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Deficiency of the complement regulator CD59a enhances disease severity, demyelination and axonal injury in murine acute experimental allergic encephalomyelitis

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There is a growing body of evidence implicating complement and, in particular, the terminal pathway (membrane attack complex; MAC) in inducing demyelination in multiple sclerosis and experimental allergic encephalomyelitis. In this paper, we examined the disease course and pathological changes in mice deficient in the major regulator of MAC assembly, CD59a, during the course of acute experimental allergic encephalomyelitis induced by immunisation with recombinant myelin oligodendrocyte glycoprotein. Disease incidence and severity were significantly increased in CD59a-deficient mice. The extent of inflammation, demyelination and axonal injury were assessed in spinal cord cross-sections from CD59a-deficient and control mice, and all these parameters were enhanced in the absence of CD59a. Areas of myelin loss and axonal damage in CD59a-deficient mice were associated with deposits of MAC, firmly implicating MAC as a cause of the observed injury. These findings are relevant to some types of human demyelination, where abundant deposits of MAC are found in association with pathology.
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Although the aetiology of the human inflammatory and demyelinating disease multiple sclerosis (MS) is uncertain, it is widely accepted that autoimmunity plays a significant role.[1] Evidence from the animal model, experimental autoimmune encephalomyelitis (EAE), implicates an autoimmune T-cell response as the initiating factor. However, in unmodified EAE, demyelination and neuronal damage are usually minor and additional factors are required to induce pathology more closely resembling the human disease.[2,3] These observations have led to the suggestion that other aspects of the immune system may be responsible for myelin damage in models and humans, and has provoked the hypothesis that different mechanisms of pathology are operating in different subsets of MS patients, or even within the same patient.[4] In addition to myelin damage, direct axonal injury occurs in both EAE and MS,[5] and axonal loss correlates with persistent functional deficit.[6] The mechanisms leading to axonal damage are not well understood.

There is now a substantial body of evidence implicating complement (C) as a myelinolytic agent both *in vitro* and *in vivo* in humans and in experimental animals. Early *in vitro* models of demyelination using cerebellar explant cultures showed that the demyelinating component of sera from animals with EAE was heat labile, a classical characteristic of the C system.[7] Central nervous system (CNS) myelin and the myelin-producing oligodendrocytes activate the classical pathway of C *in vitro*[8,9] and C activation products are deposited in and around areas of demyelination in experimental demyelination and MS.[10,11] In rat EAE,

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C depletion ameliorates disease,[12] and in an antibody-mediated demyelinating form of EAE (ADEAE), induced by injection of antibodies to myelin oligodendrocyte glycoprotein (MOG) at the onset of clinical signs in EAE, C is essential for demyelination.[13,14] Recent studies in C3 gene-targeted mice have introduced an element of confusion. One study reported a protective effect of C3 deficiency on levels of disease, inflammation and demyelination in MOG peptide (amino acids 35–55)-induced EAE,[15] whereas a second study found no effect of C3 deficiency in this model, bringing into question the importance of C.[16] Importantly, the former study utilised mice backcrossed to C57BL/6, whereas the latter used mice on a mixed C57BL/6 × 129/Sv background; the observed differences are therefore likely to be strain dependent.

Although C activation generates numerous activities that might contribute to myelin damage, including the opsonic fragments of C3, the chemotactic peptides C3a and C5a and the cytolytic MAC, the identity of the myelin-damaging factors remains unclear. An essential role of the MAC was suggested from early *in vitro* studies of myelin destruction using sera depleted in individual C components.[8,17] Our recent studies in C6-deficient rats, which are unable to form the MAC but have otherwise normal C systems, demonstrated that in the absence of MAC formation, demyelination and axonal injury were absent and clinical disease reduced in the ADEAE model.[18] These results, strongly implicating MAC in myelin loss, were subsequently confirmed by others.[19]

