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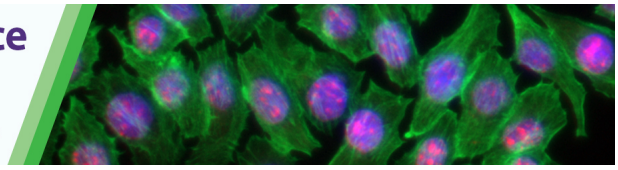

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Cutting Edge: C3, a Key Component of Complement Activation, Is Not Required for the Development of Myelin Oligodendrocyte Glycoprotein Peptide-Induced Experimental Autoimmune Encephalomyelitis in Mice[1]

Divina M. Calida,* Cris Constantinescu,† Enkhmaa Purev,[2]* Guang-Xian Zhang,* Elvira S. Ventura,* Ehud Lavi,‡ and Abdolmohamad Rostami[3]*

Experimental autoimmune encephalomyelitis (EAE), an inflammatory demyelinating disease of the CNS, is regarded as an experimental model for multiple sclerosis. The complement has been implicated in the pathogenesis of multiple sclerosis. To clarify the role of C in mouse EAE, we immunized mice deficient in C3 ($C3^{-/-}$) and their wild-type ($C3^{+/+}$) littermates with myelin oligodendrocyte glycoprotein peptide 35–55. $C3^{-/-}$ mice were susceptible to EAE as much as the $C3^{+/+}$ mice were. No differences were found for the production of IL-2, IL-4, IL-12, TNF-α, and IFN-γ between $C3^{+/+}$ and $C3^{-/-}$ mice. This finding shows that C3, a key component in C activation, is not essential in myelin oligodendrocyte glycoprotein peptide-induced EAE in mice. ***The Journal of Immunology,*** **2001, 166: 723–726.**

The C system is a complex array of plasma proteins and associated cell membrane proteins essential for humoral immune defenses. The C system may be activated by three pathways, the immune complex-triggered "classical" activation route, the immune complex-independent lectin route, and the alternative activation pathway. Initiation of activation by any of these pathways leads to the formation of multimolecular enzymes, which cleave and activate the third (C3) and the fifth (C5) components of C. Following activation of C5, the cytolytic membrane attack complex (MAC)[4] or terminal C component (TCC) consisting of activated C5 (C5b), C6, C7, C8, and C9 is generated. The resulting TCC activation leads to promotion of phagocytosis and direct lysis of certain pathogens, production of anaphylatoxins, clearance of immune complexes, regulation of adaptive immunity, and elimination of self-reactive B cells (1–3).

Several reports indicate that C activation may play an essential role in the mediation and maintenance of inflammatory reactions in the nervous system (4–8). In addition, C has been implicated in the process of demyelination, and myelin components themselves can activate C (9). Several studies suggest the participation of C in diseases such as multiple sclerosis (MS) and the Guillain-Barré syndrome (GBS), the pathological hallmark of which is inflammation and demyelination. TCC has been shown in actively demyelinating areas of the CNS in MS (10) and appears in serum, cerebrospinal fluid, and peripheral nerve of patients with GBS (11).

Experimental autoimmune encephalomyelitis (EAE) and neuritis (EAN) are useful models for studying the immunopathogenesis of MS and GBS, respectively (12). They are T cell-mediated autoimmune diseases induced by active immunization with myelin components or by transfer of neuroantigen-reactive T cells. In EAE and EAN, several studies involving rats have used experimental manipulations that reduce C activation to investigate their effect on disease. Cobra venom factor (CVF), an extract of Naja naja venom, prevents formation of TCC by binding factor B and thus forming stable C3/C5 convertase and depletes C. CVF led to divergent clinical and histopathological outcomes (4–8). CVF suppressed acute and mild but not hyperacute EAE (5) and mild

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[4] Abbreviations used in this paper: MAC, membrane attack complex; EAE, experimental autoimmune encephalomyelitis; MOG, myelin oligodendrocyte glycoprotein; pMOG, MOG peptide 35–55; $C3^{-/-}$, C3 knockout; $C3^{+/+}$, C3 wild-type; TCC, terminal C component; MS, multiple sclerosis; GBS, Guillain-Barré syndrome; EAN, experimental autoimmune neuritis; CVF, cobra venom factor.

