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Corticosteroid Treatment Of Experimental Autoimmune Encephalomyelitis In The Lewis Rat Results in Loss of Vβ 8.2$^+$ And Myelin Basic Protein-Reactive Cells from the Spinal Cord, with Increased Total T-Cell Apoptosis but Reduced Apoptosis of Vβ8.2$^+$ Cells

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Abstract

We have studied the effects of corticosteroid treatment on the numbers of lymphocytes obtained from the spinal cords of Lewis rats with acute experimental autoimmune encephalomyelitis (EAE) induced by inoculation with myelin basic protein (MBP) and adjuvants. Flow cytometric studies showed that treatment with dexamethasone (4 mg/kg) 8–12 h prior to study on day 14 after inoculation resulted in a reduction in the numbers of CD5$^+$, TCR$\alpha\beta^+$ and Vβ8.2$^+$ cells in the spinal cord. Limiting dilution analysis indicated that dexamethasone treatment 12 h prior to study on day 12 after inoculation reduced the frequencies of MBP-reactive and interleukin-2-responsive lymphocytes in the spinal cord to low levels, but reduced the frequency of concanavalin-A-responsive lymphocytes to a lesser extent. Using propidium iodide staining of nuclear chromatin we also studied lymphocyte apoptosis. Greater numbers of apoptotic cells were found in the cells extracted from the spinal cords of rats, examined on day 14, that had been treated 1–12 h previously with dexamethasone, than in saline-treated controls. This increased level of apoptosis was observed in the CD5$^+$ and TCR$\alpha\beta^+$ cell populations. At 1–4 h after dexamethasone treatment there was a reduction in the selective apoptosis of Vβ8.2$^+$ cells that normally occurs during spontaneous recovery from EAE. Therefore apoptosis of Vβ8.2$^+$ cells cannot explain the reduction in the numbers of Vβ8.2$^+$ cells and MBP-reactive cells in the CNS after dexamethasone treatment. By 8–12 h after dexamethasone treatment the selectivity of the apoptotic process was restored. These studies suggest that a reduction in the number of T-lymphocytes in the central nervous system contributes to the beneficial effects of corticosteroids in EAE.

Keywords: apoptosis; corticosteroids; experimental autoimmune encephalomyelitis; multiple sclerosis; recovery; T-lymphocytes

1. Introduction

Experimental autoimmune encephalomyelitis (EAE) is mediated by CD4$^+$ T-lymphocytes and can be induced in susceptible animals by active inoculation with myelin antigens and adjuvants or by the passive transfer of T-lymphocytes sensitized to these antigens. EAE is the best available animal model of multiple sclerosis. Recovery from EAE is associated with loss of T-lymphocytes from the central nervous system (CNS) (Matsumoto and Fujiwara, 1987; McCombe et al., 1992; Zeine and Owens, 1993) and apoptosis of these lymphocytes in the CNS (Pender et al., 1991 and Pender et al., 1992; Schmied et al., 1993). In the Lewis rat, the encephalitogenic lymphocytes in EAE induced by inoculation with myelin basic protein (MBP) (MBP–EAE) are predominantly Vβ8.2$^+$ (Offner et al., 1993; Tsuchida et al., 1993; Imrich et al., 1995). In MBP–EAE induced by the passive transfer of Vβ8.2$^+$ MBP-specific

T-lymphocytes or by active inoculation with purified MBP and adjuvants, we have shown that the disease-relevant Vβ8.2$^+$ MBP-reactive T-lymphocytes are selectively eliminated from the CNS by apoptosis (Tabi et al., 1994 and Tabi et al., 1995; McCombe et al., 1996). The aim of the present study was to determine whether exogenous corticosteroids, which are able to suppress the clinical signs of EAE and which are widely used in the treatment of multiple sclerosis, lead to a reduction in the number of Vβ8.2$^+$ lymphocytes and MBP-reactive T-cells in the CNS in EAE, and whether this is associated with increased T-cell apoptosis. To simplify the analysis, we examined the effects on T-lymphocytes of a single dose of dexamethasone.

2. Experimental

2.1. Animals

Lewis rats (JC strain; 20 female, 167 male), aged 8–10 weeks, were obtained from the animal breeding facility 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 (CSL, Melbourne, Australia) containing 4 mg/ml *Mycobacterium butyricum* (Difco). 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

Rats were examined every morning. Weakness was assessed using the system of Pender (1986). Tail weakness was assessed by holding the animal by the base of the tail and observing tail movement. It was graded as follows: 0=no weakness; 1=weakness of the distal part of the tail only, the distal tail failing to curl around the examiner's finger; 2=weakness of the whole tail but with the proximal tail still able to be erected vertically against gravity; 3=severe weakness with only a flicker of tail movement; 4=complete flaccid paralysis of the tail. Hindlimb weakness was graded as follows: 0=no weakness; 1=slight dragging of toes of both hindfeet; 2=severe dragging of both hindfeet but not the rest of the hindlimbs; 3=severe dragging of both hindlimbs, often with both hindlimbs displaced to one side of the body; 4=total flaccid paralysis of the hindlimbs. The forelimbs were assessed in a similar way to the hindlimbs. The scores for the tail, hindlimbs and forelimbs were added to obtain a total clinical score (maximum=12).

