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Myelin oligodendrocyte glycoprotein-specific antibodies from multiple sclerosis patients exacerbate disease in a humanized mouse model

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ABSTRACT

Myelin oligodendrocyte glycoprotein (MOG) is exposed on the outer surface of the myelin sheath, and as such, represents a possible target antigen for antibodies in multiple sclerosis (MS) and other demyelinating diseases. However, despite extensive analyses, whether MOG-specific antibodies contribute to pathogenesis in human MS remains an area of uncertainty. In the current study we demonstrate that antibodies derived from adult MS patients exacerbate experimental autoimmune encephalomyelitis (EAE) in 'humanized' mice that transgenically express human FcγRs (hFcγRs). Importantly, this exacerbation is dependent on MOG recognition by the human-derived antibodies. The use of mice that express hFcγRs has allowed us to also investigate the contribution of these receptors to disease in the absence of confounding effects of cross-species differences. Specifically, by engineering the Fc region of MOG-specific antibodies to modulate FcγR and complement (C1q) binding, we reveal that FcγRs but not complement activation contribute to EAE pathogenesis. Importantly, selective enhancement of the affinities of these antibodies for specific FcγRs reveals that FcγRIIA is more important than FcγRIIIA in mediating disease exacerbation. These studies not only provide definitive evidence for the contribution of MOG-specific antibodies to MS, but also reveal mechanistic insight that could lead to new therapeutic targets.

1. Introduction

Much evidence indicates that autoreactive antibodies contribute to pathogenesis in demyelinating diseases such as multiple sclerosis (MS), motivating the development of efficacious therapies to specifically target the antibody response in this disease [1–4]. Of the antigens that constitute myelin, myelin oligodendrocyte glycoprotein (MOG) has attracted much attention since the extracellular domain of this protein is exposed on the outer lamella of the myelin sheath [5,6], thereby providing a target for antibody-mediated attack. However, despite considerable interest in the analysis of MOG-specific responses [7–11], fundamental questions remain concerning the role of MOG-specific antibodies in pathogenesis. For example, assays to assess MOG-specific antibodies have produced variable results, most likely due to differences in the

Abbreviations: AUC, area under the curve; CDC, complement dependent cytotoxicity; CNS, central nervous system; EAE, experimental autoimmune encephalomyelitis; HC, healthy control; HEL, hen egg lysozyme; MOG, myelin oligodendrocyte glycoprotein; MS, multiple sclerosis; WT, wild type.

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source and configuration of antigen used [12]. Thus, although there is consensus concerning the need for binding to conformational epitopes on MOG for an antibody to participate in disease in rodent models [7,9,13–15], the contribution of antibodies of this specificity to MS is controversial [12]. Addressing this issue is expected to not only provide mechanistic insight into antibody-mediated pathogenesis in MS and other demyelinating diseases, but also to identify diagnostic and therapeutic targets.

By contrast with several other neuroantigens [e.g. myelin basic protein (MBP)] used to induce experimental autoimmune encephalomyelitis (EAE) in rodent models through induction of encephalitogenic T cells in the absence of a humoral response [16–19], MOG-derived peptides and recombinant MOG (extracellular domain) induce T cell and antibody responses [20–22]. Interestingly, however, EAE induced by immunization of C57BL/6 mice with recombinant rodent (rat/mouse) MOG protein (extracellular domain) or rodent MOG peptide residues 35–55 (MOG35-55) is B cell-independent, whereas immunization with recombinant human MOG (hMOG; extracellular domain) induces EAE that is both B and T cell-dependent [20,21,23,24]. Although B cell deficient mice are relatively resistant to hMOG-induced EAE [20,21,23,24], disease can be induced in these mice by immunization with recombinant hMOG to induce a MOG-specific T cell response followed by the transfer of serum from hMOG-immunized wild type mice [15]. By contrast, immunization of the mice with ovalbumin prior to antibody/serum transfer does not result in EAE [15]. Further, the $CD4^+$ T cell response against the immunodominant epitope of hMOG, hMOG35-55, which differs from rodent MOG35-55 by substitution of Ser42 by proline, is significantly less encephalitogenic than that induced by rodent MOG35-55 immunization [20]. In this context, we have also observed exacerbation of EAE in wild type C57BL/6 mice by induction of mild disease through immunization with hMOG35-55 peptide, followed by the delivery of MOG-specific antibodies [25]. Such transfer models therefore provide instructive systems for the investigation of the contribution of MOG-specific antibodies to pathogenesis.