Autoantibodies against MOG have been found on disintegrating myelin around axons in acute MS lesions and in a marmoset model of demyelinating EAE, suggesting that some forms of MS may closely resemble the ADEAE model, driven by anti-MOG antibody and C.[20] C activation products and vesicles suggestive of MAC damage to oligodendrocytes were found in MS cerebrospinal fluid and brain[11,21–23] and MAC was localised to the edges of active plaques in MS brain, suggesting a close association with ongoing pathology.[2] Recently, a multicentre study of a large collection of active MS lesions defined four different types of pathology. The most common, accounting for over 50% of cases, was characterised by high levels of immunoglobulin and MAC deposition at areas of active myelin destruction.[4] A similar MAC-positive pattern of pathology has been defined in a recent study of patients with neuromyelitis optica, a condition closely related to MS.[24]

The evidence described above points to a role for the MAC in causing demyelination in EAE and in MS. CD59, a ubiquitously expressed cell surface complement regulatory protein, exists to protect self cells from damage caused by the activation of the C cascade by blocking the formation of the MAC.[25] CD59 might therefore be an important resistance factor in protecting against MAC-mediated demyelination. In the mouse, the situation is complicated by a gene duplication event, creating two forms of CD59, the first described, termed CD59a being broadly distributed whereas the second, CD59b is likely expressed only in testis.[26–28] We have recently generated CD59a-deficient mice (CD59a−/−) by targeted gene deletion.[29] These mice are healthy apart from mild intravascular haemolysis and haemoglobinuria. Here, we describe the effects of this deficit on the course of acute EAE. Disease was induced in CD59a−/− mice and littermate controls using the recombinant extracellular Ig-like domain of MOG ($rMOG^{IgD}$) as immunogen, the disease course was monitored and major neuropathologic correlates of disease, namely inflammation (CNS infiltration), demyelination and axonal injury, compared. CD59a−/− mice had more severe clinical disease at all time points and much increased inflammatory cell infiltration, demyelination and axonal injury. These findings provide further support for the hypothesis that MAC is a critical factor in demyelination and axonal injury in models and humans, and suggest that the expression of CD59 in the CNS might be a key protective factor.

Materials and methods

Animals

CD59a−/− (129/Sv × C57 Bl/6 J) mice and wild-type littermates were generated in-house.[29] Both the parental strains and mice on the mixed 129/Sv × C57Bl/6J background have previously been shown to be susceptible to the induction of EAE using MOG-derived peptide.[30] Mice 8-week old were used in all experiments. Only male mice were used in the present study as they have a serum haemolytic C activity 10–12 times greater than that of female mice on this genetic background.[29] Animals were maintained according to Home Office guidelines.

Induction of EAE

Recombinant rat MOG^{IgD} ($rMOG^{IgD}$) consisting of amino acids 1–125 of the extracellular immunoglobulin domain of MOG, was generated as described previously.[31] On day 0, mice were immunised subcutaneously at a single site close to the base of the tail with 200 μl of an emulsion containing 200 μg $rMOG^{IgD}$ in complete Freund's adjuvant (Becton Dickinson, Oxford, UK) supplemented with 2.5 mg/ml *Mycobacterium tuberculosis* H37 Ra (Difco Labs, Detroit, MI, USA). Mice also received 200 ng pertussis toxin intraperitoneally (Sigma, Poole, Dorset, UK) in phosphate-buffered saline (PBS) on days 0 and 2. Mice were weighed and monitored daily for signs of clinical disease, scored as follows: 1, tail atony; 2, hind-limb weakness; 3, hind-limb paralysis; 4, moribund; 5, dead. Animals

that attained a clinical score of 3 or 4 at monitoring were killed immediately to conform to home office guidelines. All remaining animals were killed at day 16 for harvest of tissues.

