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Review

Transient decomplementation of mice delays onset of experimental autoimmune encephalomyelitis and impairs MOG-specific T cell response and autoantibody production

Nóra Terényi[a], Nándor Nagy[c], Krisztián Papp[b], József Prechl[b], Imre Oláh[c], Anna Erdei[a,b,*]

[a] Department of Immunology, Eötvös Loránd University, H-1117 Budapest, Hungary
[b] Immunology Research Group of the Hungarian, Academy of Sciences at Eötvös Loránd University, H-1117 Budapest, Hungary
[c] Department of Human Morphology and Developmental Biology, Faculty of Medicine, Semmelweis University, H-1094 Budapest, Hungary

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ABSTRACT

Multiple sclerosis (MS) is the most common inflammatory and demyelinating disease of the central nervous system. In both MS and its animal model experimental autoimmune encephalomyelitis (EAE), it is thought that infiltrating $CD4^+$ T cells initiate an inflammatory process and collect other immune effectors to mediate tissue damage. The pathophysiology of the disease however remains unclear. Here we focus on the role of the complement system in the pathomechanism of EAE, employing mice with transiently depleted complement activity achieved by a single injection of cobra venom factor (CVF) 2 days before the induction of the disease. Our results show that in decomplemented C57BL/6 mice immunized with myelin oligodendrocyte glycoprotein (MOG) peptide 35–55, the onset of the disease is significantly delayed. In SJL/J mice which develop a relapsing-remitting form of EAE after injection with proteolipid protein (PLP) peptide 139–151, the attenuation of both phases could be observed in CVF-treated animals. In C57BL/6 mice the level of MOG specific autoantibodies and their complement activating capacity evaluated on day 21 were found significantly reduced in animals transiently decomplemented before induction of the disease. The *in vitro* response of T cells isolated from the lymph nodes of MOG-immunized animals at the onset of EAE was also investigated. We found that the proliferative capacity of MOG-specific T lymphocytes derived from CVF treated animals is significantly reduced, in agreement with the histology of the spinal cords showing a decreased infiltration of $CD4^+$ T cells in these mice. Our data suggest, that lack of systemic complement at the time of induction of EAE delays the onset and attenuates the course of the disease most probably via diminishing the response of MOG-specific T cells and production of autoantibodies.

1. Introduction

Multiple sclerosis (MS) is the most common inflammatory autoimmune disease of the central nervous system (CNS). Immune response against the oligodendrocytes destroys their product, the myelin sheath. These lesions and the following axonal damage cause very diverse sensual and vegetative symptoms, depending on the site of the immune attack. Experimental autoimmune encephalomyelitis (EAE) is a widely used animal model of MS (Baxter, 2007). It is believed that Th1 type regulatory T cells induce the inflammatory process and activate the effector cells, such as cytotoxic T cells, macrophages, resident astrocytes and microglia cells (Martin et al., 1992; Sospedra and Martin, 2005). More recently the possible role of Th17 cells was also described (Bettelli et al., 2007) but still remain some open questions regarding the pathogenesis of the disease (Steinman, 2008).

The complement system is known to play an important role not only in the effector phase, but also in the initiation and regulation of adaptive responses (Pepys, 1974; Kerekes et al., 1998; Heyman, 2000; Carroll, 2004; Hawlisch and Kohl, 2006). The role of complement proteins in the pathogenesis of MS and EAE has been investigated for a long time; the results however are still contradictory. Some investigators using C3 KO mice found, that complement is not required for the development of EAE (Calida et al., 2001) or at least C4 is not essential (Boos et al., 2005). Nevertheless many other investigations have proven that complement has an important role in the course of this disease. These studies were carried out in C3 KO mice (Nataf et al., 2000), with rats decomplemented with cobra venom factor (Linington et al., 1989), with transgenic mice expressing sCrry in the nervous system (Davoust et al., 1999) and by using

* Corresponding author at: Department of Immunology at Eötvös Loránd University, H-1117 Budapest, Hungary. Tel.: +36 1 381 2175; fax: +36 1 381 2176.
E-mail address: anna.erdei@freemail.hu (A. Erdei).

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sCR1 treated rats (Piddlesden et al., 1994). So far no investigations of EAE have been carried out using cobra venom factor-treated mice. Cobra venom factor (CVF) is widely used for transient decomplementation of mice and rats. This complement activating protein in cobra venom is a C3b-analogue, which is able to bind to factor B and generate a relatively stable C3/C5 convertase. This leads to the activation of the complement cascade in CVF-treated animals and results in the elimination of C3 and C5 from the circulation. Normal levels of C3 will be restored in a week (Vogel et al., 2004).

The role of autoantibodies in MS is still controversial; yet one of the most important diagnostic hallmarks is the presence of oligoclonal Igs and plasma cells in cerebrospinal fluid. There is a growing number of evidence supporting the view that B cells and antibodies play a role in the pathogenesis of MS (Corcione et al., 2004; Urich et al., 2006). As experiments with B cell depleted animals show less clear results, the role of Igs seems to strongly depend on the experimental system (Lyons et al., 1999).

In the present study our aim has been to reveal how lack of complement at the initiation of EAE influences the onset and course of the disease. As it is known for long that complement is involved in both T-dependent and T-independent antibody responses, we compared the amount of MOG-specific antibodies in normal and CVF-treated mice, after induction of EAE. In addition to the levels of these autoantibodies, their complement activating ability was also assessed, using an antibody-complement (AbC) array system, developed recently at our department (Papp et al., 2007; Papp et al., 2008). Finally, the proliferative capacity of MOG-specific lymph node T-cells derived from normal and decomplemented animals has also been compared.