Despite extensive analyses [7–13], the effector functions of MOG-specific antibodies or other neuroantigen-specific antibodies that participate in damaging myelin in EAE or MS remain poorly defined. Complement deposits have been detected in autopsy samples from MS patients, and combined with the observation that most MOG-specific antibodies in patients are of the IgG1 isotype [12], this has led to the suggestion that complement activation contributes to disease. Further, a study demonstrating that MOG-specific antibodies of the (mouse) IgG2a class are more active in EAE-exacerbation than mouse IgG1 antibodies was interpreted to be due to the higher activity of mouse IgG2a in complement dependent cytotoxicity (CDC) [26]. However, both human IgG1 and mouse IgG2a antibodies are also active in FcγR-mediated functions such as antibody-dependent cell-mediated phagocytosis (ADCP) [27–29], although analyses in Fcγ receptor (FcγR)-deficient mice have led to mixed results concerning the role of FcγRs in pathogenesis [30–32]. In this context, the divergence of FcγRs between rodents and man has provided a significant limitation for the translation of studies of FcγR-mediated function in mice to humans [33]. For example, the affinities of IgG subclasses for cognate FcγRs do not show equivalence across species [33–35]. The cross-species differences have motivated the generation of a mouse strain in which the mouse FcγRs have been deleted and human FcγRs expressed as transgenes (hFcγR mice) [34], to result in a suitable model to assess human FcγR activity. These mice have been used in the current study to investigate antibody-mediated pathogenesis in a hMOG35-55 immunization/transfer model of EAE.

We demonstrate that purified polyclonal IgG preparations from patients with MS exacerbate EAE in hFcγR transgenic mice. Importantly, this increase in disease can be ablated by targeted depletion of the MOG-specific antibodies. In combination with the use of Fc engineering to modulate the effector function activity of MOG-specific antibodies, we demonstrate that FcγR-activity, but not CDC, is critical for EAE exacerbation. Collectively, our studies demonstrate that MOG-specific antibodies from MS patients can contribute to pathogenesis in a humanized FcγR mouse model, in addition to providing mechanistic insight into diseases involving neuroantigen-specific autoantibodies.

2. Materials and methods

2.1. Mice

C57BL/6 mice transgenically expressing hFcγRs [34] were generously provided by Drs. Jeffrey V. Ravetch and Patrick Smith (Rockefeller University, NY, USA). Mice were bred in a specific pathogen-free facility at the University of Texas Southwestern Medical Center or Texas A&M University and were handled in compliance with institutional policies and protocols approved by the corresponding Institutional Animal Care and Use Committees. 7–10 week old mice were used in experiments.

