Commentary:

Sorting the wheat from the chaff: identifying demyelinating components of the myelin oligodendrocyte glycoprotein (MOG)-specific autoantibody repertoire

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Myelin oligodendrocyte glycoprotein (MOG) is the only myelin protein known to initiate a demyelinating autoantibody response in EAE, an animal model for multiple sclerosis (MS). The pathophysiological significance of MOG-specific autoantibodies in MS is, however, controversial, as high titer antibody responses to MOG are also found in many patients with non-demyelinating neurological diseases. In this issue of the *European Journal of Immunology*, von Büdingen et al. demonstrate that demyelination in a primate model of MOG-induced EAE is mediated by MOG-specific antibodies directed against discontinuous, rather than linear, MOG epitopes. This functional segregation of pathogenic vs. non-pathogenic autoantibodies in terms of epitope specificity may be crucial to understand the relevance of MOG-specific responses in human disease. This commentary discusses these findings in the context of the structure and immunobiology of MOG, and their implications with respect to antibody-mediated demyelination in MS.

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1 Humoral responses in the pathogenesis of multiple sclerosis

Loss of immunological self-tolerance to myelin antigens in genetically susceptible individuals plays a major role in the pathogenesis of multiple sclerosis (MS), a complex inflammatory demyelinating disease of the central nervous system (CNS) [1]. MS is often discussed as a purely T cell mediated disease, but there is increasing indirect evidence that antibody-dependent mechanisms are also involved in disease pathogenesis. Immunopathological studies identified immunoglobulins and complement-activation products deposited on the surface of intact myelin sheaths at the active edge of demyelinating lesions, as well as the presence of opsonized myelin debris that is cleared from the lesions by activated macrophages [2–7]. Plasma exchange can also dramatically reduce clinical disease activity in a subset of MS patients [8, 9]. This provides further circumstantial evidence that humoral factors can be involved in disease pathogenesis, but their mode of action remains obscure.

The tacit assumption made on the basis of immunopathological findings is that this mode of action involves autoantibodies recognizing determinants exposed on the surface of the myelin sheath. Immunopathological studies indicate that the clinical significance of antibody-mediated demyelination in this response varies between patients. There is no evidence at all that antibody is involved in lesion formation in 20–40% of patients [5, 6], whilst in some subtypes of MS such as Devic’s disease (neuromyelitis optica) antibody-mediated effects may play a dominant role in disease pathogenesis [7]. There is also a wide variation in the clinical response of MS...
anti-inflammatory disease of the CNS, with very limited transfer of encephalitogenic T cell lines is essentially an ADCC-dependent effector mechanisms trig- gered by the antibody binding to MOG exposed at the membrane surface [18]. However, the relative impor- tance of the MOG-specific antibody response with respect to demyelination and clinical disease is critically dependent on the species under investigation.

In rats and marmosets, EAE induced by the adoptive transfer of encephalitogenic T cell lines is essentially an inflammatory disease of the CNS, with very limited demyelination [13, 14, 18–20]. This is not an antigen-specific phenomenon, as the adoptive transfer of T cells specific for different myelin antigens [e.g. MOG, MBP, proteolipid protein (PLP) or myelin-associated glycoproteins (MAG)] or non-myelin antigens (e.g. S100) triggers a similar inflammatory non-demyelinating disease in the rat [18]. In both species, the formation of large demyelinating lesions similar to those seen in MS is an antibody-dependent phenomenon. The dissociation of the effector mechanisms responsible for inflammation and demyelina-

2 Anti-MOG antibodies in EAE and MS

The demyelinating potential of MOG-specific autoanti- bodies was confirmed in vivo by adoptive transfer in rats [13, 14], mice [14, 15] and primates [16] with EAE and in vitro using myelinated cultures of CNS tissue [17]. In animals with EAE induced by the adoptive transfer of myelin basic protein (MBP)-specific T cells [13, 14], or by active immunization with MBP [14], with MOG [15] or with white-matter homogenate [16], the intravenous injection of MOG-specific antibodies enhanced demyelination and inflammation, resulting in a dramatic increase in dis- ease severity. This response is mediated by a combina- tion of complement and antibody-dependent cell cyto- toxicity (ADCC)-dependent effector mechanisms trig- gered by the antibody binding to MOG exposed at the membrane surface [18]. However, the relative impor- tance of the MOG-specific antibody response with respect to demyelination and clinical disease is critically dependent on the species under investigation.

