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The effect of cyclosporin A on the adoptive transfer of experimental allergic encephalomyelitis in the Lewis rat

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SUMMARY

Experimental allergic encephalomyelitis (EAE) can be adoptively transferred in Lewis rats with spleen cells from immunized animals, after culture with concanavalin A or myelin basic protein (MBP). The effect of the immunosuppressive drug cyclosporin A (CsA) on the *in vitro* and *in vivo* steps of the cell transfer has been investigated. Clinical signs of EAE were completely suppressed by CsA in rats immunized with MBP in Freund's complete adjuvant and spleen cells from these animals, cultured with the antigen, did not transfer the disease. The incidence of transferred disease was also reduced, if recipients were treated with CsA, although a higher dose of drug than that needed to suppress active EAE was required. In both instances complete suppression of EAE was only accomplished for the period of dosing, although the clinical signs of disease which appeared after withdrawal of the drug were significantly reduced in severity. These results imply that an immune response in the host animal is a prerequisite for adoptive transfer of EAE or suggest that CsA can regulate the action of lymphocytes already primed.

INTRODUCTION

The fungal metabolite cyclosporin A (CsA) which has selective anti-lymphocytic activity in both humoral and cell-mediated allergic reactions (Borel *et al.*, 1976; Wiesinger & Borel, 1979), has been found to suppress the autoimmune disease experimental allergic encephalomyelitis (EAE) in guinea pigs (Bolton & Cuzner, 1980), rats (Levine & Sowinski, 1977; Borel, 1976) and primates (Borel, personal communication).

In the guinea pig clinical signs of EAE have been passively transferred to normal syngeneic recipients by large numbers of sensitized lymph node cells (Stone, 1961), whereas in the Lewis rat it has only been possible to transfer histological signs of disease (Paterson & Hanson, 1969). Relatively small numbers of spleen cells have been shown to transfer full clinical signs of EAE if cultured with the mitogen concanavalin A (Con A) (Panitch & McFarlin, 1977) or with the antigen myelin basic protein (MBP), prior to transfer (Richert *et al.*, 1979).

Paterson & Hanson (1969) described the inhibition of EAE in actively sensitized Lewis rats given single or multiple injections of cyclophosphamide. They also showed that passive transfer of

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histological disease was completely inhibited using lymph node cells from cyclophosphamide-treated donors. In addition, recipients treated with the drug before or after injection of sensitized donor cells failed to develop histological signs of EAE.

Similarly, we have examined the effect of CsA on the adoptive transfer of EAE in the Lewis rat. Spleen cells, when cultured in the presence of CsA, did not transfer clinical signs of disease. Likewise, recipients treated with CsA only developed reduced clinical signs of EAE after withdrawal of the drug.

MATERIALS AND METHODS

Experimental animals. Female Lewis rats (100–150 g) obtained from Bantin and Kingman Ltd (Aldborough, Hull) were fed FFG (M) diet with water *ad lib.*

Induction of active EAE. EAE was induced in the rat by injecting a water-in-oil emulsion containing 50 μg guinea pig MBP made according to the method of Deibler, Martenson and Kies (1972) and 500 μg *Mycobacterium tuberculosis* (DIFCO $H_{37}Ra$) into the front footpads (0·05 ml per footpad).

Induction of adoptive EAE with cultured spleen cells. At day 12 after induction of active EAE the rats were killed and their spleens removed, chopped over a fine wire mesh and washed with medium. RPMI 1640 (GIBCO) with 2 mM L-glutamine, 100 units penicillin and 100 μg streptomycin ml^{-1} buffered with sodium bicarbonate and HEPES was used throughout to wash and culture the cells. The spleen cells were placed in culture at a concentration of 2×10^6 cells ml^{-1} in 75-cm^2 Falcon tissue-culture flasks containing 5% heat-inactivated fetal calf serum (Seralab) and 10^{-5} M 2-mercaptoethanol. In addition, the mitogen Con A (1 μg ml^{-1}) or the antigen MBP (0·1 μg ml^{-1}) was added. The spleen cells were cultured for 72 hr at 37°C in an atmosphere of 5% CO_2 and identical cell samples were pooled. Two-hundred-μl aliquots were placed in groups of six in round-bottom microculture plates. The samples were labelled with 1 μCi ^{3}H-thymidine (2·0 Ci $mmol^{-1}$, The Radiochemical Centre, Amersham, England) incubated for a further 4 hr and harvested with a semi-automatic cell harvester (Mash II harvester, Microbiological Associates Inc.), onto Whatman grade GF/A paper. The radioactivity on the filter discs was counted in a Packard Tricarb Scintillation Counter (Model 2405). The stimulation index was equal to the ratio of a mean number of counts/min^{-1} of cultures containing mitogen/antigen to that of cultures with no mitogen/antigen.