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but not severe EAN (7). Soluble C receptor type 1 (sCR1) ameliorated EAE (13), but its effect in EAN was less significant than that of CVF (14). Thus, although these studies suggest a role for C in EAE and EAN, the magnitude of this role and whether C is required for development and clinical expression of EAE is not known. The only modest effect of the above manipulations may be explained by inherent difficulties associated with their use such as short half-life, limited diffusibility due to high m.w., and high immunogenicity (13, 15–17).

In this study, we investigated the role of C in mouse EAE by using mice deficient in the key component of the C cascade, C3. EAE induced by myelin oligodendrocyte glycoprotein (MOG) is currently considered to be the closest to MS clinically (relapsing-remitting with accumulating deficits), histologically (inflammation and demyelination), and immunologically (evidence for a role for both T cells and Abs). In addition, although MOG is present in myelin in smaller amounts than other proteins (18, 19), it is an Ag against which a large number of lymphocytes react in MS patients (20–22). Therefore, we used the MOG-induced EAE model in this study.

Materials and Methods

Ten-week-old B6×129/F_1 C3 wild-type ($C3^{+/+}$) and C3 knockout ($C3^{-/-}$) mice were provided by Dr. John Lambris, University of Pennsylvania. Sex- and age-matched wild-type littermates were used in the experiments.

Induction of EAE and scoring

Seven $C3^{+/+}$ and seven $C3^{-/-}$ mice were each injected s.c. with 400 μg MOG peptide 35–55 (pMOG) in CFA containing 2.5 mg/ml *Mycobacterium tuberculosis* over two sites at the back. Pertussis toxin (500 ng; List Biological Laboratories, Campbell, CA) was given i.p. on days 0 and 2 post immunization. Peptide synthesis was provided by the Protein Chemistry Laboratory of the Medical School of the University of Pennsylvania supported by core grants of the Diabetes and Cancer Centers (DK-19525 and CA-16520). EAE scoring was performed on a scale of 0–5 as previously described (12).

Histopathological assessment

Brains and spinal cords were harvested on day 31 and embedded in paraffin; then, sections were stained with hematoxylin and eosin for assessment of inflammation and with Luxol Fast Blue for assessment of demyelination. Additional sections of spinal cord were embedded in plastic and stained with toluidine blue. Two investigators unaware of the experimental groups to which the tissues belonged assessed inflammation and demyelination as follows. For inflammation: −, none; +/− a few inflammatory cells; +, organization of perivascular infiltrates; ++ to +++, increasing severity of perivascular cuffing with extension into the adjacent tissue. For demyelination: 0, none; + rare foci, ++, a few areas of demyelination; +++, large (confluent) areas of demyelination.

Immunohistochemical assessment of IL-12 in the CNS during EAE

Selected frozen sections of brains and spinal cords isolated at day 31 were fixed in acetone and stained using rat anti-mouse IL-12 mAbs and the rat alkaline phosphatase (APAAP) system (Dako, Carpinteria, CA), and fast red (Sigma, St. Louis, MO) as alkaline phosphatase substrate. Sections were counterstained with hematoxylin and the reaction (compared with secondary Ab alone) was classified as follows: −, none; +, faint; ++ moderate; +++, intense; ++++, very intense.

ELISA for cytokines

ELISA (Endogen, Boston, MA) for IL-2, IL-4, IL-12, TNF-α, and IFN-γ were performed in supernatants of 10 μg/ml pMOG-stimulated lymphocyte and spleen cultures, and sera from $C3^{-/-}$ and $C3^{+/+}$ mice according to the manufacturer's instructions.

Statistical analysis

The Mann-Whitney *U* test was used for comparing clinical courses. We used ANOVA and Student's *t* test comparing degrees of inflammation and demyelination. A value of $p \leq 0.05$ was considered significant.

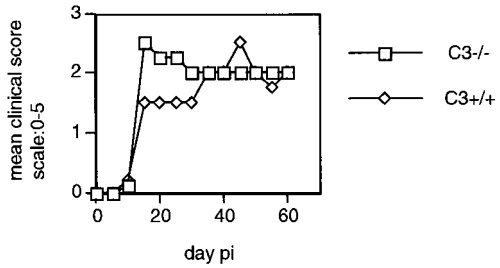

FIGURE 1. Clinical course of EAE in $C3^{-/-}$ and the wild-type mice housed in a conventional environment. The animals were immunized with 400 μg pMOG in CFA and 500 ng pertussis toxin given on days 0 and 2 post immunization. Both groups displayed chronic EAE with similar disease severity; however, peak of disease was observed earlier in $C3^{-/-}$ mice.

