Effects of cyclosporin A treatment on clinical course and inflammatory cell apoptosis in experimental autoimmune encephalomyelitis induced in Lewis rats by inoculation with myelin basic protein

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Abstract

Experimental autoimmune encephalomyelitis (EAE) was induced in Lewis rats by inoculation with myelin basic protein (MBP) and adjuvants. Rats were treated with second daily injections of saline or cyclosporin A (CsA) from the day of inoculation. Saline-treated rats had an acute episode of disease followed by clinical recovery. Rats treated with CsA 16 or 32 mg/kg had minimal signs of EAE at the usual time after inoculation, but developed signs of disease after treatment was ceased. Rats treated with CsA 8 mg/kg had a delayed first episode of disease and then developed a relapsing or a chronic persistent course of disease. CsA 4 mg/kg delayed the onset of disease. To study the effects of CsA on the inflammatory infiltrate, cells were extracted from the spinal cords of rats with EAE, 16 h after a single injection of CsA or saline. Extracted cells were labelled with antibodies to T cells, CD11b/c (macrophages/microglia), CD95 (Fas) and Fas ligand. CsA 4 mg/kg did not alter the composition of the inflammatory infiltrate. Treatment with higher single doses of CsA caused a dose-dependent decline in the percentage of T cell receptor (TCR) $\alpha\beta^+$ cells in the inflammatory infiltrate. All doses of CsA caused a significant increase in the number and percentage of cells that were apoptotic. CsA treatment caused an increase in the percentages of $CD5^+$ and $TCR\alpha\beta^+$ cells that were apoptotic. There was a decline in the percentage of apoptotic T cells that were $V\beta8.2^+$, compared to the percentage of non-apoptotic T cells that were $V\beta8.2^+$, in CsA treated rats compared to saline-treated controls. This suggests that, while CsA treatment caused a non-specific increase in the overall level of T cell apoptosis in the spinal cord, it abrogated the selective apoptosis of $V\beta8.2^+$ encephalitogenic T cells that normally occurs during spontaneous recovery from acute EAE.

Author Keywords: apoptosis; Cyclosporin A; encephalomyelitis; immunoregulation; T lymphocyte

1. Introduction

Cyclosporin A (CsA) is a cyclic peptide with immunomodulatory properties. CsA acts within the cell by binding to cyclophilin, an immunophilin. This results in inactivation of calcineurin, and suppression of transcription of genes, including those for cytokines and cytokine receptors (Flanagan et al., 1991). Immunophilins also have neuroprotective abilities (Sabatini et al., 1997). CsA is used as an immunosuppressive agent in organ transplantation and in the treatment of autoimmune diseases. In multiple sclerosis, CsA treatment has been of modest benefit (Multiple Sclerosis Study Group, 1990). In some situations, CsA can provoke autoimmune disease. One example of CsA-induced autoimmunity is the graft versus host disease that occurs in CsA-treated animals after syngeneic bone marrow transplantation (Hess et al., 1994). CsA in high doses suppresses clinical signs of experimental autoimmune encephalomyelitis (EAE) (Bolton et al., 1982; Polman et al., 1988) which is an animal model of multiple sclerosis. In Lewis rats, treatment with low-dose CsA (4 mg/kg) converts EAE induced by inoculation with guinea pig spinal cord from an acute to a chronic relapsing disease (Polman et al., 1988; Pender et al., 1990; McCombe et al., 1994). The relapsing disease produced by

treatment with CsA may result from the suppression of the immunoregulatory mechanisms that normally prevent further episodes of disease. In EAE, such regulatory mechanisms may include suppressor cells (Karpus et al., 1992), downregulatory cytokines, and apoptosis of T cells in the central nervous system (CNS) (Pender et al., 1991 and Pender et al., 1992; Schmied et al., 1993; Tabi et al., 1994 and Tabi et al., 1995; McCombe et al., 1996a).

The present study was performed in two parts. Firstly, we assessed the effects of CsA treatment on the clinical course of EAE induced by inoculation with myelin basic protein (MBP), to determine whether this is similar to the effects of CsA on EAE induced by inoculation with whole spinal cord. Secondly, we studied the effects of a single dose of CsA on the inflammatory infiltrate extracted from the spinal cord of rats with EAE. Because apoptosis is involved in the spontaneous recovery of rats from acute EAE, we also assessed the effects of CsA on apoptosis of inflammatory cells in the spinal cord in EAE.

2. Methods

2.1. Induction of EAE

Male Lewis rats (JC strain), aged 8–10 weeks, were obtained from the Central Animal Breeding House of The University of Queensland. 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. On day 0, rats were inoculated in one hind footpad with 0.1 ml emulsion. The total dose of MBP was 50 $^\mu$g/rat. We assessed weakness of the tail, hindlimbs and forelimbs. The degree of weakness of each region was separately graded on a scale of 0 (no weakness) to 4 (total paralysis) as previously described (Pender, 1986). The scores from each region were added together to give a total clinical score (maximum total clinical score=12).