2.2. Antibodies

The following antibodies were used in the current study: Brilliant Violet 421 (BV421)-labeled anti-human CD16B (CLB-gran11.5), BV421-labeled mouse IgG2a, κ, isotype control (G155-178), FITC-labeled anti-mouse Ly-6C (AL-21) and PerCP/Cy5.5-labeled anti-mouse CD11b (M1/70) were purchased from Becton-Dickinson. Alexa Fluor 647 (A647)-labeled anti-human CD64 (10.1), A647-labeled anti-human CD16 (3G8), A647-labeled mouse IgG1, κ, isotype control (MOPC-21), BV421-labeled anti-mouse F4/80 (BM8), PE/Cy7-labeled anti-mouse CD11b (M1/70), Alexa Fluor 488-labeled anti-mouse $I\text{-}A^b$ (AF6-120.1), PE-labeled anti-mouse CD86 (GL-1) and PerCP-labeled anti-mouse CD45 (30-F11) were purchased from Biolegend. APC-labeled anti-human CD32A (IV.3) and APC–labeled mouse IgG2b, κ, isotype control (MPC-11) were purchased from Boster Biological Technology. PE-labeled anti-mouse F4/80 (BM8) was purchased from eBioscience. A647-labeled goat anti-mouse IgG2b was purchased from Life Technologies. Anti-human CD32B (X63-21/7.2) [36] was purified using protein G-Sepharose from supernatants of hybridoma cells that were generously provided by Dr. Mark Hogarth (Burnet Institute, Melbourne, Australia).

2.3. Recombinant antibodies and MOG

Chimeric MOG-specific antibodies were generated by fusing the V_H and V_L domain genes from previously described MOG-specific hybridomas (1005 and 1011) [25] with the human IgG1-derived C_H and C_L (Cκ) genes, respectively. The following mutations were introduced into the Fc region of the chimeric heavy chain genes to modulate binding to hFcγRs [37–40] or C1q [41] using standard molecular biology methods: G236A/S239D/I332E (ADE), A330L/I332E (LE), G236A, D265A and K322A. Chimeric heavy and light chain genes were cloned into pcDNA™3.4-TOPO® vector (Life Technologies) for expression. Antibodies were expressed by transiently transfecting Expi293™ cells (Life Technologies) with chimeric heavy and light chain expression constructs using the Expi293™ expression system kit (Life Technologies). Antibodies were purified from culture supernatants using protein G-Sepharose. The purified antibodies were analyzed using an analytical size exclusion chromatography column (Superdex™ 200-15/150 GL, GE Healthcare). Monoclonal MOG-specific mouse IgG1 (8-18C5 [42])

was expressed and purified as described previously [25]. Clinical grade wild type (WT) human IgG1, trastuzumab (Herceptin®), was obtained from the pharmacy at the University of Texas Southwestern Medical Center. D1.3 (monoclonal anti-hen egg lysozyme IgG1, [43]) was purified using lysozyme-Sepharose [44] from hybridoma culture supernatants.

A baculoviral stock for the expression of the extracellular domain (residues 1–121) of hMOG [23,45] was kindly provided by Drs. Anne Cross and Jeri-Anne Lyons (Washington University School of Medicine, MO, USA). Sf9 insect cells (Life Technologies) were infected with hMOG baculoviral stock to generate high-titer virus stocks, which were subsequently used to infect High Five insect cells (Life Technologies) and recombinant protein purified from the culture supernatant using Ni^{2+}-NTA agarose (Qiagen).

2.4. Purification of polyclonal IgG from patient sera

Archived sera from patients diagnosed with MS and healthy controls were obtained from the Neurosciences Biorepository at the University of Texas Southwestern Medical Center (TX, USA) under protocols approved by the Texas A&M University Institutional Review Board (IRB protocol # IRB2015-0083D) and the University of Texas Southwestern Medical Center Institutional Review Board (IRB protocol # 022011-211). 200 μl serum from each patient was diluted into 10 ml PBS (Lonza) and IgG purified using protein G-Sepharose.