Tissue Processing and Analysis

Animals were killed by perfusion with cold 4% paraformaldehyde in PBS via the aorta, while under terminal anaesthesia. Brains and spinal cords were removed, postfixed in 4% paraformaldehyde in PBS at 4°C overnight, washed in PBS and embedded in paraffin wax. Analysis and quantitation of inflammation, demyelination and axonal injury were performed by a blinded observer at the Brain Research Institute, University of Vienna as described previously.[18] Paraffin sections (6 μm) were cut from cervical spinal cord and stained with haematoxylin and eosin for analysis of inflammation (average number of perivascular inflammatory infiltrates per spinal cord cross-section), luxol fast blue/cresyl violet (LFB/CV) for analysis of demyelination (arbitrary scale; 0, no demyelination; 2, perivascular demyelination; 4, extensive perivascular and subpial demyelination with formation of confluent plaques), Bielchowsky's silver stain for axonal loss (0, no axonal loss; 2, few perivascular fibres lost; 4, profound axonal loss in lesions), immunocytochemical staining of β-APP for analysis of acute axonal injury (average number of APP-positive axonal profiles per spinal cord cross-section) and immunocytochemical staining for C9/MAC (rabbit polyclonal against rat C9, strongly cross-reactive with mouse C9) for analysis of MAC deposition (0, no deposits; 2, detectable (weak) immunostaining in lesions; 4, strong positive immunostaining in lesions).

For the detection of CD59a and CD59b, paraffin wax-embedded sections of spinal cord were dewaxed by washing in xylene three times (5 min each), and dehydrated by soaking through an ascending series of ethanol concentrations. Epitope retrieval was achieved by microwaving (750 W; 30 min) in 0.2% citric acid pH 6.0. After cooling to room temperature, endogenous peroxidase was quenched by incubation in 0.3% H_2O_2 in PBS for 15 min. Sections were washed in PBS, soaked in PBS/1% bovine albumin (PBS/BSA), then incubated with either the rat mAb mCD59a.1 or the mouse mAb mCD59b.1 (each at 10 μg/ml in PBS/BSA) for 30 min at room temperature in a humidity chamber. After washing in PBS, sections were incubated under the same conditions with a 1:100 dilution in PBS of donkey anti-rat Ig HRPO (Jackson Labs; West Grove, PA, USA) or rabbit anti-mouse Ig HRPO (Sigma) as appropriate. After washing in PBS, sections were developed with 0.05% 4,4-diaminobenzidine (Sigma) in PBS/0.02% H_2O_2. Sections were lightly counterstained in haematoxylin prior to mounting and viewing.

For the detection of CD59a by immunofluorescence, spinal cords were removed from animals and snap-frozen in isopentane chilled on dry ice. Spinal cord sections (10 μm) were cut using a cryotome and mounted onto glass slides. After overnight drying, sections were fixed in ice-cold acetone for 5 min, and left to dry at room temperature for 30 min. Sections were then washed in PBS and PBS/1% BSA, before incubating with primary antibody for 1 h at room temperature. Following washing in PBS and PBS/1% BSA, sections were then incubated in FITC-conjugated secondary antibody, 1 h at room temperature. Nuclei were stained with DAPI during the final wash in PBS. Coverslips were then applied with Vectorshield (Vector Labs, Peterborough, UK).

Statistical Analysis

All comparisons of nonparametric data (clinical score, demyelination, axonal loss, C9/MAC immunoreactivity) were performed using the Mann–Whitney *U* nonparametric tests. Student's *t*-test was applied to parametric data (inflammation, APP staining) and Fisher's exact test was used for comparison of disease incidence. Two-tailed *P*-values are quoted throughout.

Results

CD59a Expression in the Mouse Brain

In the CD59a+/+ mice, the vascular endothelium was strongly stained for CD59a throughout the brain and cord. Parenchyma of the cerebral cortex showed weak, diffuse staining in white matter, whereas in the cerebellum, CD59a was localized to white matter and the molecular layer, while Purkinje cells and the granular layer were negative, essentially as reported previously.[28,29] In the spinal cord, there was extensive staining in the white matter, while the central grey matter was only weakly stained, shown more clearly by immunofluorescence, a more sensitive technique for the detection of weak expression (Figure 1a and c). CD59a−/− mice showed no specific staining with the mAb in the brain or cord, even when stained by immunofluorescence, confirming the deficiency (Figure 1b and d). Staining for CD59b using monoclonal antibody (mAb) mCD59b.1 was negative in the brain and cord from CD59a+/+ and CD59a−/− mice, although testis sections processed in a similar manner stained with this mAb (not shown).