2. Methods

2.1. Animals, EAE induction

Female, 6–8 weeks old C57BL/6 and SJL/J mice, purchased from Charles River Laboratories were used. All animal experiments were in accordance with national regulations and were authorized by the ethical committee of the institute. C57BL/6 mice were injected *s.c.* on day 0 with 100 μg of Myelin Oligodendrocyte Glycoprotein 35–55 peptide (MOG, MEVGWYRSPFSRVVHLYRNGK) and SJL/J mice with 100 μg Proteolipid Protein peptide 139–151 (PLP, HSLGKWLGHPDKF) emulsified in CFA containing 4 mg/ml Mycobacterium tuberculosis (Chondrex Inc.). The peptides were synthesized by Gábor Tóth (University of Szeged, Hungary). In addition to the peptides, on days 0 and 2 mice were given *i.p.* 200 ng pertussis toxin (List Biological Laboratories).

Clinical signs of EAE were assessed daily using the following standard scale: 0, no clinical signs; 1, loss of tail tone; 2, incomplete hind limb paralysis; 3, complete hind limb paralysis; 4, moribund; 5, death. Animals with score 4 for more than 2 days were sacrificed. Representative of two to four independent experiments is shown.

2.2. Decomplementation

For the decomplementation of mice Cobra Venom Factor (CVF from Naja naja, Aczon SpA) was used. Mice were injected *i.p.* with 50 μg CVF in physiological saline 2 days before the induction of EAE.

2.3. Histological and immunocytochemical procedures

For cryostat sections the spinal cord and the lymphoid organs were excised, covered with liver for cryoprotection and snap-frozen in liquid nitrogen. Ten-micron-thick frozen sections were collected on poly-L-lysine-coated slides (Sigma–Aldrich), fixed in cold acetone, and air dried. For light microscopy the tissue samples were fixed in buffered 4% glutaraldehyde (Polysciences Inc.) solution for 24 h and postfixed in 1% osmium tetroxide (Polysciences) solution for 2 h. After rehydration in graded ethanol, the tissue samples were embedded in Polybed/Araldite 6500 mixture (Polyscience).

Immunocytochemistry was performed on cryostat sections according to standard techniques. Briefly, after rehydration in PBS, the acetone fixed sections were incubated with primary antibodies for 45 min, followed by biotinylated rabbit anti-rat IgG (Vector Laboratories). The endogenous peroxidase activity was quenched by treatment of the sections with 3% hydrogen peroxide (Sigma–Aldrich) for 10 min. Rigorous washing was followed by incubation of avidin-biotinylated peroxidase complex (Vector, Vectastain Elite ABC kit) at room temperature. The binding sites of the primary antibodies were visualized with 4-chloro-1-naphthol (Sigma–Aldrich). To identify macrophages rat anti-mouse CD11b mAb (clone: M1/70.15) was used. For T-cell staining anti-CD4 mAb (clone: YTS 091.1.2) was used. Appropriate isotype control antibodies were used at the same concentration. All antibodies were purchased from ImmunoTools GmbH.

2.4. Bone marrow derived dendritic cells (BMDC)

Six-week-old female C57BL/6 mice were sacrificed and their femurs were removed. Bone marrow was washed out with 5 ml of RPMI medium. Red blood cells were depleted by ammonium chloride treatment for 1 min at room temperature. Cells were cultured at 10^6/ml in RPMI medium, containing 10% of X63 cell (kindly provided by Dr. A. Rolink, University of Basel, Switzerland) supernatant, as the source of GM-CSF. Cytokine was added on every second day and BMDC were used as antigen-presenting cells (APC) on day 7.

2.5. T cell proliferation assays

Cells were isolated from the draining lymph nodes of mice injected with MOG peptide after 15 or 39 days of induction, as indicated. For the FACS-analysis lymph node cells were stained with CFSE (Molecular Probes). As APC spleen cells or BMDC were used. The ratio of APC and lymphocytes was 1:10. MOG 35–55 peptide was used at a concentration of 50 μg/ml. Cells were cultured in RPMI-1640 supplemented with 10% FCS, 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 0.05% 2-mercaptoethanol (Sigma–Aldrich). Proliferation was assessed either by FACS (BD LSR II), or by measuring the incorporation of ^{3}H thymidin, as indicated. In the case of CFSE-labeled cells data were analyzed by FCS Express.

2.6. Antigen array

For testing the antibody response a protein array system was used. PLP 139–151 and MOG 35–55 peptides (both 5 mg/ml), myelin basic protein (MBP, 1 mg/ml) and MOG (0.6 mg/ml; kindly provided by Dr. H. Wekerle, Martinsried, Germany) were used. Fourfold dilution series were applied in triplicates, using Calligrapher miniarrayer (BioRad), and samples were printed onto home-made nitrocellulose coated glass slides. All materials were diluted in PBS-azide. The generation of microarray data is described elsewhere in detail (Papp et al., 2007; Papp et al., 2008). Briefly, dried arrays were rinsed for 15 min in PBS just before use, and then incubated with sera of mice in a humidified chamber at 37 °C degrees for 60 min. The reaction was terminated by washing the array with 0.05% Tween 20 containing PBS. Slides were then incubated in a mixture of detecting antibodies with gentle agitation for 30 min at room temperature in the dark. FITC conjugated goat anti-mouse C3 F(ab)$_2$ and Alexa Fluor 647 labeled goat anti-mouse IgG (H + L) (Molecular Probes) were diluted in 5% BSA, 0.05% Tween 20 containing PBS for 10,000 and 5000 times, respectively. Following washing and drying, slides were scanned on a Typhoon Trio + Imager (Amersham Bioscience) following standard protocols. Data were analyzed with ImageQuantTL (Amersham Bioscience) software. Fluorescence