Antibody response in MOG35–55 induced EAE

Lalive d'Epinay, Patrice; Molnarfi, Nicolas; Benkhoucha, Mahdia; Weber, Martin S.; Santiago-Raber, Marie-Laure

Journal of Neuroimmunology 240-241 (2011) 28–33

Contents lists available at SciVerse ScienceDirect

Journal of Neuroimmunology

journal homepage: www.elsevier.com/locate/jneuroim

Antibody response in $MOG_{35–55}$ induced EAE

Patrice H. Lalive [a,b,c], Nicolas Molnarfi [a], Mahdia Benkhoucha [a,b], Martin S. Weber [d], Marie-Laure Santiago-Raber [a,*]

[a] Department of Pathology and Immunology, Faculty of Medicine, University of Geneva, Geneva, Switzerland
[b] Department of Neurosciences, Division of Neurology, University Hospital of Geneva, Geneva, Switzerland
[c] Department of Genetics and Laboratory Medicine, Laboratory Medicine Service, University Hospital of Geneva, Geneva, Switzerland
[d] Department of Neurology, Technische Universitat Munchen, Munich, Germany

ARTICLE INFO

Article history:
Received 30 June 2011
Received in revised form 13 September 2011
Accepted 15 September 2011

Keywords:
Experiemntal Autoimmune Encephalomyelitis
Multiple Sclerosis
B cell response
Anti-DNA
Anti-RNA
Anti-MOG

ABSTRACT

Neurological deficit in experimental autoimmune encephalomyelitis (EAE) and multiple sclerosis is widely considered to be a consequence of synergistic T and B cell responses to central nervous system (CNS) antigens. We show that mice immunized with encephalitogenic myelin oligodendrocyte glycoprotein ($MOG_{35–55}$) peptide develop significant serum levels of anti-MOG antibodies in parallel with disease progression. Furthermore, EAE mice developed antibodies against DNA and RNA, a serological hallmark observed in autoimmune diseases such as systemic lupus erythematosus. The presence of anti-nucleic responsive B cells and antibodies during EAE may highlight a previously unappreciated mechanism in the pathogenesis of CNS autoimmunity.

1. Introduction

Multiple sclerosis (MS) is a relatively common disease with a complex etiology involving central nervous system (CNS) inflammation, demyelination and axonal damage. In occidental countries, MS affects about 0.1% of the population and is the first cause of disability of non-traumatic origin in young adults (Sospedra and Martin, 2005). While MS has traditionally been considered to be a T-cell-mediated disease, there is an increasing body of evidence that B cells and autoantibodies may play a pathogenic role in this disease, providing a rationale for therapeutic approaches targeting B cells and B cell derived components of the immune system. B cells and plasma cells are commonly found in active MS plaques and the presence of cerebrospinal fluid-specific antibodies (oligoclonal bands) remains a hallmark finding in the diagnosis of MS (Noseworthy et al., 2000). In addition, myelin-specific antibodies have been identified in areas of vesicular demyelination (Genain et al., 1999), suggesting that they directly promote CNS damage, at least in a subset of MS patients (Lucchinetti et al., 2000). Among these antibody responses, those directed against conformational epitopes of myelin-oligodendrocyte glycoprotein (MOG) are considered to be potentially pathogenic in MS (Lalive et al., 2006; Menge et al., 2007; Lalive, 2008; Lalive et al., 2011; Weber et al., 2011).

Experimental autoimmune encephalomyelitis (EAE) is an animal model that recapitulates many clinical and pathological features of human MS. Immunization of C57BL/6 mice with MOG peptide amino acid (aa) 35–55 ($MOG_{35–55}$) alone has been shown to induce a chronic demyelinating disease predominantly driven by $CD4^+$ T-cell-mediated immunity. The significance of myelin-specific antibodies in mediating myelin loss in this EAE model is controversial (Lalive, 2008). As only B cell receptor (BCR) binding by native antigens initiates BCR signaling and a program of B-cell activation that ultimately results in B-cell differentiation and antibody production, it has generally been considered that production of MOG autoantibodies does not to take place in $MOG_{35–55}$-induced EAE in a meaningful manner (Slavin et al., 2001). A small body of evidence however points to a possible contribution of myelin-reactive antibodies to $MOG_{35–55}$-induced EAE pathogenesis, and in particular T cell-dependent IgG1 and IgG2a antibody responses (Zhang et al., 2004); first, the benefit of B cell-depletion in MOG-induced EAE was associated with decreased antibody titers against MOG peptide (Matsushita et al., 2006); second, transfer of anti-MOG serum from $MOG_{35–55}$-immunized mice has been demonstrated to increase severity of EAE in WT mice (Du and Sriram, 2002). These studies imply that MOG peptide-specific antibodies and/or anti-MOG serum may be pathogenic in this strain, as it seems to be the case in several mouse and rat strains (Bernard et al., 1997). According to some (Ichikawa et al., 1996a) but not all (Adelmann et al., 1995) investigations, Lewis rats immunized with $MOG_{35–55}$ can develop multifocal demyelinating disease despite the demonstration that these animals do not develop conformation-dependent anti-MOG antibodies (Ichikawa et al., 1996a, 1996b).