2.2. CsA treatment

For studies of the effects of CsA treatment on the clinical course of EAE, rats were given intraperitoneal injections of saline or CsA every second day, commencing on the day of inoculation, for periods of 21–42 days. For studies of the effects of CsA on the cells extracted from the spinal cord, rats were given a single injection of CsA, at an interval of 16 h before sacrifice on either day 14 or day 15 after inoculation.
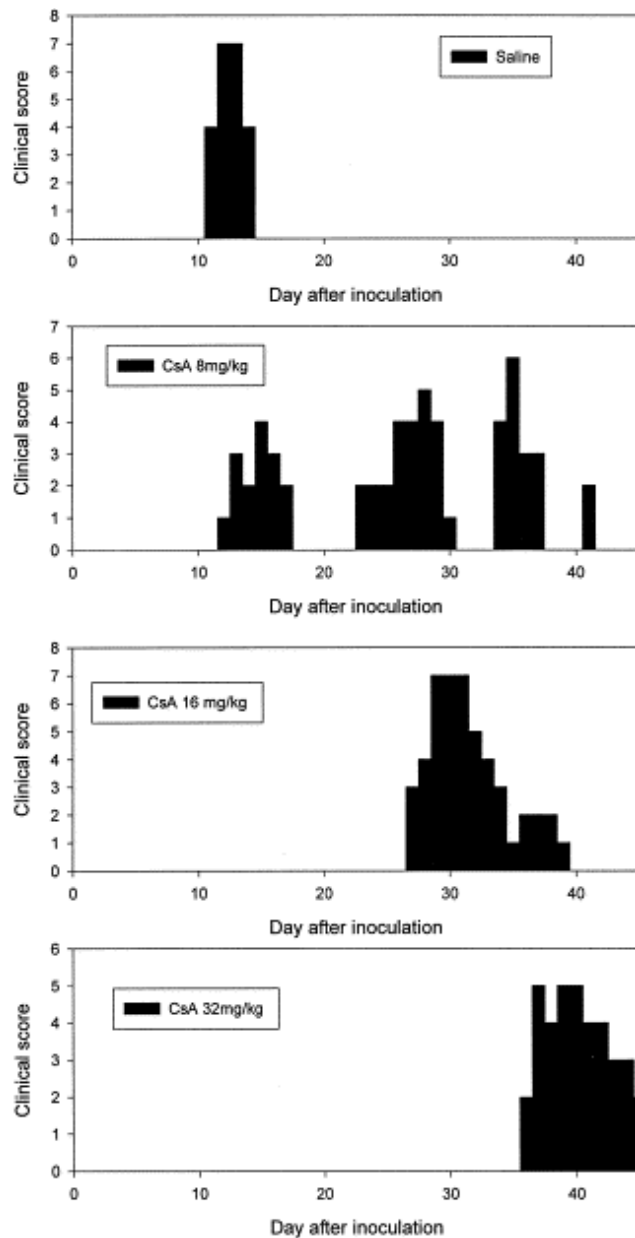
2.3. Extraction of cells from spinal cord

As described previously (McCombe et al., 1996b), cells were isolated from the spinal cords of anaesthetized rats perfused with ice-cold saline. The spinal cord was removed by insufflation, weighed, and a single cell suspension in ice-cold RPMI 1640 containing 1% foetal calf serum (FCS) was prepared. The cell suspension was mixed with isotonic Percoll (Percoll: Hank's balanced salt solution 9:1) in a 3:2 ratio (density 1.052) in 50 ml centrifuge tubes and centrifuged for 25 min at 640×g at 4°C. The myelin layer and supernatant were discarded, while the last 9 ml supernatant and the cell pellet were retained. The cell pellet was resuspended, transferred to a conical 10 ml centrifuge tube, underlaid with 1 ml Ficoll and centrifuged for 20 min at 600×g at 4°C. Cells were harvested from the interface.

2.4. Antibodies

We used the following primary antibodies: OX19 (Dallman et al., 1984) (CD5); R73 (Hünig et al., 1989) (TCR$\alpha\beta$); R78 (Torres Nagel et al., 1993) which labels Vβ8.2$^+$ T cells (the predominant encephalitogenic cells in MBP-EAE) (Imrich et al., 1995); OX42, which labels CD11b/c+ cells (macrophages and microglia); and rabbit polyclonal antibodies specific for rat CD95 (Fas) (Santa Cruz) and rat CD95 (Fas) ligand (Santa Cruz Biotechnology). The secondary antibodies were fluorescein isothiocyanate (FITC)-conjugated rabbit anti-mouse IgG F(ab′)2 (STAR 41) (Serotec) and FITC-conjugated goat anti-rabbit IgG (ab′)2 (Rockland, Gilbertsville, PA).

2.5. Labelling of cells and flow cytometric analysis

Cells were labelled for flow cytometric analysis, using our previously described methods (McCombe et al., 1996b). Aliquots of 105–106 cells were washed with a 1:1 solution of serum in phosphate-buffered saline (PBS) containing 1% FCS and 0.1% sodium azide, then incubated with primary antibodies in PBS/azide for 30 min at 4°C. Samples were washed and incubated with secondary antibody for 30 min at 4°C in the dark. Cells were washed twice with PBS and were resuspended in 1 ml of ice-cold 50% ethanol and fixed overnight at 4°C. The following day, the cells were washed in PBS. The cell pellets were resuspended in an appropriate volume (100–300 μl) of propidium iodide (PI)-staining solution (freshly prepared by diluting stock solution [5 mg/ml RNase/250 μg/ml PI in 0.1 M PBS), pH 7.4/0.1 mM EDTA] 1:4 with PBS/azide). Samples were kept on ice, in the dark, and analyzed using a Becton Dickinson FACScan. Flow cytometric data were analyzed using Lysis II Software (Becton Dickinson). The percentages of antibody-positive cells were determined from histograms of FITC fluorescence intensity. Background fluorescence, obtained by labelling the cells with isotype-control antibodies and FITC-conjugated secondary antibody, was subtracted from the test values. To analyze the proportions of cells undergoing apoptosis, or in G1/G0 or S/G2/M phase, histograms of PI fluorescence were obtained, as described by Telford et al. (1991). Apoptotic events were defined as those having lower fluorescence than the sharply defined G0/G1 peak (White et al., 1998b). Events with higher fluorescence than this peak were defined as being in S/G2/M phase. Events with a low level of red (PI) fluorescence were not collected. Electronic compensation for two-colour analysis ensured unchanged FITC distribution following PI labelling of DNA.