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3 Antibodies to discontinuous MOG epitopes are responsible for demyelination in the marmoset model of MOG-induced EAE

MOG is a quantitatively minor type I membrane glyco- protein that is preferentially incorporated into the outer- most surface of the myelin sheath [37] where a single extracellular immunoglobulin-like domain — MOG immunoglobulin domain (MOG\textsuperscript{Ig}) — is exposed to the extracellular milieu [38–40]. It is this domain of the mole- cule that is recognized by demyelinating MOG-specific antibodies. Unlike the major encephalitogens MBP and PLP, MOG protein is not expressed at detectable levels outside the nervous system [41]. The mature protein is sequestered from the immune system within the immuno-privileged environment of the CNS, and is therefore unable to induce either central or peripheral tol- erance. Naive MOG\textsuperscript{Ig}-reactive clones therefore enter
and persist in the normal immune repertoire, clinical tolerance being maintained by clonal ignorance — the physical/functional segregation of potentially pathogenic MOG-specific T and B cell clones, in the periphery, from their cognate antigen within the CNS [42, 43].

It is therefore perhaps not surprising that active immunization with MOG\textsuperscript{Igd} induces a complex autoimmune response and severe EAE in susceptible species/strains [18]. The encephalitogenic MOG-specific T cell response is an absolute requirement to initiate disease, but its ability to mediate demyelination and induce an MS-like pathology varies between species [18]. As discussed earlier, extensive tissue damage can occur in mouse models of EAE in the absence of a functional B cell response, whereas the induction of widespread demyelination in rat and marmoset models of EAE is antibody-dependent. It was recognized some time ago that the MOG-specific antibody response recognizes both linear and discontinuous MOG\textsuperscript{Igd} epitopes, but whereas the demyelinating potential of the response to discontinuous epitopes is well established [44, 45], the significance of antibody responses to linear epitopes was obscure.

Von Büdingen and colleagues [36] isolated antibodies specific for either linear or discontinuous MOG\textsuperscript{Igd} epitopes, by immunoadfinity chromatography, and then compared their pathogenic potential by adoptive transfer into marmosets with sub-clinical EAE induced by immunization with MBP. Antibodies to discontinuous epitopes triggered extensive demyelination and increased the burden of lesions within the CNS, as demonstrated previously [13, 14, 16] using a conformation-dependent MOG-specific mAb [44]. In contrast, antibodies to linear MOG\textsuperscript{Igd} peptide epitopes did not initiate the formation of large demyelinating lesions, indicating that they were unable to recognize and bind to the native protein embedded in the myelin membrane in vivo. Unfortunately, the use of primates limited the size of the experimental groups (n=2), and pathology was assessed at different times over a period 2–7 days. It was therefore not possible to control for possible differences in lesion burden at the time of transfer, or in the rate of lesion formation induced by the two antibody populations. Nevertheless, the broad conclusions of this study — demyelinating antibody responses are directed against discontinuous, rather than linear epitopes — are in agreement with results obtained in other species [46].

Von Büdingen et al. point out that although antibodies to linear peptide epitopes did not induce extensive demyelination, the burden of CNS lesions in the animals injected with these antibodies was higher than in the two controls that received naive marmoset Ig\textsubscript{G}. The sample size is too small to make any definitive conclusions but this observation does raise the possibility that antibodies bind to MOG epitopes that are generated during the MBP-induced inflammatory response. If this is the case, this antibody response (together with antibodies recognizing other exposed determinants) may influence lesional pathology by accelerating the clearance of myelin debris from the lesions by activated macrophages.

However, the MOG-specific antibody response in patients with MS recognizes a far more complex repertoire of linear epitopes [33, 47] than marmosets [36] or rodents [26–28, 46, 48] do. This peptide-specific response in man does not recognize the native protein [33] and is therefore unlikely to mediate demyelination in vivo, but further studies are required to determine whether or not these responses have any other pathological significance. There is now a pressing need to develop clinically useful assays that will selectively detect the pathogenic component of the anti-MOG response in MS patients, and to understand what determines the balance between the response to linear (benign) and discontinuous (demyelinating) MOG B cell epitopes.

