Autoantibody-boosted T-cell reactivation in the target organ triggers manifestation of autoimmune CNS disease

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Multiple sclerosis (MS) is caused by T cells that are reactive for brain antigens. In experimental autoimmune encephalomyelitis, the animal model for MS, myelin-reactive T cells initiate the autoimmune process when entering the nervous tissue and become reactivated upon local encounter of their cognate CNS antigen. Thereby, the strength of the T-cellular reactivation process within the CNS tissue is crucial for the manifestation and the severity of the clinical disease. Recently, B cells were found to participate in the pathogenesis of CNS autoimmunity, with several diverse underlying mechanisms being under discussion. We here report that B cells play an important role in promoting the initiation process of CNS autoimmunity. Myelin-specific antibodies produced by autoreactive B cells after activation in the periphery diffused into the CNS together with the first invading pathogenic T cells. The antibodies accumulated in resident antigen-presenting phagocytes and significantly enhanced the activation of the incoming effector T cells. The ensuing strong blood–brain barrier disruption and immune cell recruitment resulted in rapid manifestation of clinical disease. Therefore, myelin oligodendrocyte glycoprotein (MOG)-specific autoantibodies can initiate disease bouts by cooperating with the autoreactive T cells in helping them to recognize their autoantigen and become efficiently reactivated within the immune-deprived nervous tissue.

autoimmunity | EAE | autoantibodies | multiple sclerosis | B cells

T cell-driven autoimmune processes directed against CNS antigens underlie the pathogenesis of multiple sclerosis (MS) and its animal model experimental autoimmune encephalomyelitis (EAE) (1). Myelin-reactive T cells become activated and differentiate in the periphery and then enter the nervous tissue and are reactivated upon local encounter of their cognate CNS antigen (2, 3). This autoaggressive T-cell response eventually leads to the recruitment of other immune cells and tissue destruction. However, several findings also support the view that B cells contribute to the pathogenesis of this T cell-driven autoimmune process (4). This contribution is indicated by the presence of locally produced antibodies (oligoclonal bands) within the CNS tissues (5), by decorations of the nervous structures with antibodies and complement (6), by the presence of meningeal B-cell follicles in progressive MS (7, 8), and also by the therapeutic effects of plasmapheresis or anti-CD20 monoclonal antibody application: i.e., autoantibody- and B cell-directed therapies, respectively (9, 10). Disease-modifying effects of myelin-specific B cells were also found in EAE (11–13). Several potential mechanisms are currently under discussion to account for the disease-promoting effects of B cells. These mechanisms include their ability to present antigen to T cells (14, 15), to generate a general "bystander activation" via the production of proinflammatory cytokines, especially IL-6 (16) and/or GM-CSF (17), and to induce an antibody/complement-mediated attack of myelin that exacerbates structural damage in autoimmune CNS lesions (18, 19). Part of the puzzle is that B cells have also been observed to have a disease-dampening effect via their release of antiinflammatory cytokines, specifically IL-10 and/or IL-35 (20, 21).

Using an integrative approach of intravital imaging, genetics, and functional characterization, we here studied the interactions of T and B cells in the course of EAE. We found that the presence of autoantigen-specific B cells potently promotes the manifestation of the autoimmune disease. We show that these disease-inducing effects are mediated, not by B cells per se, but by their specific soluble products. Myelin-directed autoantibodies in the CNS tissue were found to trigger the disease-causing inflammatory process by concentrating myelin antigens in phagocytes, thus increasing their capacity to present the autoantigen. Consequently, the myelin-reactive T cells that scan the tissue for their cognate antigens are stimulated and more easily reach the threshold for clinically relevant reactivation within the CNS tissue.

Results

Myelin Oligodendrocyte Glycoprotein-Specific B Cells Positively Influence the Initiation and Manifestation of CNS Inflammation and Clinical Disease. Myelin oligodendrocyte glycoprotein (MOG)-specific T cells (T_{MOG}) (22) were transferred alone or together with MOG-specific

Significance

Although T cells are the main players in autoimmune CNS inflammation, the role of B cells is being increasingly appreciated. We here investigated possible scenarios of how B cells could participate in the initiation of autoimmune CNS disease. We show that myelin-reactive autoantibodies accumulate in CNS-resident phagocytes, thereby concentrating myelin antigens in these cells and increasing the cells' capacity to present the autoantigen to invading myelin-reactive T cells. Consequently, these T cells are stimulated and more easily reach the threshold for clinically relevant reactivation within the CNS tissue. This previously unidentified mechanism is of potential clinical relevance because it provides a scientific explanation for immune processes leading to disease initiation and induction of relapses in multiple sclerosis and other autoimmune CNS disorders.

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The authors declare no conflict of interest.

