THE ROLE OF COMPLEMENT IN
THE PATHOGENESIS OF EXPERIMENTAL
ALLERGIC ENCEPHALOMYELITIS

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SUMMARY

The role of complement in the pathogenesis of demyelination and inflammation has been investigated
in a synergistic model of acute experimental allergic encephalomyelitis (EAE) in the Lewis rat.
Depletion of serum complement with cobra venom factor (CVF) suppressed the clinical expression
of acute inflammatory EAE induced either by immunization with 50 μg guinea pig basic protein
(MBP) in Freund's complete adjuvant, or by the passive transfer of 10⁷, but not 5 x 10⁷ MBP
activated spleen cells. Despite the suppression of clinical disease in actively induced EAE, treatment
with CVF only had a significant effect on the severity of CNS inflammation in early disease (12
days postimmunization) when the number of inflammatory foci was reduced by 35%. Three days
later this difference had resolved and no significant difference could be detected in the severity of
CNS inflammation, although control animals exhibited severe disease, the CVF treated group being
clinically normal.

Demyelination in these models is initiated by systemic injection of the antimyelin oligodendrocyte
glycoprotein (MOG) monoclonal antibody, 8-18C5, which in vitro lyses oligodendrocytes in a dose,
Fc and complement-dependent manner and in vivo induces extensive CNS demyelination in rats
with EAE. Treatment with CVF reduced the ability of this antibody to initiate demyelination in
vivo and furthermore, its F(ab)² fragment had no effect on the clinical course of EAE and was
unable to initiate demyelination in normal animals. Complement-dependent mechanisms are
therefore involved both in the clinical expression of acute inflammatory lesions and in the
pathogenesis of antibody-mediated demyelination in EAE.

INTRODUCTION

Experimental allergic encephalomyelitis (EAE) has been extensively investigated
as a model of multiple sclerosis (MS) and a number of studies have indirectly
implicated the involvement of complement in the pathogenesis of both diseases.
Analyses of cerebrospinal fluid indicate that complement activation occurs within
the central nervous system (CNS) in MS (Morgan et al., 1984a; Sanders et al.,
1986) and both complement (Lumsden, 1971) and terminal component complex

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(TCC) (Compston et al., 1987) have been demonstrated immunohistochemically in the CNS of MS patients.

Complement is also believed to play an important role in the pathogenesis of antibody-mediated demyelination in chronic relapsing EAE (CREAE) in the guinea pig. CREAE is induced by immunizing juvenile animals with an emulsion of syngeneic spinal cord tissue in Freund's complete adjuvant. Sera from CREAE animals contain a wide spectrum of antmyelin antibodies some of which recognize myelin surface epitopes (Lassmann et al., 1981; Raine et al., 1981; Brosnan et al., 1983; Schwerer et al., 1984; Linington and Lassmann, 1987) and are able to initiate complement-dependent demyelination both in vitro (Dubois-Dalcq et al., 1970; Lebar et al., 1976; Liu et al., 1983; Bradbury et al., 1984) and in vivo (Brosnan et al., 1977; Lassmann et al., 1981). However, humoral immune responses to myelin antigens play little or no part in the pathogenesis of acute EAE which is mediated by an encephalitogenic T cell response (Ortiz-Ortiz and Weigle, 1976; Ben-Nun et al., 1981) and in the rat model, is associated with minimal primary demyelination (Lassmann et al., 1988). In the acute disease, the clinical signs are generally considered to be manifestations of blood-brain barrier dysfunction and CNS oedema (Hirano et al., 1970; Juhler et al., 1984; Kerlero de Rosbo et al., 1985; Goldmuntz et al., 1986; Sedgwick et al., 1987). However, despite this apparent absence of humoral effector mechanisms in acute models of EAE (Seil et al., 1968), depletion of serum complement with cobra venom factor (CVF) can suppress or delay the onset of EAE, without decreasing the extent of CNS inflammation (Levine et al., 1971; Pabst et al., 1971; Morariu and Dalmasso, 1978). These results suggest that serum complement is required for the clinical expression of acute EAE and that clinical disease is not directly mediated by the infiltrating inflammatory cells. Conversely, CVF has no effect on the induction, course or severity of hyperacute EAE in the rat (Levine et al., 1971), and C4 deficient guinea pigs are also susceptible to the disease (Morariu and Dalmasso, 1978).