2.4. Dexamethasone or saline treatment

Dexamethasone (David Bull Laboratories, Mulgrave, Victoria) was administered by subcutaneous injection at a dose of 4 mg/kg. Control rats were given the same volume of normal saline or were untreated. In a small clinical study of the effects of daily dexamethasone treatment, rats were given daily dexamethasone or saline injections for 5 days from the day of onset of signs. For the limiting dilution and flow cytometry studies, rats were given a single dose of dexamethasone or saline, 1–12 h before study on the morning of day 12 or day 14 after inoculation.

2.5. Extraction of cells from the spinal cord

Cells were extracted from the pooled spinal cords of groups of rats that had been untreated, saline-treated or dexamethasone-treated. As shown in Table 1, 2–6 groups of rats were studied for each type of treatment. Using our previously described methods (Tabi et al., 1994), cells were isolated from the spinal cords of rats perfused with ice-cold saline under anaesthesia. The entire spinal cords were removed by insufflation and weighed, and a single cell suspension of the pooled spinal cords in ice-cold RPMI containing 1% foetal calf serum was prepared by passage 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. The cells were then passed through a nylon wool column.

Table 1
Details of rats used in flow cytometry studies

Experiment	Number of groups studied	Total number of rats studied	Mean total clinical score on day before study (day 11 or day 13)	Mean total clinical score [a] on day of study
Rats studied on day 12				
Controls (no treatment)	3	15	3.4 ± 1.9	4.5 ± 2.3
Saline				
Treated 12 h before study	2	10	1.8 ± 1.9	2.8 ± 2.0
Dexamethasone				
Treated 12 h before study	2	11	1.7 ± 1.0	1.8 ± 1.7
Rats studied on day 14				
Controls (no treatment)	3	14	4.5 ± 2.3	5.3 ± 2.7
Saline				
Treated 12 h before study	3	13	4.5 ± 1.8	5.5 ± 2.0
Dexamethasone				
Treated 1 h before study	4	19	5.5 ± 2.0	6.3 ± 2.0
Treated 2 h before study	6	31	4.7 ± 2.2	5.2 ± 2.2
Treated 3 h before study	3	17	6.1 ± 2.3	6.8 ± 2.3
Treated 4 h before study	3	15	4.3 ± 1.5	5.6 ± 2.1
Treated 8 h before study	5	28	5.2 ± 2.2	7.4 ± 2.7
Treated 12 h before study	3	14	4.8 ± 2.7	6.3 ± 3.5

[a] The mean total clinical score is the mean of the scores of each rat given a particular type of treatment.
For the flow cytometry study the cells from the spinal cords of groups were pooled.

2.6. Monoclonal antibodies

Mouse monoclonal antibodies specific for CD5 (OX19), the $\alpha\beta$ T-cell receptor (R73) and CD45 (OX1) were obtained from Dr J. Sedgwick (Sydney, Australia). Antibody to Vβ8.2 (Torres Nagel et al., 1993) was kindly provided by Dr T. Hünig (Würzburg, FRG). Mouse IgG1 κ (Dako) was used as an isotype control antibody. The secondary antibody was fluoroscein isothiocyanate (FITC)-conjugated F(ab′)2 rabbit anti-mouse IgG (STAR 41) (Serotec).

2.7. Labelling of cells and flow cytometric analysis

Using our previously described methods (Tabi et al., 1994), cells were labelled with monoclonal antibodies or permeabilized and labelled with propidium iodide for analysis of apoptosis according to the method described by Telford et al. (1991). Briefly, aliquots of 10^5–10^6 cells were washed with a 1:1 solution of serum in phosphate-buffered saline (PBS)

containing 1% foetal calf serum and 0.1% sodium azide, incubated with primary antibodies in PBS/azide for 30 min at 4°C. Cells were washed, incubated with secondary antibody for 30 min at 4°C in the dark and then washed twice with PBS to remove proteins in solution. Cells were resuspended in 1 ml of ice-cold 50% ethanol and left to fix overnight at 4°C. The following day, the ethanol was removed by washing the cells in PBS, and the cell pellets were resuspended in an appropriate volume (100–300 μl) of propidium iodide staining solution (freshly prepared by diluting stock solution [5 mg/ml RNase/250 μg/ml propidium iodide 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 analysed using a Becton Dickinson FACScan within 1 h.