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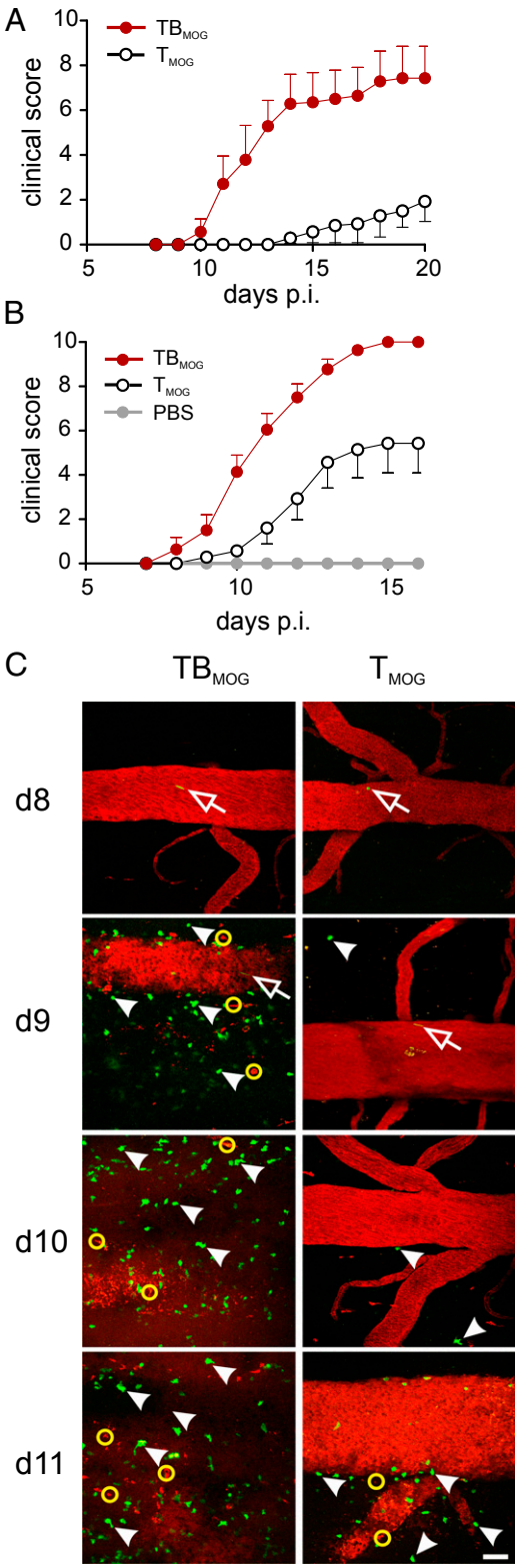

Fig. 1. MOG-specific B cells accelerate CNS inflammation and disease. (*A*) Accelerated manifestation of EAE in T-/B-MOG mice. Active EAE in T-/B-MOG (TB_{MOG}) compared with T-MOG (T_{MOG}) mice using a low pathogenic immunization protocol (*Materials and Methods*). Clinical score is shown. $n = 7$ from two independent experiments. (*B*) Acceleration of EAE in T-/B-MOG-OTII mice. Active EAE in OTII mice after transfer of $T_{MOG} \pm B_{MOG}$ cells. PBS injection was used as control. $n = 6$–14 from three independent experiments. (*C*) Accelerated T-cell CNS infiltrations in T-/B-MOG mice. 2-PM recordings of $T_{MOG\text{-}GFP}$ and $B_{MOG\text{-}RFP}$ cells in leptomeninges and the adjacent spinal cord parenchyma at the indicated time points p.i. The blood vessels were visualized by 2,000-kDa Texas Red dextran. (Scale bar: 50 μm.) Open arrow heads, intraluminal T cells; filled arrow heads, extravasated T cells; yellow circles, red phagocytes that ingested dextran leaking through the BBB. $B_{MOG\text{-}RFP}$ cells were not detectable in the CNS tissues.

B cells (B_{MOG}) (13) into C57BL/6J mice. The animals were immunized 48 h later with MOG peptide or protein (amino acids 35–55 or amino acids 1–125, respectively). Independently of the antigenic stimulus, in the presence of B_{MOG} cells, disease onset occurred earlier, and the incidence and severity of clinical deficits were increased (Table S1, Exps. 1 and 2). The disease-promoting effect of autoreactive B cells became even more evident when the animals were immunized under "suboptimal" activation conditions (Fig. 1*A*, and Table S1, Exp. 3). To exclude a potential contribution of endogenous MOG-reactive T or B cells in the WT mice, T_{MOG} and B_{MOG} cells were transferred into recipient mice with a restricted T-cell receptor (TCR) and B-cell receptor (BCR) repertoire unable to raise brain-specific immune responses: namely, OTII hosts harboring ovalbumin (OVA)-specific T cells or cross-breeds between OTII and B1.8 mice (23) additionally containing (4-hydroxy-3-nitrophenyl)acetyl (NP)-specific B cells (B_{NP}). Both OTII and OTII/B1.8 mice developed a markedly accelerated and aggravated disease course after transfer of T_{MOG} and B_{MOG} cells (Fig. 1*B*, and Table S1, Exps. 4–6). Notably, nonspecific B-cell activation was not sufficient to promote clinical EAE: when OTII hosts that had received T_{MOG} and B_{NP} cells were immunized with $MOG_{35–55}$ combined with NP-OVA or with a MOG-NP fusion peptide, a massive stimulation of the B_{NP} cells was evoked, as indicated by a substantial rise in NP-reactive antibodies (Fig. S1*A*). However, despite this strong B_{NP}-cell response, there was no significant influence on the clinical course of EAE (Table S1, Exps. 7 and 8).

