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Microglial Fc receptors mediate physiological changes resulting from antibody cross-linking of myelin oligodendrocyte glycoprotein

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Abstract

Antibodies to myelin oligodendrocyte glycoprotein (MOG) have been implicated in Multiple Sclerosis demyelination through activation of complement and/or macrophage-effector processes. We presented a novel mechanism, whereby MOG on oligodendrocytes, when cross-linked with anti-MOG and secondary antibody resulted in its repartitioning into lipid rafts, and changes in protein phosphorylation and morphology. Here, we show that similar events occur when anti-MOG is cross-linked with Fc receptors (FcRs) present on microglia but not with complement. These results indicate that FcRs are endogenous antigen/antibody cross-linkers *in vitro*, suggesting that FcRs could be physiologically relevant *in vivo* and possible targets for therapy in Multiple Sclerosis.

Keywords: Oligodendrocytes; Myelin; MOG; Microglia; Multiple Sclerosis; Complement; Fc receptors

1. Introduction

Multiple Sclerosis (MS) is an inflammatory demyelinating disease affecting the central nervous system (CNS). It is characterized by areas of demyelination, often with associated axonal degeneration, causing severe, generally irreversible, functional consequences (Lassmann, 2004). Inflammation is mediated by T cells, leading to local activation of microglia and impairment of the integrity of the blood brain barrier (Noseworthy et al., 2000). In addition, B-cell responses are implicated in MS pathogenesis. For example, immunoglobulin deposits and opsonized myelin debris are detected at the active edge of demyelinating lesions (Genain et al., 1999; Lassmann, 2004; von Budingen et al., 2004), and plasma exchange dramatically reduces clinical disease in a subset of patients (Kieseier and Hartung, 2003). In particular, antibodies to myelin oligodendrocyte glycoprotein (MOG), a highly encephalitogenic glycoprotein exposed to the extracellular environment on the outer lamella of the myelin sheath (Linington et al., 1984), are found in the cerebrospinal fluid and in disintegrating myelin around axons in lesions of acute MS patients (Genain et al., 1999). Although there is still some controversy on the specificity of this antibody response in MS patients, the role of anti-MOG in those patients with pattern II demyelination has been clearly demonstrated (Lassmann et al., 2001).

We have proposed a novel mechanism for anti-MOG-induced demyelination, wherein cross-linking MOG on the surface of oligodendrocytes (OLs) in culture with demyelinating antibodies against MOG followed by secondary cross-linking antibody, rapidly (minutes) and sequentially induces (a) repartitioning of MOG into detergent insoluble microdomains characteristic of lipid rafts, (b) alterations in the phosphorylation state of key proteins related to a cellular stress response and cytoskeletal instability, and (c) dramatic changes in cell morphology including a retraction of cell processes (without triggering cell death) (Marta et al., 2003; Marta et al., 2005a). These results were observed by using either a monoclonal antibody against the extracellular domain of MOG (Marta et al., 2003; Marta et al., 2005a) or with pathogenic anti-MOGs purified from mice with a B-cell mediated EAE induced by immunization with human MOG, but not with non-pathogenic anti-MOGs from mice with a B-cell independent EAE induced by immunization with rat MOG (Marta et al., 2005b). Recognizing the important implications of these data for understanding B-cell mediated disease in MS, we sought to identify endogenous activating cross-linkers of anti-

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MOG/MOG complexes that would be present in human MS brain, testing the hypothesis that molecules capable of binding to the Fc portion of pathogenic IgGs can mimic the effects triggered by a secondary cross-linking antibody. We focused on microglial Fc receptors (FcRs) and complement, Fc-binding components identified as effectors of antibody-mediated demyelination (Lassmann, 2004; Noseworthy et al., 2000).

2. Materials and methods

2.1. Cell culture

Rat mixed primary cultures were prepared and maintained, and enriched populations of either mature OLs or microglia were obtained by a differential adhesion protocol (Bansal et al., 1996; Pfeiffer et al., 1993); purified OLs were grown in modified N2 medium (serum-free) for 6–7 days to obtain MOG-expressing OLs (Bottenstein and Sato, 1979; Gard and Pfeiffer, 1989). Freshly prepared microglia were resuspended in modified N2 medium for OL exposure (see below).

2.2. MOG cross-linking (Marta et al., 2003; Marta et al., 2005a,b)

Purified OLs or mixed primary cultures were incubated with anti-MOG mAb 8-18C5 (IgG1) (Schluesener et al., 1987) (156 μg/ml; C. Linington, Aberdeen, Scotland) or anti-MOG Z12 (IgG2a) (Piddlesden et al., 1993) (10 μg/ml; S. Amor-P. Smith, Rijswijk, NL) for 15 min at 37 °C. MOG/anti-MOG complexes were then treated with either goat anti-mouse IgG, complement components or microglia (see below). Some mixed primary cultures were only exposed to anti-MOG 8-18C5 for 15, 30 or 60 min without any further cross-linking.

