kept on ice in the dark and analyzed within 1 h. Immunofluorescence and DNA analysis were performed on a Becton Dickinson FACSCalibur using CellQuest software. Electronic compensation for three-colour analysis ensured unchanged FITC and PE distributions following PI labelling of DNA. For each sample, 40000 events were scored. To avoid detecting nuclear debris, events with a low level of PI fluorescence were not collected (see Fig. 1B). For surface and intracellular antigen labelling, the Ig-control sample values were subtracted from all other sample values to remove FITC and PE background fluorescence. Apoptotic events were defined as those having lower PI fluorescence than the sharply defined G_0/G_1 peak.

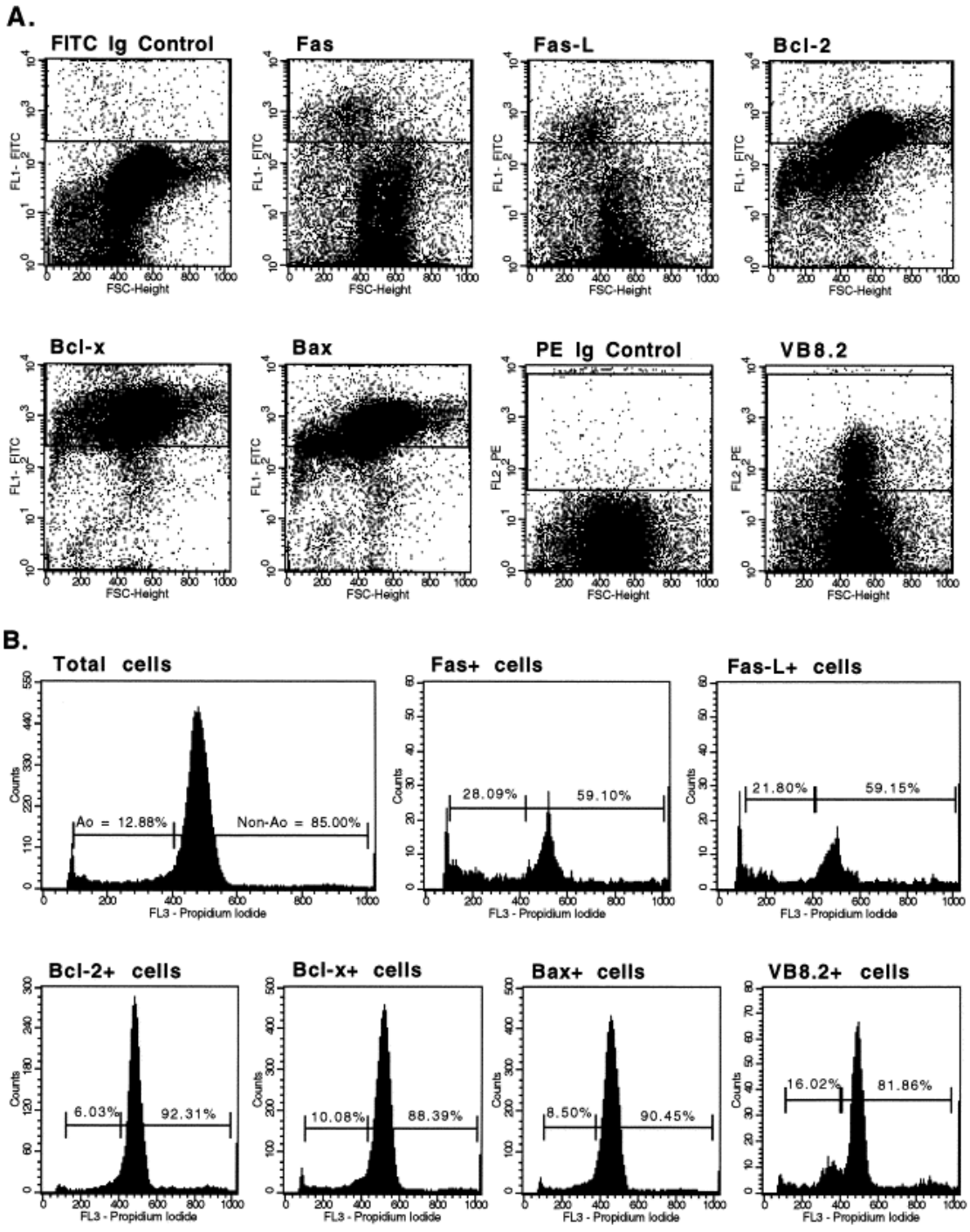


Fig. 1. (A) Representative flow cytometric plots of apoptosis-regulating protein and Vβ8.2 expression by CNS-infiltrating cells extracted from the spinal cords of rats with EAE 14 days after inoculation. The upper boxed area in each plot defines the positively labelled cells. (B) DNA staining profiles of the total inflammatory cell population (total cells) and the positively labelled populations shown in A.

