INHIBITION OF AUTOIMMUNE NEUROPATHOLOGICAL PROCESS BY TREATMENT WITH AN IRON-CHELATING AGENT

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Experimental allergic encephalomyelitis (EAE)[1] is induced in Lewis rats by a single injection of guinea pig spinal cord homogenate emulsified with complete Freund's adjuvant (GPSCH-CFA). The typical symptoms are weight loss of 5–10% beginning on day 8 or 9, followed by moderate to severe hind limb paralysis commencing on days 12–14 and lasting for an average of 8 d. Recovery is always complete by day 28. Some aspects of EAE are considered to mimic human multiple sclerosis. In both diseases (1), inflammatory lesions are found predominantly in the white matter of the brain and spinal cord, the cellular infiltrate consisting principally of T lymphocytes of the delayed-type hypersensitivity (DTH)/helper subset and monocyte/macrophages (2, 3). The DTH/helper T cells tend to be located at the periphery of the lesion, extending into the surrounding normal tissue (4, 5). This distribution pattern, together with the fact that EAE can be transferred to naive animals by sensitized T cells of the DTH/helper type (6–8), suggests that such T lymphocytes initiate the disease process.

The initial invasion of T cells in the central nervous system on day 5 or 6 is followed by the appearance of macrophages (4): macrophages are essential both for the induction (9) and pathological effects (10) of EAE, and activated macrophages are able to destory myelin (11). Activated macrophages liberate many factors, including hydrogen peroxide and superoxide (12), which may react in the presence of free (non-heme) iron to give the highly toxic hydroxyl radical (·OH) by a series of reactions summarized by the overall equation (13):

$$H_2O_2 + O_2^{-} \xrightarrow{\text{iron catalyzed}} O_2 + \cdot OH + OH^{-}.$$

The generation of ·OH in a lipid-rich environment, such as myelin, may cause

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[1] *Abbreviations used in this paper:* Con A, concanavalin A; CT, clotting time; DFOM, desferrioxamine B mesylate; DTH, delayed-type hypersensitivity; EAE, experimental allergic encephalomyelitis; GPSCH-CFA, guinea pig spinal cord homogenate dispersed in complete Freund's adjuvant; HIFCS, heat-inactivated fetal calf serum; LNC, lymph node cells; LPS, lipopolysaccharide B; MBP, myelin basic protein; MPCA, macrophage procoagulant assay; ·OH, hydroxyl radical, PEC, peritoneal exudate cells; RCT, reaction clotting time.

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extensive damage by producing a chain reaction of lipid peroxidation, macrophage chemotaxis, and free radical generation. In addition, the free iron necessary to catalyze the production of hydroxyl radicals may be available in lesions of EAE and multiple sclerosis as a result of blood extravasation, hemolysis, and subsequent hemoglobin breakdown. It is thus possible that free radical–induced tissue damage could contribute significantly to the pathology of the disease process (14).

With this mechanism in mind, we have examined the effect of desferrioxamine B mesylate (DFOM) on the inflammatory lesions of EAE. As a chelating agent with a particularly high affinity for Fe^{3+}, DFOM will compete successfully for free or loosely bound iron, which is then excreted. More tightly bound iron such as that in hemoglobin is not affected (15). We report here that treatment with DFOM dramatically suppresses both the clinical symptoms and pathology of EAE in Lewis rats. The improved clinical status of the DFOM-treated animals is reflected in the diminished severity of inflammatory lesions in the central nervous system, and in reduced DTH T cell responses measured in vitro. The fact that DFOM has seen extensive clinical use, with few side effects, raises the possibility that treatment protocols involving iron chelation may be beneficial as a relatively mild immunosuppressive procedure for the management of multiple sclerosis and other inflammatory/degenerative processes.

Materials and Methods

Rats. Female Lewis/JC rats were bred at the John Curtin School of Medical Research. They were used at 8–10 wk of age and weighed ~120–150 g.

Induction of EAE. A 20% suspension of guinea pig spinal cord homogenate was emulsified in an equal volume of Freund's incomplete adjuvant (Commonwealth Serum Laboratories, Melbourne, Australia) containing 4 mg/ml *Mycobacterium butyricum* (Difco Laboratories, Inc., Detroit MI). This inoculum is designated GPSCH-CFA. Rats were injected with 0.2 ml, divided between the footpads of all four feet.