2.6. Statistical analysis

Statistical analysis was carried out using analysis of variance (ANOVA) with Sigmastat 2.0 (Jandel Scientific). Results for observations at different doses of CsA were then compared with results for saline by the Tukey test.

3. Results

3.1. Effect of second daily CsA treatment on the clinical course of EAE

A summary of the effects of CsA treatment is shown in Table 1, and examples of the clinical course of individual rats are shown in Fig. 1. Rats treated with saline for 21–42 days had a first episode of disease commencing around day 10. Some saline-treated rats had a second episode of disease, but none had a third episode of disease or chronic disease. Rats treated with CsA 4 mg/kg had a clinical course of disease similar to that of saline-treated rats, except that the onset of signs of disease was delayed. Second daily treatment with CsA 8 mg/kg caused a mild delay of the first episode of disease and then multiple relapses or a chronic persistent course of disease in 50% of the rats. In this group, rats in the first episode of disease generally had weakness of the tail and hindlimbs, while in the later episodes of disease there was weakness of the tail and forelimbs, but not the hindlimbs. Second daily treatment with CsA 16 or 32 mg/kg prevented the development of disease during the course of treatment, although the rats later developed signs of disease after CsA treatment was ceased.

Table 1
Effects of second daily CsA treatment on clinical course of disease

Treatment (duration)	Number of rats	First episode				Second episode				Third episode				Chronic persistent disease
		Incidence of disease	Mean (SEM) day of onset	Mean max. severity in affected rats	No. of deaths	Incidence of disease in remaining rats	Mean day of onset	Mean max. severity in affected rats	No. of of deaths	Incidence of disease in remaining rats	Mean day of onset	Mean max. severity in affected rats	No. of deaths	Incidence
Controls														
Saline (day 21)	17	100%	10 (1)	7	1	30%	23	4	0	0%				0%
Saline (day 29)	5	100%	10 (1)	7	0	60%	23	4	0	0%				0%
Saline (day 42)	5	100%	11 (1)	8	1	0%			0	0%				0%
CsA treated														
4 mg/kg (day 29)	5	100%	12 (1)	6	0	20%	27	4	0	0%				0%
4 mg/kg (day 42)	5	100%	14 (1)	8	0	80%	27	4	2	0%				0%
8 mg/kg (day 21)	10	90%	14 (2)	7	0	80%	30	6	0	30%	34	5	0	20%
16 mg/kg (day 21)	10	100%	27 (8)	3	1									0%
32 mg/kg (day 21)	10	100%	31 (9)	5	1									0%

Fig. 1. Effects of CsA treatment on the clinical course of disease, showing the clinical course of representative rats treated with second daily injections of saline, CsA 8, 16 and 32 mg/kg from the day of inoculation until day 22. The rat treated with saline had a single episode of disease, the rat treated with CsA 8 mg/kg had a delayed onset of disease followed by relapses, while the rats treated with CsA 16 and 32 mg/kg failed to develop disease until treatment had been ceased.

3.2. Effect of a single dose of CsA on spinal cord inflammatory cells

The details of rats in the study are shown in Table 2. A single dose of CsA had no significant effect on the severity of disease or on the number of cells extracted from the spinal cord, compared to treatment with saline. The proportions and numbers of cells of different types are shown in Table 3. Treatment with CsA 4 mg/kg or 8 mg/kg had no significant effect on the composition of the cell population. The highest dose (CsA 32 mg/kg) caused a significant decline in the percentages of $TCR\alpha\beta^+$ lymphocytes and $V\beta 8.2^+$ cells and an increase in the percentage of $CD11b/c^+$ cells (macrophages/microglia) in the inflammatory infiltrate. There was a significant decline in the number of $TCR\alpha\beta^+$ lymphocytes but no change in the number of $CD11b/c^+$ cells. There was no change in the percentage of CD95 (Fas)$^+$ or CD95 (Fas) ligand$^+$ cells in the inflammatory infiltrate.

Table 2
Details of rats used in FACS study

Treatment	Number of groups	Mean (SEM) day of onset of signs	Mean (SEM) clinical score	Mean (SEM) number of extracted cells/g of spinal cord
Rats studied on day 14				
Saline	8	10.9 (1.3)	5.4 (2.4)	54.7 (20.1)
CsA 4 mg/kg	8	12.2 (1.1)	4.2 (2.6)	63.8 (51)
Rats studied on day 15				
Saline	8	10.0 (1.0)	4.1 (1.7)	31.8 (4.3)
CsA 8 mg/kg	8	10.6 (1.1)	5.1 (1.7)	40.2 (10.7)
CsA 16 mg/kg	7	10.5 (0.8)	4.8 (1.5)	33.6 (9.4)
CsA 32 mg/kg	8	10.0 (0.5)	4.6 (1.9)	32.8 (11.0)