2.7. Statistical analysis

Percentages of the different inflammatory cell populations which were apoptotic were compared using analysis of variance (ANOVA) to compare all the groups on each day simultaneously, followed by Student's *t*-test to compare each cell population against the total inflammatory cell population.

3. Results

3.1. Course of disease

Rats developed neurological signs of EAE 10–12 days after inoculation. The peak of disease was on days 12–14, after which the rats gradually recovered. The mean total clinical scores at the time of study are shown in Table 1. On each day after inoculation we analyzed the cells from 2–4 groups of rats, with each group containing the pooled spinal cord inflammatory cells extracted from 5–7 rats.

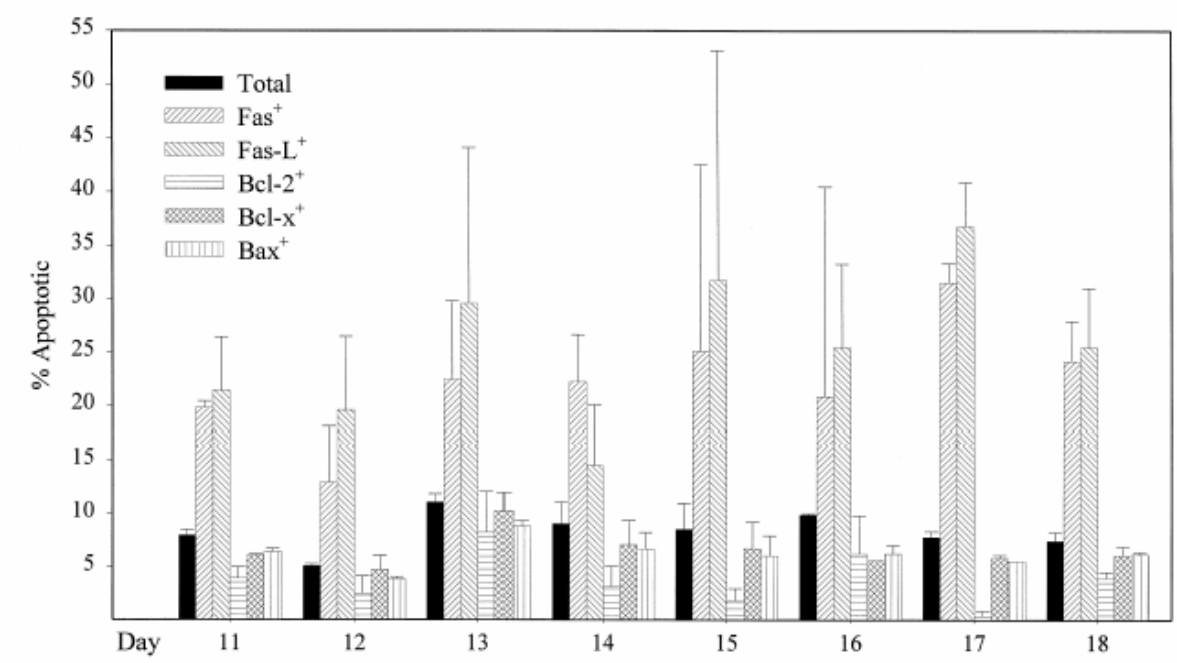
Table 1
Clinical details of rats with EAE studied on days 11–18 after inoculation

Day of study	Total number of rats	Number of groups studied	Mean total clinical score on day of study	Mean day of onset of EAE
11	13	2	2.6	10.3
12	13	2	5.0	10.1
13	26	4[a]	4.2	10.9
14	20	3	4.3	10.2
15	20	3[b]	3.8	11.0
16	14	2[c]	1.0	11.2
17	12	2[d]	1.4	11.3
18	14	2	0.1	10.2

[a]For Vβ8.2$^+$, Bax$^+$ and Bcl-x$^+$ cells, the number of groups studied on day 13 was 3.
[b]For Vβ8.2$^+$ cells, the number of groups studied on day 15 was 2.
[c]For Bcl-x$^+$ cells, the number of groups studied on day 16 was 1.
[d]For Bax$^+$ cells, the number of groups studied on day 17 was 1.