Clinical Assessment. Clinical symptoms were assessed on a scale of 0–5, as follows: 0, asymptomatic; 1, weight loss, flaccid tail; 2, ataxia, slow righting reflex; 3, paraparesis, incontinence; 4, paraplegia or quadriplegia, no voluntary limb movement; 5, moribund or death.

Histology. Brains and spinal cords were fixed in formal saline, embedded in paraffin, and stained with hemotoxylin and eosin or with periodic acid-Schiff.

Administration of Desferrioxamine. Desferrioxamine B mesylate (Desferal) was kindly donated by Ciba-Geigy Australia, Ltd., Lane Cove, New South Wales. Since Desferal is excreted rapidly in vivo (16), it was administered via an osmotic pump (type 2ML1; ALZA Corp., Palo Alto, CA) that was implanted subcutaneously. The drug was reconstituted in water at 250 mg/ml, and the pumps, (with a 2-ml capacity), delivered ~70 mg Desferal per rat per day over a period of 7 d, at a continous rate of 10 μl/h. The levels of DFOM (17) in the plasma of one rat measured on days 1, 5, and 8 after implantation of the pump were 12, 15, and 10 μg/μl, respectively. In all experiments, EAE controls inoculated with GPSCH-CFA were sham operated. At the end of the treatment, the pumps were removed and the rats were observed for a further 14 d. For in vitro studies, saturated DFOM was prepared by mixing equimolar amounts of DFOM with $FeCl_3$, since DFOM binds Fe^{3+} in a 1:1 ratio.

Preparation of Cell Suspensions. Peritoneal exudate cells (PEC) were induced by intraperitoneal injection of thioglycollate medium (Difco Laboratories, Inc.) 3 d before sampling. The cells were obtained from anesthetized animals by washing out the peritoneal cavity with 20 ml cold Hanks' balanced salt solution. This allowed repeated sampling of individual rats. Since the presence of erythrocytes may result in false positives in the

procoagulant assay, erythrocytes were removed from the PEC preparations by layering on Isopaque-Ficoll (Pharmacia, Uppsala, Sweden). <1% red cells remained after separation. Lymph node cells (LNC) were prepared by disaggregation of popliteal lymph nodes into RPMI. The cells were washed, filtered through nylon gauze (20 μm; Simons Ltd., Sydney), and resuspended in RPMI 1640 (Gibco Laboratories, Grand Island, NY) containing 5% heat-inactivated fetal calf serum and antibiotics.

Macrophage Procoagulant Assay (MPCA). The MPCA assay (18, 19) depends on specifically sensitized T cells recognizing antigen presented on appropriate stimulator cells. These lymphocytes then release lymphokine(s), which causes the production of procoagulant on the surface of the macrophages. It is this procoagulant that is measured by the capacity to reduce the clotting time of plasma. All media used in this assay were first filtered through a Zetapor filter (American Machine and Foundry Co. (Australia) Pty. Ltd., Chatswood, Australia) to remove endotoxin. Peritoneal cells (2×10^6) were incubated with or without myelin basic protein (MBP), 10 μg/ml in 0.5 ml RPMI containing 5% heat-inactivated fetal calf serum. After 16 h incubation at 37°C, 5% CO_2, the cells were washed twice and resuspended in 200 μl of Hanks' balanced salt solution. 100 μl of cell suspension was mixed with an equal volume of rat plasma (prepared as described by Geczy and Meyer [19]) and, upon addition of 100 μl of $CaCl_2$ (0.03 M), the clotting time (CT) was estimated using a BBL fibrinometer (Becton, Dickinson & Co., Sunnyvale, CA). The percent reduction in clotting time (RCT) was estimated as follows: Percent RCT = [(CT without antigen) − (CT with antigen)/(CT without antigen)] × 100. Lymph node cells were incubated with MBP (10 μg/ml) at 2×10^6/ml in RPMI, 5% heat-inactivated fetal calf serum (HIFCS), plus 10^{-4} M 2-mercaptoethanol. After 72 h, the lymphokine-rich supernatant was removed and diluted 1:5. A 0.3-ml aliquot was then incubated for 16 h with 4×10^5 PU5 cells, a continuous macrophage line that produces procoagulant factor upon stimulation with lymphokine (18), and the cells were assayed as above.