2.3. Cross-linking with microglia

Following anti-MOG treatment, OLs were incubated with a suspension containing microglia (number of microglia/OLs=0.5, 1, 2, 4 final ratio) for different intervals of time (15–60 min), or with microglia (ratio=2) that had been previously incubated with anti-CD16/CD32 (1–10 μg/ml, mouse BD Fc Block™, BD Biosciences, Palo Alto, CA) (McMahon et al., 2002) for 30 min at 37 °C. In some experiments, anti-MOG 8-18C5 was pre-crosslinked with microglia for 30 min at 37 °C and then the mixture was added to OL cultures for an additional 30 min at 37 °C.

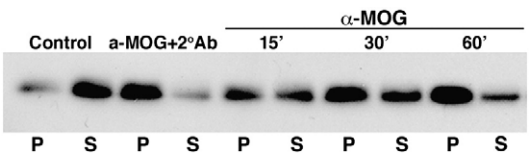

Fig. 1. MOG repartitioning occurs independently of a secondary cross-linking antibody in mixed primary cultures. MOG immunoblot shows repartitioning of MOG into a TX-100 insoluble fraction (P) after treatment with anti-MOG 8-18C5 (15 min, 37 °C) followed by anti-mouse IgG (15 min, 37 °C) (α-MOG+2 Ab), or after treatment with anti-MOG alone for 15, 30 or 60 min. MOG is TX-100 soluble (S) under control conditions. The results shown are characteristic of three independent experiments.

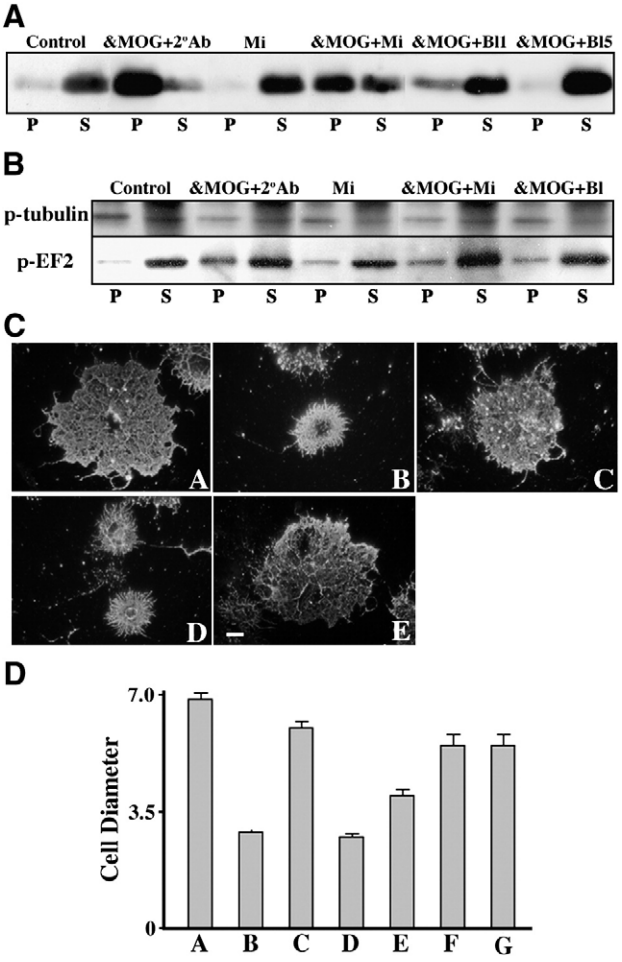

Fig. 2. Fc Receptors mediate MOG cross-linking effects. Immunoblots show (A) MOG repartitioning into a TX-100 insoluble fraction (P) and (B) dephosphorylation of β-tubulin and increased phosphorylation of EF-2 in the pellet fraction, and (C) O4 staining shows process retraction (quantification of cell diameters shown in D) in OLs after treatment with anti-MOG 8-18C5 followed by either anti-mouse IgG (α-MOG+2 AB, CB, DB) or microglia (α-MOG+Mi, CD, dD), but not after 30 min incubation with microglia alone (Mi, CC, DC) or anti-MOG plus microglia previously blocked with 1 (α-MOG+Bl1, dE), 5 (α-MOG+Bl5, CE, DF) or 10 (DG) μg/ml of anti-CD32/CD16. Under control conditions, MOG is mostly TX-100 soluble (S) and cells show normal morphology (CA, DA). Bar, 5 μm. DD vs. DE $P<0.05$, DD vs. DF. $P<0.005$ (Student's t-test). The results shown are characteristic of three independent experiments.

2.4. Cross-linking with complement components

Following anti-MOG, anti-MAG (mouse IgG1; R Quarles, NIH, Md, USA) (Marta et al., 2004) or anti-galactocerebroside (mouse IgM, O1, 1:25) treatment (15 min, 37 °C), OLs were incubated with 10–50 μg/ml of C1q (Sigma, St. Louis, MI) or 1:60 complement sera from guinea pig (Sigma) for 15, 30 or 60 min at 37 °C. To deplete cholesterol, OLs were incubated with methyl-β-cyclodextrin (5 mM, Sigma, St Louis, MI) for 15 min at 37 °C prior to antibody and complement treatments.

2.5. Immunofluorescence microscopy

To examine changes in morphology, mature OLs were stained with O4 (live cells) or with anti-myelin basic protein