* Corresponding author at: Department of Pathology and Immunology, Faculty of 1Medicine, University of Geneva, 1 Rue Michel Servet, 1211 Geneva, Switzerland. Tel.: +41 22 3794698; fax: +41 22 379 5746.
E-mail address: marie.santiago@unige.ch (M.-L. Santiago-Raber).

0165-5728/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jneuroim.2011.09.005

In general, antibodies may exert their pathogenic function in CNS autoimmune diseases via various mechanisms. Those include (i) antigen binding on target cells leading to complement activation and destruction of the target, (ii) immune complex (IC) recognition through the Fc-gamma receptor (FcγR) expressed on phagocytic cells leading to the phagocytosis of the antigen–antibody complex and destruction of the tissue (iii) antibody-dependent cellular cytotoxicity (ADCC) in which IC recognition by cells of the innate immune system leads to the release of inflammatory components by the effector cell mediating cytotoxicity and lysis of the target, and (iv) specific blockade of receptor-ligand interactions (reviewed in (Berer et al., 2010)). In addition, antibodies are also capable of targeting self-antigen such as nucleic acid-protein complexes that, after endocytosis, can activate pro-inflammatory endosomal Toll-like receptor (TLR) pathways in cells of the innate immune system and B cells (Marshak-Rothstein and Rifkin, 2007; Santiago-Raber et al., 2009) as observed in autoimmune diseases such as systemic lupus erythematosus. While anti-nucleic acid antibody levels in systemic lupus erythematosus patients correlate with disease severity, nucleic acids have been identified as a major antigenic target of CNS IgG in MS.

In this study, we investigated MOG-specific antibody responses in EAE immunized with $MOG_{35–55}$. In this EAE model, widely considered as B cell-independent, we first demonstrated the ability of B cells to mount a MOG peptide-specific antibody response in a time-dependent manner. Antibody production was closely associated with B cell activation. In addition to the increase of total IgG, we observed for the first time the generation of specific antibodies against DNA and RNA, two important triggers involved in the specific activation of essential components (Toll-like receptor 9 and 7) of the innate immune system (Prinz et al., 2006). Taken together, our experimental findings on hand, point towards a so far underappreciated and potentially pathogenic role of anti-nucleic acid antibodies in this model of CNS autoimmunity.

2. Materials and methods

2.1. Ethics statement

Animal experiments described in the present study have been approved by the Ethics Committee for Animal Experimentation of the Faculty of Medicine, University of Geneva (protocol ID number: 1005/3537/3).

2.2. Mice and induction of EAE

Female C57BL/6J (B6) mice were obtained from local breeding. Mice were kept under pathogen-free conditions and used at 8–10 weeks of age. For active immunization, B6 females were immunized with $MOG_{35–55}$ (100 μg) as described (Benkhoucha et al., 2010). Animals received pertussis toxin (300 ng per mouse) on days 0 and 2 after immunization. Mice were assigned clinical scores daily from 0 to 5 (Benkhoucha et al., 2010). Mice were bled at day 0 (before immunization) and days 7, 14 and 21 after immunization. Sera were frozen at −20 °C until use.

2.3. Serological assays

Serum levels of total IgG, IgM and IgG autoantibodies against RNA and heat-denatured DNA (ssDNA) were determined by ELISA as previously described (Santiago-Raber et al., 2010). Results for IgM and IgG are expressed in μg/ml or mg/ml, respectively by referring to a standard curve obtained with a mouse serum with known IgG and IgM concentrations (Miles Laboratories). For autoantibodies, RNA and ssDNA (10 μg/ml) were coated to ELISA plates pre-coated with poly-L-lysine (Sigma-Aldrich). Then, the plates were incubated with 1/100 diluted serum samples, and the assay was developed with alkaline phosphatase-labeled goat anti-mouse IgG. Results are expressed in units per milliliter in reference to a standard curve derived from a serum pool of lupus prone MRL-Fas^{lpr} mice. Anti-$MOG_{35–55}$ antibodies were quantified by an ELISA kit (AnaSpec Inc.) following the instructions of the manufacturer.

2.4. Flow cytometric analysis

Flow cytometry was performed using three- or four-color staining and analyzed with a FACSCalibur (BD Biosciences, San Jose, CA). The following antibodies were used: anti-CD11b (M1/70), anti-B220 (RA3-6B2), anti-CD4 (GK1.5), anti-CD69 (H1.2 F3), and anti-I-A (Y-3P). Staining was performed in the presence of saturating concentration of 2.4 G2 anti-FcγRII/III mAb.

2.5. Immunohistochemistry

Spleens were embedded in Tissue-Tek O.C.T. compound (Miles, Provenance) and snap-frozen in liquid nitrogen. Five micrometers frozen sections were stained with PE-labeled anti-IgM mAb (1B4B1; Southern Biotechnology) and FITC-conjugated peanut agglutinin (PNA; Sigma-Aldrich,) which stains germinal center B cells, in the presence of 2.4G2 anti-FcγRII/III mAb.

2.6. Statistical analysis

Analysis of serological parameters was performed by the ANOVA test. Analysis of spleen $CD69^+$ $B220^+$ B cell frequencies was performed by the Mann–Whitney *U*-test. Probability values <5% were considered significant.