Characterization of Effectors. The lymphocytes that respond in the procoagulant assay were characterized using monoclonal antibodies specific for rat T cell subsets. The W3/25 (helper/DTH) and OX8 (cytotoxic) monoclones were kindly supplied by Dr. Alan Williams, Sir William Dunn School of Pathology, Oxford. LNC were incubated (30 min at 4°C) with these monoclonal antibodies, washed three times in PBS, incubated with fluorescein-conjugated goat anti-mouse IgG (CSL, Melbourne), and washed again three times in PBS. They were then separated on a fluorescence-activated cell sorter (FACS IV; B-D FACS Systems, Sunnyvale, CA) using a 488-μm excitation wavelength, and, after culture for 72 h with MBP, supernates were assayed for procoagulant activity using the PU5 cell line.

Measurement of T Cell Proliferation by Thymidine Uptake. LNC were suspended in RPMI, 5% HIFCS, plus PSN, and incubated at 37°C in 5% CO_2 in air for 24 h, in 96-well Linbro trays at 2×10^5 cells/0.2-ml well. Reagents were added at the following concentrations: Con A, 1 μg/ml; MBP, 5 μg/ml; DFOM, 10 μg/ml; $FeCl_3$, 0.1 mM. After 24 h, 0.2 μCi [^{3}H]thymidine in 24 μl was added to each well and incubated at 37°C in 5% CO_2 for 5 h. Cells were harvested onto filter papers using a Titertek cell harvester, transferred to vials containing 5 ml scintillation fluid (xylene/PPO), and counted in a Tri-carb liquid scintillation spectrometer (Packard Instrument Co., Downers Grove, IL)

Results

Effect on Clinical EAE. Treatment of GPSCH-CFA-sensitized rats with DFOM before the onset of symptoms either completely abrogated the weight loss associated with clinical EAE (days 0–7, Fig. 1 *a*), or greatly reduced the magnitude of such weight loss (days 8–15, Fig. 1 *b*). The same general effect was seen in rats given the drug from day 11 to 18 (Fig. 1 *c*). Also, clinically affected rats that were treated on days 14–21 regained weight more rapidly than did the untreated controls (Fig. 1 *d*). Administration of DFOM to normal rats caused no change in normal weight gain.

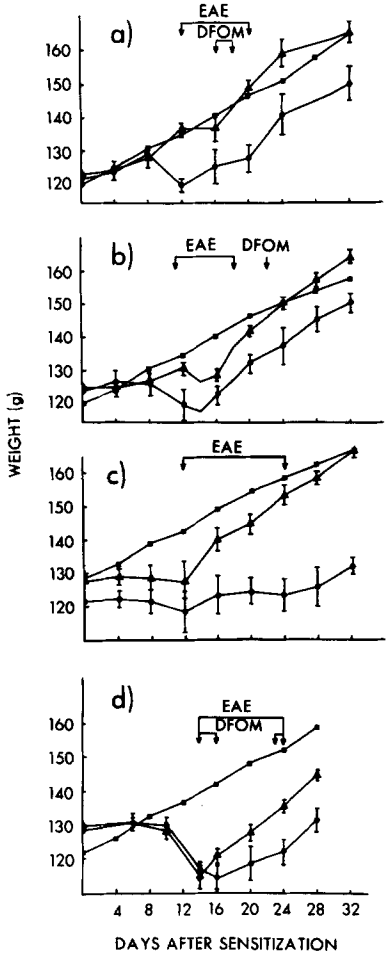

FIGURE 1. Rats were given DFOM on days 0–7 (*a*), days 8–15 (*b*), days 11–18 (*c*), and days 14–21 (*d*). The groups were: (▲) GPSCH-CFA primed and treated with DFOM; (●) GPSCH-CFA primed and untreated; (■) unprimed and given DFOM. The duration of clinical symptoms is shown by the arrows for GPSCH-CFA-primed, treated (DFOM) and untreated (EAE) rats.

The above patterns were repeated in both the incidence and severity of clinical impairment observed in all groups of rats that were dosed with DFOM (Table I). Some neurological symptoms were seen in a few animals subsequent to withdrawal of the drug (groups A, D, and H), but these were generally mild and of reduced duration. In addition, the clinically affected rats that were given DFOM showed rapid improvement during the course of treatment (compare G and H, Table I).