Results

Clinical and histopathological assessment

Both $C3^{+/+}$ and $C3^{-/-}$ mice developed disease with 100% incidence. The onset of the EAE manifestations was slightly later in $C3^{-/-}$ than in $C3^{+/+}$ mice housed in the conventional environment (15.3 vs 13.1 days, $p = 0.01$) (Fig. 1). The mean maximal score was 2.5 in both groups. There was no significant clinical and histopathological difference between the two groups (Tables I and II). The course of EAE was chronic in both groups, with no remission and no clear relapses.

Because the development of more severe EAE in $C3^{-/-}$ mice was somewhat surprising, we wanted to determine whether the environment in which the mice were kept influenced the results. Therefore, we repeated the experiment using animals kept in a germ-free environment. Both $C3^{-/-}$ and $C3^{+/+}$ developed EAE with 100% incidence. Disease onset and severity were observed. $C3^{-/-}$ mice had significantly higher mortality (of 50%) compared with 0% in the wild-type counterparts. There was no mortality among the $C3^{-/-}$ mice housed in the regular environment. The disease had a trend to higher severity in $C3^{-/-}$ mice both clinically and histopathologically (Fig. 2, and Tables III and IV).

IL-12 immunohistochemistry

Because ligation of C receptors on macrophages suppresses production of IL-12 (23, 24) and because IL-12 is a key cytokine for EAE, we postulated that absence of C receptor signaling in $C3^{-/-}$ mice leads to enhanced IL-12 expression and thus to EAE, potentially more severe than in $C3^{+/+}$ counterparts. Therefore, we performed immunohistochemical staining for IL-12 in spinal cords of $C3^{-/-}$ and $C3^{+/+}$ mice with EAE. At the stage investigated we did not detect any IL-12 in the CNS of mice, whereas spleens stained mildly positive.

Cytokine determination in the supernatants of lymph nodes and spleen cells, and sera of $C3^{+/+}$ and $C3^{-/-}$ mice with EAE

We found no differences in production of IL-2, IL-4, IL-12, TNF-α, and IFN-γ in the supernatants from cultures of lymph node

Table I. *Clinical profile of pMOG-induced EAE in $C3^{-/-}$ mice and wild-type control in conventional environment*[a]

Mouse Strain	*n*	Incidence	Mean Onset (day)	Mean Maximum Clinical Score
$C3^{-/-}$	7	7/7	15.29	2.5
$C3^{+/+}$	7	7/7	12.13	2.5

[a] $C3^{-/-}$ mice developed disease 3 days later than the controls ($p < 0.01$). Both groups showed similar severity of disease.

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Table II. *Histopathological examination in $C3^{-/-}$ and $C3^{+/+}$ mice with EAE housed in conventional environment*[a]

Mouse Strain	Mean Maximum Clinical Score	Inflammation Grade (incidence)				Demyelination Grade (incidence)			
		0	+	++	+++	0	+	++	+++
$C3^{-/-}$	2.5	0	4/6	2/6	0	0	3/6	3/6	0
$C3^{+/+}$	2.5	0	1/4	3/4	0	1/4	0	2/4	1/4

[a] Moderate to severe inflammation and demyelination was observed after histopathological examination of Luxol Fast Blue-stained brain and spinal cord of C3 wild-types and -deficient mice. Histopathological difference between the two groups of mice was not statistically significant.

and spleen cells. Sera from $C3^{+/+}$ or $C3^{-/-}$ mice also showed no difference in the production of these cytokines.