To find out more about the nature of the disease-promoting properties of B_{MOG} cells, we used intravital two-photon laser scanning microscopy (2-PM) of the lower thoracic/lumbar spinal cord tissue: i.e., a preferentially affected CNS tissue during MOG-induced EAE in C57BL/6J mice (24). T_{MOG} and B_{MOG} cells expressing green or red fluorescent protein (GFP or RFP), respectively, were recorded from days 8–11 after immunization (p.i.): i.e., shortly before disease onset and during the development of the acute phase of EAE. The $T_{MOG\text{-}GFP}$ cells were clearly visible in the blood stream at days 8–9 p.i. in both groups: i.e., animals that had received $T_{MOG\text{-}GFP}$ cells alone (T-MOG mice) or $T_{MOG\text{-}GFP}$ cells together with $B_{MOG\text{-}RFP}$ cells (T-/B-MOG mice) (Movies S1 and S2). In T-/B-MOG mice, $T_{MOG\text{-}GFP}$ cells rapidly accumulated within the leptomeningeal milieu already at day 9, accompanied by a clear disruption of the blood–brain barrier (BBB) even before the animals showed any clinical symptoms (Fig. 1*C*, and Movie S2). In contrast, in T-MOG mice, only a few extravasated $T_{MOG\text{-}GFP}$ cells were detectable, and massive infiltration of $T_{MOG\text{-}GFP}$ cells and BBB leakage occurred significantly later, starting at day 11 p.i. (Fig. 1*C* and Movie S3). This B_{MOG} cell-mediated acceleration in T-cell infiltration into the leptomeninges and the CNS parenchyma was confirmed and quantified by flow cytometry (Fig. S1*B*). Notably, B_{MOG} cells were not found within the inflammatory CNS lesions (Fig. 1*C* and Fig. S1 *C* and *D*).

B_{MOG} Cells Do Not Influence the Priming and Differentiation of T_{MOG} Cells in Secondary Lymphoid Organs. B_{MOG} cells were able to present MOG antigen to T_{MOG} cells in vitro (Fig. S1*E*), suggesting that the acceleration of T_{MOG} cell entry into the CNS could be due to enhanced T-cell activation in the periphery. We therefore next recorded the activation of $T_{MOG\text{-}GFP}$ cells in the draining lymph nodes (LNs), 10–50 h after immunization. Our 2-PM studies revealed a significant reduction in the velocity of $T_{MOG\text{-}GFP}$ cells, with a minimum velocity being reached 30–40 h p.i (Fig. S2*A*, *Left*). This reduction in velocity was accompanied