Flow cytometric data was analyzed using Lysis II Software (Becton Dickinson). The percentage of antibody-positive cells was determined from histograms of FITC fluorescence intensity. Background fluorescence, obtained by labelling the cells with isotype control antibodies and FITC, was subtracted from the test values. To analyse levels of apoptosis, histograms of propidium iodide fluorescence were obtained. Debris with low levels of fluorescence was excluded by gating. Apoptotic events were defined as those having lower fluorescence than the sharply defined G_0/G_1 peak (Telford et al., 1991). We have previously shown by cell sorting that cells with low propidium iodide fluorescence characteristic of apoptosis also have DNA fragmentation characteristic of apoptosis. When there were many apoptotic events, the G_0/G_1 peak was defined in control samples.

2.8. Enrichment of Vβ8.2$^+$ cells in the apoptotic lymphocyte populations

To estimate the selectivity of apoptosis of Vβ8.2$^+$ T-cells, we compared the percentage of apoptotic T-lymphocytes that were Vβ8.2$^+$ to the percentage of non-apoptotic T-lymphocytes that were Vβ8.2$^+$ using our previously described methods (Tabi et al., 1994; McCombe et al., 1996). This comparison was expressed as a mean ratio for each type of treatment and represents the enrichment of Vβ8.2$^+$ cells in the apoptotic lymphocyte populations. We assumed that the Vβ8.2$^+$ cells in the CNS in EAE are CD5$^+$ and TCR $\alpha\beta^+$. For each group of rats that had received similar treatment, the ratios of the percentage of apoptotic cells that were Vβ8.2$^+$ to the percentages of apoptotic cells that were CD5$^+$ or TCR$\alpha\beta^+$ were calculated. In a similar fashion, the ratios of the non-apoptotic cells that were Vβ8.2$^+$ to the percentages of non-apoptotic cells that were CD5$^+$ or TCR$\alpha\beta^+$ were calculated. The enrichment of Vβ8.2$^+$ cells in the apoptotic compared to the non-apoptotic T-cell population was then calculated by dividing the ratio for the apoptotic cells by the ratio for the non-apoptotic cells. For each type of treatment, we then calculated the mean enrichment.

2.9. Limiting dilution analysis

A suspension of cells was prepared from the spinal cord as described above. Plastic adherent cells were removed by incubating the CNS cell suspensions at 10^5–10^6 cells/ml in 100 mm diameter polystyrene Petri dishes (Corning) for 45 min at 37°C. Non-adherent cells were collected by vigorously pipetting the cells and rinsing the dishes with RPMI 1640 supplemented with 216 mg/l L-glutamine (Gibco), 100 IU penicillin, 100 μg/ml streptomycin (Sigma), 0.1 mM Na-pyruvate (Gibco), non-essential amino acids (Gibco), 36 mg/l L-asparagine (Sigma), 5×10^{-5} M β-mercaptoethanol (Sigma), 5 μg/ml Fungizone (Squibb) and 5% heat-inactivated horse serum (Trace Biosciences). Limiting dilution analysis was carried out as previously described (Tabi et al., 1995). Briefly, 12–24 replicates of increasing numbers of cells extracted from the CNS and 5×10^5 irradiated thymic cells as antigen-presenting cells were incubated with 15 μg/ml MBP, 50 U/ml mouse recombinant interleukin-2 (IL-2) (Boehringer Mannheim) or 5 μg/ml Concanavalin-A (Con-A) (Sigma). Control wells

contained the same numbers of spinal cord cells as the MBP or IL-2 stimulated wells and 5×10^5 irradiated thymic cells in the absence of antigen or mouse recombinant IL-2. For the Con-A stimulated proliferation, the control wells contained irradiated thymic cells alone with Con-A. MBP and IL-2 stimulated wells and their controls were supplemented with 10 U/ml IL-2 (MLA 144 supernatant) 24 h after the commencement of the assay and were incubated for 7 days. Con-A stimulated cultures and their controls were not supplemented with IL-2 and were incubated for 5 days. For the last 12 h of incubation, ^{3}H-thymidine was added to the wells. Wells were scored as positive if the thymidine incorporation was at least twice background.

3. Results

3.1. Clinical effects of daily dexamethasone treatment

Rats with MBP–EAE that were given daily injections of dexamethasone from the day of onset of signs had less severe clinical signs and earlier onset of clinical recovery than rats treated with daily saline injections.

3.2. Clinical effects of a single dose of dexamethasone prior to study on day 12 or day 14

The clinical scores of rats used for the flow cytometry study are shown in Table 1. The rats were examined on the morning of each day and the clinical scores are shown for the day before study and the day of study. Dexamethasone injections were given 1–12 h before sacrifice for study on the mornings of day 12 or 14 after inoculation. Treatment with dexamethasone 12 h prior to day 12 appeared to reduce the progression of clinical signs of disease from day 11 to day 12. Treatment with dexamethasone 12 h prior to day 14 did not arrest progression of clinical signs of disease from day 13 to day 14.

