A Study on Demyelinating Effect of Galactocerebroside in Experimental Allergic Encephalomyelitis

Shin Kwang Khang*, M.D., Je G. Chi, M.D., Sang Kook Lee, M.D.

Department of Patholgy, Faculty of Medicine, Hallym University, and Department of Pathology, College of Medicine, Seoul National University*

An experimental allergic encephalomyelitis was induced by bovine myelin basic protein (MBP) and bovine galactocerebroside (GC) on male guinea pigs. Animals were divided into five experimental and one control groups. Among the five experimental groups, three were inoculated with 75 ug, 150 ug and 300 ug of MBP, respectively, to see the dose dependency of demyelination. The fourth group was inoculated with mixture of 75 ug of MBP and 180 ug of GC and the fifth group with 180 ug GC. All inocula was injected intradermally in emulsion state mixed with equal amount of complete Freund adjuvant. Control group was injected with adjuvant only.

Clinical symptoms began to appear from 15th day after inoculation and animals were sacrificed on maximum neurologic deficit or 4 to 5 days after the onset of symptoms. Demyelination was observed in 6 out of 8 animals inoculated with MBP/GC mixture, while only 3 out of 24 animals inoculated with various dosage of MBP showed demylination. The difference was statistically significant. Serum antibodies to MBP and GC were measured by ELISA method. All of the eight animals inoculated with MBP/GC mixture and two animals inoculated with GC had low titer of anti-GC antibodies, while all animals inoculated with MBP, MBP alone or MBP/GC mixture, had high titer of anti-MBP antibodies.

Therefor it is concluded that the demyelination is augmented by GC and is not significantly dose-dependent on MBP.

Key Words: Myelin basic protein, Galactocerebroside, Demyelination

INTRODUCTION

Toward the end of the 19th century, iatrogenic, sometimes fatal paralytic accidents occurred that were apparently related to immunization with the Pasteur rabies vaccine, which was prepared in rabbit central nervous system tissue. This event raised the possibility of development of paralytic disease by repeated administration of central nervous system tissue. In 1933

Address for Correspondence: Shin Kwang Khang, M.D.,
Department of Pathology, Kangdong Sacred Heart Hospital,
445, Gil-Dong, Kangdong-Ku, Seoul 134-701, Korea

and 1935, Rivers et al. produced the first animal model of experimental allergic encephalomyelitis (EAE) by repeated injection of normal brain tissue in monkeys over prolonged period. The development of adjuvants in the 1940s led to the shortening of the interval between injection of antigen and development of signs, which enabled development of highly reporducible models of EAE in several species. Among many species of experimental animals, guinea pig has been most widely used (Waksman & Adams, 1962 ; Raine & Stone, 1977 ; Raine et al., 1978) and guinea pig spinal cord has been known to be the most potent antigen (Stone et al., 1969 ; Canto et al., 1977).

The basic pathological changes of experimental

allergic encephalomyelitis are demyelination and inflammation. These pathologic changes together with clinical course and immunologic aspects are similar to multiple sclerosis (Mehta et al., 1981; Waksman & Adams, 1962), the prototype of human demyelinating disease, and has been contributed to the study for autommune demyelination (Grundke-Iqbal & Bornstein, 1980 ; Ackermann et al., 1981).

Experimental allergic encephalomyelitis has been believed to be related to T cell mediated cellular immunity (Ortiz-ortiz & Weigle, 1976), which was supported by those facts that EAE could be transferred passively by T lymphocytes (Paterson, 1960 ; Mokhtarian et al., 1984) and demyelination was preceded by invasion of the tissue by lymphocytes (Waksman & Adams, 1962). But possibility of a role for humoral immunity was postulated by the observations that cellular infiltration was preceded or accompanied by alteration in vascular permeablity (Oldstone & Dixon, 1968). Development of monoclonal antibody technology also demonstrated IgG in white matter before clinical signs were manifested (Traugott et al., 1981, 1982). They also stated that macrophages and B cells were observed at the center of the active lesion, while T cells were observed along the margin of the lesion. These observations strongly support the participation of humoral immunity in autoimmune demyelination together with demonstration of antibodies to myelin in serum and CSF (Appel & Bornstein, 1964 ; Gonatas et al., 1974 ; Raine et al., 1981a ; Glynn et al., 1986).

Early animal models of EAE were induced by crude emulsion of brain and spinal cord. But late in 1960s, the structure of the myelin basic protein (MBP) was clarified and MBP alone was found to be able to induce clinical EAE (Kibler & Shapira, 1968 ; Martenson et al., 1970). Since then, MBP has been accepted as major antigen responsible to immune-mediated demyelination. But in guinea pig, both clinical signs and histologic changes of MBP-induced EAE were rather weaker than those induced by brain or spinal cord tissue and demyelination was not observed by usual encephalitogenic dose of MBP (Raine et al., 1978, 1981b). Furthermore, antiserum against MBP failed to induce demyelination in vitro (Seil et al., 1968 ; Raine et al., 1981a). So participation of other myelin component was suspected and attention was focused on myelin lipids (Raine et al., 1981a, b ; Moore et al., 1984). The candidate most likely important appeared to be the glycolipid galactocerebroside (GC), since antiserum to GC could induce demyelination in CNS culture with concurrent inhibition of myelin regeneration (Seil et al., 1968 ; Fry & Bornstein, 1974

; Raine et al., 1981a). Demyelination in vivo was also demonstrated when sensitized with MBP and GC together (Raine et al., 1981b). Other myelin lipids also enalbed to induce demyelination, but GC has been most potent (Moore et al., 1984).