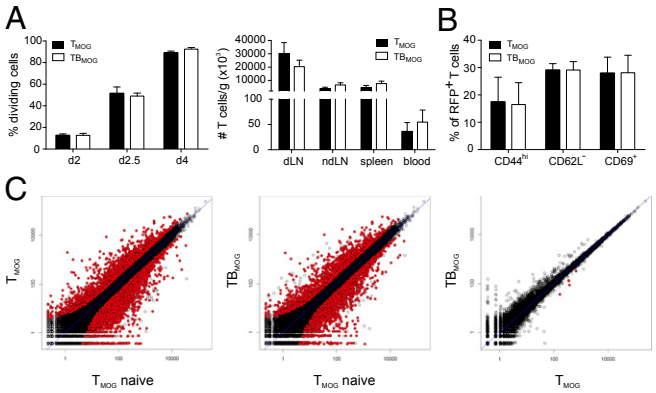

Fig. 2. T_{MOG} cell priming is not changed in the presence of B_{MOG} cells. T_{MOG} cells in the draining LNs were analyzed (*A* and *B*) in the priming phase (day 2–4 p.i.) or (*C*) from the spleen briefly before disease onset at day 9 p.i. (*A*) T_{MOG} cell proliferation in draining LNs of T-/B-MOG (TB_{MOG}) and T-MOG (T_{MOG}) mice. (*Left*) Percentage of dividing cells analyzed by flow cytometry of $T_{MOG\text{-}RFP}$ cells labeled by carboxyfluoresein succinimidyl ester (CFSE) as mean ± SEM. $n = 6$–10. (*Right*) Absolute numbers of $T_{MOG\text{-}RFP}$ cells at day 4 p.i. Mean ± SEM. $n = 5$–10. (*B*) Analysis of activation markers. $T_{MOG\text{-}RFP}$ cells analyzed at day 4 p.i. for the indicated membrane proteins by flow cytometry (% of positive $T_{MOG\text{-}RFP}$ cells). $n = 4$. (*C*) RNAseq transcriptome analyses. Transcriptomes of effector T_{MOG} cells sorted from spleens of T-MOG and T-/B-MOG mice 9 d p.i. were compared with each other and also with nonprimed T_{MOG} cells ($n = 3$). Gene expression levels of effector T cells from T-MOG mice plotted against those of T-/B-MOG mice (*Right*) or naive T_{MOG} cells (*Left*) or T-/B-MOG mice-derived effector T_{MOG} cells vs. naive T_{MOG} cells (*Center*). Differentially expressed genes (red).

by a strong enlargement of T-cell size (Fig. S2 *B* and *C*). At 30 h p.i., T_{MOG}-cell divisions became detectable (Fig. S2*D* and Movie S4). Stable contacts of $T_{MOG\text{-}GFP}$ cells with B cells were observed in the presence of B_{MOG} but not B_{NP} cells (Fig. S2*E*). These T-B_{MOG} pairs were in constant motion, the B_{MOG} cells seemingly dragging their T-cell partners behind them (Fig. S2*E* and Movie S5) (25). Activation of B_{MOG} cells was indicated by an up-regulation of MHC II and CD86 (Fig. S2*F*). Surprisingly, despite these intense T-cell/B-cell interactions, we did not observe any significant differences in T_{MOG}-cell deceleration and enlargement in T-/B-MOG vs. T-MOG or T-MOG/B-NP animals (Fig. S2 *A*, *Right* and *B* and *C*). Moreover, there was no significant change in their proliferation (Fig. 2*A*, Fig. S2*D*, and Fig. S3 *A* and *B*) or in the expression levels of activation markers (CD44, CD69, and CD62L) or proinflammatory cytokines (IFNγ, IL-17, and GM-CSF) tested at the peak of activation and immediately before the onset of CNS invasion: i.e., days 4 and 9 p.i., respectively (Fig. 2*B* and Fig. S3 *C*–*E*). There was also no difference in the expression levels of Bcl-6, CXCR5, ICOS, Il-21, and PD-1, indicating that the T_{FH} differentiation of T_{MOG} cells was not changed in the presence of B_{MOG} cells (Fig. S3*F*). A quantitative deep sequencing analysis was performed on the transcriptomes of T_{MOG} cells from T-MOG and T-/B-MOG mice in comparison with naive T_{MOG} cells. T_{MOG} cells from T-MOG and T-/B-MOG mice had virtually identical differentiation programs, chemokine and cytokine profiles, cell locomotion genes, and activation levels. As expected, the profiles of the activated effector T_{MOG} cells were completely distinct from naive T_{MOG} cells before transfer (Fig. 2*C* and Fig. S4). The data up to this point indicated that B_{MOG} cells did not enter the CNS lesions nor did they change the initial T_{MOG}-cell activation and differentiation.

MOG-Specific Antibodies Trigger the Initiation of Clinical EAE. We next addressed the possibility that B_{MOG} cells initiate and aggravate EAE by their ability to produce MOG-specific autoantibodies (MOG AAbs) that are indeed detectable by days 4–5 p.i. in T-/B-MOG mice (Fig. S5*A*). Interestingly, B_{MOG} cells deficient for the transcription factor X-box binding protein 1 (XBP-1), which is required for the differentiation of B cells into antibody-producing plasma cells (26), lost the capacity to accelerate EAE (Table S1, Exps. 9 and 10). XBP-1–deficient B_{MOG} cells retained their antigen presentation capacity (Fig. S5*B*) but failed to produce significant amounts of MOG AAbs (Fig. S5*C*) (26).

To directly test the disease-promoting potential of MOG AAbs, we i.v. injected sera from preimmunized MOG-BCR or NP-BCR knock-in mice into immunized recipient animals. In fact, the serum containing MOG-antibody but not NP-antibody or serum obtained from T-/B-MOG-XBP-1^{deficient} mice significantly accelerated disease onset (Table S2, Exps. 1–3). Very similar findings were obtained when a purified monoclonal anti-MOG antibody (MOG mAAb; 8.18-C5) (27) was transferred instead of the serum (Fig. 3*A* and Table S2, Exp. 4). Interestingly, a late infusion of the serum containing MOG AAb [i.e., after peripheral T_{MOG} cell priming (day 8 p.i.)] exerted disease-triggering effects identical to those of early infusion (day 5 p.i.), suggesting that the AAbs acted in the CNS rather than in the periphery. Leading from these results, we injected serum or the purified MOG mAAb intrathecally (i.t.) 8 d p.i.: the AAb application fully reproduced the clinical findings (Table S2, Exps. 5 and 6). Similar disease-accelerating effects were seen when the MOG mAAb was transferred i.v. or i.t. during transfer EAE: i.e., in animals that had received fully primed and differentiated pathogenic T_{MOG} effector cells for disease induction (Table S2, Exps. 7 and 8). Not surprisingly, transfer of effector T cells into MOG-BCR knock-in mice that constitutively display high MOG AAb titers also resulted in an earlier and aggravated disease course (Table S2, Exp. 9). Importantly, human anti-MOG AAb-containing immunoglobulins retrieved from a patient suffering from an acute autoimmune CNS attack fully reproduced the disease-accelerating effects whereas irrelevant human immunoglobulins did not show any influence on the disease course or severity (Fig. 3*B* and Table S2, Exp. 10).