3. Results

3.1. Development of $MOG_{35–55}$-induced EAE is associated with generation of a humoral response

As conflicting results exist in the literature regarding the activation of B cells in $MOG_{35–55}$-induced EAE, we sought first to monitor B cell-antibody production following active immunization with this myelin peptide antigen. To investigate whether serum production of IgG and IgM could be detected in $MOG_{35–55}$-induced EAE, sera were sequentially obtained at day 0 (D0), D7, D14 and D21 (peak disease) from B6 mice ($n=18$) immunized with $MOG_{35–55}$ (Fig. 1A), and tested by ELISA for total IgG and IgM. As depicted in Fig. 1B, the level of total IgM was slightly modified over time until EAE peak ($p<0.05$). In contrast, we observed a significant increase of total IgG levels from D14 ($3.51+/-1.83$ mg/ml; mean +/− SD) to D21 ($6.29+/-3.74$ mg/ml) post-immunization when compared to D0 ($1.15+/-0.46$ mg/ml) ($p<0.0001$) (Fig. 1C).

3.2. Active immunization with $MOG_{35–55}$ generates a specific anti-$MOG_{35–55}$ peptide response

To further examine whether active immunization with $MOG_{35–55}$ elicits anti-$MOG_{35–55}$ antibodies, sera were isolated from mice with EAE ($n=18$) at D0, D7, D14 and D21 post-immunization, and examined by ELISA. As depicted in Fig. 2, we observed significantly increased levels of IgG anti-$MOG_{35–55}$ from D14 ($3360+/-6815$ ng/ml) up to D21 ($5336+/-8787$ ng/ml) post-immunization when compared to sera isolated at D0 (undetectable) ($p<0.01$). Based on an arbitrary cut-off of mean at D0 + 3 SD, sera were considered negative for anti-$MOG_{35–55}$ antibodies for all mice at D0 and D7 post-immunization, whereas sera isolated from 16/18 mice (89%) and 18/18 mice (100%) were positive for anti-$MOG_{35–55}$ antibodies at D14 and D21, respectively. These data demonstrate that immunization with $MOG_{35–55}$ triggers activation of autoreactive MOG-specific B cells.

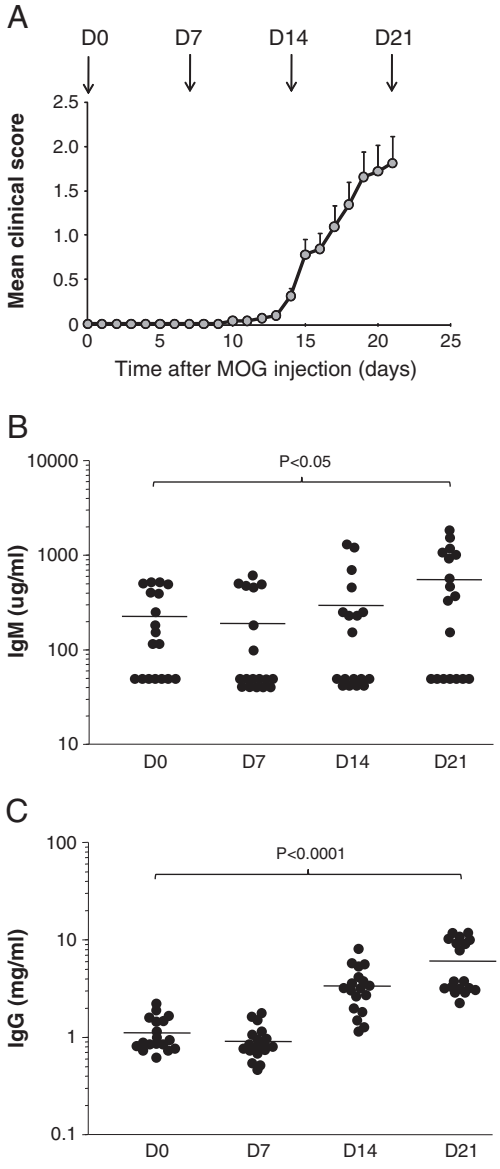

Fig. 1. MOG_{35-55} elicits an IgG antibody response in EAE mice. (A) Means +/− SEM of clinical scores during MOG-induced EAE established in 18 B6 females. Serum levels of total IgM (B) and IgG (C) were determined in MOG_{35-55}-induced EAE B6 mice (n = 18) at different time points (arrows). Each symbol represents an individual animal. Results are expressed as μg/ml (IgM) and mg/ml (IgG). Horizontal lines indicate the mean values.