These conflicting results reflect the difficulties inherent in comparing data obtained using experimental models of EAE which differ significantly in their mode of induction, clinical course and pathological response. Furthermore, the in vivo half-life of the active CVF-factor B complex is short and measurable haemolytic serum complement activity returns after 3-4 days, thereby limiting interpretation of results obtained with this reagent to the acute, predominantly inflammatory phase of EAE.

Recently an acute demyelinating form of EAE has been established in which the significance of complement in the clinical expression and pathogenesis of CNS inflammation and demyelination can be reliably determined (Lassmann et al., 1988; Linington et al., 1988). Intravenous injection of encephalitogenic MBP specific T cells into Lewis rats induces an acute inflammatory response in the CNS and compromises blood-brain function. During this period systemic injection of a CNS myelin specific monoclonal antibody (Mab) 8-18C5 (Linington et al.,
1984) results in the formation of large confluent areas of demyelination (Lassmann et al., 1988; Linington et al., 1988). Similar results can also be obtained with this Mab when EAE is actively induced using purified MBP as the encephalitogen (Schluessner et al., 1988).

We have now investigated the requirement for serum complement in the pathogenesis of this synergistic model of acute demyelinating EAE. The results provide evidence that complement is not only involved in the pathogenesis of antibody-mediated demyelination, but that it also plays an important role in the clinical expression of low grade CNS inflammation.

METHODS

Animals and reagents

Lewis rats, weighing 190–220 g were obtained from Bantin and Kingman, UK. Myelin basic protein was purified from whole guinea pig brain and stored lyophilized at -70°C. Cobra venom factor was isolated from lyophilized venom (Naja naja kaouthia, Sigma Chemical Co., Poole, Dorset, UK) by the method of Vogel and Müller-Eberhard (1984). The purified CVF was free of phospholipase A2 activity and was functionally active both in vivo and in vitro. The monoclonal antibody 8-18C5, (specific for the myelin oligodendrocyte glycoprotein (MOG)) and control polyclonal mouse IgG were purified from mouse ascitic fluid and serum respectively, by ammonium sulphate precipitation and ion exchange chromatography (Linington et al., 1988). F(ab)2' fragments were prepared from purified IgG by pepsin digestion and exhaustive dialysis (Parham, 1986). The purity of these reagents was determined by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) under both reducing and nonreducing conditions (Laemmli, 1970). Enzyme linked immunosorbant assays (ELISA) and Western blotting techniques (Linington and Lassmann, 1987), using antibodies specific for mouse IgG (heavy and light chains) and mouse F(ab)2', failed to demonstrate intact mouse IgG in the F(ab)2' preparation, whereas this reagent still reacted with MOG. Normal human serum was obtained from laboratory personnel and was depleted of C9 by passage over a column of monoclonal antihuman C9 antibody coupled to Sepharose 4B (Morgan et al., 1984b).

Oligodendrocyte culture

Oligodendrocytes were prepared from the optic nerves of 7 day old Wistar rats (Raff et al., 1983). Cells were cultured on polylysine-coated coverslips in Dulbecco's modified Eagle's medium supplemented with 0.5% fetal calf serum, gentamicin (25 µg/ml), bovine insulin (5.0 µg/ml), human transferrin (100 µg/ml), progesterone (60 ng/ml), putrescine (16 µg/ml), selenium (40 ng/ml), thyroxine (400 ng/ml) and tri-iodothyronine (300 ng/ml). Oligodendrocytes in these cultures were detected by indirect immunofluorescence using mouse monoclonal antibodies specific for galactocerebroside (GC: Ranscht et al., 1982) or MOG (Linington et al., 1984), and polyclonal rabbit antisera specific for MBP (prepared by B.P.M.). Astrocytes were visualized using rabbit antiglial fibrillary acidic protein (GFAP) antiserum (a generous gift from Dr M. L. Cuzner). Appropriate fluorescein and rhodamine antimouse and antirabbit IgG conjugates were purchased from Sigma. Cells were fixed with 2% paraformaldehyde for 15 min followed by 10 s in ethanol/acetic acid (95/5 % v/v) at -20° C to visualize MBP and GFAP. Staining for GC and MOG was performed on live cells at room temperature in the presence of 0.05% sodium azide. The cultures routinely consisted of > 90% oligodendrocytes as determined using these cell markers. Cell viability was determined using propidium iodide and cells examined with a Zeiss universal inverted fluorescence microscope.
Induction of EAE