Meanwhile, sensitization with MBP in higher doses above the usual encephalitogenic dose also induced demyelination in guinea pig (Moore et al., 1985), and demyelination was demonstrated in animals other than guinea pig by MBP alone (Alvord et al., 1980 ; Mokhtarian et al., 1984). So it is still uncertain which component is the major target in autoimmune demyelination. We tried this study to attempt compare the dose related demyelinating effect of MBP with that of GC.

METERIAL AND METHODS

Animal Groups and inocula

A total of 40 adult male guinea pigs, weighing 400-500gm, was used. Animals were housed in groups two to four per cage and fed chow, cabbage and water ad libitum.

Animals were devided into 5 experimental groups and 1 control group. EAE was induced by bovine myelin basic protein (MBP) and bovine galac tocerebroside (GC) supplied by Sigma in lyophilized powder. All inocula were delivered by single intradermal injection of 0.5 ml in 5 sites in nuchal area and back.

The composition of inocula and number of animals in each group summarized in Table 1. are as follows:

MBP group; Three groups are included and each group consists of 8 animals. Each animal received 0.5ml of an emulsion consisting of 0.25ml of normal saline containing 75ug, 150ug or 300ug of bovine MBP in 0.25ml of complete Freund adjuvant (CFA). Lyophilized powder of MBP was dissolved in saline and homogenized with equal amount of CFA.

MBP/GC group; This group consists of 8 animals. Each animal. was injected with 0.5ml of an inocula

Table 1. Animal groups and inocula

Group	Inocula	No. of Animals
A	MBP 75 ug/CFA	8
B	MBP 150 ug/CFA	8
C	MBP 300 ug/CFA	8
D	MBP 75 ug/GC 180 ug/CFA	8
E	GC 180 ug/CFA	4
F	CFA	4

Note: MBP ; myelin basic protein
      GC ; galactocerebroside
      CFA ; complete Freund adjuvant

consisting of 75ug of MBP and 180ug of GC in CFA. GC was suspended in saline containing MBP by sonication and this mixture was homogenized with CFA so that each 0.5ml emulsion contained 180ug of GC and 75ug of MBP.

GC group: This group consists of 4 animals and each animal received 0.5ml of an emulsion containing 180ug GC only.

Control group: This group consists of 4 animals and received 0.5ml of emulsion made of 0.25ml of normal saline and equal amount of CFA.

Clinical evaluation

Animals were weighed and examined daily. Clinical signs were recorded on the following scale: grade 0, no clinical sign; grade 1, non-specific signs such as sudden weight loss and decrease of physical activity; grade 2, mild paraparesis; grade 3, moderate to severe paraparesis, grade 4, quadriparesis, grade 5; moribund.

Morphologic evaluation

Animals were sacrificed when they had reached maximum neurologic deficit and those animals without obvious progression of neurologic deficit were sacrified 4 to 5 days after the onset sign. Anesthesia was done by intraperitoneal injection of chloral hydrate (40mg/100gm B.W. in 40% solution). After opening the chest cage, left ventricle was punctured to obtain blood for ELISA. Right after blood sampling, animals were perfused with about 300ml of phosphate buffered saline and then 500ml of fixative using 3 way valves (3.5% paraformaldehyde and 0.2% glutaraldehyde in PBS). The perfusate returned through a hole in the right atrium. En bloc resection of brain and spinal cord was done and postfixed in above fixative for about 1 hour. For paraffin block, tissue sections were obtained from cerebrum, cerebellum, brain stem and spinal cord. Spinal cord was sectioned at 2cm intervals from medulla. They were fixed in 10% neutral formalin, dehydrated in graded alcohols, embedded in paraffin and stained with H-E and LFB. For preparation of epon block, thin slices were taken from cervical, thoracic, lumber and sacral levels of spinal cord. These were fixed in 2.5% glutaraldehyde solution, osmicated, dehydrated in graded alcohols, rinsed in propylene oxide, embedded in epon, and one-micrometer thick epoxy sections from each level were stained with toludine blue.

These paraffin and epoxy sections were examined blindly without knowledge of the animals or group from which they were taken. Slides were examined for the degree of inflammation and demyelination. In-
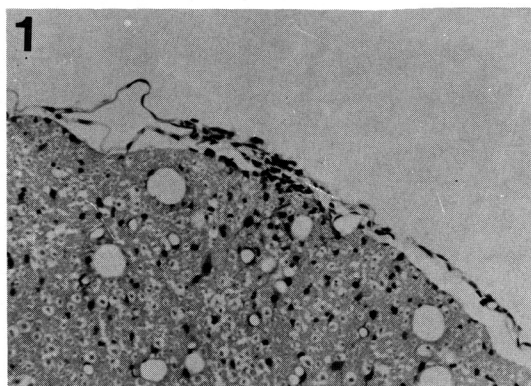