4 Structural analysis of MOG–antibody interactions

The solution of the crystal structures of MOG\textsuperscript{Igd} [49, 50] and its complex with the chimeric antigen-binding fragment (Fab) of the demyelinating MOG-specific monoclonal antibody 8-18C5 [47] has recently provided an insight into the structural constraints imposed on the demyelinating antibody response. The three-dimensional structure of MOG\textsuperscript{Igd} allows us to identify those solvent-exposed amino acids that may provide targets for autoantibody-mediated demyelination [49, 50]. These regions of the molecule include two peptide sequences (amino acids 35–55 and 63–87) that encompass highly exposed protruding loops, and which were previously identified as epitopes that are potentially recognized by antibodies that cause demyelination [26, 28, 45, 48] (Fig. 1A).

Even more informative was identification of the antibody-binding site recognized by the demyelinating mAb 8-18C5 [49] (Fig. 1B). This antibody recognizes an immunodominant epitope, or one of a cluster of closely related immunodominant epitopes targeted by the demyelinating response to MOG in the mouse [45]. Analysis of the MOG–antibody complex reveals that it binds to the upper surface of MOG\textsuperscript{Igd}, interacting mainly with four distinct regions of the molecule — the N terminus of the protein, and its BC-, C'C''- and FG-loops. The dominant contribution to antibody binding is made by the FG-loop.
Fig. 1. Molecular characterization of demyelinating MOG epitopes. (A) Putative linear MOG epitopes (35–55) and (63–87) are depicted in the molecular structure of the extracellular domain of MOG which is shown as a ribbon diagram. The β-sheets are depicted in yellow, amino acids 35–55 including the highly exposed region 40–47 are colored light green and green, respectively; and amino acids 63–87 that encompass the protruding DE-loop are shown in light blue and blue. (B) The discontinuous MOG epitope recognized by the mouse antibody 8-18C5. Amino acids involved in binding 8-18C5 are colored red. In addition, the molecular surfaces of those amino acids are depicted that combine to form the conformation-dependent epitope recognized by this mAb. Data are obtained from the analysis of the crystal structures of rat MOGIgd and its complex with a Fab fragment derived from the demyelinating MOG-specific mAb 8-18C5 [49].

(AMINO ACIDS 101–108) THAT IS COMPLETELY BURIED IN A CAVITY MADE BY THE HEAVY-CHAIN COMPLEMENTARITY-DETERMINING REGIONS. THIS LOOP ACCOUNTS FOR 65% OF TOTAL SURFACE AREA OF MOG\textsuperscript{Rat} THAT INTERACTS WITH THE ANTIBODY-BINDING SITE. CRUCIALLY, THE CONFORMATION OF THE FG-LOOP IS HIGHLY STRAINED AND IS ONLY MAINTAINED BY THE SURROUNDING AMINO ACIDS OF THE CORRECTLY FOLDED PROTEIN MOLECULE [49]. THIS PROVIDES A SIMPLIFIED EXPLANATION FOR THE FAILURE OF PEPTIDE MAPPING USING LINEAR PEPTIDES TO DETECT THIS ANTIGENIC REGION, AND THE INABILITY OF PEPTIDES CONTAINING THIS SEQUENCE TO TRIGGER A PATHOGENIC AUTOANTIBODY RESPONSE, AS IN ISOLATION THIS LINEAR PEPTIDE SEQUENCE WILL BE UNABLE TO ADOPT THE APPROPRIATE CONFORMATION.

### 5 Regulation of the response to discontinuous MOG B cell epitopes

Analysis of the fine specificity of the human antibody response to MOG reveals a distinct bias in favor of antibodies that recognize linear as opposed to discontinuous epitopes [33]. In the context of neurological disease, proteases involved in disease pathogenesis and antigen processing may bias determinant spreading in favor of a non-pathogenic antibody response by disrupting the structural organization of MOG\textsuperscript{Rat} and eliminating discontinuous epitopes such as that recognized by mAb 8-18C5 [49]. This may explain the high frequency of antibody responses recognizing linear MOG peptides, but not the intact molecule, in patients with MS [23]. However, the composition of the MOG-reactive antibody repertoire is also clearly influenced by both genetic and environmental influences [47, 52].