Active EAE was induced following the injection of 100 μl of a 1:1 emulsion containing 50 μg MBP in phosphate buffered saline (PBS) and 50 μl Freund's complete adjuvant (CFA; Difco) into the hind footpads. Passive transfer of EAE was carried out as previously described (Linington et al., 1988). Rats immunized using the protocol for active EAE were killed at the onset of clinical disease, their spleens removed and single cell suspensions prepared. Spleen cells were cultured for 3 days in RPMI 1640 containing 5% fetal calf serum in the presence of MBP (10 μg/ml) at a density of 2 x 10^5 leucocytes/ml; cells were collected by centrifugation, washed, counted and resuspended in PBS. Naive syngeneic recipients were then injected in the tail vein with the appropriate number of cells in 1 ml of PBS. All animals were weighed and examined daily for clinical signs of EAE and scored on the scale: 0.5, partial loss of tail tone; 1.0, complete loss of tail tone; 2.0, hindlimb weakness; 3.0, hindlimb paralysis; 4.0, moribund; 5.0, dead.

Rats were decomplemented by intraperitoneal (i.p.) injection of 1 μg/g body wt. purified CVF in Tris-saline. Antibody and F(ab)2' fragments were administered by injection into the tail vein in 1 ml of PBS.

Animals were prepared for histology by perfusion through the aorta with 4% paraformaldehyde in PBS. Tissue was then immediately removed and fixation continued in the same reagent for 24 h. Tissue was embedded in paraffin and multiple sections cut from various levels of the spinal cord and brain. Sections were stained with haematoxylin-eosin or luxol fast blue in order to assess inflammation and demyelination.

RESULTS

Decomplementation in the Lewis rat by CVF

A single i.p. injection of CVF (1 μg/g body weight) abolished haemolytic serum complement activity for at least 48 h. Low level (0-3% of control) complement activity was detected in the sera of 25% of animals 72 h after injection of CVF. Thereafter, serum haemolytic activity rose slowly reaching 10% of control values by 7 days and was fully restored in all animals 2 weeks after CVF.

![Fig. 1. Effect of a single injection of CVF (1 μg/g body weight) on the serum haemolytic complement activity of Lewis rats (n = 4, error bars denote ±1 SD).](https://academic.oup.com/brain/article-abstract/112/4/895/319840)
treatment (fig. 1). A second injection of CVF, 4 days after the first, ensured that serum complement activity was completely abolished for a total period of 6 days (data not shown).

**Influence of CVF on the course of actively induced EAE**

Preliminary experiments confirmed earlier reports (Levine et al., 1971; Pabst et al., 1971) that CVF treatment influences the clinical course of actively induced EAE. Treatment of Lewis rats with CVF on days 0 and 10 after immunization with MBP in CFA entirely abolished the clinical signs of EAE, although in some CVF treated animals recovery of serum complement activity coincided with transient weight loss. In order to avoid the possibility that CVF treatment influenced the development of the encephalitogenic immune response to MBP, the initial CVF injection was subsequently omitted. A single injection of CVF on day 10, 24-48 h before the expected onset of disease, suppressed the development of clinical signs in an experimental group of 10 rats (fig. 2). No clinical evidence of EAE subsequently developed in CVF treated rats studied for up to 30 days postimmunization (p.i.) with MBP/CFA, even though haemolytic serum complement returned to control levels by day 18.