3.3. Autoantibodies directed against nucleic acid (RNA and ssDNA) are produced during MOG_{35-55}-induced EAE

As data indicate that antibodies against nucleic acid contribute to the severity of experimental models of autoimmunity, we hypothesized that a similar mechanism of action could take place in EAE pathogenesis. Detections of anti-RNA and anti-ssDNA specific antibodies were assessed by ELISA in the sera of EAE mice following disease induction. As shown in Fig. 3A, anti-RNA IgG were detected in sera of EAE mice at D14 (4.65 +/− 5.35 U/ml) and D21 (7.23 +/− 5.05 U/ml) ($p<0.0001$) when compared to D0 (1.62 +/− 0.87 U/ml). Based on an arbitrary cut-off of mean at D0 + 3 SD, sera were considered negative for anti-RNA antibody for all mice at D0 and D7, whereas sera obtained from 5/18 mice (28%) and 14/18 mice (78%) were positive at D14 and D21 post-immunization, respectively. Moreover, we observed the presence of anti-ssDNA IgG in sera of EAE mice at D14 (1.53 +/− 2.67 U/ml) and D21 (2.55 +/− 2.63 U/ml) ($p<0.001$), but not

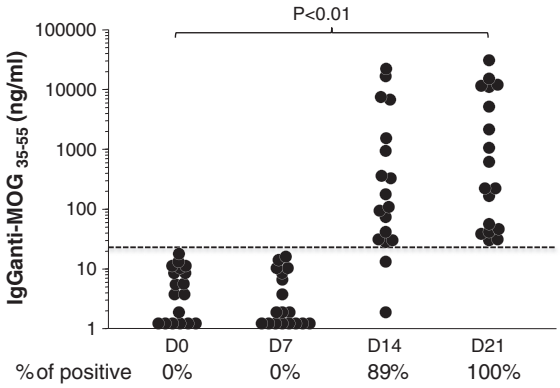

Fig. 2. Elevated anti-MOG_{35-55} IgG antibodies correlate with MOG_{35-55} induced EAE development. Serum levels of anti-MOG_{35-55} IgG antibodies were determined in MOG_{35-55} induced EAE mice (n = 18) at different time points. Each symbol represents an individual animal. Results are expressed as ng/ml. p value obtained by ANOVA is indicated. An arbitrary cut-off of positivity (mean at D0 + 3 SD) is indicated by the horizontal dotted line. Percentage (%) of positive mice is indicated for each time point.

at D0 (0.56 +/− 0.09 U/ml) ($p<0.001$) (Fig. 3B). Based on an arbitrary cut-off of mean at D0 + 3 SD, sera were considered negative for anti-ssDNA antibody for all mice at D0, positive for 2 mice (11%) at D7, whereas sera from 7/18 mice (44%) and 16/18 mice (89%) were positive for anti-ssDNA antibodies at D14 and D21 post-immunization, respectively.

3.4. MOG_{35-55} promotes B-cell activation

While reports indicate that, in contrast to native protein, peptide antigen do not trigger BCR activation, our data suggest that active immunization with MOG_{35-55} is a sufficient signal for B cell activation as determined by an antibody response. To further directly examine activation of B cells, frequencies of B cells ($B220^+$) expressing the activation marker CD69 were analyzed in the spleen of MOG_{35-55}-induced EAE at peak disease. As shown in Fig. 4A (left panel), we found an increased frequency of CD69-expressing B cells in mice with EAE when compared to control mice (3.0 +/− 0.6% [% $B220^+$ B cells +/− SD] vs 1.0 +/− 0.2% ($p = 0.0001$; n = 5/group)). In line with these results, we further observed an up-regulation of MHC class II molecule expression by B cells at the peak of the disease (Fig. 4A right panel), confirming the activated status of B cells.

As the germinal center (GC) reaction of antigen-activated B lymphocytes is the hallmark of an antibody-mediated immune response, we further analyzed the composition of B cell follicles (IgM^+) by immunofluorescence in the spleen at the peak of EAE severity using a PNA staining. Our results demonstrate the presence of germinal center B cells in secondary lymphoid organs of mice with EAE, while such staining was absent in B6 wild-type control mice (Fig. 4B).

As data indicate that activated B cells migrate within the CNS, we next evaluated the presence of $B220^+$ B cells in the spinal cord of EAE mice at peak disease. As shown in Fig. 4C (left panel), our results demonstrate a significant percentage of B cells in the spinal cord of mice with EAE (11.7 +/− 3.9 [% total nucleated cells +/− SD] vs. undetectable (n = 5/group)). B cells present in the CNS compartment were also expressing a high level of MHC class II molecules (Fig. 4C middle panel). As expected, a significant proportion of $CD11b^+$ cells (myeloid lineage including macrophages and dendritic cells) and $CD4^+$ T cell was also observed in the spinal cord of EAE mice during peak disease in comparison to control mice (Fig. 4C right panel).

4. Discussion

In this study, we investigated the presence of auto-antibodies in MOG_{35-55}-induced EAE, a model widely considered B cell-independent.