3.2. Apoptosis of cells expressing apoptosis-regulating proteins

To determine the susceptibility to apoptosis of cells expressing different apoptosis-regulating proteins, we employed simultaneous flow cytometric analysis of surface and intracellular antigens and DNA content. Cells expressing Fas or Fas-L tended to be smaller in size (FSC) than cells not expressing these proteins (Fig. 1A). This is explained by the apoptotic shrinkage of Fas$^+$ cells and Fas-L$^+$ cells which, as the DNA profiles show (Fig. 1B), have an increased susceptibility to apoptosis compared to the total inflammatory cell population. In contrast, Bcl-2$^+$ cells tended to be larger than Bcl-2$^-$ cells, which is explained by the relative resistance of Bcl-2$^+$ cells to apoptosis (Fig. 1B). The proportions of Fas$^+$ cells and Fas-L$^+$ cells undergoing apoptosis were higher than the proportion of total inflammatory cells undergoing apoptosis on every day from day 11 to day 18 after inoculation (Fig. 2), indicating that Fas$^+$ cells and Fas-L$^+$ cells are highly vulnerable to apoptosis throughout the course of EAE. The resistance of Bcl-2$^+$ cells to apoptosis was also evident throughout the course of EAE (Fig. 2). The expression of Bcl-x or Bax did not appear to influence susceptibility to apoptosis (Fig. 1B and Fig. 2).



ANOVA *P*-value	0.004	0.082	0.001	0.001	0.116	>0.5	<0.001	0.004
t test *P*-values								
Fas$^+$ vs Total	0.058	0.266	0.051	0.016	0.219	>0.5	0.014	0.052
Fas-L$^+$ vs Total	0.046	0.105	0.008	0.174	0.111	0.369	0.009	0.045
Bcl-2$^+$ vs Total	0.313	>0.5	>0.5	0.149	>0.5	>0.5	0.121	0.471
Bcl-x$^+$ vs Total	>0.5	>0.5	>0.5	>0.5	>0.5	ND	>0.5	>0.5
Bax$^+$ vs Total	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	ND	>0.5

Fig. 2. Percentages of CNS-infiltrating cells expressing apoptosis-regulating proteins that were apoptotic in rats with EAE on days 11–18 after inoculation. Cells were extracted from the spinal cord and immunostained for flow cytometry analysis. The mean and population standard deviation of the proportion of positively stained cells that was apoptotic on each day were then calculated. The ANOVA *P*-values for the overall comparison of the responses of the six cell populations on each day, together with the *P*-values (Student's *t*-test) for the comparisons of selected pairs of cell populations, are shown directly below the day to which they refer (ND=not determined because of insufficient data).

The vulnerability of Fas$^+$ cells and Fas-L$^+$ cells to apoptosis was also indicated by enrichment of Fas$^+$ cells and Fas-L$^+$ cells in the apoptotic compared to the non-apoptotic populations (Table 2). The proportion of Fas$^+$ cells in the apoptotic population was 3.0–6.4 times the proportion of Fas$^+$ cells in the non-apoptotic population. Even more strikingly, the proportion of Fas-L$^+$ cells in the apoptotic population was 4.2–15.9 times the proportion in the non-apoptotic population. In contrast to Fas$^+$ cells and Fas-L$^+$ cells, Bcl-2$^+$ cells were consistently under-represented in the apoptotic population (Table 2), indicating that they are protected against apoptosis. The proportions of cells expressing Bcl-x or Bax were similar in the apoptotic and non-apoptotic populations.