Histological examination revealed that despite the absence of clinical disease, CVF treated animals had extensive perivascular cuffs of inflammatory cells.

![Figure 2](https://academic.oup.com/brain/article-abstract/112/4/895/319840)
Fig. 3. A, spinal cord section of a rat with actively induced EAE, 15 days p.i. when killed. This animal exhibited severe hindlimb paralysis. B, as in A but treated with CVF 10 days p.i. This animal was clinically well when killed. Note the typical perivascular location of the inflammatory infiltrates in these sections. C, spinal cord section from an animal with actively induced EAE injected i.v. with the 8-18C5 Mab, 15 days p.i. Note the extensive areas of subpial and confluent perivascular myelin loss indicated by the arrow heads. D, as in C, but decomplemented on day 10 p.i. Note the absence of demyelination despite white matter inflammation. Luxol fast blue. Bars 500 μm. E–H on facing page.
Fig. 3. e-h. higher magnification of the lesions indicated by the arrows in a-d, respectively. Luxol fast blue. Bars 100 μm.
throughout the CNS (fig. 3). Although there were no obvious differences in the intensity, number or distribution of inflammatory infiltrates seen in animals with clinical EAE and those treated with CVF at the height of clinical disease (15 days p.i.), CVF treatment did reduce the number of inflammatory foci present in the lumbar spinal cord by 35% ($P < 0.005$) during the onset of clinical disease (12 days p.i.; Table 1). Staining with luxol fast blue revealed no demyelination at the level of resolution obtained in this light microscopic study (fig. 3). These observations indicate that CVF treatment acts to dissociate the clinical and histological signs of EAE.

### Table 1. The Influence of CVF Treatment on CNS Inflammation During the Course of Actively Induced EAE*

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<thead>
<tr>
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<th>Number of inflammatory foci/spinal cord section</th>
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<tr>
<td></td>
<td>EAE controls</td>
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<tr>
<td></td>
<td>(mean ± SD)</td>
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<tr>
<td>Day 12</td>
<td>8.5 ± 2.6 (n = 4)</td>
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<tr>
<td>Day 15</td>
<td>5.1 ± 3.1 (n = 4)</td>
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* Rats with actively induced EAE were perfused with paraformaldehyde both at the onset (day 12 p.i.) and at the height of clinical disease (day 15 p.i., see fig. 2). Multiple sections were prepared from the lumbar region of each spinal cord and stained with haematoxylin-eosin. A minimum of 19 sections from each animal were counted in order to determine the mean density of inflammatory foci/spinal cord cross-section. CVF treatment reduced the number of inflammatory foci by 35% ($P < 0.005$, Student's t test) at the onset of disease, although there was no significant difference subsequently. As the animals for each time point were taken from different experiments, no significance should be placed on the apparent reduction in the number of infiltrates between days 12 and 15 p.i.

**Action of CVF on passively transferred EAE**

The effect of CVF treatment was also investigated in a passive transfer model of EAE in which disease was induced by the transfer of activated MBP specific T cell blasts derived from the spleens of MBP immunized rats. This model offers the advantage that activation of the effector T cell population occurs in vitro and cannot therefore be influenced by CVF treatment of the recipients. Furthermore, the clinical severity of the disease was readily manipulated by varying the number of activated spleen cells transferred.

The injection of $10^7$ MBP activated spleen cells induced a mild monophasic form of EAE in which animals lost weight and developed hindlimb weakness (fig. 4A). In contrast, injection of $5 \times 10^7$ cells/animal resulted in all animals being moribund within 5 days of cell transfer (fig. 4B). The ability of CVF treatment to suppress clinical signs of EAE in this model was dependent on the dose of MBP activated spleen cells used. The milder clinical disease induced with $10^7$ spleen cells was suppressed by injection (i.p.) of CVF 24 h after cell transfer (fig. 4C). The onset of clinical disease was delayed in the CVF treated animals and the mean
clinical score reduced from 2 (SD = 0) to 0.5 (SD = 0.55, n = 6 for both groups). Once again, despite suppressing clinical disease, CVF had no obvious effect on CNS inflammation in animals examined 5 days after cell transfer. In contrast to these observations, CVF treatment had no effect on severe EAE induced with $5 \times 10^7$ spleen cells, even when serum complement activity was further depleted by a second injection of CVF 3 days after cell transfer and 24–48 h before the onset of clinical disease (fig. 4D).