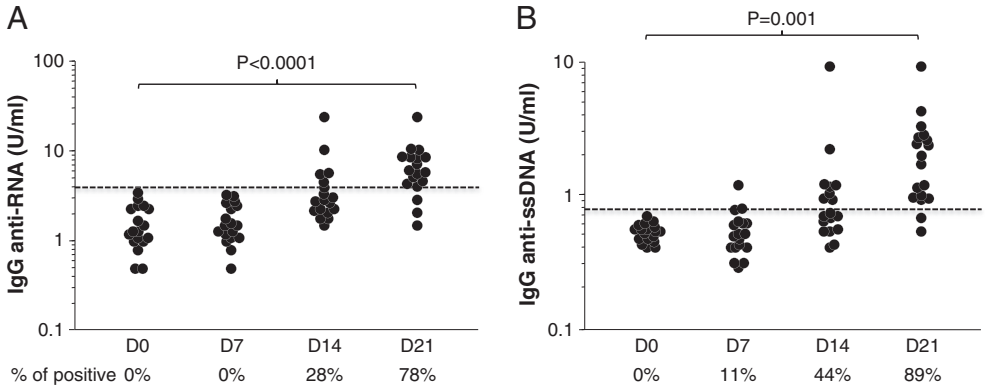

Fig. 3. MOG_{35-55}-induced EAE is associated with serum anti-nucleic autoantibody titers. Serum levels of anti-RNA (A) and anti-ssDNA (B) autoantibodies, were determined in MOG_{35-55}-induced EAE mice (n = 18) at different time points. Each symbol represents an individual animal. Results are expressed as U/ml. p values obtained by ANOVA are indicated. An arbitrary cut-off of positivity (mean at D0 + 3 SD) is indicated by the horizontal dotted line. Percentage (%) of positive mice is indicated for each time point.

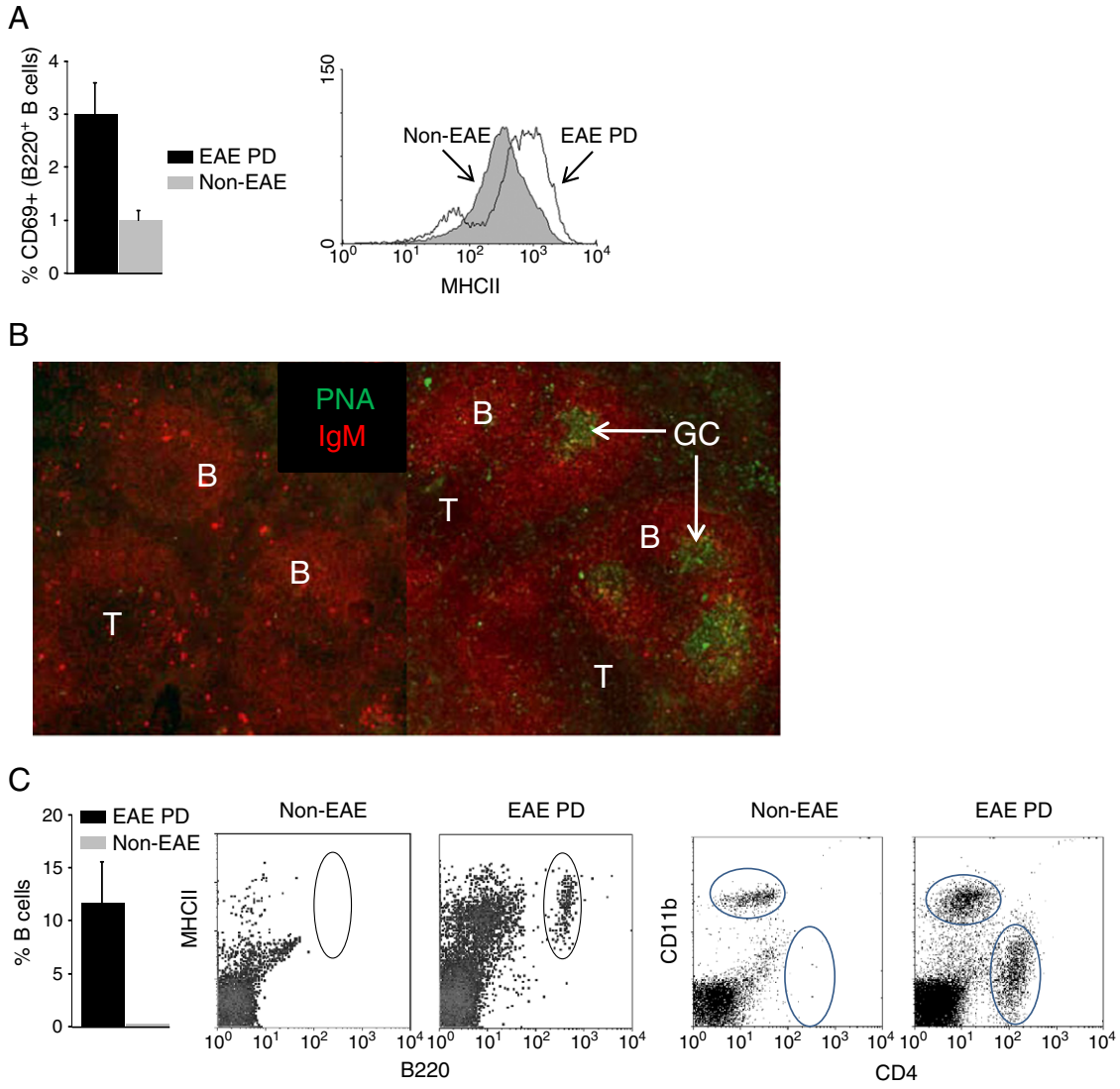

Fig. 4. EAE is characterized by increased B cell activation within lymphoid organ and CNS. (A) Expression of CD69 and MHC class II molecules by splenic B cells from EAE mice at peak disease (PD) or from control naive mice. Percentages of CD69-positive $B220^+$ cells (means of five mice ± SD) are shown. For MHC class II staining, representative results from one of 5 histograms are shown. (B) Spleen sections from both EAE mice at peak disease (PD) and control naive mice were stained with PE-labeled anti-IgM (red) and FITC-labeled PNA (green). Representative results obtained from 5 mice in each group are shown. Histological images indicate several germinal centers (GC) only within the splenic B cell zone (B) of EAE mice, but not of control naïve mice. T, T cell zone. (C) Percentage of $B220^+$ B cells (means of five mice ± SD) within spinal cords of EAE mice at peak disease and control naive mice are shown (left panel). Representative flow cytometry results from spinal cords indicating percentage of $B220^+$-$MHCII^+$ B cells (middle panel), or $CD4^+$ T cells and $CD11b^+$ cells (right panel), from EAE mice at peak disease and control naive mice. One representative of 5 individual mice is shown.