2.5. Surface plasmon resonance analyses

Equilibrium dissociation constants for the binding of chimeric antibodies (c1005 and c1011) to recombinant hMOG and mouse MOG (mMOG) were determined using surface plasmon resonance (BIAcore T200; GE Healthcare) and previously described methods [46]. CM5 chips were coupled with recombinant hMOG or mMOG to densities of ~1500 RU followed by injection of antibodies at various concentrations (0.1–800 nM) at a flow rate of 10 μl/min using PBS pH 7.4 (Lonza), 0.01% (v/v) Tween-20 as running buffer. Flow cells were regenerated at the end of each cycle using 0.1 M glycine, 0.15 M NaCl, pH 2.8 buffer. The equilibrium dissociation constants (K_Ds) for the interactions of the antibodies with hMOG/mMOG were determined using a 1:1 interaction model and custom written software [46,47]. The obtained K_D values are apparent, rather than absolute, due to the avidity effects associated with bivalent binding of antibody to immobilized MOG. To analyze the effects of Fc mutations on FcγR binding, CM5 chips were coupled with recombinant hFcγRs (R&D Systems) to densities of ~1000–2500 RU and antibodies were injected at a flow rate of 10 μl/min in PBS pH 7.4 (Lonza), 0.01% (v/v) Tween-20 at a concentration of 1500 nM.

2.6. ELISA

Binding of polyclonal human IgG samples and 8-18C5 (mouse monoclonal MOG-specific antibody [42]) to hMOG was analyzed by ELISA. 96-well plates (Nunc) were coated with recombinant hMOG at a concentration of 10 μg/ml at room temperature for 1 h, followed by addition of antibodies at a concentration of 5 μg/ml. Bound antibodies were detected using anti-human IgG (Fc-specific) or anti-mouse (heavy and light chain-specific) antibody conjugated to HRP (Sigma-Aldrich). The isotypes of the MOG-specific IgGs present in human sera were determined using a human IgG isotyping kit (eBioscience) following a standard protocol with recombinant hMOG-coated 96-well plates (Nunc). MOG-coated plates were incubated with IgG samples at a concentration of 50 μg/ml. Bound MOG-specific antibodies were detected using anti-human isotype specific antibody conjugated to HRP (eBioscience).

2.7. Binding analyses using flow cytometry

The binding of polyclonal IgG from human sera to mMOG, and inhibition of binding of these human antibodies by 8-18C5, was analyzed using a transfected EL4 lymphoma cell line (EL4 cells) expressing full length mMOG on the cell surface (EL4-MOG cells). EL4 cells and EL4-MOG cells were kindly provided by Dr. Gurumoorthy Krishnamoorthy (Max Planck Institute of Neurobiology, Martinsreid, Germany) [14]. EL4 and EL4-MOG cells (1×10^6) were incubated with 25 μg/ml human IgG in the presence or absence of 5-fold excess of 8-18C5 or control IgG (D1.3) on ice. Bound human antibodies were detected using DyLight 650-labeled goat anti-human IgG (10 μg/ml; Abcam). Samples were analyzed by flow cytometry (Accuri; Becton-Dickinson) and data processed using FlowJo software (FlowJo).

2.8. Exacerbation of EAE in hFcγR mice

Female hFcγR mice were immunized subcutaneously with 100 μg (per mouse) hMOG35-55 peptide (CS Bio) emulsified with complete Freund's adjuvant (CFA; Sigma-Aldrich) supplemented with an additional 4 mg/ml heat-inactivated *Mycobacterium tuberculosis* (strain H37RA, Becton-Dickinson) at four sites in the flanks. On days 0 and 2 post-immunization, 200 ng of pertussis toxin was administered intraperitoneally. The mice were monitored for disease progression and scored daily for clinical signs of EAE. On day 15 post-immunization, mice were sorted into groups based on EAE scores on each day prior to and including day 15, resulting in equal numbers of mice with similar disease course in each group using a cost function (implemented in MATLAB). Groups of mice were injected intravenously with either 250 μg polyclonal IgG from human sera or 200 μg chimeric antibodies (c1011 or c1005), their mutated derivatives or isotype control (trastuzumab). Mice were monitored and scored daily until day 30. The scoring system for disease activity was as described previously [48]: 0, no paralysis; 1, limp tail; 2, moderate hind limb weakness; 3, severe hind limb weakness; 4, complete hind limb paralysis; 5, quadriplegia; and 6, death.