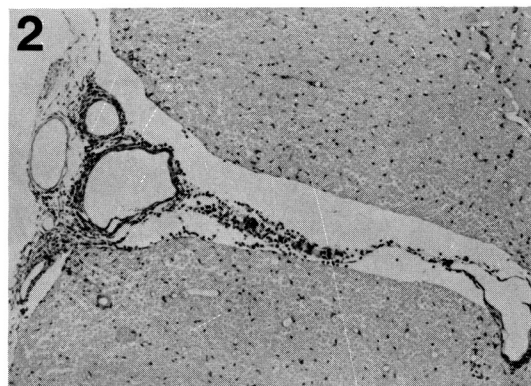Fig. 1. Inflammation (±). A few inflammatory cells in leptomeninges (H&E×200).

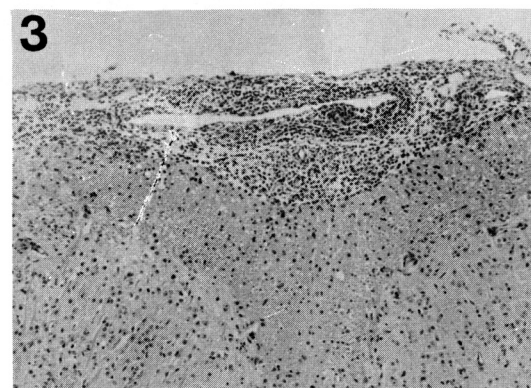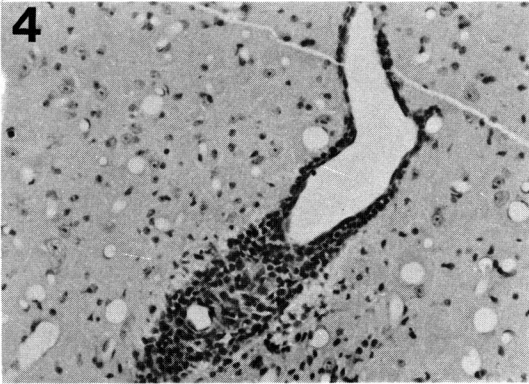Fig. 2. Inflammation (+). Perivascular cuffing in leptomeninges (H&E ×100).

Fig. 3. Inflammation (++). Severe inflammatory cell infiltration in subarachnoid space (H&E ×100).

flammation was rated as follows; (−), no inflammatory cells; (±), a few inflammatory cells in leptomenings; (+), more inflammatory cells in leptomeninges with perivascular condensation; (++), severe leptomen-

Fig. 4. Inflammatory cells in Virchow-Robin space. Inflammatory cells in Virchow-Robin space with early parenchymal infiltration (H&E ×200).
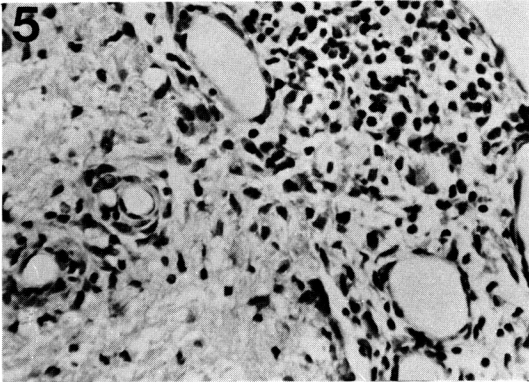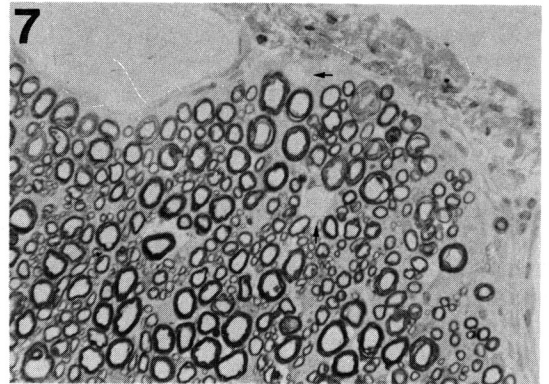

Fig. 5. Inflammation (+++). Localized parenchymal infiltration of inflammatory cells (H&E ×400).
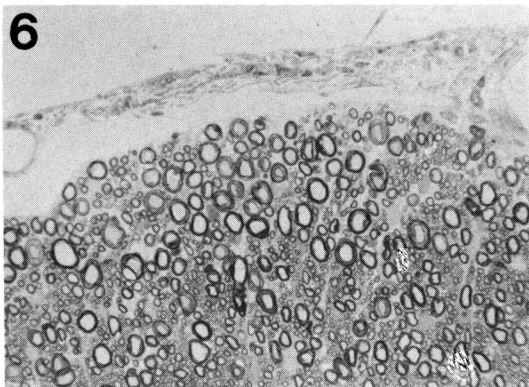
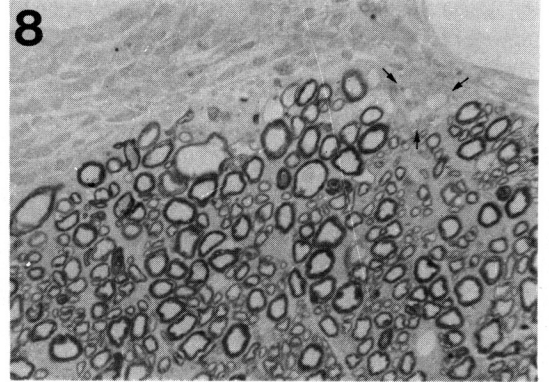**Fig. 6** Axons with intact myelin sheath (Epon section, toluidine blue ×400).