![Fig. 4. Influence of CVF treatment on the clinical course of passively induced EAE. EAE was induced by the transfer of either $10^7$ (A, C) or $5 \times 10^7$ (B, D) MBP activated spleen cells on day 0 (n = 6 in each case). This was followed 24 h later by an i.p. injection either of CVF (C, D) or an equal volume of Tris/saline (A, B). These injections were repeated 72 h later in groups B and D. A further group of animals also received an i.v. injection of 8-18C5 Mab on day 3 (C, n = 4, open circles). The SEM did not exceed 15% of the mean clinical score with the exception of those CVF treated animals with transient disease on day 5 (C, closed circles).](https://academic.oup.com/brain/article-abstract/112/4/895/319840)

**Complement dependent oligodendrocyte lysis by the 8-18C5 Mab in vitro**

The mechanism by which the anti-MOG Mab, 8-18C5, mediates demyelination in vivo (Linington et al., 1988) was investigated in vitro. Oligodendrocytes maintained in culture for several days express the MOG epitope recognized by the 8-18C5 Mab on their surface (N.J.S., unpublished results). Addition of varying concentrations of the 8-18C5 Mab to the cultured cells, in the presence of fresh human serum as a source of complement, resulted in a dose dependent lysis of oligodendrocytes. Antibody-mediated damage to the target cells was quantitated by fluorescence microscopy using the criterion of propidium iodide exclusion (fig. 5).
Inactivation of complement either by heating the serum at 56° C for 20 min or depletion of C9 by passage over an anti-C9 antibody column abolished the lytic potential of the Mab, although in the latter case lysis was restored following reconstitution with purified human C9. These experiments demonstrate that the antibody mediates oligodendrocyte damage via the complement cascade, ultimately resulting in the formation of membrane attack complex. This was further demonstrated in experiments using 8-18C5 F(ab)2'. Although the F(ab)2' fragment bound to the oligodendrocyte surface, determined by fluorescence microscopy, it failed to mediate oligodendrocyte lysis (fig. 5) indicating that lysis is mediated by the Fc C1q binding domain via the classical activation pathway.

**Complement depletion inhibits antibody mediated demyelination in vivo**

Previous studies have demonstrated that i.v. injection of the monoclonal antibody 8-18C5 before the onset of both actively and passively induced EAE, increases disease severity and initiates widespread perivascular and subpial demyelination (fig. 3; Schluesener *et al.*, 1987; Lassmann *et al.*, 1988; Linington *et al.*, 1988). We have confirmed these observations and used this model of EAE to determine the role of complement in acute antibody-mediated demyelination in vivo.

Rats (n = 6) were injected with 8-18C5 11 days after sensitization with MBP to induce acute demyelinating EAE (fig. 2b). Although treatment with CVF protected these animals from the potentially lethal effect of the Mab, the antibody
did induce clinical disease, that is, loss of weight and tail tone, followed by hind limb paralysis (fig. 2D). Histologically, the induction of clinical signs was associated with demyelination although this was reduced in comparison with animals not treated with CVF (fig. 3).

Similarly, no demyelination was observed in animals with EAE induced with either $10^7$ or $5 \times 10^7$ MBP sensitized spleen cells and treated both with CVF and 8-18C5 Mab, 3 and 4 days after the cell transfer, respectively. Control CVF treated animals with EAE injected with polyclonal mouse IgG in place of 8-18C5 Mab remained clinically well and exhibited no CNS demyelination.