To evaluate the activity of MOG-specific antibodies in MS patient-derived IgG in exacerbating EAE, polyclonal IgG isolated from an MS patient (MS-3) serum was depleted of MOG-specific antibodies by incubation with EL4-MOG cells. Briefly, 10^7 EL4-MOG cells were incubated with 1.5 mg polyclonal IgG (MS-3) with rotation for 1 h at 4 °C, followed by pelleting of the cells by centrifugation and harvesting of supernatant. Similarly, EL4 cells were used as a control. The supernatants were evaluated for depletion of hMOG-specific antibodies by ELISA, as described in section 2.6. The disease-exacerbating potential of EL4-MOG-treated and EL4-treated IgG was determined by transferring 250 μg IgG into immunized hFcγR mice, as described above.

2.9. Pharmacokinetic studies

Antibodies were labeled with iodine-125 (^{125}I; Perkin Elmer) as described previously [49]. Male hFcγR mice were fed 0.1% Lugol (Sigma-Aldrich) in water for 72 h before intravenous injection in the tail vein with ^{125}I-labeled chimeric antibodies (10–15 μg per mouse). Whole body radioactivity counts were measured using an Atom Lab 100 dose calibrator (Biodex) at the indicated time points. Radioactive counts were normalized with respect to the value obtained immediately following the injection and the normalized data against time was plotted. The area under each of these curves was calculated using Graphpad prism (Graphpad Software).

2.10. Immunohistochemical analyses of spinal cords

Mice were immunized with hMOG35-55 and on day 15, patient-derived IgG, c1011 or control IgG (human IgG1, trastuzumab) delivered as above. Three days following antibody delivery, the mice were perfused with heparin/PBS, euthanized and spinal cords harvested. Isolated spinal cords were fixed in 10% Neutral Buffered Formalin (NBF) for 24 h and transferred to 70% ethanol. Following tissue processing using standard methods, the samples were embedded in paraffin, sectioned at 4 μm, and stained with a regressive hematoxylin and eosin (H&E) protocol by the histology laboratory in the Department of Veterinary Pathobiology at Texas A&M University. Stained sections were imaged using an Axio Observer microscope (Zeiss) with 10X air-based objective.

2.11. Flow cytometry analyses using hFcγR mice

To analyze the expression of FcγRs on macrophages in hFcγR transgenic mice, spleens were isolated and used to generate single cell suspensions by mechanical disruption and forcing the cells through 70 μm strainers (Becton-Dickinson). Splenic cell suspensions were depleted of erythrocytes using red blood cell lysis buffer and stained with fluorescently-labeled antibodies specific for mouse Ly-6C, F4/80, CD11b, hFcγRI (CD64), hFcγRIIA (CD32A), hFcγRIIIA/B (CD16) and hFcγRIIIB (CD16B). To analyze the expression of hFcγRIIB (CD32B), splenic cell suspensions were initially incubated with unlabeled antibody specific for hFcγRIIB (mouse IgG2b), followed by staining with fluorescently-labeled goat anti-mouse IgG2b and anti-mouse Ly-6C, F4/80 and CD11b antibodies.

Mice were immunized with hMOG35-55 and on day 15, patient-derived IgG or control IgG (human IgG1, trastuzumab) delivered as above. Mice were perfused with heparin/PBS, euthanized and spinal cords isolated 3 days following antibody delivery. Single cell suspensions were generated using the same method as for spleens. Mononuclear cells from spinal cord cell suspensions were obtained using Percoll (1131 g/ml, GE Healthcare) gradients. Briefly, cells were washed with 37% Percoll [2118 g, room temperature, 15 min] and suspended in 30% Percoll which was then layered over 70% Percoll and centrifuged at 2118 g for 15 min at room temperature. Following centrifugation, the cells at the interface were collected, washed and stained with antibodies specific for CD45, CD11b, F4/80, I-A^b, CD86 and hFcγRIIA.