3.3. Numbers of lymphocytes in the spinal cord after dexamethasone treatment

The numbers of lymphocytes in the spinal cords of rats were studied on days 12 and 14 at varying times after a single dose of dexamethasone, after saline treatment or after no treatment. On day 12 (see Fig. 1), rats that had been treated 12 h previously with dexamethasone (4 mg/kg) had few $CD5^+$ cells and few $V\beta8.2^+$ cells in the spinal cord compared to those that had received no treatment or had received saline 12 h previously.

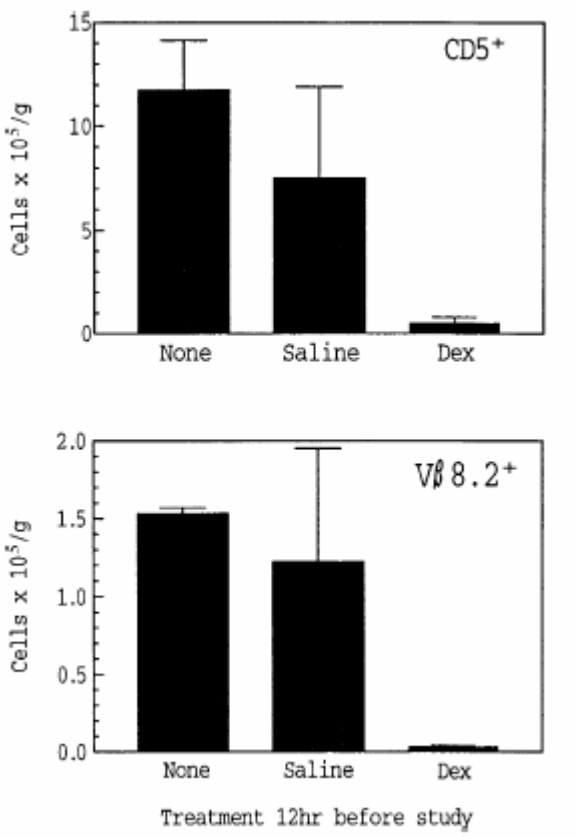

Fig. 1. Numbers of $CD5^+$ and $V\beta8.2^+$ cells/g of spinal cords of rats on day 12 after inoculation with MBP and adjuvants. The numbers of lymphocytes were measured by flow cytometry. 12 h prior to study on the morning of day 12, rats were given no treatment, saline treatment or dexamethasone (4 mg/kg) treatment (dex=dexamethasone).

In rats studied on day 14, we compared the effects of no prior treatment, treatment with saline 12 h previously and treatment with dexamethasone 1, 2, 3, 4, 8 and 12 h prior to study. Fig. 2 shows the numbers of $CD5^+$ cells, $TCR\alpha\beta^+$ cells and $V\beta8.2^+$ cells in untreated rats, rats treated with dexamethasone and rats treated with saline. The numbers of $CD5^+$ cells, $TCR\alpha\beta^+$ cells and $V\beta8.2^+$ cells had declined considerably by 8 h after dexamethasone treatment. At earlier time points (1–4 h), the decline mainly affected the $V\beta8.2^+$ population.

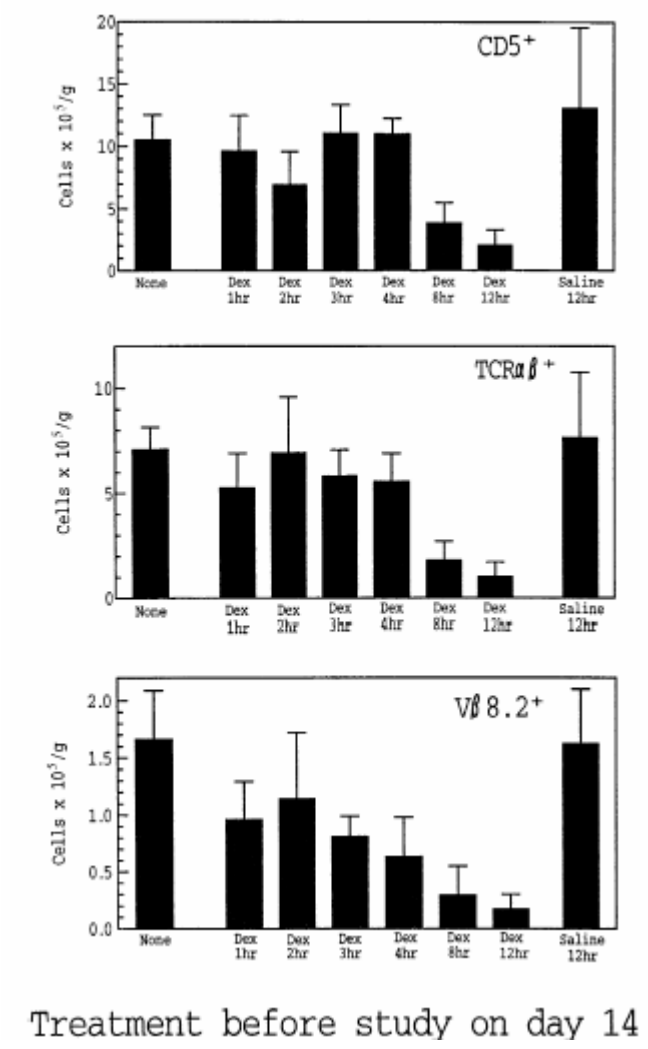

Fig. 2. Numbers of $CD5^+$, $TCR\alpha\beta^+$ and $V\beta 8.2^+$ cells/g of spinal cord of rats on day 14 after inoculation with MBP and adjuvants. The numbers of lymphocytes were measured by flow cytometry. Rats were given no treatment, saline treatment 12 h prior to study, or dexamethasone treatment 1–12 h prior to study on day 14 (dex=dexamethasone).