The impression gained from the histological analysis of sections of medulla and spinal cord from rats treated early with DFOM was that the severity of inflammatory process was minimal when compared with that recognized in the EAE-primed controls (Fig. 2). Relatively few lesions were observed, and the extent of cellular invasion was slight. Also, it was noted that sections from those animals that had already developed symptoms at the commencement of treatment

TABLE I

Effect of DFOM on the Duration and Severity of Symptoms of EAE

Group*	No. in group	Treatment		Days after sensitization							
		DFOM	Days	12	14	16	18	20	22	24	28
A	5	+	0–7	0	0	5 (1.0)	0	0	0	0	0
B	5	–		0	5 (3.0)‡ 1–4	5 (3.5) 2–4	5 (2.0)	2 (0.5)	0	0	0
C	5	+	8–15	0	0	0	0	0	1 (1.5)	0	0
D	5	–		3 (3.3) 3–4	3 (4.0) 2–4	4 (3.0) 2–4	2 (1.0)	2 (2.0) 1–3	1 (3.0)	0	1 (1.0)
E	5	+	11–18	0	0	0	0	0	0	1 (0.5)	0
F	5	–		3 (1.0)	4 (2.0) 1–3	3 (1.7) 1–3	3 (1.0)	2 (1.0)	2 (2.0) 1–3	0	0
G	10	+	14–21	0	2 (1.5) 1–2	8 (1.4) 1–2	3 (1.0)	0	0	1 (1.0)	0
H	10	–	14–21	0	2 (1.5) 1–2	7 (2.0) 1–3	6 (2.7) 2–3	2 (1.5) 1–3	3 (2.3) 1–4	2 (0.8)§ 1–2	0

* Osmotic pumps delivering 70 mg of DFOM per day were implanted into groups of five rats. The controls were sham-operated. The results for days 14–21 are cumulated data for two groups of five animals: the two animals that relapsed on day 24 were from the same group.

‡ The results are given as: number of clinically affected animals, the mean clinical score for those with neurological signs (in parentheses) and, on line below, the range of clinical scores where these varied.

§ These rats were examined daily from days 22–28, and showed symptoms only on days 24.

showed evidence of numerous old lesions, with fibrin deposition but little cellular infiltration. The few cells that were present had the general appearance of macrophages.

Patterns of Lymphocyte Responsiveness. The initial hypothesis was that DFOM may limit the severity of EAE by removing free iron necessary for generating hydroxyl radicals. However, we also considered the possibility that the drug might have direct effects on lymphocyte function. The analysis of this aspect was facilitated by the availability of the procoagulant assay for MBP-specific T cells (Table II). Reactive lymphocytes can be detected in both lymph node and peritoneal exudate populations from rats primed with GPSCH-CFA (Tables II and III). Effector function is apparently mediated by T cells of the DTH/helper subset (Table III).

PEC were taken at intervals from the rats that had been examined clinically (Fig. 1, Table I) and were tested in the procoagulant assay. The level of response was depressed for PEC sampled from 1 to 3 d after the cessation of DFOM treatment (groups A and B, day 10; C and D, day 16; E and F, day 21; Table IV). This diminution in activity was least apparent for the rats that were clinically affected when drug treatment was initiated on day 14, though evidence of suppressed lymphocyte function was still apparent (groups G and H, day 21; Table IV). However, by day 32 (Table IV), PEC from all groups of rats given DFOM showed levels of procoagulant activity that were comparable to those seen in the EAE controls. Thus, the capacity of lymphocytes to respond to MBP is completely restored within 11 d of the cessation of drug treatment (Table IV). This may reflect that GPSCH-CFA is likely to persist as a depot antigen for several weeks after injection. Even so, recovery of lymphocyte responsiveness did not lead to the development of severe symptoms in any group, and 12 of the 15 animals that were given DFOM from day 8 onwards remained completely free of the disease after treatment (groups D, F, and H, Table I).