Table 3
Effect of CsA on numbers and percentages of labelled cells in the inflammatory infiltrate

| Treatment	$CD5^+$ cells		$TCR\alpha\beta^+$ cells		$TCR\,V\beta8.2^+$ cells		$CD11b/c^+$ cells		Fas^+ cells		Fas ligand$^+$ cells	
	% positive (SEM)	No. (SEM) of cells/g	% positive (SEM)	No. (SEM) of cells/g	% positive (SEM)	No. (SEM) of cells/g	% positive (SEM)	No. (SEM) of cells/g	% positive (SEM)	No. (SEM) of cells/g	% positive (SEM)	No. (SEM) of cells/g
Rats studied on day 14												
Saline (day 14)	29.1 (5.4)	15.8 (4.2)	16.2 (3.7)	9.2 (3.6)	3.3 (1.3)	2.1 (1.5)	39.3 (7.5)	23.6 (8.4)	31.6 (8.2)	18.0 (4.1)	29.1 (6.4)	16.9 (5.2)
CsA (day 14)	31.8 (6.0)	19.6 (15.7)	16.0 (6.2)	8.8 (5.5)	2.5 (1.5)	1.5 (1.3)	38.1 (11.3)	27.7 (29.3)	23.7 (9.3)	17.8 (20.3)	23.5 (6.4)	16.4 (16.2)
t test	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Rats studied on day 15												
Saline	23.3 (7.4)	7.4 (2.6)	14.2 (5.1)	4.5 (1.7)	3.3 (1.7)	1.1 (0.7)	56.9 (6.7)	18.2 (3.6)	37.8 (7.4)	12.1 (2.9)	22 (11)	7.1 (3.9)
CsA 8 mg/kg	27.9 (2.9)	11.2 (3.2)	10.1 (3.3)	4.2 (2.2)	2.2 (0.7)	0.9 (0.4)	58.4 (5.8)	23.8 (7.7)	40.2 (7.4)	15.9 (7.3)	40.2 (6.0)	6.8 (1.6)
CsA 16 mg *P* (cf. saline)	22.5 (9.9)	7.0 (1.9)	7.5 (1.7) $p < 0.05$	2.5 (0.9)	2.8 (3.6)	0.75 (0.7)	65.2 (5.7)	21.8 (5.9)	40.2 (3.3)	13.2 (4.7)	20.5 (9.0)	6.2 (2.4)
CsA 32 mg *P* (cf. saline)	20.3 (4.7)	7.2 (2.4)	5.7 (2.2) $p < 0.05$	2.1 (0.9) $p < 0.05$	1.3 (0.7) $p < 0.05$	0.5 (0.3)	66.1 (6.0) $p < 0.05$	23.7 (8.0)	46.9 (8.7)	16.9 (7.1)	36.8 (2.6)	14.9 (2.5)
ANOVA	NS	NS	$p = 0.001$	$p = 0.02$	$p = 0.044$	NS	$p = 0.02$	NS	NS	NS	NS	NS

3.3. Effect of a single dose of CsA on apoptosis

In the total cell population, treatment with 4 mg/kg (on day 14) did not alter the percentage of cells that were apoptotic (data not shown). Treatment with CsA 8, 16 or 32 mg/kg (on day 15) produced a significant, dose-dependent increase in the number and percentage of cells that were apoptotic (Fig. 2). Table 4 shows the effects of CsA on apoptosis of cells labelled with different antibodies. Treatment with CsA 4 mg/kg did not alter the percentages or numbers of the different cell populations that were apoptotic. Treatment with CsA 8, 16 and 32 mg/kg caused a significant increase in the percentage of CD5+ cells undergoing apoptosis. There was increased apoptosis of TCR$\alpha\beta^+$ cells after the 16 and 32 mg/kg doses, although this was statistically significant only for the 16 mg/kg dose. CsA treatment did not alter the percentage of Fas$^+$ or Fas ligand$^+$ cells that were apoptotic.

Fig. 2. Number of cells per gram of spinal cord and percentage of the extracted cells that were apoptotic cells, in G0/G1 and G2/S/M phase on day 15 after treatment with saline or CsA 8, 16 or 32 mg/kg. In a dose-dependent fashion, there was a significant increase in the percentage of cells that were apoptotic after CsA treatment, and an increase in the number of apoptotic cells, although this was only significant for the highest dose of CsA. The increase in the percentage of cells that were apoptotic was accompanied by a decrease in the percentage of cells that were in G0/G1 phase.

Table 4
Effect of CsA on numbers and percentages of labelled cells that were apoptotic