The pooled spleen cells were centrifuged at 1,800 rev min^{-1} for 10 min at room temperature, counted and the concentration adjusted to 2×10^7 viable cells/ml^{-1} for injection into the rat tail vein. Cell viability was assessed using nigrosin dye exclusion.

Neurological assessment of EAE. Clinical signs of EAE were graded as follows: 1. flaccid tail; 2. hind limb hypotonia; and 3. hind limb paralysis and incontinence.

Results are represented as the average maximum recipient response. This is the mean of the maximum clinical scores shown by each animal.

Histological assessment of EAE. Sections from the first 4-cm length of cervical spinal cord were fixed in 10% formal saline, cut, stained with haematoxylin–eosin and the number of inflammatory lesions quantified by light microscopy.

CsA. CsA was kindly donated by Dr J.F. Borel (Sandoz Laboratories, Switzerland). For *in vitro* work 2 mg CsA was dissolved in 0·8 ml of ethanol and 0·2 ml of Tween 80. The solution was diluted to the required concentration with tissue culture medium.

For *in vivo* work CsA was prepared daily and was dissolved in olive oil (vehicle) by heating to 60°C for 2 hr. CsA or the vehicle was orally administered to rats via a 1-ml syringe and stomach tube.

RESULTS

Suppression of active EAE with CsA

Rats were treated from the time of immunization with CsA at a daily dose of 25 mg kg^{-1} body

weight for 12 days. This dose was found to be effective in suppressing the clinical signs of EAE in guinea pigs in a previous study (Bolton & Cuzner, 1980) and was based on a dose–response curve for Lewis and Wistar rats (Borel *et al.*, 1976). Undosed and oleate-treated rats showed clinical signs of EAE from 11–17 days after immunization whereas the CsA-treated animals showed no signs of disease at this time. However, the CsA-treated rats did show full clinical signs of EAE between days 22 and 31 after immunization (i.e. 11–20 days after dosing with CsA was stopped) (Table 1), but the number of inflammatory lesions in the spinal cord was significantly reduced ($P < 0{\cdot}01$).

Suppression of adoptively transferred EAE by treatment of donor rats with CsA

Spleens were removed from the three groups of rats, treated as described above, 12 days after immunization with MBP/FCA and the cells cultured with MBP. At the time of removing the spleens only the rats in the control groups showed clinical signs of EAE, and only their cultured spleen cells transferred clinical disease to recipients (Table 2).

Treatment in vitro of spleen cells with CsA

Spleen cells from adult Lewis rats were taken 12 days after immunization when the animals showed clinical signs of EAE. Only spleen cells cultured with antigen or mitogen transferred clinical signs of EAE to the recipients. However, when CsA was added to the cell cultures for 72 hr, together with either the antigen or mitogen the cells failed to transfer any clinical signs of disease (Table 3). A concentration of 1 $\mu g\ ml^{-1}$ CsA was selected, as higher levels, although not toxic, interfered with cellular proliferation and reduced the yield of lymphocytes. If CsA was added to the cultures for 3 hr immediately prior to the cell transfer, the recipient rats were susceptible to full clinical signs of EAE.

Cellular proliferation, assayed by the uptake of tritiated thymidine, was significantly depressed ($P < 0{\cdot}001$) in cultures containing mitogen and CsA compared with those containing mitogen alone.

The counts of cells cultured in the presence of antigen were less than those of the unstimulated cultures and did not appear to be affected by CsA.

Table 1. Treatment of rats with CsA for 12 days after immunization

Treatment	Average maximum response		No. animals with clinical and histological signs of disease	Mean lesion no./4-cm length of spinal cord (±s.d.)
	Days 11–17	Days 22–31		
None	2·7	0	6/6	80·5±41·8
Oleate	2·7	0	6/6	n.d.
CsA 25 mg kg^{-1} day^{-1}	0	2·8	6/6	18·6±16·2

Table 2. The effect of CsA treatment on the ability of cultured spleen cells to adoptively transfer EAE

Donor treatment daily	No. cells transferred intravenously* ($\times 10^7$)	Average maximum recipient response	No. animals with clinical signs of disease
None	2	3·0	6/6
Oleate	2–4	2·8	8/8
CsA†	2–4	0	0/9

* MBP concentration in culture 0·1 $\mu g\ ml^{-1}$.