CD59a Deficiency Exacerbates Clinical Disease in EAE

In two separate experiments, EAE was induced in CD59a−/− and littermate control CD59a+/+ mice by immunisation with $rMOG^{IgD}$ emulsified in complete Freund's adjuvant. Recombinant MOG^{IgD} was chosen rather than the commonly used encephalitogenic

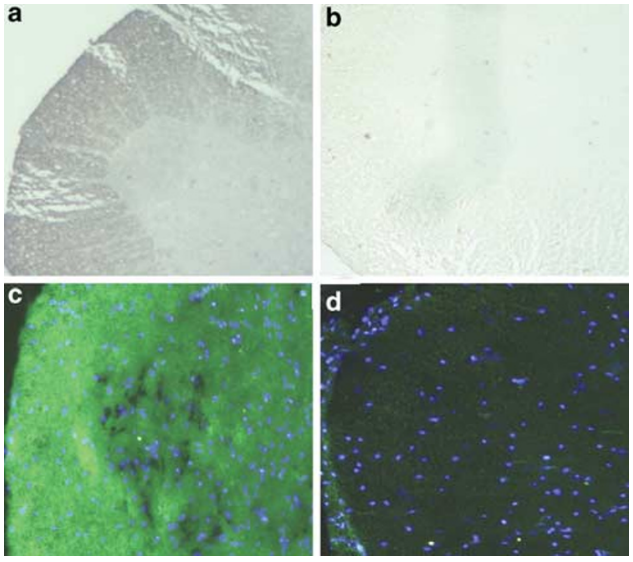

Figure 1 Distribution of CD59a in mouse cervical spinal cord. Sections from CD59a+/+ (a,c) and CD59a−/− (b,d) mice were stained with the anti-CD59a mAb mCD59a.1. In (**a** and **b**), paraformaldehyde-fixed tissue was stained using an immunoperoxidase protocol as detailed in methods. In (**c** and **d**), acetone-fixed tissue was stained using an immunofluorescence protocol as described. CD59a was strongly expressed in the white matter, but was barely detectable in grey matter by immunoperoxidase staining in CD59a+/+ mice; immuofluorescence staining revealed the weak grey matter expression of CD59a. No specific staining was observed by either protocol in CD59a−/− animals. (**c** and **d**) were counterstained with DAPI to reveal cell nuclei and provide a means of imaging the negative sections. Final magnification: ×40.

MOG peptide 35–55, as in our hands peptide-induced disease was variable and irreproducible in the C57/BL×129/Sv F1 mice. Further, there is growing evidence that demyelinating anti-MOG antibodies are directed against conformation-dependent epitopes present on the rMOG^{IgD},[32] and thus immunisation with peptides may not efficiently induce a demyelinating antibody response. Clinical scores and other clinical parameters during rMOG^{IgD}-induced EAE in CD59a−/− and CD59a+/+ mice in the two experiments are summarised in Figure 2 and Table 1. In the CD59a−/− group ($n=8$ in experiment 1; 7 in experiment 2), disease signs were first apparent between days 11 and 13 (12.8±0.8 experiment 1; 12.2±0.5 experiment 2) and 100% of mice developed clinical disease. The peak average disease score was 2.95 (±0.46 s.e.m.) in experiment 1 and 3.5 (±0.55 s.e.m.) in experiment 2. In contrast, only 1/8 (12.5%; experiment 1) and 3/7 (42%; experiment 2) of the CD59a+/+ mice developed disease, commencing on day 13 in all four symptomatic animals. The disease score did not exceed 2 in any symptomatic CD59a+/+ animal. In both experiments, disease incidence and maximal clinical scores were significantly different between the CD59a−/− and CD59a+/+ mice.