Genes within the MHC selectively censor the ability of H2-b mice to mount an antibody response to discontinuous (demyelinating) rat MOG\textsuperscript{Rat} epitopes, whilst leaving the response to linear epitopes intact. This effect could involve protease activities encoded within the MHC, the selective deletion of MOG-reactive B cell clones mediated by structural homologues of MOG, or even strain-specific differences in the expression of MOG outside the CNS [46]. However, irrespective of the mechanism involved it is highly sensitive to changes in the amino acid sequence of MOG. This led to some confusion, as it influences the susceptibility of B cell-deficient mice to EAE induced by either rat or human MOG\textsuperscript{Rat}.

The amino acid sequence of MOG\textsuperscript{Rat} is highly conserved between rodents and man, and both proteins induce severe EAE in C57BL/6 mice [22]. The relative importance of antibody-dependent mechanisms in the pathogenesis of the disease induced by the two proteins is, however, very different. As described above, C57BL/6 mice are unable to mount a pathogenic autoantibody response following active immunization with rat MOG\textsuperscript{Rat} [46] and, not surprisingly, in the absence of a pathogenic B cell response, susceptibility to rat MOG\textsuperscript{Rat}-induced EAE is B cell independent in this mouse strain [20, 21]. This is not the case when disease is induced by human MOG\textsuperscript{Hum} [15, 21]. This is due to differences in the amino acid sequences of rat and human MOG\textsuperscript{Hum} that modify the composition/function of the immune response [21]. First, unlike the rodent protein, human MOG\textsuperscript{Hum} does induce a pathogenic antibody response in C57BL/6 mice that cross-reacts with the mouse protein to enhance demyelination and clinical disease \textit{in vivo} [15]. However, the substitution of proline for serine at position 42 within the immunodominant T cell epitope reduces the pathogenicity of the T cell arm of the immune response [21]. As a consequence, antibody-dependent mechanisms are the major factor determining clinical disease activity in C57BL/6 mice immunized with human MOG\textsuperscript{Hum} and susceptibility is clearly B cell dependent [15, 21].

These observations demonstrate that relatively minor changes in amino acid sequence can dramatically influ-
ence the relative importance of the immune effector mechanisms involved in the pathogenesis of autoimmune disease. It is clearly very important to use autologous autoantigens when studying the regulation of autoimmunity and autoaggression. With this caveat in mind it should be noted that von Büdingen et al. [36] investigated the marmoset response to recombinant rat MOG<sup>ld</sup>. It remains to be demonstrated whether or not the antibody response to the autologous marmoset protein will exhibit the same combination of epitope specificities induced by recombinant rat MOG.

### 6 Detecting antibody responses to discontinuous MOG epitopes in EAE and MS

The results presented by von Büdingen et al. [36] demonstrate that it is essential to develop assays that will differentiate between responses to pathogenic (discontinuous) and “irrelevant” (linear) MOG epitopes if we are to identify demyelinating MOG-specific autoantibodies in EAE and MS. Unfortunately, simple ELISA using recombinant protein expressed in bacteria are unable to differentiate between the two responses [30]. This is even the case after extensive re-folding and purification of recombinant MOG<sup>ld</sup>, probably because the antigen denatures during ELISA protocols (C. Linnington et al., unpublished data).

However, the simplest criterion defining the demyelinating response is that it must recognize the native extracellular domain of MOG as it is expressed in vivo on the myelin surface. This can be reproduced in vitro using MOG-transfected cell lines, and FACS-based assays can then be used to identify the pathogenic component of the antibody response binding to the cell surface [33, 44, 46]. This approach can clearly differentiate between responses to discontinuous and linear MOG epitopes. As demonstrated in Fig. 2 this technique differentiates between responses to linear (non-pathogenic), and discontinuous (demyelinating) epitopes in the rat, and was also used to identify cytolytic antibody response to MOG in murine models of MOG-induced EAE [46]. Using this approach it may also be possible to confirm the association between demyelination in MBP/PLP-induced EAE in the marmoset with the development of a MOG-specific antibody response [53].

This cell-based approach to identify potential pathogenic components within a polyclonal/polyspecific antibody response to MOG is relatively powerful. However, it should be noted that in MS patients, antibodies to linear epitopes were present in all donors, whereas potentially pathogenic responses to the native extracellular domain of the protein were only detected in <5% of patients [33]. Is this low value due to a lack of sensitivity of the assay, rapid absorption of demyelinating antibodies in the CNS, or are other myelin antigens the dominant target for demyelinating autoantibodies in MS? We are confident that these questions will be answered in the near future and will have a considerable impact on the clinical management of MS.

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