Discussion

Despite multiple lines of evidence implicating the C system in the nervous system inflammation and demyelination, it is not clear whether C is absolutely required for the development of EAE. Isolated C component deficiencies have been reported in patients with MS. Hypocomplementemia, with a persistent or fluctuating pattern, has been reported in a substantial proportion of patients with MS (25). However, this has been attributed to a sustained C activation, and it is not clear whether genetic absence of C3 can coexist with MS. Similarly, although C is involved in EAE, the development of this disease in the absence of this pivotal component of C activation has so far not been investigated. MS immunopathology displays a remarkable heterogeneity (26) and has been grouped into four distinct patterns; of these patterns, I and II are similar, showing T cell and macrophage infiltration but only the latter shows abundant Ig and activated C deposition. Pattern I resembles T cell-mediated EAE, and pattern II resembles T cell-mediated, Ab-enhanced EAE (27). The other two patterns, III and IV, are suggestive of primary oligodendrocyte dystrophy, implicating virus- or toxin-induced demyelination rather than autoimmunity (26). These patterns demonstrate that the inflammatory demyelination in MS lesions is not necessarily accompanied by local C deposition.

Our results show that absence of C3, therefore virtual functional inactivation of the C system, does not abrogate the development of MOG-induced EAE in mice. Moreover, disease was of similar severity, or even showed a tendency to be more severe, in $C3^{-/-}$ mice. However, it is possible that in other EAE models C plays a role. For example, in rats, where terminal C activation is more vigorous than in mice, Ab-mediated demyelination is a more pathogenic mechanism. In mice, EAE induced by MOG peptide does not require B cells but EAE induced by whole protein does (28).

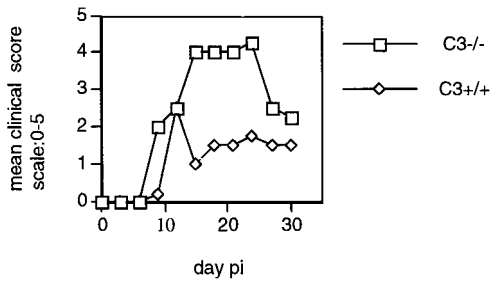

FIGURE 2. Clinical course of pMOG-induced EAE in $C3^{-/-}$ and $C3^{+/+}$ mice housed in a germ-free environment. No significant difference was noted in disease onset; however, $C3^{-/-}$ mice developed more severe disease compared with the $C3^{+/+}$ mice ($p < 0.01$).

Table III. *Clinical profile of $C3^{-/-}$ and wild-type control mice immunized with pMOG in a germ-free environment*

Mouse Strain	*n*	Incidence	Mean Onset (day)	Mean Maximum Clinical Score
$C3^{-/-}$	4	4/4	9.5	4.25
$C3^{+/+}$	6	6/6	9.8	2.5

[a] $C3^{-/-}$ mice showed more severe EAE than the $C3^{+/+}$ mice ($p < 0.01$). Onset of disease was the same in both groups.

Several mechanisms have been postulated for the role of C in EAE. First, C enhances phagocytosis of particulate materials including myelin destruction during CNS inflammation. Second, C enhances Ab-dependent cell-mediated cytotoxicity, a phenomenon mediated by macrophages that is shown to be important in EAE (29, 30). The fact that macrophage depletion ameliorates EAE has been used as an argument for this possibility (5, 29, 31, 32). Third, C activation leads to release of anaphylatoxins, which stimulate the release of a large number of inflammatory products. Fourth, the MAC has direct cytolytic properties (6). Last, recent evidence shows that early activation of the C plays a role in shaping the subsequent, adaptive immune response; therefore, it may lead to a more powerful neuroantigen-specific immune response in EAE (33).

However, it appears that none of these C-dependent mechanisms is absolutely required for MOG peptide-induced murine EAE to occur. Moreover, although myelin itself is a known stimulus for C activation (9) this action is unlikely to represent a critical step in EAE. However, the fact that the EAE onset was delayed in $C3^{-/-}$ suggests that although not required, C3 contributes to the early pathologic process. It has been suggested that, although the primary effector cells in EAE are T cells and although these are important for the initiation of the autoimmune reaction and the inflammatory process, the development of anti-myelin Abs and C activation augments the severity of clinical and histopathological lesions in EAE (13, 27). The fact that we have demonstrated demyelination in the absence of the key C component indicates that other or additional mechanisms mediate demyelination as well and that the immunopathological heterogeneity shown in MS, which appears to be patient specific (26), may also apply to EAE models. In relapsing EAE, as in MS, epitope spreading plays an important role. Thus it is possible that, although C3 is not required for development of MOG peptide-induced EAE in the mice, it is required for the later stages during epitope spreading. This would also reflect the MS pathology (34).