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<th>Table 2. Comparison of the in vivo effects of the 8-18C5 antibody and its F(ab)2' fragment on the course and pathology of adoptively transferred EAE*</th>
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<td><strong>Experimental group</strong></td>
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<tr>
<td>EAE controls</td>
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<td>8-18C5</td>
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<td>8-18C5 F(ab)2'</td>
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* Animals were injected with $10^7$ MBP activated spleen cells on day 0 followed by (a) 5 mg polyclonal mouse IgG, (b) 5 mg 8-18C5, (c) 4 mg 8-18C5 F(ab)2' on day 4. Animals injected with either 8-18C5 or its F(ab)2' fragment in the absence of EAE developed no clinical or histological signs of EAE. * From time of antibody injection; † determined on day 6; ‡ inflammation, D = demyelination.

In addition to using CVF treatment to examine the importance of complement components in this model of antibody-mediated demyelination, we also assessed the ability of 8-18C5 F(ab)2' fragments to substitute for the intact Mab and initiate demyelination in vivo. In contrast to the intact antibody, injection of 8-18C5 F(ab)2' into rats with passively transferred EAE (4 mg i.v. at the onset of clinical disease) failed to modify the severity, course or pathology of the disease and did not induce demyelination (Table 2). These results confirm the in vitro study and demonstrate that the Fc region, which contains the C1 binding site, is an absolute requirement for the antibody to initiate demyelination in vivo.
DISCUSSION

In the present study, clinical signs of acute but not hyperacute EAE were suppressed after decomplementation by CVF although CNS inflammation, assessed histologically, was unchanged at the height of clinical disease. In an acute synergistic model of antibody mediated, demyelinating EAE, CVF treatment demonstrated that serum complement is also necessary for antibody-mediated demyelination in vivo.

CVF acts by binding factor B and forming a stable C3 and C5 convertase (CVF-FB), thereby rapidly hydrolyzing these components in an uncontrolled manner and depleting serum of haemolytic complement. A number of short-lived activation products (e.g., C3a, C5a), which mediate a range of biological activities including anaphylaxis and chemotaxis, are generated in this process. However, the half lives of these activation products are short (Jose, 1987) and the rat appears to suffer no immediate or long-term effects from CVF treatment other than a gross depletion of haemolytic serum complement. It is, however, unclear as to how effective CVF treatment is at depleting complement within the CNS. Monocytes have the capacity to synthesize all the complement components necessary for TCC formation (Hetland et al., 1986) and astrocytes have been reported to synthesize C3 and factor B (Lévi-Strauss and Mallat, 1987). It is therefore possible that low levels of complement may be synthesized locally in inflammatory CNS lesions and escape activation by the CVF-FB convertase complex. CVF appears to be effective for 3-4 days in the rat, after which serum complement is again detectable, effectively limiting studies of CVF mediated decomplementation to the acute phase of EAE. Although some complement deficient rat strains are available, they have not been characterized for susceptibility to EAE. We therefore consider that CVF treatment is at present the method of choice for determining the role of complement in the pathogenesis of EAE.

The initial event in the induction of EAE is recognition of the target antigen by activated T cells; this results in blood-brain barrier dysfunction and CNS infiltration by inflammatory cells (see Wekerle et al., 1986). In the rat, mild clinical EAE induced either by active immunization with MBP or the transfer of encephalitogenic T cells, is an inflammatory disease without significant demyelination (Lassmann et al., 1988; Linnington et al., 1988), in which the clinical signs are believed to depend on blood-brain barrier dysfunction and CNS oedema (Kerlero de Rosbo et al., 1985; Goldmuntz et al., 1986; Sedgwick et al., 1987). Since in EAE, CVF treatment can suppress low grade clinical disease without affecting CNS inflammation, the clinical expression of these inflammatory events within the CNS must involve a complement dependent mechanism which is not directly dependent on the inflammatory infiltrate. This hypothesis is supported by experiments in which severe clinical EAE has been induced in leucopenic rats in the absence of inflammatory cells in the CNS (Sedgwick et al., 1987).