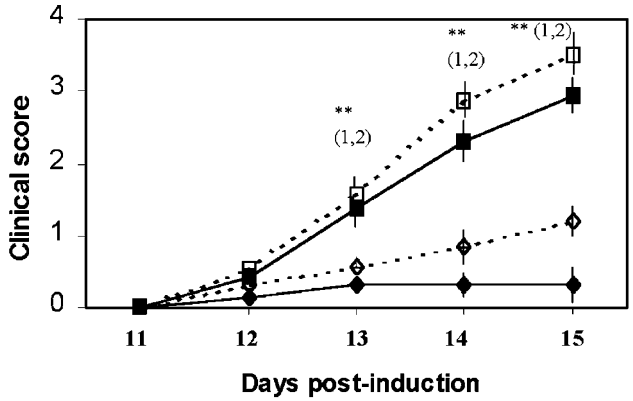

Figure 2 Clinical scores in CD59a+/+ and CD59a−/− mice. Two separate experiments were performed, experiment 1 (filled symbols) comprising eight mice per group, experiment 2 (open symbols), seven per group. Within each experiment, mice were scored daily as detailed in the materials and methods. The data represent the average clinical scores (mean±s.e.m.) from CD59a+/+ (diamonds) and CD59a−/− (squares) in each experiment; vertical bars represent s.e.m. of each data set. The two experiments were separately analysed statistically using a two-tailed Mann–Whitney *U*-test for nonparametric variables. Significant differences between the groups are indicated (**$P<0.01$, highly significant) for experiments 1 and 2.

Table 1 Clinical comparisons between CD59a−/− and CD59a+/+ mice[a]

Parameter	*CD59a−/−*	*CD59a+/+*	P-*value*
Experiment 1 (n=8)			
Clinical score day 15 (mean±s.e.m.)	2.95±0.46	0.31±0.25	0.005*
Incidence	8/8 (100%)	1/8 (12.5%)	0.005*
Day of onset	12.8±0.8	13	NA
Experiment 2 (n=7)			
Clinical score day 15 (mean±s.e.m.)	3.5±0.55	1.2±0.32	0.005*
Incidence	7/7 (100%)	3/7 (42%)	0.010*
Day of onset	12.5±0.5	13	NA

[a]Results for clinical score and day of onset are means (±s.d. where appropriate). Incidence is given as absolute numbers with percentages in parentheses. Significance of difference in clinical scores between groups was assessed using Student's unpaired T-test, and Fisher's exact test was used to assess differences in incidence between groups. Two-tailed *P*-values are quoted throughout. NA=not applicable.
*Significant differences ($P<0.05$).

Demyelination and Axonal Injury are Enhanced in CD59a-deficient Mice

In order to examine the consequences of CD59a deficiency for CNS pathology, CNS tissue was taken from mice in experiment 1 immediately after killing and processed into paraffin for analysis of inflammation, demyelination, axonal damage and C9/MAC deposition. The results are summarised in Table 2 and representative staining of transverse spinal cord sections are shown in Figures 3 and 4. The low magnification images in Figure 3 show clearly the distribution and extent of inflammatory infiltrate

Table 2 Pathological comparisons between CD59a−/− and CD59a+/+ mice[a]

Parameter	*CD59a−/−*	*CD59a+/+*	P-*value*
Experiment 1 ($n=8$) (all mean ± s.e.m.)			
Inflammation	2.4 ± 0.60	0.86 ± 0.46	0.08
Demyelination	1.6 ± 0.49	0.3 ± 0.28	0.05*
Axonal loss	1.4 ± 0.53	0.3 ± 0.53	0.08
APP immunoreactivity	64 ± 19.4	16 ± 7.07	0.05*
C9/MAC Immunoreactivity	2.6 ± 0.67	0.3 ± 0.28	0.01*

[a]Results are means (±s.d. where appropriate). Significance of differences between groups was assessed using Student's unpaired *T*-test based upon statistical advice regarding the nature of the variable.
*Significant differences ($P<0.05$).