Treatment	CD5+ cells		TCRαβ+ cells		TCR Vβ8.2+ cells		CD11b/c+ cells		Fas+ cells		Fas ligand+ cells	
	% apoptotic (SEM)	No. (SEM) of apoptotic cells/g	% apoptotic (SEM)	No. (SEM) of apoptotic cells/g	% apoptotic (SEM)	No. (SEM) of apoptotic cells/g	% apoptotic (SEM)	No. (SEM) of apoptotic cells/g	% apoptotic (SEM)	No. (SEM) of apoptotic cells/g	% apoptotic (SEM)	No. (SEM) of apoptotic cells/g
Rats studied on day 14												
Saline (day 14)	7.4 (2.8)	1.2 (0.6)	10.8 (3.4)	1.0 (0.6)	11.4 (3.8)	0.2 (0.2)	39.3 (7.5)	2.0 (1.1)	31.7 (8.2)	1.9 (0.7)	29.1 (6.4)	2.0 (1.0)
CsA (day 14)	8.8 (6.1)	1.8 (1.7)	11.2 (6.0)	0.9 (0.5)	11.0 (5.3)	0.1 (0.1)	38.1 (11.2)	3.1 (3.6)	23.7 (9.3)	2.0 (2.2)	23.5 (6.4)	1.8 (1.5)
t test	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Rats studied on day 15												
Saline	8.9 (2.8)	0.7 (0.4)	11.8 (2.8)	0.5 (0.2)	12.6 (2.8)	0.1 (0.05)	9.8 (2.8)	1.8 (0.7)	12.0 (4.4)	1.4 (0.5)	15.5 (4.8)	0.9 (0.4)
CsA 8 mg/kg	14.2 (3.2) $p < 0.05$	1.6 (0.6) $p < 0.05$	12.6 (3.2)	0.6 (0.4)	10.8 (3.2)	0.1 (0.07)	9.9 (3.2)	2.3 (0.8)	14.7 (5.0)	2.4 (1.5)	15.9 (3.0)	1.1 (0.4)
CsA 16 mg/kg P (cf. saline)	14.4 (3.6) $p < 0.05$	1.0 (0.4)	16.5 (3.0) $p < 0.05$	0.4 (0.2)	15.8 (4.3)	0.1 (0.06)	9.8 (3.9)	2.2 (1.0)	10.1 (3.2)	1.4 (0.8)	12.8 (3.3)	0.8 (0.3)
CsA 32 mg/kg P (cf. saline)	20.4 (4.7) $p < 0.05$	1.4 (0.6)	15.6 (2.9)	0.3 (0.1)	12.2 (4.8)	0.05 (0.04)	12.7 (4.3)	2.9 (1.1)	17.3 (2.4)	2.9 (1.7)	19.6 (5.5)	3.0 (1.2)
ANOVA	$p < 0.001$	$p = 0.007$	$p = 0.02$	NS	NS	NS	NS	NS	NS	NS	NS	NS

3.4. Effect of a single dose of CsA on selective apoptosis of $V\beta 8.2^+$ cells

There was no significant change in the number or percentage of $V\beta 8.2^+$ cells that were apoptotic, after a single injection of CsA (Table 4). For each experiment, we calculated the ratio of the percentage of apoptotic cells that were $V\beta 8.2^+$ to the percentage of apoptotic cells that were $TCR\alpha\beta^+$, and the ratio of the percentage of non-apoptotic cells that were $V\beta 8.2^+$ to the percentage of non-apoptotic cells that were $TCR\alpha\beta^+$. The ratio of these two values was then calculated, and used to express the enrichment of $V\beta 8.2^+$ cells in the apoptotic T cell population compared to the non-apoptotic T cell population. Using this calculation, we have previously shown enrichment of $V\beta 8.2^+$ cells in the apoptotic T cell population in the CNS in MBP-EAE induced by the passive transfer of $V\beta 8.2^+$ MBP-reactive T cells, indicating selective apoptosis of $V\beta 8.2^+$ cells (Tabi et al., 1994). Similar selective apoptosis of $V\beta 8.2^+$ cells also occurs in the CNS in EAE induced by active immunization with MBP and adjuvants (McCombe et al., 1996a), the model used in the present study. We now show that CsA treatment decreases the enrichment of $V\beta 8.2^+$ cells in the apoptotic $CD5^+$ and apoptotic $TCR\alpha\beta^+$ lymphocyte populations (Fig. 3).
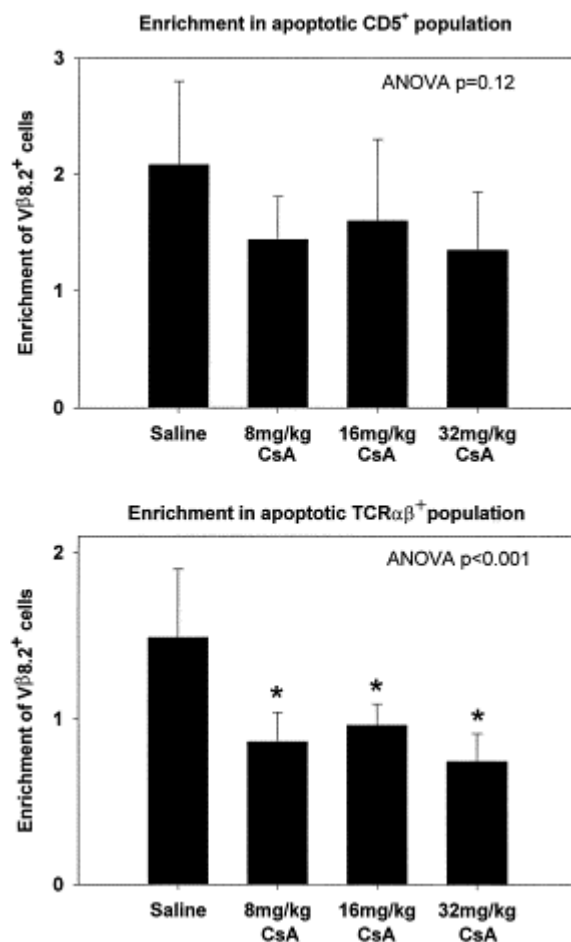
Fig. 3. Ratios of the proportion of apoptotic $CD5^+$ lymphocytes (upper panel) and apoptotic $TCR\alpha\beta^+$ lymphocytes (lower panel) that are $V\beta 8.2^+$ to the proportion of non-apoptotic $CD5^+$ lymphocytes and non-apoptotic $TCR\alpha\beta^+$ lymphocytes that are $V\beta 8.2^+$. This ratio gives an index of selective apoptosis of $V\beta 8.2^+$ cells. The ratio was reduced at all doses of CsA studied, although the results were statistically significant only for the $TCR\alpha\beta^+$ populations (calculated by ANOVA; results that are significantly different from that of the saline-treated group (Tukey test) indicated by asterisks). The decrease was largely due to an increase in the percentages of $CD5^+$ lymphocytes and $TCR\alpha\beta^+$ lymphocytes that were apoptotic without an increase in the percentage of $V\beta 8.2^+$ cells that were apoptotic.