The disease-promoting effects of MOG AAbs were not species-restricted. In Lewis rat transfer EAE induced by MOG-reactive T cells (T_{MOG} cells) that are known to have a low pathogenic potential (28, 29), the i.t. infusion of MOG mAAbs resulted in a strong acceleration and aggravation of clinical EAE (Fig. 3*C* and Table S2, Exp. 11).

Enhancement of Antigen Presentation of CNS-Derived APC by MOG AAbs and Subsequent Activation of T_{MOG} Cells. What are the mechanisms by which the MOG AAbs trigger these clinical effects? In the acute phase of EAE, MOG AAbs were reported to mediate myelin destruction (18, 19). However, in our mouse or rat models, we did not find any indication of disease-relevant MOG-AAb–induced demyelination (Fig. S5 *D*–*F*). Our findings in EAE induced by transfer of T cells reacting against the astrocytic antigen S100β were in line with these observations. $T_{S100\beta}$ cells, similarly to T_{MOG} cells, have a low pathogenicity in

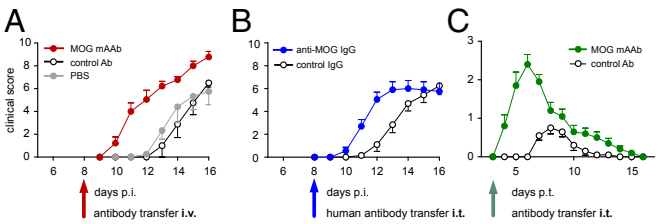

Fig. 3. MOG AAbs mediate the disease-promoting effects of B cells. (*A*) Active EAE in C57BL/6J mice after i.v. injection of MOG mAAb or of an isotype control antibody (control Ab). (*B*) Active EAE in C57BL/6J mice after i.t. injection of human IgG containing MOG AAb or control human IgG. (*C*) Effects of i.t.-applied MOG mAAb in $T_{MOG\text{-}GFP}$ cell-induced transfer EAE of Lewis rats. Clinical scores are shown. Arrows, time of antibody injection. Data are presented as mean ± SEM. $n = 4$–16 pooled from two or three independent experiments.

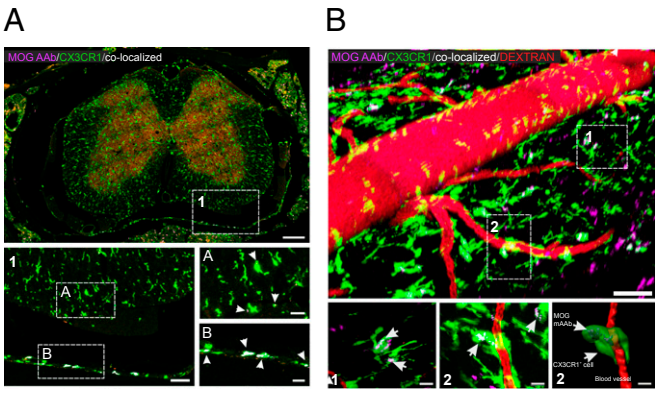

Fig. 4. Binding of MOG mAAb to CNS macrophages/microglia. (*A*) Confocal analysis of a spinal cord section of MOG-immunized CX3CR1-EGFP mice at day 8 p.i. SeTau-647–labeled MOG mAAb had been injected i.v. on day 7. Arrows indicate colocalization of CX3CR1-EGFP$^+$ cells with MOG mAAb (colocalization appears in white). Note that the colocalization signal is more pronounced with meningeal macrophages than with microglia. Green, CX3CR1$^+$ cells; red, T_{MOG} cells; magenta, SeTau-647–labeled MOG antibody. [Scale bars: overview picture (*Top*), 200 μm; higher magnification (*1*), 50 μm; and *Insets A* and *B*, 20 μm. (*B*) Intravital 2-PM imaging of naive CX3CR1-EGFP mice 24 h after i.v. injection of MOG mAAb. (Scale bars: overview, 70 μm; scale bar zoom-ins, 15 μm.) Shown is 70-kDa Texas Red dextran (red) for visualization of blood vessels. Green, CX3CR1$^+$ macrophages and microglia; magenta, SeTau-647–labeled MOG mAAb. Arrows indicate colocalization of the MOG mAAb with macrophages or microglia (white). (*Bottom Right*) A 3D reconstruction of the cell in region 2 using Imaris software.