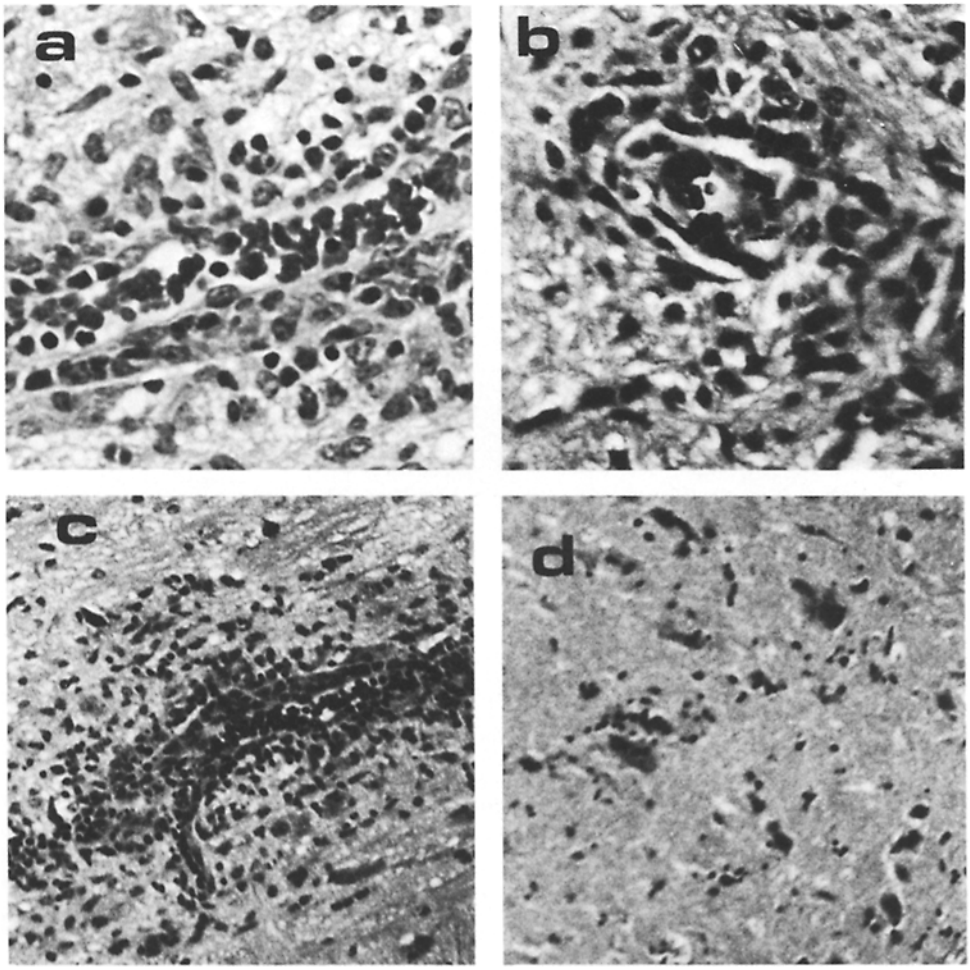

FIGURE 2. The hematoxylin and eosin–stained sections are all from the medulla of rats killed 29 d after priming with GPSCH-CFA. Those designated *a* and *c* are from untreated rats, while *b* and *d* are from animals given DFOM on days 14–21. The lesions shown in *a* and *c* are typical, while *b* and *d* illustrate the most severe inflammatory process that could be found in the DFOM-treated rats: the perivascular cuff (*b*) is the only one observed in sections from this group. × 600 for *a* and *b*; × 150 for *c* and *d*.

LNC were also tested from other rats that were subjected to the treatment schedules used for the clinical evaluation (groups A–F, Table I). These animals were killed at various times after removal of the osmotic pumps. The patterns of response seen (Table V) were generally similar to those observed for the PEC populations (Table IV).

To determine whether DFOM was inhibiting macrophage or T cell function, PEC from DFOM-treated rats were incubated with lipopolysaccharide B (LPS), which is known to directly stimulate procoagulant activity in macrophages (18). The PEC from DFOM-treated rats responded at least as well as normal PEC (Table VI). Also, the addition of LNC from untreated rats primed with GPSCH-CFA 18 d previously restored the capacity of PEC from the DFOM-treated

TABLE II

Specificity of the MPCA Assay for Peritoneal Exudate Populations

Sensitization in vivo		MPCA response (percent reduction in clotting time) on in vitro challenge with:		
Antigen	Day	MBP	OVA*	Nil
GPSCH-CFA	7	23.4	2.5	3.7
GPSCH-CFA	14	45.9	3.0	2.5
GPSCH-CFA	21	25.2	3.5	3.6
Nil	—	3.4	—	2.3

* Ovalbumin.