4. Discussion

We assessed the effects of CsA treatment on the clinical course of MBP-EAE in Lewis rats. Saline-treated rats treated followed the usual clinical course of MBP-EAE (Pender, 1988). Treatment with high doses of CsA (16 and 32 mg/kg) caused suppression of the expected first episode of disease, with clinical signs of disease developing after treatment was ceased. Previously, CsA (50 mg/kg) has been shown to suppress EAE induced by inoculation of rats, guinea pigs and monkeys with bovine spinal cord, although some animals developed disease after treatment was ceased (Bolton et al., 1982). Treatment with CsA 4 mg/kg delayed the onset of the disease. Treatment with CsA 8 mg/kg delayed the onset of disease, increased the frequency of second episodes and resulted in 50% of rats having a third episode or following a chronic persistent course. This effect is similar to the effects of low-dose CsA treatment of EAE induced by inoculation with guinea pig spinal cord (Polman et al., 1988; Pender et al., 1990) and of experimental autoimmune neuritis (EAN) induced by inoculation with purified myelin (McCombe et al., 1990). However, the dose of CsA (8 mg/kg) required to cause relapses of MBP-EAE was higher than that (4 mg/kg) required to produce relapses of EAE induced with whole spinal cord.

It is likely that the immunosuppressive actions of CsA (Hess et al., 1988) contribute to the suppression of signs of EAE. However, the ability of CsA to decrease blood–brain barrier permeability (Paul and Bolton, 1995) and the neuroprotective capacity of immunophilins (Sabatini et al., 1997) could also play a role. In earlier studies, Bolton et al. (1982) found that treatment with CsA 50 mg/kg caused a reduction in inflammation in the spinal cord of guinea pigs with EAE. In MBP-EAE, we now show that a single high dose of CsA (32 mg/kg) caused a decline in the percentage of TCR$\alpha\beta^+$ cells in the spinal cord inflammatory infiltrate. This decline in the percentage of T cells in the spinal cord could result from decreased entry of cells to the CNS or increased removal from the CNS.

To determine whether CsA treatment caused increased elimination of cells, we measured apoptosis. During spontaneous recovery from MBP-EAE, there is apoptosis of T cells (Pender et al., 1991 and Pender et al., 1992; Schmied et al., 1993) and macrophages/microglia in the CNS (Nguyen et al., 1994; White et al., 1998a). There is selective apoptosis of Vβ8.2$^+$ MBP-reactive T cells(Tabi et al., 1994 and Tabi et al., 1995; McCombe et al., 1996a), which may be due to CD95-mediated antigen-specific activation-induced apoptosis (White et al., 1998b). Apoptosis of other cells in the CNS in EAE may be due to non-antigen-specific processes such as withdrawal of growth factors or the effects of endogenous corticosteroids (Pender et al., 1992; Nguyen et al., 1994). We now show that apoptosis of inflammatory cells in the spinal cord in MBP-EAE is increased 16 h after treatment with CsA. The percentages of CD5$^+$ cells and TCR$\alpha\beta^+$ cells that were apoptotic were increased. There was no increase in apoptosis of Vβ8.2$^+$ T cells, the predominant encephalitogenic T cells in this form of EAE (Imrich et al., 1995). CsA treatment abrogated the selective apoptosis of Vβ8.2$^+$ cells. We suggest that this indicates that CsA treatment leads to an increase in non-antigen-specific T cell apoptosis at the same time as decreasing antigen-specific activation-induced apoptosis of encephalitogenic T cells.

The observed increase in total T cell apoptosis could be due to effects of CsA on non-antigen-specific apoptosis, for example by suppression of production of cytokines such as IL-2 that are necessary for cell survival. Another mechanism for increased T cell apoptosis is suggested by the finding that CsA can enhance transforming growth factor β mediated apoptosis of lymphocytes (Andjelic et al., 1997). Our observation that CsA impairs the selective apoptosis of Vβ8.2$^+$ T cells in the CNS may be explained by the known ability of CsA to reduce activation-induced apoptosis (Shi et al., 1989), by inhibiting the expression of Fas ligand (Brunner et al., 1996; Latinis et al., 1997). However, in the present study, we did not find a decline in expression of Fas or Fas ligand.