† Dose 25 mg kg^{-1} day^{-1} for 12 days after immunization.

Table 3. Culture conditions and response of rats receiving 2×10^7 spleen cells intravenously from rats immunized 12 days previously with MBP/FCA

Antigen or mitogen*	CsA for 72 hr ($\mu g\ ml^{-1}$)	CsA for 3 hr ($\mu g\ ml^{-1}$)	Average maximum recipient response	No. animals with clinical signs/total	Counts min^{-1}	Stimulation index
—	—	—	0	0/10	6,496	—
Con A	—	—	2·2	6/6	226,114	34·7
Con A	1	—	0	0/5	66,055	10·1
MBP	—	—	3·0	14/14	3,042	0·6
MBP	1	—	0	0/7	2,200	0·4
MBP	—	1	2·3	6/8	n.d.	

* Con A concentration in culture 1 $\mu g\ ml^{-1}$; MBP concentration in culture 0·1 $\mu g\ ml^{-1}$.

Table 4. The effect of CsA on rats receiving 2×10^7 sensitized spleen cells intravenously*

Recipient treatment†	Average maximum recipient response		No. animals sick/total
	Days 4–10	Days 12–18	
None	3·0	0	14/14
Oleate	3·0	0	10/10
CsA (25 mg kg^{-1})	1·4	0	8/12
CsA (50 mg kg^{-1})	0	1·3	6/11

* MBP concentration in culture—0·1 $\mu g\ ml^{-1}$.

† Cell recipients were treated daily for 11 days after cell transfer.

Treatment of recipient rats with CsA

The effect of treating recipient rats with CsA was investigated. A group of animals was dosed daily with CsA (25 mg kg^{-1} body weight) from the time of transfer of cultured spleen cells from immunized donors. Control groups received oleate alone or were not dosed. Four to 10 days after the cell transfer all three groups showed clinical signs of EAE, but the symptons of those in the group receiving CsA were greatly suppressed in comparison with those of the control groups (Table 4). When the dose of CsA was increased to 50 mg kg^{-1} body weight day^{-1} the rats showed no clinical signs of EAE whilst receiving the drug. After treatment was stopped, at a time when the control groups had completely recovered, six of the 11 rats developed clinical signs of EAE.

DISCUSSION

Cyclosporin A has been shown to suppress the autoimmune disease EAE in a number of laboratory animals. We have investigated some of the effects of CsA on the *in vitro* and *in vivo* steps involved in the adoptive transfer of EAE in order to determine how the drug might be affecting the course of the disease.

When CsA was added to the spleen cell cultures at the beginning of the *in vitro* stage in the transfer, together with either antigen or mitogen, the spleen cells were prevented from transferring

clinical signs of EAE. This was not due to cytotoxicity of CsA as viable cells were always transferred. Furthermore, addition of the drug to the cultures a few hours prior to cell transfer did not prevent the transfer of disease.

The degree of cellular proliferation, assayed by ^{3}H-thymidine uptake, was depressed in the cultures containing the mitogen and CsA. Therefore, it may be the suppression of cellular proliferation during the *in vitro* cell culture which is preventing the transfer of EAE. Holda, Welch and Swanborg (1980) have shown that nylon-wool-adherent cells, probably lymphoblastic in origin, are responsible for the transfer of EAE. However, there was no measurable proliferation in the cultures containing antigen compared with unstimulated cultures, in agreement with the findings of Richert *et al.* (1979) and Panitch (1980). Therefore, it was not possible to show any suppression when CsA was added.

Alternatively, cellular proliferation in the presence of antigen or mitogen may not be essential for the transfer of clinical disease. Recently, Panitch (1980) has shown that although cellular proliferation in the host was essential for the successful transfer of clinical signs of EAE, *in vitro* transformation of the donor cells was not required. CsA may therefore be blocking another key step in the transfer reaction.