Fig. 7. Demyelination (±). Two naked axons are observed (arrow). (Epon section, toluidine blue ×400).

Fig. 8. Demyelination (+). Naked axons in group (arrow) (Epon section, toludine blue ×400).

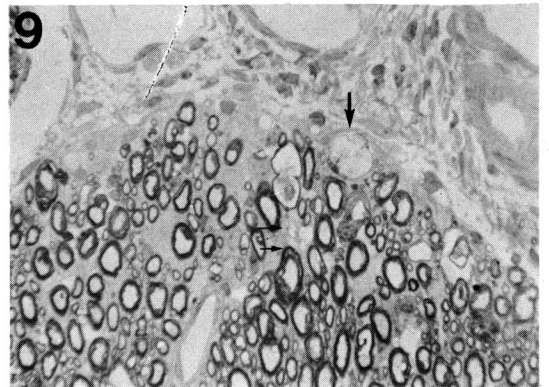**Fig. 9.** Demyelination (+) with swelling of myeline sheath (large arrow) (Epon section, toluidine blue ×400).

ingeal and perivascular infiltration in parenchyma; (+++), localized parenchymal infiltration; (++++), extensive parenchymal infiltration.

For demyelination, slides were rated; (−), no demyelinated axons; (±), a few naked axons in subpial area; (+), many scattered demyelinated axons in

subpal area; (++), groups of subpial naked axons; (+++), large plaques of demyelination extending into the white matter; (++++), extensive white matter involvement (Fig. 1-11).

Determination of antibody titers

Anti-MBP and Anti-GC antibody titers were determined by the enzyme-linked immuno-sorbent assay (ELISA). For this, 96well flat-bottomed polyvinyl-chloride plates (Linbro/Titer-Tek, Flow Laboratories) were coated with 0.1ml antigen solution per well, containing I ug of MBP or GC, respectively. MBP was dissolved in sodium carbonate buffer (pH 9.6) and GC was dissolved in absolute ethanol. After air drying, non-reacted groups were inactivated by exposure to 1% polyvinyl-pyrolidone (Sigma, M.W 40,000) for 20 minutes at room temperature. After rineses in phosphate buffered saline containing 0.5% Tween 20 (PBS/Tween, pH 7.4), antigen-coated plates were incubated overnight at 4°C with 50ul/well of serum diluted from 1:2 to 1:256 in PBS/Tween. The next day, the plates were rinsed again with PBS/Tween and then exposed to peroxidase-conjugated goat anti-guinea pig IgG (Sigma), diluted to 1:1000, for 90 minutes.

After this, a color reaction was obained in a specific antibody-containing cells by exposure to o-phenylene-diamine (40mg/100ml) in phosphate citrate buffer (pH 5.0), containing 20ul of 33% H_2O_2 for about 15 minutes.

When the serum displayed an orange reaction product, the color development was stopped by the addition of 0.4N H_2SO_4. The OD490 was read with MCC-microplate multiscanner (Flow laboratories).

RESULT

Clinical findings

Eighteen of 36 animals in experimental groups showed clinical signs between days 15 to 23 post-inoculation (PI) and rather evenly distributed. But 3 animals showed the first sign on days 29, 31 and 32 PI, respectively. Clinical signs were rather mild. Thirteen animals showed nonspecific symptoms only, such as loss of body weight and decrease of physical activity. Four showed mild paraparesis and only one in MBP/GC group showed quadriparesis. This animal suddenly lost about 10% of his body weight during a night on day 15 PI, followed by development of mild paraparesis, which had progressed to quadriparesis over the ensuing 3 days.

One animal in MBP 150ug group died suddenly on day 16 PI without development of neurologic sings,

but was excluded because postpartum examination revealed mild inflammatory reaction only.

The rate of development of clinical EAE was not significantly different among experimental groups, but the degree of signs were slightly severe in MBP 150ug and MBP/GC groups.

Morphologic findings

Inflammation was found in 25 out of 36 animals in experimental group. Eight animals were without clinical signs. Dose-dependency of MBP was not apparent both in inflammation and demyelination. But demyelination was observed in 6 out of 8 animals simultaneously inoculated with MBP and GC, while only 3 out of 24 animals in MBP groups of varying doses showed demyelination. The difference was statistically significant (P<0.01). Demyelination was observed mainly in sections from lumbar and sacral segments of spinal cord. The results within individual groups summarized in Table 2 and 3 are as follows:

MBP 75ug

Inflammatory reaction is most intensively observed in this group. Of the 8 animals 5 showed inflammatroy reaction, grade (+++) in 2 animals and grade (++) in 3 animals, 2 of the latter were without clinical signs. The 7th animal has demonstrated marked decrease of body weight on day 23 PI, but regained the weight over the ensuing 4 days. There was no inflammation. Those 2 animals with grade (+++) inflammtion showed parenchymal infiltration in sections from cerebrum, cerebellum, brain stem and all levels of sinal cord. But the infiltrats in cerebrum and cerebellum were mainly confined to paraventricular area and choroiditis was accompanied. Grade (±) demyelination was observed in sections from sacral segment of these animals.