Table 2
Expression of apoptosis-regulating proteins by the apoptotic population compared to the non-apoptotic population[a]

Day of study	Fas	Fas-L	Bcl-2	Bcl-x	Bax
11	3.0 ± 0.0	4.2 ± 0.8	0.4 ± 0.0	0.7 ± 0.1	0.7 ± 0.1
12	3.5 ± 1.0	6.5 ± 2.2	0.4 ± 0.2	0.8 ± 0.0	0.7 ± 0.1
13	3.0 ± 1.4	5.1 ± 2.9	0.6 ± 0.2	0.9 ± 0.1	0.8 ± 0.0
14	3.9 ± 1.3	4.5 ± 3.2	0.2 ± 0.1	0.8 ± 0.1	0.7 ± 0.1
15	5.4 ± 3.6	15.9 ± 17.1	0.2 ± 0.1	0.7 ± 0.1	0.6 ± 0.1
16	3.6 ± 3.6	5.8 ± 2.4	0.4 ± 0.2	0.6 ± 0.0	0.7 ± 0.1
17	6.4 ± 0.0	8.3 ± 2.1	0.1 ± 0.1	0.8 ± 0.0	0.7 ± 0.0
18	5.0 ± 1.8	5.0 ± 1.9	0.4 ± 0.1	0.9 ± 0.2	0.8 ± 0.1

3.3. Expression of apoptosis-regulating proteins by Vβ8.2$^+$ T lymphocytes

We have previously presented evidence that encephalitogenic Vβ8.2$^+$ MBP-specific T cells are selectively eliminated from the CNS by apoptosis during spontaneous recovery from EAE, induced by the passive transfer of Vβ8.2$^+$ MBP-reactive T cells (Tabi et al., 1994) or by immunization with MBP and complete Freund's adjuvant (McCombe et al., 1996a). We therefore examined the expression of apoptosis-regulating proteins by the disease-relevant Vβ8.2$^+$ T cells in the CNS, which comprised up to 10.6% of the total inflammatory cells at the peak of disease and down to 1.1% at recovery (data not shown). As illustrated in Fig. 3, the proportions of Fas$^+$ cells and Fas-L$^+$ cells in the Vβ8.2$^+$ population were 1.4–5.8 and 1.5–8.8 times, respectively, the proportions of these cells in the non-Vβ8.2$^+$ population. The proportions of Vβ8.2$^+$ cells expressing Fas or Fas-L were highest during the later recovery phase of the disease (16–18 days after inoculation). The proportions of Bcl-2$^+$, Bcl-x$^+$ and Bax$^+$ cells in the Vβ8.2$^+$ population also tended to be higher than the proportions of these cells in the non-Vβ8.2$^+$ population (results for Bcl-2$^+$ cells are shown in Fig. 3). Furthermore, the proportions of Fas$^+$Vβ8.2$^+$ cells that were apoptotic were 6.4 and 4.4 times the proportions of Fas$^-$Vβ8.2$^+$ cells that were apoptotic 13 and 14 days after inoculation, respectively (day 13 was the time of maximal apoptosis; see Fig. 2), and the proportions of Fas-L$^+$Vβ8.2$^+$ cells that were apoptotic were 2.9 and 4.9 times the proportions of Fas-L$^-$Vβ8.2$^+$ cells that were apoptotic on these days (Table 3). The proportions of Bcl-2$^+$Vβ8.2$^+$ cells that were apoptotic were 0.9 and 0.4 times the proportions of Bcl-2$^-$Vβ8.2$^+$ cells that were apoptotic on days 13 and 14 after inoculation respectively (Table 3). These results indicate that Vβ8.2$^+$ cells expressing Fas or Fas-L are highly vulnerable to apoptosis, whereas Vβ8.2$^+$ cells expressing Bcl-2 are protected against apoptosis.