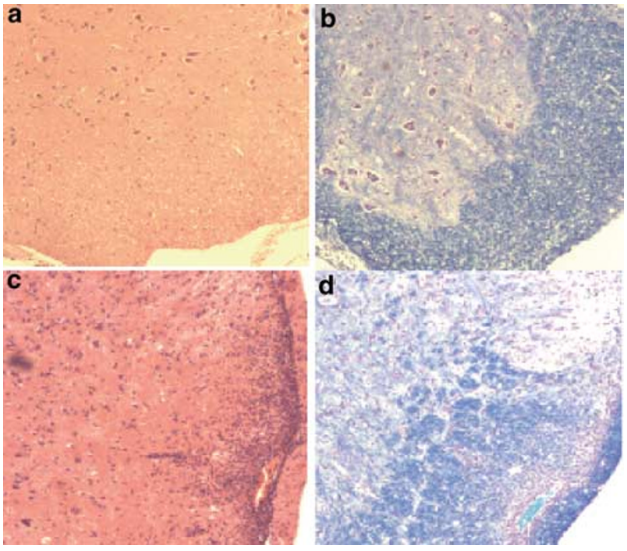

Figure 3 Inflammation and myelin loss in CD59a−/− and CD59+/+ mice. Histological analysis of semiserial paraffin sections from a quadrant of cervical spinal cord of representative CD59a+/+ (**a**, **b**) and CD59a−/− (**c**, **d**) mice. Haematoxylin and eosin staining for analysis of inflammatory cell infiltrates are shown in (**a** and **c**). Heavy subpial and perivascular infiltrates, predominantly in grey matter are seen in CD59a−/− mice, whereas typically in CD59+/+ mice, a few scattered cells were seen in grey matter. LFB/CV staining for myelin (**b**,**d**) revealed large plaques of demyelination in grey matter of CD59a−/− mice compared with essentially normal myelin in CD59a+/+ mice. Final magnification: ×40.

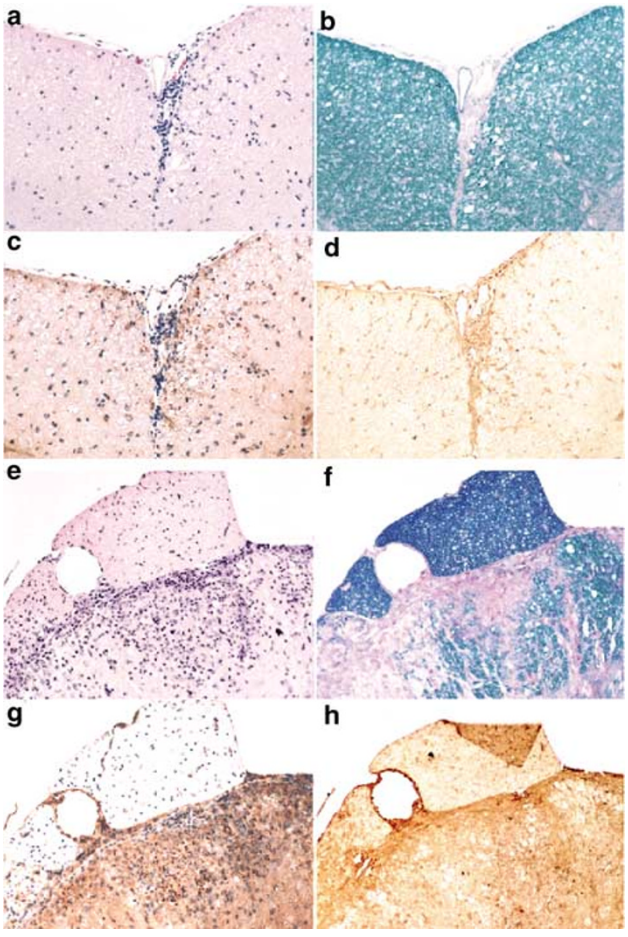

Figure 4 Detailed analysis of pathological changes in EAE. Histological analysis of semiserial paraffin sections from the grey matter of cervical spinal cord of representative CD59a+/+ (**a–d**) and CD59a−/− (**e–h**) mice. Haematoxylin and eosin staining for analysis of inflammatory infiltrate (**a**, **e**); extensive perivascular and parenchymal inflammation is obvious in CD59a−/− spinal cord (**e**) compared to minor infiltration in CD59a+/+ (**a**). Staining for myelin by LFB/CV (**b**, **f**) demonstrates extensive plaques of demyelination in CD59a−/− spinal cord (**f**, note the unaffected peripheral myelin in the dorsal root entry zone, arrowed) compared to a normal myelin staining in the CD59a+/+ cord (**b**). Immunocytochemical staining for deposition of C9/MAC (**c**, **g**) indicates extensive deposition of the MAC in CD59a−/− mice (**g**) with minimal staining in the CD59a+/+ mouse (**c**). Finally, immunocytochemical staining for APP (**d**, **h**) shows staining in the demyelinated area of the CD59a−/− mouse (**h**), indicating axonal injury. Final magnification: ×80.