MBP 150ug

Among the 8 animals in this group, inflammation was observed in 6 animals. But the intensity of the reaction was rather milder than that of the MBP 75ug group. One animal with inflammatory reaction was without clinical signs and another one animal died overnight on day 16 PI, having demonstrated only floppiness earlier, but inflammation was relatively mild. One animal, sacrificed 5 days after the onset of weight loss, showed no reaction. Two animals having demonstrated mild paraparesis showed grade (++) inflammatory reaction in sections from lumbar and sacral segments. But inflammatory reaction of those animals with nonspecific signs tend to be located in higher levels of spinal cord. Demyelination was not observed in all animals, but one with grade (++) inflamma-

Table 2. Clinical, inflammation and demyelination scores of animal groups

75 ug MBP				150 ug MBP		
Clin.	Inflam.	Demyel.		Clin.	Inflam.	Demyel.
1(32)	+++	+/−		0	+	−
0	++	−		‡	+	−
0	−	−		1(15)	−	−
0	−	−		2(29)	++	−
2(20)	+++	+/−		0	−	−
1(21)	++	−		2(20)	++	−
1(23)	−	−		1(23)	+	−
0	++	−		0	++	−

300 ug MBP				75 ug MBP/180 ug GC		
Clin.	Inflam.	Demyel.		Clin.	Inflam.	Demyel.
0	−	−		0	+/−	+/−
0	+	−		1(16)	+	+/−
1(23)	+/−	−		1(21)	−	−
1(18)	++	−		0	−	−
1(16)	+/−	−		4(15)	+	+
1(22)	+	+		0	+	+
0	−	−		2(31)	+++	++
0	+	−		1(16)	++	+

180 ug GC				CFA		
Clin.	Inflam.	Demyel.		Clin.	Inflam.	Demyel.
0	−	−		0	−	−
0	−	−		0	−	−
0	+/−	−		0	+/−	−
1(21)	+/−	−		0	−	−

Note: MBP; myelin basic protein ( ); day of onset
      GC ; galactocerebroside ‡; sudden spontaneous death
      CFA; complete Freund adjuvant

Table 3. Comparative encephalitogenecity of animal groups

Group	Inocula	Animals with	Inflammation*	Demyelination*
A	MBP 75 ug/CFA	4/8	++	−
B	MBP 150 ug/CFA	4/8	+	−
C	MBP 300 ug/CFA	4/8	+	−
D	MBP 75 ug/GC 180 ug/CFA	5/8	+	+
E	GC 180 ug/CFA	1/4	−	−
F	CFA	0/4	−	−

Note: * ; The score is group average

tion showed swelling of myelin sheath where macrophages were tightly adhered (Fig. 12).

MBP 300ug

   Inflammation was observed in 6 animals, 2 of which were without clinical signs. The degree of inflammation was rather milder than those of groups with lesser doses of MBP inocula. One animal showed scattered naked axons in subpial area.
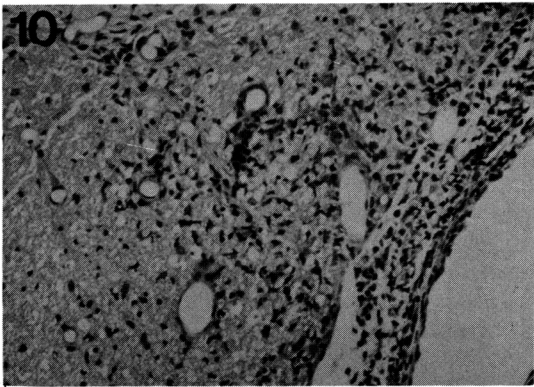
Fig. 10. Demyelination (++). Note subpial zone of demyelination (LFB-H&E ×200).
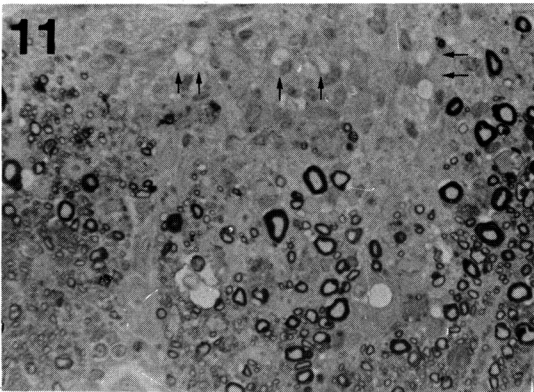

Fig. 11. Epon section of same case. Naked axons are scattered (arrow) among inflammatory cell (Epon section, toluidine blue ×400).
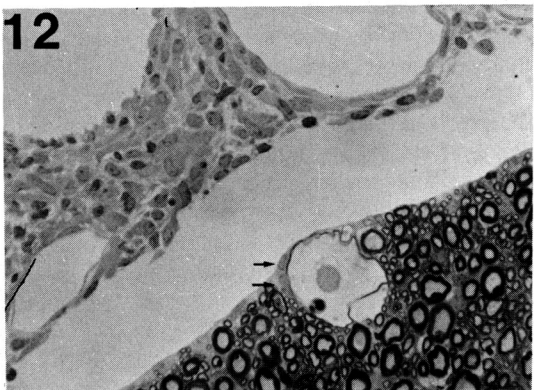

Fig. 12. A single axon with swollen myelin sheath. Macrophages are attached to the outer surface of myelin sheath (arrow) (Epon section, toluidine blue ×400).