TABLE III

*FACS Separation of T Cells Responding in the MPCA Assay**

Lymph node cells (LNC) sorted for:	Percentage of cells stained	Reduction in clotting time
	%	%
W3/25 (helper/DTH)	25.3	35.4 ± 2.1
Unstained	—	<5.0
Ox8 (cytotoxic/suppressor)	7.2	<5.0
Unstained	—	38.2 ± 3.5
Unseparated	—	48.0 ± 4.4

* The cells from rats sensitized with GPSCH-CFA 10 d previously were separated into stained (fluorescent) and unstained populations on a FACS IV. They were then, together with unseparated cells that had not been passed through the FACS, stimulated with MBP in the presence of PEC (1:10 lymph node cells) from normal mice and supernates were assayed using the PU5 macrophage line.

TABLE IV

Levels of T Cell Responsiveness in Peritoneal Exudates

Group*	Treatment		Procoagulant activity on days:‡					
	Drug	Days	7	10	14	16	21	32
A	Nil	0–7	—	12.0 ±1.7	—	—	23.4 ± 1.0	23.4 ± 2.0
B	DFOM	0–7	—	3.3 ± 1.4§	—	—	23.0 ± 1.8	18.6 ± 2.4
C	Nil	8–15	5.5 ± 1.3	—	—	21.6 ± 1.9	—	52.2 ± 2.1
D	DFOM	8–15	5.5 ± 1.1	—	—	3.1 ± 1.1§	—	54.4 ± 6.2
E	Nil	11–18	—	9.5 ± 2.6	—	—	23.7 ± 2.3	45.5 ± 2.9
F	DFOM	11–18	—	9.5 ± 2.8	—	—	<1.0	49.8 ± 2.7
G	Nil	14–21	—	—	18.7 ± 1.5	—	24.1 ± 1.8	24.4 ± 2.7
H	DFOM	14–21	—	—	19.2 ± 1.7	—	16.7 ± 3.5§	20.8 ± 3.3

* These are the same groups as those shown in Table I and Fig. 1.

‡ Mean ± SD for five animals; rats were injected i.p. with thioglycollate 3 d before sampling.

§ These differences are statistically significant ($P < 0.05$).

animals to produce procoagulant factor on stimulation with MBP protein. Conversely, LNC from EAE/DFOM rats were unable to stimulate normal macrophages in the presence of MBP. The defect resulting from DFOM treatment is thus in the lymphocyte rather than the macrophage population.

TABLE V
MPCA-promoting Activity of Lymph Node Cells

Days given DFOM	Day of sampling	Reduction in clotting time*
		%
0–7	13	9.7
Nil	13	39.0
8–15	31	51.1
Nil	31	33.7
11–18	18	14.0
Nil	18	28.7
11–18	35	49.8
Nil	35	35.8

* Supernates from cultures with MBP were assayed using the PU5 cell line.

TABLE VI
Restoration of PEC Reactivity by Addition of EAE-sensitized LNC

PEC*	LNC	MBP	LPS
Normal	None	4.1	39.3
EAE/DFOM (days 11–18)	None	1.0	42.3
EAE/DFOM (days 11–18)	EAE LNC	36.5	
EAE/DFOM (days 11–18)	Normal LNC	4.5	
EAE (day 18)	None	36.0	
Normal	EAE LNC	38.7	
None	EAE LNC	3.9	
Normal	DFOM/EAE LNC	2.4	
None	DFOM/EAE LNC	1.5	

* PEC were tested for procoagulant activity by incubation with antigen (MBP, 5 μg/ml or LPS, 0.01 μg/ml) for 16 h. LNC from EAE (day 18 PI), DFOM/EAE (DFOM days 11–18) or normal rats were added at 3 × 10^5/10^6 PEC, the optimum ratio for stimulation (unpublished results). Results are expressed as a reduction in clotting time of normal plasma.

TABLE VII
Effect of DFOM and $FeCl_3$ on T Cell Proliferation

Lymph node cells	Stimulation index*				
	Con A	MBP	MBP + DFOM	MBP + (DFOM + $FeCl_3$)	MBP + $FeCl_3$
EAE day 10	4.0	2.9	1.0	2.3	2.3
Normal	6.4	<1	<1	<1	1.1

* Results are expressed as a stimulation index relative to counts for unstimulated control cultures.