and myelin loss in CD59a−/− mice (Figure 3c and d) compared with the near-absence of inflammation and preservation of myelin in the CD59+/+ mice (Figures 3a and b). Figure 4 shows higher magnification views of white matter used for scoring of pathology. Inflammation, demyelination, C9/MAC deposition and axonal damage were all significantly increased in the CD59a−/− group compared to CD59a+/+ littermate controls. Subpial and perivascular infiltration of inflammatory cells was seen in all CD59−/− mice and, to a lesser extent, in three of the eight CD59+/+ mice (Table 2 and Figure 4a and e). In the majority of CD59a−/− mice (7/8), perivascular plaques of demyelination were obvious, whereas only one of the eight CD59a+/+ animals showed even traces of demyelination, despite the presence of inflammatory infiltrates in three of these animals. Typical results for staining of myelin using luxol fast blue/cresyl violet are shown in Figures 4b (CD59a+/+) and 4f (CD59a−/−). In the CD59a−/− mouse large, confluent zones of demyelination were obvious, whereas the myelin staining in the CD59a+/+ mouse appeared normal. A peripheral nerve root is also seen in Figure 4e–h (arrowed at top of image in Figure 4f), and here

inflammation was negligible and peripheral nerve myelin was preserved, as expected, since MOG is not expressed in peripheral nervous tissue.[33]

Axonal injury was assessed in two ways: Bielschowsky's silver staining for axons was used to assess axonal loss (Table 2) and staining for amyloid precursor protein (β-APP) was used as a marker of acute axonal injury (Figure 4d and h) with quantification of the number of APP-positive axonal profiles per spinal cord cross-section (Table 2).

Both methods demonstrated greater axonal injury in CD59a−/− mice, although only the latter measure reached statistical significance.

Figure 4 also shows immunocytochemical staining for C9/MAC (Figure 4c and g). Staining with anti-C9, used here as a surrogate marker of MAC deposition in tissue, was detectable in trace amounts in the majority of CD59+/+ mice, but was significantly more abundant in the CD59a−/− mice (Table 2). MAC deposits were found on perivascular tissue elements in the areas of inflammation and/or demyelination, suggesting that the source of the C proteins was from the blood. It was not possible using immunocytochemistry to localise MAC to the surface of specific cell types unequivocally.

Discussion

CD59 is the sole cell membrane regulator of MAC assembly in humans and other mammals.[25] The expression of CD59 protects cells from MAC-mediated damage and deficiency, as seen in the haemolytic disorder paroxysmal nocturnal haemoglobinuria (PNH), where the deficiency of CD59 (and CD55) on haemopoeietic elements renders cells susceptible to the lytic effects of the MAC.[34] CD59 expression in the brain has been examined in humans and rodents. In the rat, CD59 expression is very low in the CNS, and oligodendrocytes do not express the protein, making them extremely susceptible to C lysis *in vitro*.[35] In contrast, all brain cells in humans express CD59, albeit at low levels.[36–39] In the mouse, the situation is complicated by the presence of two genes encoding CD59 proteins. The first described, CD59a, is broadly distributed and, as shown here, is abundant in the spinal cord white matter (Figure 1). Although it is not possible to identify *in situ* the cells expressing CD59a, primary mouse astrocytes in culture strongly express CD59a, while oligodendrocytes and neurones express weakly (not shown). The expression pattern of the second, CD59b, is controversial. Some contend that CD59b is broadly distributed, and is an important MAC regulator in tissues.[40,41] However, we have developed specific mAb to analyse the expression of the two forms of mouse CD59 and find CD59b expression only in testis.[28] In particular, a thorough analysis of mouse CNS cells and sections failed to detect any staining for CD59b (Figure 1 and our unpublished data). We therefore contend that CD59a is the sole regulator of MAC assembly in the CNS in mice.