MBP 75ug/GC 180ug

Inflammation was observed in 6 out of 8 animals. Two of the 6 animals were without clinical signs. Demyelination was observed in all of 6 animals and the degree of demyelination roughly correlated with that of inflammation. One animal, which developed paraparesis and incontinence on day 31 PI was the one with maximum pathologic finding. There was grade (+++) inflammatory reaction in the brain and entire segments of the cord. Epon section from sacral segment revealed groups of subpial demyelinated axons and many macrophges (Fig. 10, 11).

GC 180ug

Two of the 4 animals showed grade (±) inflammatory reaction. Demyelination was not demonstrated.

CFA

One animal showed equivocal inflammatory reaction only.

Antibody measurement

Anti-MBP antibodies were detected in all animals inoculated with MBP with the titer above 1:256, regardless of clinical illness or pathologic findings, while none of the animal without sensitization with MBP has anti-MBP antibodies.

Anti-GC antibodies were detected in lower titer than anti-MBP antibodies. Seven out of 8 animals in MBP/GC group showed anti-GC antibody titers of 1:16 and one of 1:8. Only 2 of 4 animals in GC group showed anti-GC antibody titers of 1:256. The rest of the animals do not have anti-GC antibodies (Table 4).

Table 4. Antibody titers to MBP and GC

Inocula	Anti-MBP	Anti-GC
MBP 75 ug	1:256	1:2
MBP 150 ug	1:25.6	1:2
MBP 300 ug	1:256	1:2
MBP 75 ug/GC 180 ug	1:256	1:16
GC 180 ug	1:2	1:256*
CFA	1:2	1:2

Note: MBP ; myelin basic protein
      GC ; galactocerebroside
      CFA ; complete Freund adjuvant
      * ; Only 2 animals have positive titer

DISCUSSION

The major encephalitogenic agent has been known to be MBP. But in guinea pigs, encephalomyelitis in-

duced by MBP alone did not show demyelination, while clinical signs and inflammatory reaction had been constantly present. (Raine et al., 1978, 1981b). But the result was ambiguous, because guinea pigs inoculated with higher doses of MBP sometimes showed demyelination (Moore et al., 1985). Recently, augmentation of demyelination in EAE by myelin lipids has been reported (Raine et al., 1981a ; Moore et al., 1984).

This study attempts to compare the demyelinating effect of dose-dependency of MBP and augmentation of GC in EAE. At the same time, we measured serum antibodies to MBP and GC and tried to link these results to morphologic findings to clarify the major agent responsible for autoimmune demyelination.

Among the 32 animals inoculated with MBP, MBP alone or MBP with GC, 17 developed clinical EAE and inflammation was observed in 23 animals. Clinical signs began to appear between day 15 and 32 PI. The time of clinical onset was slightly delayed compared to result of others (Raine et al., 1981b ; Moore et al., 1984). This might be due to animal used in this study. The time of onset can be different according to strains even in Hartley guinea pigs, which is the species most widely being used in EAE (Raine & Stone, 1977) and Kim et al. (1986), who used the same animal with us, reported that the clinical signs developed later than Hartley guinea pigs. Some of their animals developed clinical signs as late as 6 weeks after inoculation.

The inflammatory reaction was rather mild in most animals and mainly confined to leptomeniges and perivascular areas. In general, the inflammatory reaction is milder in EAE induced by MBP compared to that induced by brain or spinal cord extract (Moore et al., 1984). In brain, inflammatory reaction was mainly observed along the paraventricular area sometimes accompanied by inflammation of choroid plexus, which might be related to CSF pathway and assumed to be an indirect evidence for participation of humoral immunity in EAE together with detection of antibodies in CSF and serum (Gonatas et al., 1974 : Glynn et al., 1986).

Dose-related differences in incidence rate of clinical illness and degree of inflammation was not evident among groups inoculated with varying doses of MBP. Inflammatory reaction was most severe in MBP 75ug group. Previous reports has been ambiguous. Moore et al. (1985) observed that higher doses of MBP induced more rapid clinical onset and more agressive inflammatory reaction, but Lampert and Kies (1967) were on the other side.

Among the 25 animals showing inflammatory reac-

tion, 8 were free of clinical signs. In general, the degree of inflammation correlates well with severity of clinical signs in guinea pigs (Williams & Moore, 1973 ; Levin et al., 1975 ; Raine et al., 1981b) as well as in other species (Hughes & Stedronska, 1973). In acute EAE, inflammatory cells, particularly T cells, appear in tissue 5 to 6 days before the onset of clinical signs (Traugott et al., 1981, 1982). Considering the ralatively delayed onset of clinical illness in present study, they might be sacrificed before clinical signs develop.