Low-dose CsA may cause relapses of disease by suppressing a mechanism that normally prevents further episodes of disease. We have previously shown that corticosteroid treatment of MBP-EAE increases total T cell apoptosis but reduces apoptosis of Vβ8.2$^+$ cells in the CNS, and leads to relapses of EAE (McCombe et al., 1996b; Nguyen et al., 1997). We therefore considered the possibility that CsA might induce relapses of disease by inhibiting the apoptosis of encephalitogenic T cells in the CNS. However, although we found that CsA treatment decreases the selective apoptosis of Vβ8.2$^+$ cells, there was no decline in the percentage of Vβ8.2$^+$ cells that were apoptotic. Another possible

means by which CsA treatment could lead to relapses of EAE is by impairment of production of downregulatory cytokines. One possibility is IL-10 which is present in lower amounts in the spinal cord of DA rats, which follow a chronic course of MBP-EAE, compared to Lewis rats which have an acute course of disease (Diab et al., 1997). Further studies are required to determine whether alteration of cytokine production is related to the production of relapses in the present model of relapsing MBP-EAE. In conclusion, the present study presents a model of chronic relapsing MBP-EAE in Lewis rats. It also shows that T cell apoptosis in the CNS in EAE can be modulated by the action of CsA.

Acknowledgements

References

Andjelic, S., Khanna, A., Suthanthiran, M. and Nikolic, Z.J., 1997. Intracellular Ca2+ elevation and cyclosporin A synergistically induce TGF-beta 1-mediated apoptosis in lymphocytes. *J. Immunol.* **158**, pp. 2527–2534

Bolton, C., Borel, J.F., Cuzner, M.L., Davison, A.N. and Turner, A.M., 1982. Immunosuppression by cyclosporin A of experimental allergic encephalomyelitis. *J. Neurol. Sci.* **56**, pp. 147–153

Brunner, T., Yoo, N.J., LaFace, D., Ware, C.F. and Green, D.R., 1996. Activation-induced cell death in murine T cell hybridomas. Differential regulation of Fas (CD95) versus Fas ligand expression by cyclosporin A and FK506. *Int. Immunol.* **8**, pp. 017–1026

Dallman, M.J., Thomas, M.L. and Green, J.R., 1984. MRC OX-19: a monoclonal antibody that labels rat T lymphocytes and augments in vitro proliferative responses. *Eur. J. Immunol.* **14**, pp. 260–267

Deibler, G.E., Martenson, R.E. and Kies, M.W., 1972. Large scale preparation of myelin basic protein from central nervous tissue of several mammalian species. *Prep. Biochem.* **2**, pp. 139–165

Diab, A., Zhu, J., Xiao, B.G., Mustafa, M. and Link, H., 1997. High IL-6 and low IL-10 in the central nervous system are associated with protracted relapsing EAE in DA rats. *J. Neuropathol. Exp. Neurol.* **56**, pp. 641–650

Flanagan, W.M., Corthesy, B., Bram, R.J. and Crabtree, G.R., 1991. Nuclear association of a T cell transcription factor blocked by FK-506 and cyclosporin A. *Nature* **352**, pp. 803–807

Hess, A.D., Esa, A.H. and Colombani, P.M., 1988. Mechanisms of action of cyclosporin: effect of cells of the immune system and on subcellular events in T cell activation. *Transplant. Proc.* **20** 2, pp. 29–40 Suppl.

Hess, A.D., Fischer, A.C., Horwitz, L., Bright, E.C. and Laulis, M.K., 1994. Characterization of peripheral autoregulatory mechanisms that prevent development of cyclosporin-induced syngeneic graft-versus-host disease. *J. Immunol.* **153**, pp. 400–411

Hünig, T., Wallny, H.-J., Hartley, J.K., Lawetzky, A. and Tiefenhaler, G., 1989. A monoclonal antibody to a constant determinant of the rat T cell antigen receptor that induces T cell activation. *J. Exp. Med.* **169**, pp. 73–86

Imrich, H., Kugler, C., Torres-Nagel, N., Dorries, R. and Hunig, T., 1995. Prevention and treatment of Lewis rat experimental allergic encephalomyelitis with a monoclonal antibody to the T cell receptor V beta 8.2 segment. *Eur. J. Immunol.* **25**, pp. 1960–1964

Karpus, W.J., Gould, K.E. and Swanborg, R.H., 1992. CD4^{+} suppressor cells of autoimmune encephalomyelitis respond to T cell receptor-associated determinants on effector cells by interleukin-4 secretion. *Eur. J. Immunol.* **22**, pp. 1757–1763

Latinis, K.M., Carr, L.L., Peterson, E.J., Norian, L.A., Eliason, S.L. and Koretzky, G.A., 1997. Regulation of CD95 (Fas) ligand expression by TCR-mediated signaling events. *J. Immunol.* **158**, pp. 4602–4611

McCombe, P.A., van der Kreek, S.A. and Pender, M.P., 1990. The effects of prophylactic cyclosporin A on experimental allergic neuritis (EAN) in the Lewis rat. Induction of relapsing EAN using low-dose cyclosporin A. *J. Neuroimmunol.* **28**, pp. 131–140

McCombe, P.A., de Jersey, J. and Pender, M.P., 1994. Inflammatory cells, microglia and MHC class II antigen-positive cells in the spinal cord of Lewis rats with acute and chronic relapsing experimental autoimmune encephalomyelitis. *J. Neuroimmunol.* **51**, pp. 153–167

McCombe, P.A., Nickson, I., Tabi, Z. and Pender, M.P., 1996. Apoptosis of V beta 8.2[+] T lymphocytes in the spinal cord during recovery from experimental autoimmune encephalomyelitis induced in Lewis rats by inoculation with myelin basic protein. *J. Neurol. Sci.* **139**, pp. 1–6