The CD59a gene was deleted in the mice described here (CD59a−/−), resulting in a mild PNH-like phenotype in otherwise healthy animals.[25] The induction of EAE by immunisation with recombinant $rMOG^{IgD}$ in CD59a+/+ mice on the mixed 129/sv × C57 Bl/6 J background caused a mild, inflammatory disease with low penetrance, minimal symptoms and little or no demyelination. In marked contrast, CD59a−/− littermates developed severe, paralytic disease associated with 100% penetrance, marked demyelination and axonal injury. In all CD59a−/− mice, abundant perivascular and parenchymal inflammatory infiltrates were apparent in the CNS, compared to mild inflammatory infiltrates in a minority of the CD59a+/+ mice. Confluent plaques of demyelination and florid axonal injury or loss were present in and around the infiltrates in sections from CD59a−/− mice, but were conspicuous by their absence in CD59a+/+ controls. Staining for MAC also correlated with the areas of inflammation, demyelination and axonal injury in CD59a−/− mice, establishing a direct link between the deficiency of this important regulator of the terminal pathway, MAC deposition and enhancement of inflammation and tissue injury. In summary, the absence of CD59a transforms $rMOG^{IgD}$-induced EAE in C57BL/6 × 129/Sv F1 mice from a mild inflammatory disease of low penetrance to a florid demyelinating disease with 100% penetrance and evidence of axonal damage. As the only known function of CD59a is to protect against MAC, these findings strongly support the hypothesis, developed from studies in C6-deficient rats, that MAC is a major drive to myelin and axonal damage in rodent EAE.

How is C activated in the EAE model utilised here? By analogy with the rat ADEAE model one might suggest that $rMOG^{IgD}$ induces the production of C-fixing anti-MOG antibodies that drive pathology. However, recent work has clearly shown that in mice on the H-2^b genetic background, which includes the C57/BL × 129/Sv mice used here, immunisation with rat MOG^{IgD} fail to generate antibodies reactive against properly folded *rat* MOG.[42,43] It is thus likely that C is activated in an antibody-dependent manner, secondary to other triggers. Disease induction following immunisation with rat MOG^{IgD} induced disease even in the absence of B cells and required an encephalitogenic T-cell response. Our demonstration that disease severity, demyelination and axonal damage in this model are all mediated by C activation and modulated by the MAC inhibitor CD59 significantly extend our reported findings in the antibody-driven demyelinating disease in rats, ADEAE.[18]

What are the implications for human disease? CD59 deficiency in humans is rare. Although individuals with PNH lack CD59 and other

glycolipid-anchored proteins on blood cells arising from the mutant stem cell clone, they express CD59 normally on other cells and tissues.[44] The sole described case of total CD59 deficiency presented in childhood with symptoms compatible with PNH, but with an unexplained history of neurological disease.[45] No follow-up of this patient has been reported, making impossible any conclusions regarding the effects of CD59 deficiency in the brain in humans. It is possible that chronic activation of C and formation of MAC in the brain in MS may deplete reserves of CD59, a suicide inhibitor consumed during interaction with MAC, resulting in a state of functional deficiency in areas of pathology. Indeed, acquired deficiency of CD59 has been demonstrated in Alzheimer brain and proposed to contribute to the progression of pathology.[46] Alternatively, CD59 may be rendered inactive, as suggested in a recent report report describing the presence in some MS patients of autoantibodies directed against CD55 and CD59.[47] Such autoantibodies might block C regulator function in the brain, mimicking deficiency of the regulators and contributing to demyelination. MS exhibits considerable heterogeneity in pathology with some types characterised by severe demyelination and abundant deposition of MAC.[4] Although CD59 expression has not been assessed in these cases, it is possible that a relative deficiency or increased consumption of CD59 within the CNS in some individuals predisposes to MAC-mediated pathology. Such individuals might benefit from aggressive anti-C therapy aimed at reducing the damaging effects of the MAC in the CNS.

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