Clinical Immunology (2013) **146**, 155–164

available at www.sciencedirect.com

Clinical Immunology

www.elsevier.com/locate/yclim

The complement system contributes to the pathology of experimental autoimmune encephalomyelitis by triggering demyelination and modifying the antigen-specific T and B cell response

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Received 9 August 2012; accepted with revision 9 December 2012
Available online 22 December 2012

KEYWORDS
B cells;
Complement;
Demyelination;
EAE/MS;
MP4;
T cells

Abstract So far, studies of the human autoimmune disease multiple sclerosis (MS) have largely been hampered by the absence of a pathogenic B cell component in its animal model, experimental autoimmune encephalomyelitis (EAE). To overcome this shortcoming, we have previously introduced the myelin basic protein (MBP)–proteolipid protein (PLP) MP4-induced EAE, which is B cell and autoantibody-dependent. Here we show that MP4-immunized wild-type C57BL/6 mice displayed a significantly lower disease incidence when their complement system was transiently depleted by a single injection of cobra venom factor (CVF) prior to immunization. Considering the underlying pathomechanism, our data suggest that the complement system is crucial for MP4-specific antibodies to trigger CNS pathology. Demyelinated lesions in the CNS were colocalized with complement depositions. In addition, B cell deficient J_HT mice reconstituted with MP4-reactive serum showed significantly attenuated clinical and histological EAE after depletion of complement by CVF. The complement system was also critically involved in the generation of the MP4-specific T and B cell response: in MP4-immunized wild-type mice treated with CVF the MP4-specific cytokine and antibody response was significantly attenuated compared to untreated wild-type mice. Taken together, we propose two independent mechanisms by which the complement system can contribute to the pathology of autoimmune encephalomyelitis. Our data corroborate the role of complement in triggering antibody-dependent demyelination and antigen-specific T cell immunity

Abbreviations: APC, Antigen presenting cell; B6, C57BL/6; C1qRP, C1q receptor P; C3aR, C3a receptor; C5aR, C5a receptor; CR2, Complement receptor 2; CNS, Central nervous system; CVF, Cobra venom factor; DAF, Decay-accelerating factor; EAE, Experimental autoimmune encephalomyelitis; MBP, Myelin basic protein; MOG, Myelin oligodendrocyte glycoprotein; MP4, MBP–PLP fusion protein; MS, Multiple sclerosis; OVA, Ovalbumin; PLP, Proteolipid protein; WT, Wild-type.

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1521-6616/$ - see front matter
http://dx.doi.org/10.1016/j.clim.2012.12.007

and also provide first evidence that the complement system can modify the antigen-specific B cell response in EAE and possibly MS.

1. Introduction

Multiple sclerosis (MS) is one of the most prevalent human autoimmune disorders of the central nervous system (CNS). While it was originally considered to be a primarily T cell-mediated disease, ample evidence has accumulated that B cells and autoantibodies are crucially involved [1–3].

Experimental autoimmune encephalomyelitis (EAE) has been studied for decades as an animal model for MS. However, there are only few models that entail a pathogenic B cell/antibody response. Among the murine B cell-dependent models are the human myelin oligodendrocyte glycoprotein (MOG) protein-induced EAE of C57BL/6 (B6) mice [4] and a MOG peptide 92–106 T cell receptor transgenic SJL model [5]. We have recently introduced the MP4-elicited disease on the B6 background [6]. MP4 is a fusion protein comprising the 21.5 kDa isoform of human myelin basic protein (MBP) and the three hydrophilic domains of proteolipid protein (PLP). It was originally generated as a drug candidate for MS due to its capability to induce T cell tolerance in EAE [7]. MP4-induced EAE proved to be strongly reliant on MP4-specific antibodies: B cell-deficient μMT and J_HT mice immunized with MP4 did not develop EAE — however by transfer of MP4-reactive serum disease could be restored to the level of the wild-type mice. In addition, we found antibody depositions within demyelinated CNS lesions in MP4-immunized wild-type B6 mice [8]. The mechanisms of MP4-specific antibody action have not yet been investigated. Since the blood–brain-barrier is disturbed in the disease, it can be expected that complement components have access to the CNS where they will be locally activated [9].

The involvement of the complement system has long been suggested in the pathogenesis of both EAE and MS. Histopathological characterization of MS lesions has demonstrated the deposition of antibodies and complement in the majority of patients [10] and complement activation has been proposed to be a cause of oligodendrocyte/myelin damage through the formation of the membrane attack complex and complement-mediated cytolysis [10–13]. Moreover, it has been shown that complement can modulate the induction, effector and contraction phase of the T cell response [14]. Following cognate interactions between antigen presenting cells and T cells, locally produced complement has not only been reported to function as a costimulatory molecule for T cells inducing their proliferation and cytokine production, but also to sustain viability of naïve T cells [12,15,16]. In addition, the complement system has been proposed to be involved in B cell activation and memory cell generation [17,18].

Overall, studies in EAE brought forth controversial results. While some investigators found the disease to be independent of complement [19,20], others reported attenuated disease in complement component-deficient or complement-depleted animals [21–24]. Even others suggested a neuroprotective role of complement activation and C5b-9 assembly [25–27]. These findings indicate that the complement system could take over a dual role in the disease process. There are currently no investigations on the role of the complement system in MP4-induced EAE.

The present study aimed at characterizing the involvement of complement in the B cell-/antibody-dependent MP4 model, studying both the relevance for the establishment of CNS pathology and the MP4-specific T and B cell response.

2. Materials and methods

2.1. Mice

Female C57BL/6J mice were purchased from the Harlan Laboratories (Rossdorf, Germany). Female J_HT mice were a kind gift from Ari Waisman (University of Mainz, Germany). All mice were 6–8 weeks old at the time of treatment. The mice were maintained at our local animal facilities under specific pathogen-free conditions in isolated ventilated cages. All treatments were performed according to an approved protocol and complied with the institutional guidelines.

2.2. Immunizations

The MBP–PLP fusion protein MP4/Apogen was obtained from Alexion Pharmaceuticals (Cheshire, CT). Incomplete Freund's adjuvant (IFA) was prepared as a mixture of mannide monooleate (Sigma-Aldrich, St. Louis, MO) and paraffin oil (EMScience, Gibbstown, NJ), and complete Freund's adjuvant (CFA) was obtained by mixing *M. tuberculosis H37 RA* (Difco Laboratories, Franklin Lakes, NJ) at 5 mg/ml into IFA. For active immunization, B6 or J_HT mice were immunized subcutaneously in both sides of the flank with a total dose of 200 μg MP4 or OVA in CFA. Pertussis toxin (PTX; List Biological Laboratories, Hornby, ONT, Canada) was given at 200 ng per mouse on the day of immunization and 48 h later. For passive transfer experiments, the protocol of Lyons et al. [28] was used with J_HT mice receiving four 150 μl injections of pooled antisera at 3-day intervals for a total dose of 600 μl. The donor sera had been obtained from wild-type B6 mice immunized with MP4. Clinical assessment of EAE was performed daily according to the following criteria: (0) no disease; (1) floppy tail; (2) hind limb weakness; (3) full hind limb paralysis; (4) quadriplegia; (5) death. Mice that were in-between the clear-cut gradations of clinical signs were scored intermediately in increments of 0.5.

2.3. Decomplementation

Decomplementation of mice was performed using cobra venom factor (CVF from *naja naja kaouthia*; ACZON S.p.A., Bologna, Italy). CVF is an analogue of the complement protein