Spleen cells from rats, at a time when clinical symptoms of EAE were fully suppressed by CsA, failed to transfer disease although clinical signs of EAE appeared in animals 12 days following the withdrawal of the drug. Thus, immunocompetent cells necessary for the generation of clinical signs of EAE may not have been present in the spleen of donor rats at the time of cell culture and transfer. It is possible that CsA prevents the development of immunocompetent cells as when the drug is withdrawn it takes the same time for clinical signs to appear as in untreated animals. Paterson and Hanson (1969) showed that cyclophosphamide completely suppressed EAE in Lewis rats and that cells from these animals would not transfer histological signs of disease. Their 'donor' rats, however, if left after cyclophosphamide treatment, did not develop clinical signs of EAE in contrast to the CsA-treated rats in the present study. However, if the CsA treatment is continued beyond 34 days after sensitization, symptoms of EAE appear in only 13% of the rats (Borel, personal communication).

A prerequisite for induction of adoptive EAE is the transfer of cultured sensitized spleen cells, but the disease may be substantially suppressed by treatment of recipient animals with CsA. A higher dose of CsA than that used to suppress active EAE was necessary to completely suppress the clinical signs of adoptive disease and, as in active EAE after dosing was stopped, some rats showed clinical signs of disease. It took the same length of time for symptoms to appear after withdrawal of the drug as it would have for undosed recipients. These results suggest that the transferred spleen cells are inhibited by CsA or that the drug is preventing the expression of the immune response in the host.

The results of this study show that CsA delays the appearance of immunocompetent cells in EAE, but does not suppress the development of disease completely unless treatment is continued up to the time when antigen is no longer present. CsA only prevents adoptive transfer if present during the entire culture period. It could be that the drug inhibits the development of the cells responsible for the transfer, and thereby allows unregulated suppressor cell activity (Leapman *et al.*, 1980). The active rôle of the host in the adoptive transfer of EAE is clearly demonstrated by the finding that the transfer of disease to cell recipients is also suppressed during treatment with CsA.

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REFERENCES

BOLTON, C. & CUZNER, M.L. (1980) Modification of EAE by nonsteroidal anti-inflammatory drugs. In *The Suppression of Experimental Allergic Encephalomyelitis and Multiple Sclerosis* (ed. by A.N. Davison & M.L. Cuzner), p, 189. Academic Press, London.

BOREL, J.F. (1976) Comparative study of *in vitro* and *in vivo* drug effects on cell-mediated cytotoxicity. *Immunology*, **31**, 631.

BOREL, J.F., FEURER, C., GUBLER, H.U. & STAHELIN, H. (1976) Biological effects of Cyclosporin A: a new anti-lymphocytic agent. *Agents Actions*, **6**, 468.

DEIBLER, G.E., MARTENSON, R.E. & KIES, M.W. (1972) Large scale preparation of myelin basic protein from central nervous tissue of several mammalian species. *Prep. Biochem.* **2,** 139.

HOLDA, J.H., WELCH, A.M. & SWANBORG, R.M. (1980) Autoimmune effector cells. I. Transfer of experimental allergic encephalomyelitis with lymphoid cells cultured with antigen. *Euro. J. Immunol.* **10,** 657.

LEAPMAN, S.B., FILO, R.S., SMITH, E.J. & SMITH, P.G. (1980) *In vitro* effects of Cyclosporin A on lymphocyte subpopulations. *Transplantation*, **30,** 404.

LEVINE, S. & SOWINSKI, R. (1977) Suppression of the hyperacute form of experimental allergic encephalomyelitis by drugs. *Arch. Int. Pharmacodyn.* **230,** 309.

PANITCH, H.S. (1980) Adoptive transfer of experimental allergic encephalomyelitis with activated spleen cells: comparison of *in vitro* activation by Concanavalin A and myelin basic protein. *Cell. Immunol.* **56,** 163.

PANITCH, H.S. & MCFARLIN, D.E. (1977) Experimental allergic encephalomyelitis: enhancement of cell-mediated transfer by Concanavalin A. *J. Immunol.* **119,** 1134.

PATERSON, P.Y. & HANSON, M.A. (1969) Cyclophosphamide inhibition of experimental allergic encephalomyelitis and cellular transfer of the disease in Lewis rats. *J. Immunol.* **103,** 1311.

RICHERT, J.R., DRISCOLL, B.F., KIES, M.W. & ALVORD, JR, E.C. (1979) Adoptive transfer of experimental allergic encephalomyelitis: incubation of rat spleen cells with specific antigen. *J. Immunol.* **122,** 494.

STONE, S.H. (1961) Transfer of allergic encephalomyelitis by lymph node cells in inbred guinea pigs. *Science, N.Y.* **134,** 619.

WIESINGER, D. & BOREL, J.F. (1979) Studies on the mechanism of action of Cyclosporin A. *Immunobiology*, **156,** 454.