3.4. Limiting dilution analysis of lymphocyte frequencies in the spinal cord after treatment with dexamethasone

The frequency of MBP-reactive cells was studied on days 12 and 14. Table 2 shows the frequencies of cells in groups of rats, studied on day 12, that had been given saline or dexamethasone 12 h previously. After dexamethasone treatment there was a major decline in the frequency of MBP-reactive cells while the frequency of Con-A-responsive cells remained relatively high. The frequency of IL-2-responsive cells also declined considerably after dexamethasone treatment. We performed a similar study on day 14 and found that the frequency of MBP-reactive cells in a saline-treated rat was 1/19703, while in a dexamethasone-treated rat it was 1/114262.

Table 2
Limiting dilution analysis of the frequency of MBP-reactve cells, Con-A responsive cells and IL-2 responsive cells on day 12 after inoculation with MBP and adjuvants

Experiment	Number of rats	Mean total clinical score on day 11	Mean total clinical score on day 12	Frequency of MBP-reactive cells	Frequency of Con-A responsive cells	Frequency of IL-2 responsive cells
No treatment	3	2.7	4.3	1/326	1/112	not measured
12 h after dexamethasone (first experiment)	4	2.3	3.3	< 1/137 920	1/500	not measured
12 h after dexamethasone (second experiment)	7	1.2	0.5	< 1/114 932	1/304	< 1/114 932
12 h after saline	4	1.8	2.3	1/3062	> 1/50	1/848

3.5. Numbers of apoptotic cells in the spinal cord after treatment with dexamethasone

The studies detailed above indicate that corticosteroid treatment results in a reduction in the number of T-lymphocytes in the spinal cord of rats with EAE. To determine whether the reduction in cell numbers was associated with increased levels of apoptosis, we used flow cytometry to measure numbers of apoptotic cells. We were unable to perform these studies on day 12, because insufficient cells remained after dexamethasone treatment to permit analysis of apoptosis. The percentages, on day 14, of all the cells extracted from the spinal cord that were apoptotic in untreated rats, dexamethasone-treated rats and saline-treated rats are shown in Fig. 3. The percentages of cells that were apoptotic were increased in the rats given dexamethasone 1–12 h prior to study compared to the untreated and saline-treated animals. Fig. 4 shows the percentage of cells of different types that were apoptotic in untreated rats and in rats given prior treatment with dexamethasone or saline. In untreated rats and in saline-treated rats, the percentages of Vβ8.2$^+$ cells that were apoptotic were greater than the percentages of CD5$^+$ and TCR$\alpha\beta^+$ cells that were apoptotic, indicating that there is selective apoptosis of the disease-relevant Vβ8.2$^+$ cells in this form of EAE, as we have shown previously (McCombe et al., 1996). One to 8 h after dexamethasone treatment there were increases in the percentages of CD5$^+$ cells and TCR$\alpha\beta^+$ cells that were apoptotic. The percentage of Vβ8.2$^+$ cells that were apoptotic was decreased 1–4 h after dexamethasone but was increased at 8–12 h.