One micrometer-thick epoxy sections were good enough for evaluation of individual axons. Immunohistochemical staining with peroxidase conjugated anti-MBP antiserum was tried, but the result was not satisfactory because detailed examination for demyelination of individual fiber was impossible. For the examination of subtle change, 1 micrometer epoxy section was far superior. Plastic embedding with water soluble resin might be a good alternative, which enables embedding of larger specimen and many kinds of staining for myelin.

There was distinct difference in demyelination between MBP goups and MBP/GC group. Only 3 out of 24 animals sensitized with varying doses of MBP alone showed demyelination, while 6 out of 8 animals inoculated with MBP and GC showed demyelination. The result was statistically significant ($P < 0.01$). Among the MBP groups, there was no significant differences in demyelination according to dose of MBP inoculated. Thus, demyelination was augmented by GC, but was not significantly dose-related on MBP.

Detection of anti-GC antibodies in all of the animals of MBP/GC group would be an evidence that these antibodies play an important role in autoimmune demyelination. The rather weak expression of demyelination in present study might be related to low titer of anti-GC antibodies. Formation of high titer of anit-GC antibodies usually requires repeated sensitization (Raine et al., 1981a ; Moore et al., 1984). So it is assumed that repeated injection of GC would result in stronger expression of demyelination.

Demyelination was not observed in animals inoculted with GC alone. Serum against GC could induce demyelination in vitro (Raine et al., 1981a) and serum against MBP failed to demyelinate in CNS culture (Seil et al., 1968 ; Raine et al., 1981a). But in vivo study, sensitization to both MBP and GC is required. From these facts it would be speculated that major target in autoimmune demyelination is the GC and MBP may play a role at the stage before anti-GC antibody induced demyelination.

Assuming that both cellular and humoral immunity

are participated in autoimmune demyelination, the first possible mechanism might be T cell-dependent B cell immunity. Identification of T cells prior to appearance of B cells in tissue and distribution pattern of T cells and B cells in the lesion may lead to the speculation that primary T cell sensitization to MBP serves to recruit T cells to the CNS and a B cell response to GC. This speculation is further supported by the fact that T cells located along the periphery of the lesion are mainly composed of helper T cells (Traugott et al., 1983). Recently, it was postulated that major target in cell mediated cytotoxicity is glycolipid (Nudelman et al., 1982 ; Mühlradt et al., 1984). So GC, one of the major glycolipid in myelin, may be the final target in antibody dependent cell-mediated cytotoxic reaction. Another possible speculation is that MBP acts as a simple carrier protein for lipid hapten GC. In this sense, chemical conjugation of MBP· and GC prior to sensitization will induce better results.

REFERENCES

Ackermann HP, Ulrich J, Heitz U: *Experimental allergic encephalomyelitis, exudate and cellular infiltrates in the spinal cord of Lewis rats. Acta Neuropathol (Berl). 54:149-152, 1981.*

Alvord Jr EC, Shaw CM, Hruby S, et al: *Chronic relapsing experimental allergic encephalomyelitis induced in monkeys with myelin basic protein. J Neuropathol Exp Neurol. 39:338, 1980.*

Appel SH, Bornstein MB: *The application of tissue culture to the study of experimental alllergic encephalomyelitis. II serum factors responsible for demyelination. J Exp Med. 119:303-312, 1964.*

Campbell B, Vogel PJ, Fisker E, et al: *Myelin basic protein administration in multiple sclerosis. Arch Neurol. 29:10-15, 1973.*

Dal Canto MC, Fujinami RS, Paterson PY: *Experimental allergic encephalomyelitis in suckling Lewis rats, comparison with the disease in adult animals. Lab Invest. 37:395-405, 1977.*

Davis RL, Robertson DM: *Textbook of neuropathology. Williams & Willkins, Baltimore. 468-547, 1985.*

Fry JM, Bornstein MB: *Cerebroside antibody inhibits sulfatide synthesis and myelination and demyelinates in cord tissue culture. Science. 183:540-542, 1974.*

Glynn P, Weedon D, Cuzner MC: *Chronic experiemental autoimmune encephalitis; Circulating autoantibodies bind predominantly determinants expressed by complexes of basic protein and lipids of myelin. J Neurol Sci. 111-123, 1986.*

Gonatas NK, Gonatas JO, Stieber A, et al: *The significance of circulating and cell-bound antibodies in experimen-tal allergic encephalomyelitis. Am J Pathol. 116:529-457, 1974.*

Grundke-Iqbal I, Bornstein MB: *Multiple sclerosis; Serum gamma globulin and demyelination in organ culture. Neurology. 30:749-754, 1980.*

Hughes RAC, Stedronska J: *The susceptibility of rat strains to experimental allergic encephalomyelitis. Immunology. 24:879,884, 1973.*

Kibler RF, Shapira R: *Isolation and properties of an encephalitogenic protein from bovine, rabbit and human central nervous system tissue. J Biol Chem. 243:281-286, 1968.*

Kim WH, Chi JG, Lee SK: *An observation of blood-brain barrier change and immunohistopathologic findings in experimental allergic encephalomyelitis. Kor J Pathol. 20:277-286, 1986.*