McCombe, P.A., Nickson, I., Tabi, Z. and Pender, M.P., 1996. Corticosteroid treatment of experimental autoimmune encephalomyelitis in the Lewis rat results in loss of V beta 8.2[+] and myelin basic protein-reactive cells from the spinal cord, with increased total T cell apoptosis but reduced apoptosis of V beta 8.2^{+} cells. *J. Neuroimmunol.* **70**, pp. 93–101

Multiple Sclerosis Study Group, 1990. Efficacy and toxicity of cyclosporin in chronic progressive multiple sclerosis: a randomized, double-blinded, placebo-controlled clinical trial. *Ann. Neurol.* **27**, pp. 591–605

Nguyen, K.B., McCombe, P.A. and Pender, M.P., 1994. Macrophage apoptosis in the central nervous system in experimental autoimmune encephalomyelitis. *J. Autoimmun.* **7**, pp. 145–152

Nguyen, K.B., McCombe, P.A. and Pender, M.P., 1997. Increased apoptosis of T lymphocytes and macrophages in the central and peripheral nervous systems of Lewis rats with experimental autoimmune encephalomyelitis treated with dexamethasone. *J. Neuropathol. Exp. Neurol.* **56**, pp. 58–69

Paul, C. and Bolton, C., 1995. Inhibition of blood–brain barrier disruption in experimental allergic encephalomyelitis by short-term therapy with dexamethasone or cyclosporin A. *Int. J. Immunopharmacol.* **17**, pp. 497–503

Pender, M.P., 1986. Ascending impairment of nociception in rats with experimental allergic encephalomyelitis. *J. Neurol. Sci.* **75**, pp. 317–328

Pender, M.P., 1988. The pathophysiology of myelin basic protein-induced acute experimental allergic encephalomyelitis in the Lewis rat. *J. Neurol. Sci.* **86**, pp. 277–289

Pender, M.P., Stanley, G.P., Yoong, G. and Nguyen, K.B., 1990. The neuropathology of chronic relapsing experimental allergic encephalomyelitis induced in the Lewis rat by inoculation with whole spinal cord and treatment with cyclosporin A. *Acta Neuropathol.* **80**, pp. 172–183.

Pender, M.P., Nguyen, K.B., McCombe, P.A. and Kerr, J.F., 1991. Apoptosis in the nervous system in experimental allergic encephalomyelitis. *J. Neurol. Sci.* **104**, pp. 81–87

Pender, M.P., McCombe, P.A., Yoong, G. and Nguyen, K.B., 1992. Apoptosis of alpha beta T lymphocytes in the nervous system in experimental autoimmune encephalomyelitis: its possible implications for recovery and acquired tolerance. *J. Autoimmun.* **5**, pp. 401–441

Polman, C.H., Matthaei, I., de Groot, C.J., Koetsier, J.C., Sminia, T. and Dijkstra, C.D., 1988. Low-dose cyclosporin A induces relapsing remitting experimental allergic encephalomyelitis in the Lewis rat. *J. Neuroimmunol.* **17**, pp. 209–216

Sabatini, D.M., Lai, M.M. and Snyder, S.H., 1997. Neural roles of immunophilins and their ligands. *Mol. Neurobiol.* **15**, pp. 223–239

Schmied, M., Breitschopf, H., Gold, R., Zischler, H., Rothe, G., Wekerle, H. and Lassmann, H., 1993. Apoptosis of T lymphocytes in experimental autoimmune encephalomyelitis. Evidence for programmed cell death as a mechanism to control inflammation in the brain. *Am. J. Pathol.* **143**, pp. 446–452

Shi, Y.F., Sahai, B.M. and Green, D.R., 1989. Cyclosporin A inhibits activation-induced cell death in T cell hybridomas and thymocytes. *Nature* **339**, pp. 625–626

Tabi, Z., McCombe, P.A. and Pender, M.P., 1994. Apoptotic elimination of Vβ8.2$^+$ cells from the central nervous system during recovery from experimental autoimmune encephalomyelitis induced by the passive transfer of Vβ 8.2$^+$ encephalitogenic T cells. *Eur. J. Immunol.* **24**, pp. 2609–2617

Tabi, Z., McCombe, P.A. and Pender, M.P., 1995. Antigen-specific down-regulation of myelin basic protein-reactive T cells during spontaneous recovery from experimental autoimmune encephalomyelitis: further evidence of apoptotic deletion of autoreactive T cells in the central nervous system. *Int. Immunol.* **7**, pp. 967–973

Telford, W.G., King, L.E. and Fraker, P.J., 1991. Evaluation of glucocorticoid-induced DNA fragmentation in mouse thymocytes by flow cytometry. *Cell. Prolif.* **24**, pp. 447–459

Torres Nagel, N.E., Gold, D.P. and Hünig, T., 1993. Identification of rat TCRβ-V8.2, 8.5, and 10 gene products by monoclonal antibodies. *Immunogenetics* **37**, pp. 305–308

White, C.A., McCombe, P.A. and Pender, M.P., 1998. Microglia are more susceptible than macrophages to apoptosis in the central nervous system in experimental autoimmune encephalomyelitis through a mechanism not involving Fas (CD95). *Int. Immunol.* **10**, pp. 935–941

White, C.A., McCombe, P.A. and Pender, M.P., 1998. The roles of Fas, Fas ligand and Bcl-2 in T cell apoptosis in the central nervous system in experimental autoimmune encephalomyelitis. *J. Neuroimmunol.* **82**, pp. 47–55