One possibility includes direct cytotoxic effects of proinflammatory cytokines such as TNF on oligodendrocytes (35, 36). The fact that C3 and, therefore the integrity of the C system, may have a protective effect against TNF effects has also been supported by studies showing an enhanced production of TNF as well as IL-1β

Table IV. *Histopathological examination in $C3^{-/-}$ and $C3^{+/+}$ mice with pMOG-induced EAE housed in a germ-free environment*[a]

Mouse Strain	Mean Maximum Clinical Score	Inflammation Grade (incidence)				Demyelination Grade (incidence)			
		0	+	++	+++	0	+	++	+++
$C3^{-/-}$	4.25	0	0	1/2	1/2	0	0	2/4	0
$C3^{+/+}$	2.5	0	4/4	0	0	2/4	2/4	0	0

[a] $C3^{-/-}$ showed more severe inflammation ($p < 0.01$) and demyelination ($p < 0.05$).

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associated with increased susceptibility to endotoxin challenge in $C3^{-/-}$ mice (37). A similar mechanism may explain the increased severity of EAE in germ-free $C3^{-/-}$ mice compared with $C3^{+/+}$ mice in the same environment. Although we did not find differences between C3-deficient and -sufficient mice in the production of several cytokines including TNF by spleen and lymph node cells, it is possible that other time points on investigating their expression in the CNS may have revealed differences. Although Ab-dependent cell-mediated cytotoxicity may represent a pathogenic mechanism in EAE (32), the integrity of the C pathway is not required. The fact that the macrophage depletion ameliorates EAE (30) may reflect elimination of an important source of Ag presentation, costimulatory signals, and/or cytokines.

IL-12 is an essential cytokine for EAE development and presentation. C receptor ligation on macrophages leads to specific down-regulation of IL-12. Therefore, we postulated that the absence of C3 might up-regulate IL-12. Although we found no difference in IL-12 production in the peripheral immune system, the possibility still remains that IL-12 up-regulation occurred in the peripheral immune system of $C3^{-/-}$ mice at the other stages of disease that we did not study. Despite the fact that we did not immunohistochemically detect IL-12 in the CNS of $C3^{-/-}$ or $C3^{+/+}$ mice with EAE during the stage of investigation, such an explanation remains plausible. Despite IL-12 being required for EAE, its up-regulation in EAE CNS is early and very transient (38). Also in MS, it has been detected only during acute, active disease (39).

$C3^{-/-}$ mice may retain the MAC (37). However, it is unlikely that this explains the retained susceptibility to EAE of $C3^{-/-}$ in our study. Preliminary experiments in our laboratory have shown that C5-deficient mice are also mildly susceptible to EAE (D. M. C. and A. R., unpublished observation). In conclusion, we show that genetic deficiency of C3, the key link in the C activation cascade, is not required for development of MOG peptide-induced EAE and its features of inflammation and demyelination.