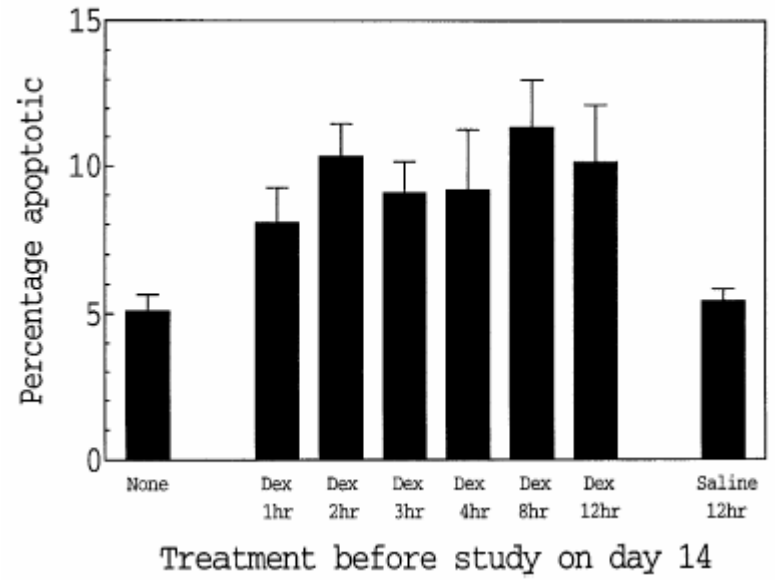

Fig. 3. Percentages of the cells extracted from the spinal cord that were apoptotic on day 14 after inoculation of rats with MBP and adjuvants. Rats were given no treatment, saline treatment 12 h prior to study, or dexamethasone treatment 1–12 h prior to study on day 14. In the rats that received dexamethasone treatment 1–12 h prior to study, the percentages of cells that were apoptotic were increased compared to the untreated and saline-treated rats (dex=dexamethasone).

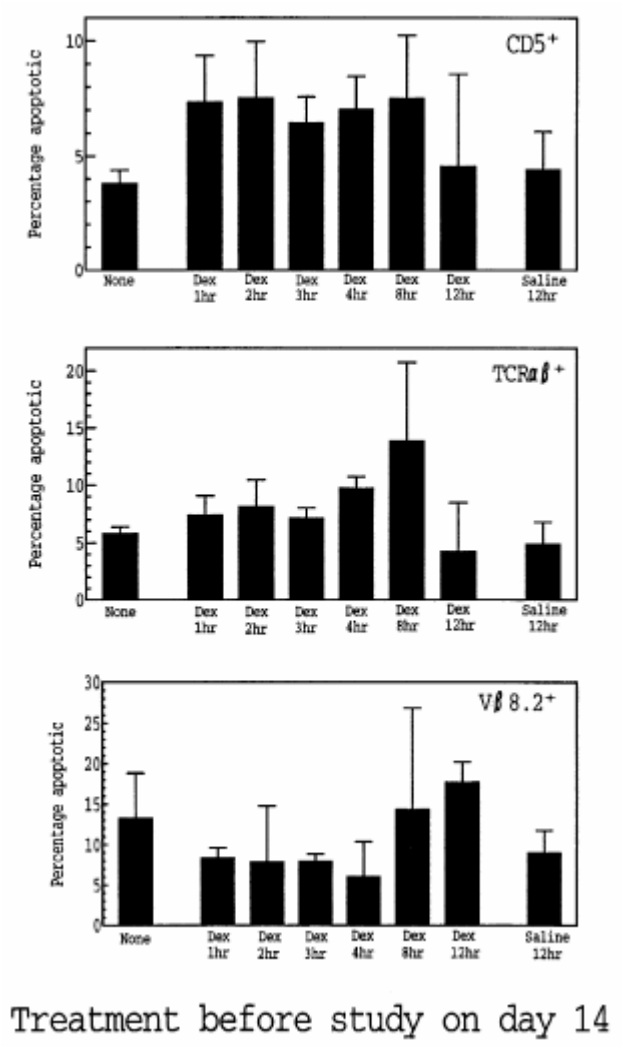

Fig. 4. Percentages of $CD5^+$, $TCR\alpha\beta^+$ $V\beta8.2^+$ cells that were apoptotic in the spinal cord of rats studied on day 14 after inoculation with MBP and adjuvants. Rats were given no treatment, saline treatment 12 h prior to study, or dexamethasone treatment 1–12 h prior to study on day 14 (dex=dexamethasone).

To analyse further the effects of dexamethasone on the selective apoptosis of $V\beta8.2^+$ cells, we calculated the enrichment of $V\beta8.2^+$ cells in the apoptotic, compared to the non-apoptotic $CD5^+$ and $TCR\alpha\beta^+$ populations (Fig. 5). In untreated rats and in saline-treated rats, there was a 2–3 fold enrichment of $V\beta8.2^+$ cells in the apoptotic population, once again indicating selective apoptosis of these disease-relevant cells. At 1–4 h after dexamethasone treatment the enrichment of $V\beta8.2^+$ cells in the apoptotic populations was reduced to a level of unity (that is, no enrichment). By 8–12 h the enrichment was restored to that observed in saline-treated controls.