For both spleens and spinal cords, stained cells were analyzed by flow cytometry using a LSRFortessa (Becton-Dickinson). Flow cytometry data were processed using FlowJo software (FlowJo).

2.12. Statistical analyses

For disease data, statistical analyses were carried out using two-way ANOVA and Tukey's multiple comparison test. Statistical analyses for ELISAs to analyze the depletion of anti-MOG IgG and pharmacokinetic data (AUCs) were performed using one-way ANOVA and Tukey's multiple comparison test. Tests of statistical significance for pairwise comparisons were carried out using two-tailed Student's *t*-test. Graphpad prism (Graphpad Software) was used for all the above analyses. *p* values of less than 0.05 were taken to be significant.

3. Results

3.1. Development of an antibody-mediated model of EAE in hFcγR mice

We recently developed an EAE model in which the passive transfer of mouse monoclonal MOG-specific antibody exacerbates disease in C57BL/6 mice immunized with hMOG35-55 [25,50]. In the current study, this passive EAE model was humanized by employing hFcγR mice (C57BL/6 background) [34]. hFcγR mice lack murine FcγRs and express hFcγRI, hFcγRIIA^{R131}, hFcγRIIB^{I232}, hFcγRIIIA^{F158}, and hFcγRIIIB under the control of their endogenous human regulatory elements [34]. Importantly, in these humanized mice FcγR expression patterns recapitulate those reported in humans, and the FcγR-mediated effector functions are normal [34]. However, although the expression patterns of hFcγRs have been characterized for the majority of hematopoietic cell types in these mice [34], macrophages that are known to contribute to inflammation in EAE models [51] were not included in these analyses. We therefore used flow cytometry to investigate the surface expression levels of hFcγRI, hFcγRIIA, hFcγRIIB, hFcγRIIIA/B and hFcγRIIIB. All FcγRs were expressed by both F4/80^{hi}CD11b^{lo} and F4/80^{lo}CD11b^{hi} macrophages, although for F4/80^{lo}CD11b^{hi} cells several sub-populations with different levels of FcγR expression were observed (Fig. S1).

To assess the ability of MOG-specific antibodies to exacerbate EAE in hFcγR mice, two mouse monoclonal MOG-specific antibodies (1005, 1011) described in our earlier study [25] with different affinities for hMOG and mouse MOG (mMOG) were chimerized using the human IgG1-derived constant region. The MOG-specific antibody 1011 competes with the well-characterized mouse antibody, 8-18C5 [42], for binding to mMOG. The interaction of 8-18C5 with MOG has been shown to be dependent on residues 103,104 in the FG loop of this protein, which is exposed at the tip of the immunoglobulin-like domain [9,52]. By contrast, antibody 1005 does not compete with 8-18C5 or 1011 for MOG binding [25], demonstrating that 1005 and 1011 recognize distinct epitopes. The affinities of the chimerized antibodies for binding to hMOG and mMOG were similar to those of their parent mouse antibodies [25], with chimeric 1005 (c1005) exhibiting a ~10- and ~100-fold lower affinity for hMOG and mMOG, respectively, compared with chimeric 1011 (c1011; Supplementary Table 1) [25]. To investigate whether these chimeric MOG-specific antibodies could exacerbate EAE, hFcγR mice were immunized with hMOG35-55 and 15 days later, c1011, c1005 or human IgG1 control (Trastuzumab; human HER2-specific) were delivered intravenously. The delivery of c1011 or c1005 exacerbated the disease, demonstrating the pathogenicity of these chimeric antibodies in hFcγR mice (Fig. 1A and B).