A further experiment was done in vitro to establish whether the effect of DFOM was indeed mediated via the removal of loosely bound iron. Lymph node

cells from EAE-primed rats were incubated in vitro with MBP. The resultant proliferative response was completely inhibited by incorporating DFOM in the medium (Table VII). However, the inhibitory effect of DFOM was substantially reversed when the drug was saturated with Fe^{3+} before adding it to the culture, indicating that DFOM suppresses lymphocyte responses by chelating loosely bound iron.

Discussion

Our results show that the severity of EAE is greatly diminished in rats given the iron-chelating agent DFOM. The effects range from complete prevention of the development of neurological symptoms to more rapid recovery in rats that are already clinically affected. The clinical pattern is broadly reflected in the extent of histopathological changes. Recovery of lymphocyte responsiveness after the cessation of therapy does not lead to the development of severe disease.

The protective effect of DFOM in EAE is also directly related to the level of responsiveness for lymphocytes of the DTH/helper subset, taken from both lymph nodes and the peritoneal cavity. The most likely reason for the reduction in the severity of the disease is thus that DFOM is a potent immunosuppressive agent for DTH effector T cells (6). The finding that DFOM suppresses lymphocyte proliferation in vitro and the fact that this is reversed by addition of $FeCl_3$ indicates that the depressive effect may operate via removal of loosely bound iron; further experiments are in progress so that a definitive conclusion of this type can be made. Iron is also thought to be necessary for other aspects of lymphocyte function, including recirculation and homing (20). Dietary iron deficiency is associated (in various antigen systems) with reduced delayed hypersensitivity (20), failure of sensitized lymphocytes to bind to target cells (22), and decreased DNA synthesis (23).

Incubation with DFOM appeared to have little effect on macrophages in vitro, in that it did not inhibit the production of procoagulant in response to endotoxin stimulation or phagocytosis of zymosan particles (unpublished results) at drug concentrations that depress lymphocyte proliferation. However, macrophages are well supplied with lysosomal iron (24). Thus, the relatively short assay time and concentrations of DFOM used may not have been sufficient to cause any inhibition of function.

Apparently, DFOM is acting primarily on lymphocytes. The observation that a more rapid resolution of the disease was seen in rats that were treated after the development of symptoms indicates that these effects may operate in the site of pathology. Decreased production of procoagulant in inflammatory lesions might be expected to reduce the deposition of fibrin in white matter in EAE (25). Chelation and removal of free iron from sites of inflammation may also prevent free radical–induced lipid peroxidation and production of chemotactic factors resulting from tissue damage. An excess of free iron in the CNS is known to contribute directly to such processes, and to the development of gliosis (26).

It is of interest that iron deposits in the form of hemosiderin, which can be mobilized by DFOM (27), have been found in multiple sclerosis plaques (28). In fact, in one study, iron levels in such plaques were found to be approximately five times the level found in normal nervous tissue (29). There is thus some

reason to think that, both from the aspect of removing iron from the brain and of reducing lymphocyte stimulation, DFOM might merit a trial as a therapeutic agent in multiple sclerosis. The drug is relatively nontoxic, having been used for many years to treat both iron overload and iron poisoning (30, 31) and, more recently, the tissue damage of long-term hemodialysis (32). Our in vitro results also suggest that drugs that remove free iron may be of general value as mild immunosuppressive agents to inhibit autoreactive, or rejection processes involving lymphocytes of the DTH/helper subset.

Summary

Lewis rats that are primed with guinea pig spinal cord homogenized in complete Freund's adjuvant (GPSCH-CFA) develop overt symptoms of experimental allergic encephalomyelitis (EAE). Treatment with the iron-chelating agent, desferrioxamine B mesylate (DFOM), at various times before the onset of EAE, dramatically suppressed both the severity and duration of disease. When DFOM was administered to rats soon after the development of neurological signs, a rapid recovery occurred, though mild, transient symptoms could be seen ~1 wk after withdrawal of the drug. Treatment with DFOM was always accompanied by a diminution of T cell responsiveness on the part of the delayed-type hypersensitivity/helper subset and, on histological examination, an absence of inflammatory cells from lesions. Iron is believed to influence both the migration and function of immune effector cells. It can also act as a catalyst in the formation of free radicals, which are highly toxic agents causing tissue damage in sites of inflammation. The mechanisms underlying the effect of DFOM on the severity of EAE, and the possible implications for treatment of multiple sclerosis are discussed.

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