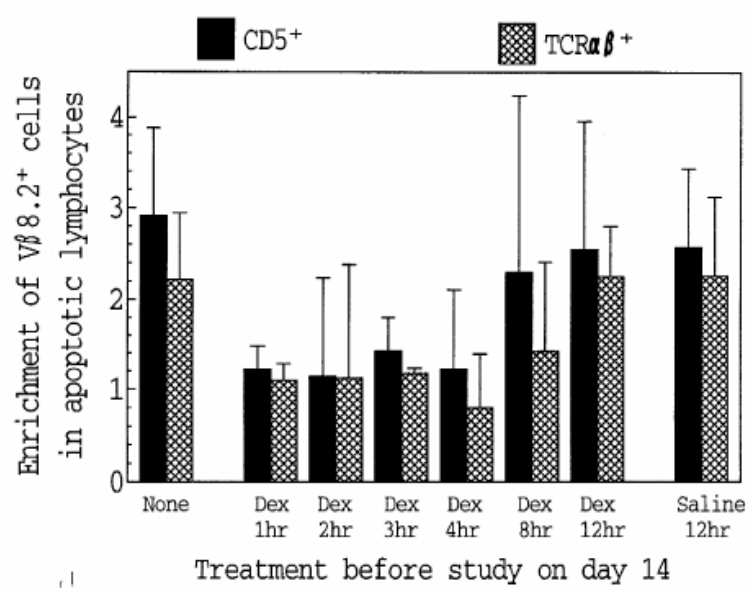

Fig. 5. Enrichment of Vβ8.2$^+$ cells in the apoptotic CD5$^+$ and TCR$\alpha\beta^+$ populations of cells from the spinal cords of rats studied on day 14 after inoculation with MBP and adjuvants. Rats were given no treatment, saline treatment 12 h prior to study, or dexamethasone treatment 1–12 h prior to study on day 14 (dex=dexamethasone).

Fig. 6 shows the numbers of cells of different types that were apoptotic on day 14 at different times after dexamethasone treatment. In rats given dexamethasone 1–4 h previously, there was an increase in the number of apoptotic CD5$^+$ cells and a decrease in the number of apoptotic Vβ8.2$^+$ cells. The number of apoptotic TCR$\alpha\beta^+$ cells was increased 4 h after dexamethasone treatment. At 8 and 12 h after dexamethasone treatment, the numbers of CD5$^+$, TCR$\alpha\beta^+$ and Vβ8.2$^+$ apoptotic cells were all reduced, reflecting the reduced numbers of cells remaining in the CNS (see Fig. 2).

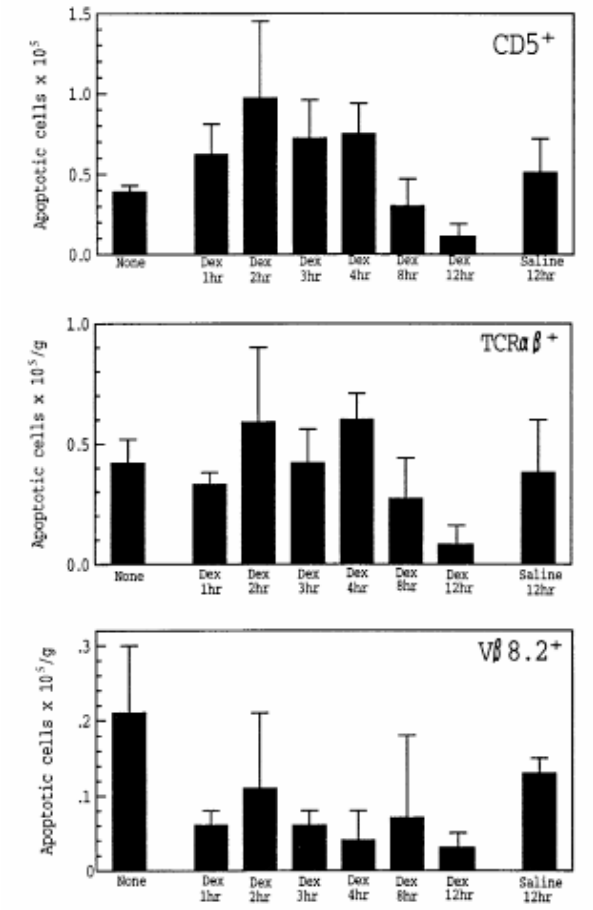

Fig. 6. Numbers of apoptotic $CD5^+$, $TCR\alpha\beta^+$ and $V\beta8.2^+$ cells/g of spinal cord of rats studied at day 14 after inoculation with MBP and adjuvants. Rats were given no treatment, saline treatment 12 h prior to study, or dexamethasone treatment 1–12 h prior to study on day 14. (dex=dexamethasone).

4. Discussion

The development of EAE in Lewis rats is associated with infiltration of the nervous system by T-lymphocytes and macrophages (Hickey et al., 1983; McCombe et al., 1992), followed by demyelination of the central nervous system and the nerve roots. We have recently shown that recovery from passively transferred and actively induced MBP–EAE is associated with a selective elimination of $V\beta8.2^+$ MBP-reactive T-cells from the spinal cord by apoptosis (Tabi et al., 1994 and Tabi et al., 1995; McCombe et al., 1996) and have suggested that this apoptosis may represent activation-induced cell death of T-lymphocytes in the target organ of this autoimmune disease (Pender et al., 1992; Tabi et al., 1994 and Tabi et al., 1995).