The mechanism by which complement acts to induce the clinical expression of
low grade CNS inflammation is uncertain. However, as the encephalitogenic T cell response induces blood-brain barrier dysfunction (Sedgwick et al., 1987), serum complement will gain access to the CNS during acute EAE. In vitro, isolated CNS myelin (Vanguri et al., 1982; Silverman et al., 1984) and more importantly, intact cultured rat oligodendrocytes (N. J. Scolding et al., unpublished results) can activate complement in the absence of myelin specific antibodies. This mechanism, however, does not result in gross demyelination in acute inflammatory EAE (Lassmann et al., 1988; Linington et al., 1988), suggesting that either protection mechanisms act (Ramm et al., 1983; Carney et al., 1985; Morgan et al., 1987) to inhibit complement-mediated demyelination in vivo, or any damage is sublytic. Nevertheless, activation of complement and the deposition of sublytic levels of TCC on the oligodendrocyte/myelin complex may have a direct effect on cell metabolism, stimulating the production and release of leukotriene B₄ by oligodendrocytes (Shirazi et al., 1987). Furthermore, significant amounts of inflammatory mediators such as C3a and C5a would be released, further increasing vascular permeability and stimulating infiltrating inflammatory cells to produce potentially harmful biological compounds (prostaglandins, toxic oxygen metabolites and lysosomal enzymes) (Rother et al., 1985; Jose, 1987). CVF treatment would obviously suppress this response.

Serum complement is not an absolute requirement for the clinical expression of CNS inflammation in EAE. Large doses of encephalitogenic spleen cells override the suppressive effect of CVF and induce severe clinical disease in the absence of normal levels of serum complement. This form of EAE is associated with intense CNS inflammation, in which axonal damage and secondary demyelination are notable features (Lassmann et al., 1988), and it appears to be a better model of acute disseminated encephalomyelitis than MS. The importance of complement-dependent mechanisms for the clinical expression of CNS inflammation therefore appears to be inversely proportional to the severity of the inflammatory insult.

In contrast to its involvement in the inflammatory aspect of EAE, the role of complement in the pathogenesis of acute antibody-mediated demyelination is far clearer. We have formally demonstrated that the monoclonal antibody, 8-18C5 lyses mature oligodendrocytes in vitro in a complement and Fc dependent manner. However, CVF treatment failed to abolish antibody-mediated demyelination completely in vivo. In this situation, demyelination and the associated clinical signs of disease may reflect either opsonization of myelin by antibody followed by Fc receptor-mediated macrophage attack, the activation of complement synthesized locally within the CNS by monocytes or astrocytes, or the biological consequences of Cl, C2, C4 activation, as these components of the complement system are unaffected by CVF treatment.

In vivo, demyelination initiated by the 8-18C5 Mab is intimately associated with macrophages (Lassmann et al., 1988; Linington et al., 1988) and, furthermore, depletion of the macrophage population in vivo will suppress EAE and inhibit demyelination (Brosnan et al., 1981). These results indicate that antibody-mediated
demyelination in EAE may be initiated by at least two mechanisms, the action of which may be synergistic, namely direct complement-mediated lysis and Fc-mediated macrophage attack. Antibody-dependent complement activation on the myelin surface will release complement derived macrophage activators and opsonize the membrane with C3b, for which macrophages express a specific receptor. In addition, TCC deposition may promote an influx of calcium (see Campbell, 1987), activating calcium-dependent phospholipases and neutral proteases (Banik et al., 1985) and inducing vesicular disruption of myelin (Schlaepfer, 1977), thereby destabilizing the membrane and increasing its susceptibility to external attack by macrophage-derived enzymes (Cammer et al., 1986). These mechanisms would account for the patterns of myelin destruction seen in EAE, vesicular dissolution of myelin adjacent to macrophages, invasion of macrophages into myelin sheaths and phagocytosis of myelin debris, sometimes in association with coated pits (Lassmann et al., 1988).

The results of the present study have demonstrated that complement plays an important role in the clinical expression and pathogenesis of both inflammation and demyelination in EAE. However further experiments are required fully to characterize the role of complement in vivo, in particular the relative importance of TCC formation and complement-derived inflammatory mediators, in the complex sequence of events which leads to cellular damage in the CNS and neurological dysfunction in EAE.

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