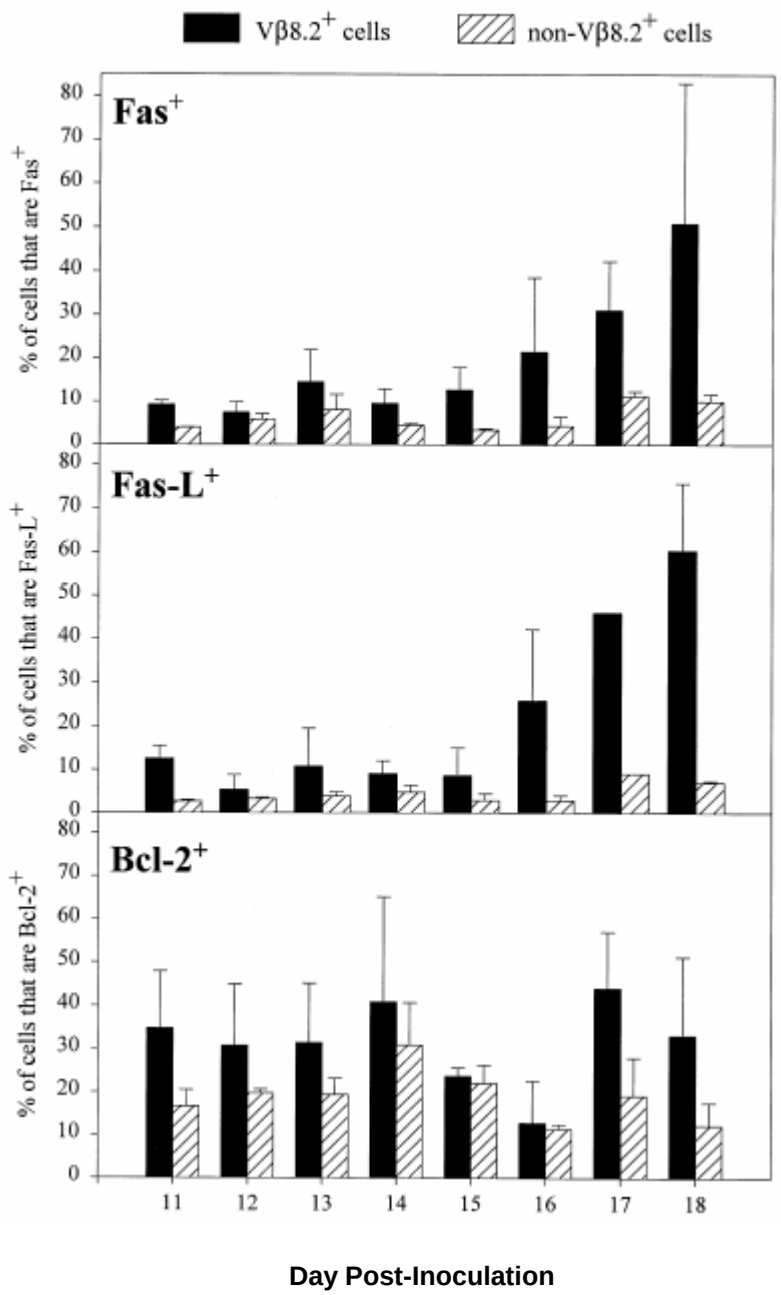


Fig. 3. Percentages of Vβ8.2$^+$ and non-Vβ8.2$^+$ CNS-infiltrating cells expressing apoptosis-regulating proteins in rats with EAE on days 11–18 after inoculation.

Table 3
Susceptibility of Vβ8.2$^+$ cells to apoptosis 13 and 14 days after inoculation

Apoptosis-regulating protein	Day	Percentage of Vβ8.2$^+$protein$^+$ cells that were apoptotic[a]	Percentage of Vβ8.2$^+$protein$^-$ cells that were apoptotic[a]	Mean ratio[b]
Fas	13	31.2 ± 8.8	7.1 ± 4.3	6.4 ± 4.3
	14	19.1 ± 9.9	7.0 ± 5.7	4.4 ± 3.8
Fas-L	13	24.6 ± 18.0	8.1 ± 0.6	2.9 ± 2.1
	14	14.5 ± 2.9	3.2 ± 1.1	4.9 ± 1.2
Bcl-2	13	9.0 ± 4.6	10.1 ± 4.0	0.9 ± 0.3
	14	6.6 ± 5.8	19.0 ± 17.4	0.4 ± 0.0

[a] Mean and population standard deviation.
[b] For each experiment the percentage of apoptotic cells in the population expressing Vβ8.2 and the apoptosis-regulating protein was divided by the percentage of apoptotic cells in the population which were Vβ8.2$^+$ but which did not express the protein. The mean and population standard deviation of these values were then calculated.