In the present experiments, we have studied the effects of corticosteroids on T-cells in the nervous system in EAE. Endogenous corticosteroid production is thought to be important in spontaneous recovery from EAE (Levine et al., 1980; MacPhee et al., 1989). Therapy with exogenous corticosteroids has also been shown to be of clinical benefit in EAE (Kibler, 1965; Vogel et al., 1972). We found that daily high-dose corticosteroid treatment of rats with MBP–EAE leads to less severe clinical signs and earlier recovery from signs of disease. To simplify the assessment of the effects of exogenous dexamethasone on the T-cells in the CNS in MBP–EAE, we used rats treated with a single dose of dexamethasone. In these rats there was a

reduction in the numbers of T-lymphocytes, including Vβ8.2$^+$ and MBP-reactive lymphocytes, in the spinal cord. Such corticosteroid-induced reduction in T-cell infiltration of the nervous system could lead to clinical benefits by reducing ongoing demyelination, thus allowing the remyelination which normally occurs during recovery from EAE (Pender, 1989) to restore neurological functions.

The action of glucocorticoids is mediated by a cytoplasmic receptor molecule that enters the nucleus and has multiple effects on gene activation (de Waal, 1994). Corticosteroids have many effects on inflammation and immune function, such as effects on vascular endothelium, adhesion molecule expression, cytokine production, T-cell activation and T-cell apoptosis (de Waal, 1994). The number of T-lymphocytes in the CNS at any time will be influenced both by the entry of new cells into the CNS, by apoptosis of T-lymphocytes within the CNS and by the exit of lymphocytes from the CNS. Thus, the reduction in the numbers of lymphocytes in the CNS in EAE after corticosteroid treatment could result from a number of different mechanisms, including peripheral effects on T-cell activation, reduction of entry of T-lymphocytes into the CNS and by increased T-cell apoptosis. Because of the importance of T-cell apoptosis in the CNS in spontaneous recovery from EAE, we studied the effects of corticosteroids on apoptosis. We measured apoptotic cells in the CNS after dexamethasone treatment and found that the percentage of T-lymphocytes that were apoptotic was increased compared to controls. We did not attempt to assess peripheral effects of dexamethasone. Corticosteroids are known to cause apoptosis of mature T-lymphocytes in vitro (Nieto and Lopez-Rivas, 1989; Zubiaga et al., 1992; Tuosto et al., 1994), and an increase in T-lymphocyte apoptosis in the CNS in EAE after dexamethasone treatment is consistent with these known effects of corticosteroids.

After corticosteroid treatment there was an early decline in the percentage of Vβ8.2$^+$ cells that were apoptotic and in the number of apoptotic Vβ8.2$^+$ cells in the CNS. Furthermore, at 1–4 h after dexamethasone treatment, Vβ8.2$^+$ cells were not selectively eliminated by the apoptotic process, as they are during spontaneous recovery from EAE. Therefore, the early reduction in the number of Vβ8.2$^+$ cells in the CNS cannot be explained by increased apoptosis of these cells. The marked reduction in the number of Vβ8.2$^+$ cells and in the frequency of MBP-reactive cells in the CNS after dexamethasone treatment may result from reduced entry of these cells into the CNS or from their increased exit. By 8–12 h after dexamethasone treatment the apoptotic elimination of Vβ8.2$^+$ cells was again selective.

We have suggested that the T-cell apoptosis that occurs during spontaneous recovery from EAE is due to activation-induced apoptosis which is dependent on engagement of the T-cell receptor in the CNS (Pender et al., 1992; Tabi et al., 1994 and Tabi et al., 1995). Glucocorticoids antagonize activation-induced apoptosis, probably by reducing the expression of the Fas ligand (Zacharchuk et al., 1990; Iseki et al., 1991; Yang et al., 1995), and we suggest that such an effect occurs after corticosteroid treatment of EAE and explains the reduction in the selective apoptosis of Vβ8.2$^+$ cells. Indeed, the observation that the selective apoptosis of Vβ8.2$^+$ cells is steroid-sensitive is support for the suggestion that this apoptosis is activation-induced.

This study of corticosteroid treatment of EAE may have implications for the treatment of multiple sclerosis. High dose methylprednisolone therapy accelerates recovery from relapses of multiple sclerosis (Durelli et al., 1986; Milligan et al., 1987). In optic neuritis, low-dose oral prednisone does not accelerate clinical recovery and may lead to an increased risk of relapse, whereas treatment with high dose methylprednisolone accelerates recovery and may reduce the risk of relapse (Beck et al., 1992). The present study suggests that corticosteroid treatment may temporarily inhibit the selective apoptosis of disease-producing T-cells in the CNS in inflammatory demyelinating disease. It is not known whether apoptosis of disease-

causing T-cells occurs in the CNS in multiple sclerosis. Indeed, we have hypothesized that a failure of this mechanism may predispose to the development of chronic autoimmune disease (Tabi et al., 1995). However, if activation-induced apoptosis of disease-producing T-cells in the CNS has a role in recovery from episodes of multiple sclerosis, impairment of such apoptosis by corticosteroid therapy would be undesirable. The dose and timing of corticosteroid therapy might then be important in determining whether this therapy is beneficial or harmful in multiple sclerosis.

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