Lewis rats due to suboptimal reactivation within the CNS (28). The i.t. infusion of MOG mAAb did not influence the clinical course or severity of S100β-EAE whereas a boosting of local $T_{S100β}$-cell reactivation by i.t. injection of S100β antigen clearly aggravated the disease (Table S3, Exp. 1). These data indicate that the disease-enhancing effect of the MOG mAAb depended on the matching of the CNS antigen between the respective antibody and disease-inducing effector T-cell specificity. Accordingly, Abs directed against a neuronal antigen (i.e., beta amyloid) did not impact on the clinic of MOG-EAE or S100β-EAE in mice or rats (Table S3, Exps. 1, 2, and 4).

MOG AAbs became detectable in the cerebrospinal fluid (CSF) with the first occurrence of T cells in the leptomeningeal milieu (Fig. S5*G*). When analyzing the distribution of a fluorescently labeled MOG-AAb, we found that, during this initial phase of CNS inflammation, the antibody did not bind at myelin structures but accumulated in leptomeningeal macrophages and adjacent parenchymal microglia (Fig. 4, Fig. S5*H*, and Movie S6). These cells were recently shown to be the first antigen-presenting cells (APCs) for myelin-reactive T cells during EAE (30–33). Therefore, we hypothesized that MOG-AAbs enhance T_{MOG}-cell activation by virtue of their high affinity binding to cognate antigens, that concentrates even minute amounts of MOG to CNS APCs and thus facilitates MOG antigen presentation in the nervous tissues. We indeed found that in vitro cultured microglia presented myelin antigen to T_{MOG} cells significantly more efficiently in the presence of MOG mAAb (Fig. 5*A*) and that ex vivo-isolated CNS-resident phagocytes from immunized MOG mAAb-treated mice stimulated effector T_{MOG} cells more efficiently than their counterparts from control Ab-treated mice (Fig. 5*B*). Furthermore, effector T cells in early EAE CNS lesions of animals treated with MOG mAAbs produced higher levels of proinflammatory cytokines than in controls (Fig. 5*C*), which became particularly clear in $T_{MOG\text{-}GFP}$ cell-induced EAE of Lewis rats. The i.t. infusion of MOG mAAb induced a strong increase of the proinflammatory cytokine levels of the $T_{MOG\text{-}GFP}$ cells within the CNS (Fig. 5*D* and Fig. S6 *A* and *B*). Consecutively, the numbers of $T_{MOG\text{-}GFP}$ cells recruited into the CNS were massively increased (Fig. 5*E* and Fig. S6*C*), and the clinical disease was accelerated and aggravated (Fig. 3*C* and Table S2, Exp. 11). These clinical effects were accompanied by a strongly boosted inflammatory milieu in the nervous tissues, with a significant recruitment of immune cells, which resembled what was observed after EAE induction with highly pathogenic AAb-independent effector T cells (Fig. S6 *D–F*) (28). There is the possibility that immune complexes could unspecifically stimulate CNS-resident APCs to increase their antigen presentation capacity or enhance their release of proinflammatory factors, such as chemokines. However, i.t. application of a CNS-irrelevant immune complex consisting of NP-OVA complexed with anti-NP Abs could not reproduce the disease-enhancing effects of MOG AAb in mouse or rat EAE (Table S3, Exps. 1, 3, and 5). Furthermore, the antigen presentation capacity of microglial cells did not change in the presence or absence of NP-OVA–anti-NP Ab complexes (Fig. 5*A*).

Discussion

Our data demonstrate that autoantigen-specific B cells have the potential to substantially contribute to the pathogenesis of CNS autoimmune disease. They not only accelerate disease onset and aggravate established clinical disease, but they also precipitate manifest disease in the situation of subclinical inflammation. These clinical effects could not be explained by any influence of MOG-specific B cells on T-cell proliferation, activation, or cytokine production during activation in the periphery as has been postulated earlier (14–16). However, it should be kept in mind that, in our model, limited numbers of MOG-reactive T and B cells were