4. Discussion

In the present study we have shown that CNS-infiltrating cells expressing Fas or Fas-L were highly vulnerable to apoptosis during the course of acute EAE, whereas Bcl-2-expressing cells appeared to be relatively protected against apoptosis. Vβ8.2$^+$ T cells, which constitute the predominant encephalitogenic MBP-reactive T cell population in the Lewis rat (Chluba et al., 1989; Burns et al., 1989; Imrich et al., 1995), expressed Fas and Fas-L more frequently than did other inflammatory cells in the CNS. Furthermore, Vβ8.2$^+$ cells expressing Fas or Fas-L were highly vulnerable to apoptosis compared to Vβ8.2$^+$ cells not expressing these proteins. These findings account for the previously demonstrated susceptibility of Vβ8.2$^+$ T cells to apoptosis in the CNS during recovery from acute EAE (Tabi et al., 1994; McCombe et al., 1996a). The present findings support our hypothesis that autoreactive T cells are eliminated from the CNS by activation-induced apoptosis (Pender et al., 1992; Tabi et al., 1994 and Tabi et al., 1995), which is mediated in previously activated T cells by the interaction of Fas and Fas-L (Bossu et al., 1993; Russell and Wang, 1993; Russell et al., 1993; Alderson et al., 1995). The ligation of Fas on the surface of the T cell can be mediated by Fas-L on the same T cell or by Fas-L expressed by other cells (Brunner et al., 1995; Dhein et al., 1995). Only a low proportion of the cells expressing the death inhibitor Bcl-2 underwent apoptosis compared to the total spinal cord inflammatory cell population, indicating that expression of Bcl-2 protects against apoptosis. Bcl-2 is known to inhibit Fas-mediated apoptosis in some cell types (Itoh et al., 1993; Yoshino et al., 1994; Takayama et al., 1995; Lacronique et al., 1996). Recently it has been shown that there are two distinct apoptotic pathways downstream of Fas, one of which is blocked by Bcl-2, with the relative contributions of these pathways varying among different cells (Yang et al., 1997). We found no evidence that Bcl-x or Bax influences the susceptibility of CNS inflammatory cells to apoptosis. As the expression of all of these apoptosis-regulating molecules is increased in activated T cells (Broome et al., 1995b; Brunner et al., 1995; Dhein et al., 1995; Ju et al., 1995), and yet only Fas$^+$ and Fas-L$^+$ cells selectively undergo apoptosis, these results indicate that Fas/Fas-L interactions, and not merely cellular activation per se, are responsible for T cell apoptosis in the CNS in EAE.

Expression of these proteins may be modulated by cellular interactions in the CNS. In EAE, previously activated MBP-reactive T cells entering the CNS may re-encounter MBP peptides on the surface of bone marrow-derived cells such as macrophages or microglia (Craggs and Webster, 1985; Frei et al., 1987; Myers et al., 1993), or on parenchymal cells such as astrocytes (Fontana et al., 1984; Myers et al., 1993). T cell receptor cross-linking, even in the absence of costimulation, increases Fas expression and induces Fas-L expression on the T cells (Brunner et al., 1995; Dhein et al., 1995; Ju et al., 1995; van Parijs et al., 1996). T cell activation also results in an increase in Bcl-2 protein (Broome et al., 1995a), but this is dependent upon continued exposure to interleukin-2 (IL-2) (Deng and Podack, 1993; Broome et al., 1995a; Mueller et al., 1996) and other cytokines which signal through the γ-chain of the IL-2 receptor (Akbar et al., 1996). If autoreactive T cells re-encounter antigen presented by non-professional APCs such as microglia or astrocytes that are unable to deliver costimulatory signals, the production of IL-2 will wane. This would then lead to a downregulation of Bcl-2 expression and may predispose the T cells to Fas-mediated apoptosis.

The proportions of Vβ8.2$^+$ T cells expressing Fas or Fas-L increased markedly during the later recovery phase of the disease. This may be due to enhanced activation of autoreactive T cells newly entering the CNS. Increased activation of autoreactive T cells in the CNS might occur as a result of an increased availability of myelin antigens following demyelination and the increased microglial expression of class II major histocompatibility complex molecules that occurs during and after recovery from EAE (Matsumoto et al., 1986; McCombe et al.,

1992). This raises the possibility that activation-induced T cell apoptosis may, as we have previously suggested (Pender et al., 1992), be an ongoing process that contributes to the tolerant state that develops after an attack of acute EAE (Willenborg, 1979). Understanding this process and how it might fail in chronic autoimmune disorders may lead to a better understanding of diseases such as multiple sclerosis.

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