Our data demonstrate that direct immunization with MOG_{35-55} triggers auto-reactive B cell activation, as exemplified by elevated production of MOG_{35-55}-specific IgG antibodies. In line with these results, our data in EAE mice further indicate increased expression levels of CD69 and MHC II molecules by B cells, together with an elevated frequency of B cells within the GC of secondary lymphoid organs and the CNS. Moreover, we demonstrate to our knowledge for the first time a humoral response against nucleic acids in this EAE model.

While our results do not indicate a specific role of B cells in this EAE model, they suggest that immunization with MOG_{35-55}, the immunodominant epitope of MOG protein for T cells, is sufficient to trigger a B cell antibody response, as exemplified by the presence of high affinity switched autoantibodies during EAE pathogenesis. It is noteworthy, that among these IgG antibodies, some are specifically directed against the MOG peptide used to immunize the mice, as demonstrated by their detection at very high levels at 14 and 21 days after immunization. While some data indicate that anti-MOG peptide antibodies are not pathogenic in this EAE model (Lyons et al., 1999, 2002; Sekiguchi et al., 2009), other studies suggest that the humoral response elicited by active immunization with MOG_{35-55} could contribute to EAE pathogenesis. Some data indeed indicate that B cell depletion during MOG_{35-55}-induced EAE progression suppressed EAE symptoms, a clinical benefit which was associated with reduction of anti-MOG peptide antibody titers (Matsushita et al., 2008). The authors of that study however did not examine the role of anti-MOG antibodies on the development of EAE in their experimental system. Other data indicate that hyper immune anti-MOG antisera from $lyn^{-/-}$ mice (an important signaling molecule involved in B cells) were able to induce severe EAE in WT mice following immunization with MOG_{35-55} (Du and Sriram, 2002), suggesting a pathogenic role of anti-MOG Abs in EAE. Our results therefore suggest that autoantibodies subsequently produced upon B cell activation could contribute to some extent to disease pathogenesis and/or progression in this model.

The activation of specific TLR pathways in innate cells or B cells by immune complexes is known to facilitate autoimmune diseases, such as systemic lupus erythematosus (SLE) (Fischer and Ehlers, 2008). SLE is a prototypic autoimmune disease characterized by the production of antibodies to a diverse array of nuclear antigens. While a role for anti-nuclear antibodies in the pathogenesis of SLE is well established, recent data show that anti-DNA and anti-RNA antibodies can activate autoreactive B cells by dual engagement of the B cells receptor (BCR) and TLR9 or TLR7, respectively (Leadbetter et al., 2002; Lau et al., 2005).

Likewise in our study, antibodies against nucleic acids were positive at low levels in EAE mice. In general, anti-nucleic acid antibodies are a secondary feature of tissue damage by the release of nuclear molecules by apoptotic or necrotic cells in areas of inflammation, such as those observed in the CNS of EAE animals. The induction of antibodies against nucleic acids in EAE mice may eventually cause more damage owing to the chronic activation of TLR7 and/or TLR9, as described in other disease models such as SLE. Specifically, internalization of endogenous immune complexes containing autoantibodies and RNA or DNA through FCγRIIB on B cells and dendritic cells is believed to be an important mechanism in the maintenance of autoimmune responses (reviewed by Kawasaki et al., 2011).

In EAE, TLR9 pathway is needed for disease induction and $TLR9^{-/-}$ animals are protected from disease progression (Marta et al., 2005; Prinz et al., 2006). In regards to our data, one can propose that in EAE, TLR9 and/or TLR7 stimulation induced by DNA- and RNA-associated antigens, respectively, may play a major role in the activation of dendritic cells and B cells, and could contribute to the propagation of inflammatory reactions in affected tissues. In support to our findings, early reports have indicated the presence of antibodies to DNA and RNA in the CSF and serum from patients with MS (Schuller et al., 1978) and that anti-DNA antibodies are a major component of intrathecal B cell responses in MS (Williamson et al., 2001). Overall, our data therefore suggest that anti-nucleic acid antibodies may promote important neuropathologic mechanisms in chronic inflammatory disorders such as MS and Lupus.

In this study, we found no consistent correlation between the serum levels of myelin-specific or anti-nucleic acid antibodies and clinical severity that could account for their pathogenicity. While our results suggest that their presence may not be essential in the pathogenesis of MOG_{35-55}-induced EAE, it is also possible the large variation of serological measures between animals, together with the heterogeneity of the disease and the limited number of animals tested, might account for this observation.