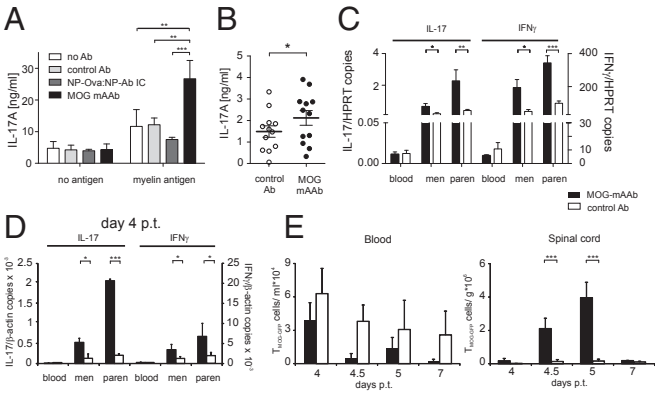

Fig. 5. Enhanced antigen presentation of CNS-derived APCs in the presence of MOG AAb. (*A*) MOG mAAbs enhance antigen presentation capacity of microglia. IL-17A release of effector T_{MOG} cells cocultured with microglia in the absence or presence of MOG mAAb, isotype control antibody, or irrelevant NP-Ova:NP-Ab immune complex (IC) without exogenous antigen or with addition of myelin particles. Mean ± SEM of ELISA data. n = 4–6. (*B*) MOG mAAbs enhance antigen presentation of CNS APCs. IL-17A release of TH17 effector T_{MOG} cells cocultured with leptomeningeal APCs isolated from immunized C57BL/6J mice treated i.t. with MOG mAAb or isotype control antibody (control Ab). Mean ± SEM of ELISA data. n = 12. No exogenous antigen was added to the tissue culture. (*C*) MOG mAAbs enhance T-cell activation in the CNS in mouse EAE. Cytokine production of T_{MOG} cells after i.v. injection of MOG mAAb or control Ab during mouse transfer EAE. T_{MOG} cells were sorted from the indicated compartments shortly before disease onset (weight loss or minimal score). Quantitative PCR data presented as mean ± SEM. House-keeping gene: HPRT. n = 5–13 from 4 different experiments. (*D*) MOG mAAbs enhance T-cell activation in the CNS in rat EAE. $T_{MOG\text{-}GFP}$ cells analyzed within EAE lesions after i.t. treatment (at day 3 p.t.) with MOG mAAb or isotype control Ab. Quantitative PCR data of $T_{MOG\text{-}GFP}$ cells sorted from the indicated compartments at day 4 p.t. Mean ± SEM of 3 animals per group from 2 independent experiments. House-keeping gene: β-actin. (*E*) $T_{MOG\text{-}GFP}$ cells invade the CNS of MOG mAAb treated animals more efficiently. Absolute numbers of $T_{MOG\text{-}GFP}$ cells at the indicated time points after transfer in blood and spinal cord parenchyma measured by flow cytometry. n = 4 per group from two independent experiments.

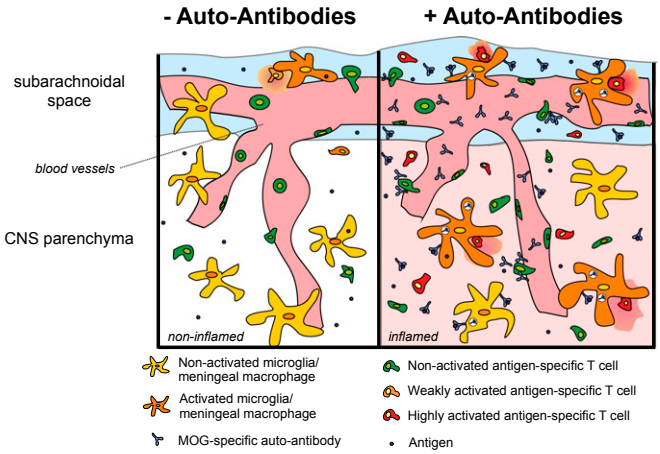

Fig. 6. Schematic depiction summarizing how MOG AAbs enhance autoimmune CNS disease. (*Left*) In the absence of AAbs, autoantigen-specific effector T cells scan the endothelial cells of the BBB. Few cells penetrate the BBB and are suboptimally reactivated by local APCs (microglia/meningeal macrophages). The reactivated T cells initiate a cascade of inflammatory events, which might eventually lead to autoimmune disease development. (*Right*) When AAbs are present in the blood circulation, they pass through the BBB along with the autoantigen-specific T cells and locally bind to their specific antigen. The antibody complexes are taken up by resident macrophages/microglia that then efficiently present the myelin antigen to the autoantigen-specific effector T cells. The enhanced reactivation of the effector T cells leads to strongly increased inflammation of the CNS parenchyma and accelerates and aggravates the clinical disease.