Lampert PW, Kies MW: *Mechanism of demyelination in allergic encephalomyelitis of pigs. An electron microscopic study. Exp Neurol. 18:210-223, 1967.*

Levine S. Sowinski R, Shaw CM: *Do neurological signs occur in experimental allergic encephalomyelitis in the absence of inflammatory lesions of the central nervous system. J Neuropathol Exp Neurol. 34:501-506, 1975.*

Martenson RF, Deibler GE, Kies MW: *Myelin basic protein of the rat central nervous system—Purification of encephalitogenic properties and amino acid compositions. Biochem Biophys Acta. 200:353-362, 1970.*

Mehta PD, Lassmann R, Wisniewski HM: *Immunologic studies of chronic relapsing EAE in guinea pigs; Similarities to multiple sclerosis. J Immunol. 127:334-338, 1981.*

Mokhtarian F, MacFarlin DE, Raine CS: *Adoptive transfer of myelin basic protein sentized T-cells produces chronic relapsing demyelinating disease in mice. Nature (Lond). 309:356-358, 1984.*

Moore GRW, Traugott U, Farooq M, et al: *Experimental autoimmune encephalomyelitis, augmentation of demyelination by different myelin lipids. Lab Invest. 51:416-424, 1984.*

Moore GRW, Traugott U, Stone SH, et al: *Dose dependency of MBP-induced demyelination in the guinea pig. J Neurol Sci. 70:187-205, 1985.*

Mühlradt PF, Bethke U, Monner DA, et al: *The glycosphingolipid globoside as serological marker on cytolytic T lymphocyte precursors and alloantigen-responsive proliferating T lymphocytes in murine spleen. Eur J Immunol. 14:852-858, 1984.*

Nudelman E, Hakomori S, Kannagi R, et al: *Characterization of a human melanoma-associated ganglioside antigen defined by a monoclonal antibody, 4.2. J Biol Chem. 257:12752-12756, 1982.*

Oldstone MBA, Dixon FJ: *Immunohistochemical study of allergic encephalomyelitis. Am J Pathol. 52:251-257,*

1968.

Ortiz-Ortiz L, Weigle WO: *Cellular event in the induction of experimental allergic encephalomyelitis in rats. J Exp Med.* 144:604-616, 1976.

Paterson PY: *Transfer of allergic encephalomyelitis by means of lymph node cells. J Exp Med.* 111:119-136, 1960.

Raine CS, Stone SH: *Chornic experimental allergic encephalomyelitis in inbred guinea pigs. NY State J Med.* 77:1693-1696, 1977.

Raine CS, Traugott U: *Supression of chronic allergic encephalmoyelitis; Relevance to multiple sclerosis. Science.* 201:495-448, 1978a.

Raine CS, Traugott U, Iqbal K: *Encephalitogenic properties of purified preperations of bovine oligodendrocytes tested in guinea pig. Brain Research.* 142:85-86, 1978b.

Raine CS, Johnson AB, Marcus DM, et al: *Demyelination in vitro. Absorption studies demonstrate that galactocerebroside is a major target. J Neurol Sci.* 52:117-131, 1981a.

Raine CS, Traugott U, Farooq M, et al: *Augmentation of immune-mediated demyelination by lipid haptens. Lab Invest.* 45:174-182, 1981b.

Raine CS. Biology of disease: *Analysis of autoimmune demyelination; Its impact upon multiple sclerosis. Lab Invest.* 50:608-635, 1984.

Rivers TM, Sprunt DH, Berry GP: *Observations on attempts to produce acute disseminated encephalomyelitis in monkeys. J Exp Med.* 58:39-54, 1933.

Rivers TM, Schwentker FF: *Encephalomyelitis accompanied by myelin destruction experimentally produced in monkeys. J Exp Med.* 61:689-702, 1935.

Seil FJ, Falk GA, Kies MW, et al: *The in vitro demyelinating activity of sera from guinea pigs sensitized with whole CNS and with purified encephalitogens. Exp Neurol.* 72:545-555, 1968.

Stone SH, Lerner EM, Goode JH Jr: *Acute and chronic autoimmune encephalitis; age, strain and sex dependency. The importance of the source of the antigen. Proc Soc Exp Biol Med.* 132:341-344, 1969.

Traugott U, Shevach E, Chiba J, et al: *Autoimmune encephalomyelitis; Simultaneous identification of T and B cells in the target organ. Science.* 214:1251-1253, 1981.

Traugott U, Shevach E, Chiba J, et al: *Chronic replapsing experimental allergic encephalomyelitis: Identification and dynamics of T and B cells within the central nervous system. Cellular Immunol.* 68:261-272, 1982.

Traugott U, Reinherz EL, Raine CS: *Multiple sclerosis; Distribution of T-cell subsets within active chronic lesions. Science.* 219:308-310, 1983.

Waksman BH, Adams RD: *A histologic study of the early lesion in experimental allergic encephalomyelitis in the guinea pig and rabbit. Am J Pathol.* 41:135-162, 1962.

Williams RM, Moore MJ: *Linkage of susceptibility to experimental allergic encephalomyelitis to the main histocompatibility locus in the rat. J Exp Med.* 138:771-782, 1973.