In conclusion, our study using the MOG_{35-55}-induced EAE model, we herein showed that autoantibodies directed against nuclear antigens are produced during disease pathogenesis as it is currently observed in SLE. Such autoantibodies are detected much earlier in the disease process but at lower levels compared to what are generally observed in lupus prone mice. It could be speculated that during EAE progression, inflammation and tissue damage may increase the availability of self nucleic acids as a source of autoantigens and may contribute to CNS autoimmunity. It would be of great interest to further investigate the role of these autoantibodies during EAE to broaden our understanding of the role of B cells and autoantibodies in the pathophysiology of MS.

Acknowledgments

This study was supported by the Swiss National Foundation (#310030-132705) and the Swiss Multiple Sclerosis Society. NM is recipient of "ECTRIMS Research fellowship exchange program" 2011. We thank Gregory Schneiter and Céline Manzin-Lorenzi for expert technical help.

References

Adelmann, M., Wood, J., Benzel, I., Fiori, P., Lassmann, H., Matthieu, J.M., Gardinier, M.V., Dornmair, K., Linington, C., 1995. The N-terminal domain of the myelin oligodendrocyte glycoprotein (MOG) induces acute demyelinating experimental autoimmune encephalomyelitis in the Lewis rat. J. Neuroimmunol. 63, 17–27.

Benkhoucha, M., Santiago-Raber, M.L., Schneiter, G., Chofflon, M., Funakoshi, H., Nakamura, T., Lalive, P.H., 2010. Hepatocyte growth factor inhibits CNS autoimmunity by inducing tolerogenic dendritic cells and CD25+Foxp3+ regulatory T cells. Proc. Natl. Acad. Sci. U. S. A. 107, 6424–6429.

Berer, K., Wekerle, H., Krishnamoorthy, G., 2010. B cells in spontaneous autoimmune diseases of the central nervous system. Mol. Immunol. 48, 1332–1337.

Bernard, C.C., Johns, T.G., Slavin, A., Ichikawa, M., Ewing, C., Liu, J., Bettadapura, J., 1997. Myelin oligodendrocyte glycoprotein: a novel candidate autoantigen in multiple sclerosis. J. Mol. Med. 75, 77–88.

Du, C., Sriram, S., 2002. Increased severity of experimental allergic encephalomyelitis in lyn−/− mice in the absence of elevated proinflammatory cytokine response in the central nervous system. J. Immunol. 168, 3105–3112.

Fischer, M., Ehlers, M., 2008. Toll-like receptors in autoimmunity. Ann. N. Y. Acad. Sci. 1143, 21–34.

Genain, C.P., Cannella, B., Hauser, S.L., Raine, C.S., 1999. Identification of autoantibodies associated with myelin damage in multiple sclerosis. Nat. Med. 5, 170–175.

Ichikawa, M., Johns, T.G., Adelmann, M., Bernard, C.C., 1996a. Antibody response in Lewis rats injected with myelin oligodendrocyte glycoprotein derived peptides. Int. Immunol. 8, 1667–1674.

Ichikawa, M., Johns, T.G., Liu, J., Bernard, C.C., 1996b. Analysis of the fine B cell specificity during the chronic/relapsing course of a multiple sclerosis-like disease in Lewis rats injected with the encephalitogenic myelin oligodendrocyte glycoprotein peptide 35–55. J. Immunol. 157, 919–926.

Kawasaki, T., Kawai, T., Akira, S., 2011. Recognition of nucleic acids by pattern-recognition receptors and its relevance in autoimmunity. Immunol. Rev. 243, 61–73.

Lalive, P.H., 2008. Autoantibodies in inflammatory demyelinating diseases of the central nervous system. Swiss Med. Wkly. 138, 692–707.

Lalive, P.H., Menge, T., Delarasse, C., Della Gaspera, B., Pham-Dinh, D., Villoslada, P., von Budingen, H.C., Genain, C.P., 2006. Antibodies to native myelin oligodendrocyte glycoprotein are serologic markers of early inflammation in multiple sclerosis. Proc. Natl. Acad. Sci. U. S. A. 103, 2280–2285.

Lalive, P.H., Hausler, M.G., Maurey, H., Mikaeloff, Y., Tardieu, M., Wiendl, H., Schroeter, M., Hartung, H.P., Kieseier, B.C., Menge, T., 2011. Highly reactive anti-myelin oligodendrocyte glycoprotein antibodies differentiate demyelinating diseases from viral encephalitis in children. Mult. Scler. 17, 297–302.