inserted in otherwise intact immune repertoires whereas most of the former studies were performed in MOG-TCR and -BCR expressing animals: i.e., where the majority of cells of the immune cell repertoires are directed against the myelin autoantigen. Despite the limited MOG-specific B-/T-cell numbers, the B-cell activation process in our model remained completely intact: i.e., B_{MOG} cells interacted with MOG-specific T cells in the draining lymph nodes and efficiently produced autoantibodies. This AAb production was found to be necessary and sufficient for the observed disease-accelerating effects of the B cells, supporting previous findings about a relevant role for AAbs in EAE pathogenesis (34–36). According to the common view, during CNS autoimmunity, the adaptive immune system attacks the brain in two hits that are driven by independent T-cell and B-cell effector mechanisms (4, 18). Thereby autoreactive T cells breach the BBB and induce the recruitment of tissue-damaging immune cells and factors. Autoantibodies, via their binding and complement-attraction capacity, can compromise the function of oligodendrocytes (37) and target a cytotoxic attack to the myelin structures, leading to extensive structural damage (18, 19, 38). Our data do not contradict this concept in general. However, in our experimental setting, which is characterized by low amounts of the provided autoantibody in contrast to previous studies (18, 19, 39), we did not find evidence for increased demyelination after autoantibody application. Mechanisms different from demyelination, such as effector cell maturation or trafficking, were postulated in the past (34), but we did not find evidence for these proposed scenarios either. Instead, our data point to a rather different effector mechanism (Fig. 6). Antibodies can foster T-cell activation by efficiently targeting and concentrating antigen to presentation-competent cells (40, 41), which should be particularly relevant in situations where (*i*) access of immune cells to the target organ is limited, (*ii*) antigen is sparsely available for presentation to T cells due to the low turnover of myelin proteins (42), and (*iii*) the conditions for presentation are suboptimal: i.e., expression of MHC and costimulatory molecules is low and no or few professional APCs are present. This constellation is clearly present during the initiation phase of autoimmunity in the highly specialized immune-deprived CNS tissue (3, 32, 43). Therefore, myelin-specific AAbs that penetrate the CSF and the meninges with the arriving effector T cells can efficiently bind to myelin proteins and accumulate in local APCs (44), thus increasing the amount of presented myelin antigens and enhancing the myelin-antigen presentation capacity of the resident phagocytes. These processes will lower the threshold for disease-relevant T-cell reactivation (Fig. 6). In fact, we recently found that increasing the amounts of presentable antigen in the CNS (e.g., by an i.t. injection of myelin antigen) boosts T-cell reactivation and disease severity (28, 32, 43). A similar function of AAbs was recently postulated in a cancer model, where naturally occurring AAbs enhanced tumor antigen presentation by dendritic cells and subsequent T-cell activation (45). "Off-target" effects of autoantibodies were also observed in a model for systemic lupus erythematosus. There, immune complexes formed by AAbs derived from systemic lupus erythematosus patients were elegantly shown to promote the migration of dendritic cells into LNs and thereby enable efficient autoantigen-specific T-cell responses within the immune specialized LN environment (46). Our data, however, indicate that immune complexes unrelated to CNS-antigens are alone not sufficient to substantially accelerate or enhance CNS autoimmune disease. In our study, human anti-MOG AAbs fully reproduced the clinical effects of their murine counterparts. These findings indicate that elevated autoantibody titers might heighten the risk for developing clinically manifested autoimmune CNS disease or subsequent disease bouts.

In summary, our data demonstrate that consecutive adaptive immune mechanisms dovetail into evoking CNS autoimmune disease. In addition to the T-/B-cell interaction in the periphery, crucial for B cells to form a sustained and high affinity antibody response, there is a second cooperative step in the early effector phase within the target organ: this time between T cells and the specific soluble products of B cells. The myelin-specific autoantibodies act indirectly by trimming resident APCs to efficiently present CNS autoantigen to the effector T cells and thereby help to initiate an efficient, though destructive, T-cell response.

Materials and Methods

For a more detailed discussion of the materials and methods, see *SI Materials and Methods*. All animal experiments were approved by the Lower Saxony state authority LAVES (Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit). The use of the patient's material was approved by the Ethic Committee of the Hospital Clinic of Barcelona, and written consent was obtained.

Immune sera. For the generation of immune sera containing high-titer antibodies against MOG or NP, MOG- or NP-specific BCR knock-in mice were immunized with 25 μg of MOG or NP-Ova, respectively, in complete Freund's adjuvant (CFA), and 2× pertussis toxin (PTX) was given i.p. on day 0 (d0) and d2. Serum was collected 2 wk after immunization. For serum transfer experiments, 100 μL of serum was injected i.v. at either day 5 or day 8 p.i., or, alternatively, 10 μL of serum was injected i.t. into the cisterna magna using a stereotactic device (Narishege) at day 8 p.i.

mAAb. MOG mAAb was purified from supernatant of the hybridoma cell line 8.18-C5 using HiTrap Protein G HP columns (GE Healthcare) following the manufacturer's protocol. Afterward, the eluted antibody was desalted using PD-10 Desalting Columns (GE Healthcare) and collected in PBS. For experiments using active EAE in mice, MOG mAAb or IgG1 isotype control (clone MOPC-21; BioXcell) was injected into C57BL/6J hosts at day 8 p.i. either i.v. (50 μg) or i.t. (10 μg). For transfer EAE, MOG mAAb or control Ab was injected i.v. at d2 posttransfer (p.t.) or i.t. at d3 posttransfer. mAAb against beta amyloid (amino acids 1–40) was obtained from antibodies-online GmbH. In rats, 30 μg of each mAAb was injected i.t.

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