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References

1. Law, S. K. A., and K. B. M. Reid. 1995. Complement. "In Focus" Series. D. Male, ed. IRC Press, Oxford University Press, Oxford.
2. Song, W.-C., M. R. Sarrias, and J. D. Lambris. 1998. Complement and innate immunity. *Immunopharmacology 49:187.*
3. Carroll, M. C. 1998. The role of complement and complement receptors in induction and regulation of immunity. *Annu. Rev. Immunol. 16:545.*
4. Feasby, T. E., J. J. Gilbert, A. F. Hahn, and M. Neilson. 1987. Complement depletion suppresses Lewis rat experimental allergic encephalomyelitis. *Brain Res. 419:97.*
5. Linington, C., B. P. Morgan, N. J. Scolding, P. Wilkins, S. Piddlesden, and D. A. S. Compston. 1989. The role of complement in experimental allergic encephalomyelitis. *Brain 112:895.*
6. Morgan, B. P. 1994. Clinical complementology: recent progress and future trends. *Eur. J. Clin. Invest. 24:219.*
7. Vriesendorp, F. T., R. E. Flynn, A. Papolla, and C. L. Koski. 1995. Complement depletion affects demyelination and inflammation in experimental allergic neuritis. *J. Neuroimmunol. 58:157.*
8. Vriesendorp, F. J., R. E. Flynn, M. R. Malone, and M. A. Pappolla. 1998. Systemic complement depletion reduces inflammation and demyelination in adoptive transfer experimental allergic neuritis. *Acta Neuropathol. 95:297.*
9. Vanguri, P., C. L. Koski, B. Silvermann, and M. L. Shin. 1982. Complement activation by isolated myelin: activation of the classical pathway in the absence of myelin specific antibodies. *Proc. Natl. Acad. Sci. USA 19:3290.*
10. Storch, M. K., S. Piddlesden, M. Haltia, M. Iivanainen, P. Morgan, and H. Lassmann. 1998. Multiple sclerosis: in situ evidence for antibody- and complement-mediated demyelination. *Ann. Neurol. 43:465.*
11. Koski, C. L. 1990. Characterization of complement-fixing antibodies to the peripheral nerve myelin in Guillain-Barré syndrome. *Ann. Neurol. 27(Suppl.):S44.*
12. Constantinescu, C. S., B. Hilliard, T. Fujioka, M. K. Bhopale, D. Calida, and A. M. Rostami. 1998. Pathogenesis of neuroimmunologic diseases: experimental models. *Immunol. Res. 17:217.*
13. Piddlesden, S. J., M. K. Storch, M. Hibbs, A. M. Freeman, H. Lassmann, and B. P. Morgan. 1994. Soluble recombinant complement receptor1 inhibits inflammation and demyelination in antibody-mediated demyelinating experimental allergic encephalomyelitis (ADEAE). *J. Immunol. 152:5477.*
14. Vriesendorp, F. J., R. E. Flynn, M. A. Pappolla, and C. L. Koski. 1997. Soluble complement receptor 1 (sCR1) is not as effective as cobra venom factor in the treatment of experimental allergic neuritis. *Int. J. Neurosci. 92:287.*
15. Jose, P. J. 1987. Complement-derived peptide mediators of inflammation. *Br. Med. Bull. 43:336.*
16. Goodfellow, R. M., A. S. Williams, J. L. Levin, B. D. Williams, and B. P. Morgan. 2000. Soluble complement receptor one (sCR1) inhibits the development and progression of rat collagen-induced arthritis. *Clin. Exp. Immunol. 119:210.*
17. Pratt, J. R., M. J. Hibbs, A. J. Laver, R. A. G. Smith, and S. H. Sacks. 1996. Effects of complement inhibition with soluble complement receptor-1 on vascular injury and inflammation during renal allograft rejection in the rat. *Am. J. Pathol. 149:2055.*
18. Linington, C., M. Webb, and P. L. Woodhams. 1984. A novel myelin associated glycoprotein defined by a mouse monoclonal antibody. *J. Neuroimmunol. 6:387.*
19. Pham-Dinh, D., M.-G. Mattei, J.-L. Nussbaum, G. Roussel, P. Pontarotti, N. Roeckel, I. H. Mather, K. Artzt, K. F. Lindahl, and A. Dautigny. 1993. Myelin/oligodendrocyte glycoprotein is a member of a subset of the immunoglobulin superfamily encoded within the major histocompatibility complex. *Proc. Natl. Acad. Sci. USA 90:7990.*
20. Wallstrom, E., M. Khademi, M. Andersson, R. Weissert, C. Linington, and T. Olsson. 1998. Increased reactivity to myelin oligodendrocyte glycoprotein peptides and epitope mapping in HLA $DR2(15)^{+}$ multiple sclerosis. *Eur. J. Immunol. 28:3329.*