Lau, C.M., Broughton, C., Tabor, A.S., Akira, S., Flavell, R.A., Mamula, M.J., Christensen, S.R., Shlomchik, M.J., Viglianti, G.A., Rifkin, I.R., Marshak-Rothstein, A., 2005. RNA-associated

autoantigens activate B cells by combined B cell antigen receptor/Toll-like receptor 7 engagement. J. Exp. Med. 202, 1171–1177.
Leadbetter, E.A., Rifkin, I.R., Hohlbaum, A.M., Beaudette, B.C., Shlomchik, M.J., Marshak-Rothstein, A., 2002. Chromatin-IgG complexes activate B cells by dual engagement of IgM and Toll-like receptors. Nature 416, 603–607.
Lucchinetti, C., Bruck, W., Parisi, J., Scheithauer, B., Rodriguez, M., Lassmann, H., 2000. Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. Ann. Neurol. 47, 707–717.
Lyons, J.A., San, M., Happ, M.P., Cross, A.H., 1999. B cells are critical to induction of experimental allergic encephalomyelitis by protein but not by a short encephalitogenic peptide. Eur. J. Immunol. 29, 3432–3439.
Lyons, J.A., Ramsbottom, M.J., Cross, A.H., 2002. Critical role of antigen-specific antibody in experimental autoimmune encephalomyelitis induced by recombinant myelin oligodendrocyte glycoprotein. Eur. J. Immunol. 32, 1905–1913.
Marshak-Rothstein, A., Rifkin, I.R., 2007. Immunologically active autoantigens: the role of toll-like receptors in the development of chronic inflammatory disease. Annu. Rev. Immunol. 25, 419–441.
Marta, C.B., Oliver, A.R., Sweet, R.A., Pfeiffer, S.E., Ruddle, N.H., 2005. Pathogenic myelin oligodendrocyte glycoprotein antibodies recognize glycosylated epitopes and perturb oligodendrocyte physiology. Proc. Natl. Acad. Sci. U. S. A. 102, 13992–13997.
Matsushita, T., Fujimoto, M., Hasegawa, M., Komura, K., Takehara, K., Tedder, T.F., Sato, S., 2006. Inhibitory role of CD19 in the progression of experimental autoimmune encephalomyelitis by regulating cytokine response. Am. J. Pathol. 168, 812–821.
Matsushita, T., Yanaba, K., Bouaziz, J.D., Fujimoto, M., Tedder, T.F., 2008. Regulatory B cells inhibit EAE initiation in mice while other B cells promote disease progression. J. Clin. Invest. 118, 3420–3430.
Menge, T., von Budingen, H.C., Lalive, P.H., Genain, C.P., 2007. Relevant antibody subsets against MOG recognize conformational epitopes exclusively exposed in solid-phase ELISA. Eur. J. Immunol. 37, 3229–3239.
Noseworthy, J.H., Lucchinetti, C., Rodriguez, M., Weinshenker, B.G., 2000. Multiple sclerosis. N. Engl. J. Med. 343, 938–952.
Prinz, M., Garbe, F., Schmidt, H., Mildner, A., Gutcher, I., Wolter, K., Piesche, M., Schroers, R., Weiss, E., Kirschning, C.J., Rochford, C.D., Bruck, W., Becher, B., 2006. Innate immunity mediated by TLR9 modulates pathogenicity in an animal model of multiple sclerosis. J. Clin. Invest. 116, 456–464.
Santiago-Raber, M.L., Baudino, L., Izui, S., 2009. Emerging roles of TLR7 and TLR9 in murine SLE. J. Autoimmun. 33, 231–238.
Santiago-Raber, M.L., Dunand-Sauthier, I., Wu, T., Li, Q.Z., Uematsu, S., Akira, S., Reith, W., Mohan, C., Kotzin, B.L., Izui, S., 2010. Critical role of TLR7 in the acceleration of systemic lupus erythematosus in TLR9-deficient mice. J. Autoimmun. 34, 339–348.
Schuller, E., Delasnerie, N., Lebon, P., 1978. DNA and RNA antibodies in serum and CSF of multiple sclerosis and subacute sclerosing panencephalitis patients. J. Neurol. Sci. 37, 31–36.
Sekiguchi, Y., Ichikawa, M., Takamoto, M., Ota, H., Koh, C.S., Muramatsu, M., Honjo, T., Agematsu, K., 2009. Antibodies to myelin oligodendrocyte glycoprotein are not involved in the severity of chronic non-remitting experimental autoimmune encephalomyelitis. Immunol. Lett. 122, 145–149.
Slavin, A.J., Soos, J.M., Stuve, O., Patarroyo, J.C., Weiner, H.L., Fontana, A., Bikoff, E.K., Zamvil, S.S., 2001. Requirement for endocytic antigen processing and influence of invariant chain and H-2M deficiencies in CNS autoimmunity. J. Clin. Invest. 108, 1133–1139.
Sospedra, M., Martin, R., 2005. Immunology of multiple sclerosis. Annu. Rev. Immunol. 23, 683–747.
Weber, M.S., Hemmer, B., Cepok, S., 2011. The role of antibodies in multiple sclerosis. Biochim. Biophys. Acta 1812, 239–245.
Williamson, R.A., Burgoon, M.P., Owens, G.P., Ghausi, O., Leclerc, E., Firme, L., Carlson, S., Corboy, J., Parren, P.W., Sanna, P.P., Gilden, D.H., Burton, D.R., 2001. Anti-DNA antibodies are a major component of the intrathecal B cell response in multiple sclerosis. Proc. Natl. Acad. Sci. U. S. A. 98, 1793–1798.
Zhang, G.X., Yu, S., Gran, B., Li, J., Calida, D., Ventura, E., Chen, X., Rostami, A., 2004. T cell and antibody responses in remitting-relapsing experimental autoimmune encephalomyelitis in (C57BL/6×SJL) F1 mice. J. Neuroimmunol. 148, 1–10.