21. Kerlero de Rosbo, N., and A. Ben-Nun. 1998. T-cell responses to myelin antigens in multiple sclerosis; relevance of the predominant autoimmune reactivity to myelin oligodendrocyte glycoprotein. *J. Autoimmun. 11:287.*
22. Kerlero de Rosbo, N., M. Hoffman, I. Mendel, I. Yust, J. Kaye, R. Bakimer, S. Flechter, O. Abramsky, R. Milo, A. Karni, and A. Ben-Nun. 1997. Predominance of the autoimmune response to myelin oligodendrocyte glycoprotein (MOG) in multiple sclerosis: reactivity to the extracellular domain of MOG is directed against three main regions. *Eur. J. Immunol. 27:3059.*
23. Marth, T., and B. L. Kelsall. 1997. Regulation of interleukin-12 by complement receptor 3 signalling. *J. Exp. Med. 185:1987.*
24. Sutterwala, F. S., G. J. Noel, R. Clynes, and D. M. Mosser. 1997. Selective suppression of interleukin-12 induction after macrophage receptor ligation. *J. Exp. Med. 185:1977.*
25. Trouillas, P., G. Aimard, F. Berthoux, and M. Devic. 1975. Multiple sclerosis with hypocomplemetaemia. *Lancet 2:932.*
26. Lucchinetti, C., W. Bruck, J. Parisi, B. Scheithauser, M. Rodriguez, and H. Lassmann. 2000. Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. *Ann. Neurol. 47:707.*
27. Linington, C., M. Bradl, H. Lassmann, C. Brunner, and K. Vass. 1988. Augmentation of demyelination in rat acute allergic encephalomyelitis by circulating mouse monoclonal antibodies directed against a myelin/oligodendrocyte glycoprotein. *Am. J. Pathol. 130:443.*
28. Lyons, J. A., M. San, M. P. Happ, and A. H. Cross. 1999. B cells are critical to induction of experimental allergic encephalomyelitis by protein but not by a short encephalitogenic peptide. *Eur. J. Immunol. 29:3432.*
29. Brosnan, C. F., M. B. Bornstein, and B. R. Bloom. 1981. The effects of macrophage depletion on the clinical and pathologic expression of experimental allergic encephalomyelitis. *J. Immunol. 126:614.*
30. Huitinga, I., N. Van Rooijen, C. J. A. De Groot, B. M. J. Uitdehaag, and C. D. Dijkstra. 1990. Elimination of macrophages infiltrating the CNS suppresses EAE in rats. *J. Exp. Med. 172:1025.*
31. Epstein, L. G., J. W. Prineas, and C. S. Raine. 1983. Attachment of myelin to coated pits on macrophages in EAE. *J. Neurol. Sci. 61:341.*
32. Lassmann, H., C. Brunner, M. Bradl, and C. Linington. 1988. Experimental allergic encephalomyelitis: the balance between encephalitogenic T lymphocytes and demyelinating antibodies determines size and structure of demyelinated lesions. *Acta Neuropathol. 75:566.*
33. Dempsey, P. W., M. E. Allison, S. Akkaraju, C. C. Goodnow, and D. T. Fearon. 1996. C3d of complement as a molecular adjuvant: bridging innate and native immunity. *Science 271:348.*
34. Raine, C. S. 1997. The Norton Lecture: a review of the oligodendrocyte in the multiple sclerosis lesion. *J. Neuroimmunol. 77:135.*
35. Selmaj, K. W., and C. S. Raine. 1988. Tumor necrosis factor mediates myelin and oligodendrocyte damage in vitro. *Ann. Neurol. 23:339.*
36. Selmaj, K., C. S. Raine, M. Farooq, W. T. Norton, and C. F. Brosnan. 1991. Cytokine cytotoxicity against oligodendrocytes: apoptosis induced by lymphotoxin. *J. Immunol. 147:1522.*
37. Fischer, M., A. Prodeus, A. Nicholson-Weller, M. Ma, J. Murrow, R. Reid, H. Warren, A. Lage, J. F. Moore, F. Rosen, and M. Carroll. 1997. Increased susceptibility to endotoxin shock in complement C3- and C4-deficient mice is corrected by C1 inhibitor replacement. *J. Immunol. 159:976.*
38. Issazadeh, S., A. Ljungdahl, B. Hojeberg, M. Mustafa, and T. Olsson. 1995. Cytokine production in the central nervous system of Lewis rats with experimental autoimmune encephalomyelitis: dynamics of mRNA expression for interleukin-10, interleukin-12, cytolysin, tumor necrosis factor α and tumor necrosis factor β. *J. Neuroimmunol. 61:205.*
39. Windhagen, A., J. Newcombe, F. Dangond, C. Strand, M. N. Woodroofe, M. L. Cuzner, and D. A. Hafler. 1995. Expression of costimulatory molecules B7-1 (CD80), B7-2 (CD86), and interleukin 12 cytokine in multiple sclerosis lesions. *J. Exp. Med. 182:1985.*

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