3.2. IgG in MS patient sera, but not healthy control sera, recognize hMOG and mMOG

To extend our studies to MOG-specific antibodies isolated from MS patients, we next analyzed the presence of hMOG-specific IgG in purified polyclonal IgG preparations from ten MS patients (age range: 23-54 years) and 2 healthy controls (aged 25 and 40 years). The use of protein G-Sepharose to isolate these IgG samples circumvents the possibility of false positives in binding assays due to non-specific binding of anti-IgG secondary/detection antibody to IgM [53], since protein G binds specifically to human IgG, but not to other immunoglobulin classes. Of these ten MS patients, seven (MS-1 to MS-7) responded to plasmapheresis and three (MS-8 to MS-10) had not undergone this treatment (Supplementary Table 2). Compared with IgG isolated from healthy controls (HC-1 and HC-2), IgG from all of the MS patients showed significant binding to hMOG (Fig. 2A), although hMOG-specific IgG signals varied amongst different patients. These differences could be due to variations in specific IgG levels and/or their affinity for binding to hMOG. Isotype analyses of the MOG-specific IgGs demonstrated that IgG1 represented the predominant or the only isotype amongst hMOG-reactive IgGs (Fig. 2B). For eight patients, levels of hMOG-specific

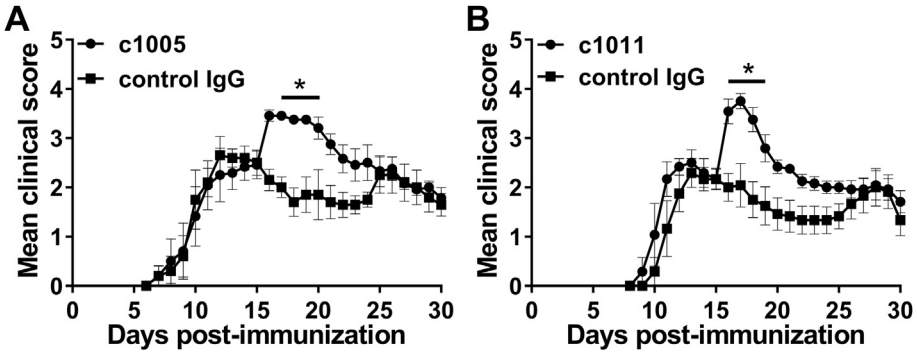

Fig. 1. Chimeric MOG-specific antibodies exacerbate EAE in hFcγR mice. (A, B) hFcγR mice (n = 5–6 mice/group) were immunized with 100 μg hMOG35-55 and treated with 200 ng pertussis toxin on days 0 and 2. On day 15, mice were sorted into equivalent groups (mean disease score of ~2) and intravenously injected with 200 μg c1005 (A), c1011 (B) or isotype control, human IgG1 (trastuzumab). Mice were scored daily for disease activity. Error bars indicate SEM and significant differences ($p < 0.05$; two-way ANOVA and Tukey's multiple comparison test) between the groups are indicated by *. Data are representative of two independent experiments.

IgG4 above background were found, whereas one patient sample (MS-4) contained levels of hMOG-reactive IgG3 that were higher than the other isotypes (Fig. 2B).

We next investigated whether the patient-derived IgG samples cross-reacted with mMOG. Conformation-dependent antibodies that recognize 'native' MOG expressed on the surface of mammalian cells have been shown to be relevant to demyelinating activity [7,9,13–15]. The binding of the IgG samples to mMOG on the surface of mMOG-transfected EL4 lymphoma cells (EL4-MOG) [14] was therefore assessed using flow cytometry. Significantly, all of the patient IgG samples, but not healthy control IgG samples, bound specifically to EL4-MOG cells (Fig. 2C and D). We also investigated whether the binding of the human IgGs overlapped with that of the mMOG-specific monoclonal antibody, 8-18C5 [42]. Consistent with an earlier report using hMOG-expressing glioma cells [13], substantial inhibition of binding to EL4-MOG cells by 8-18C5 was observed for 8 of 10 patients (Fig. 2D). IgG derived from patient MS-1 showed no significant inhibition by 8-18C5, whereas MS-2 IgG showed less inhibition compared with the other eight patient-derived IgG samples.

3.3. MOG-specific IgG in MS patient sera exacerbates disease in hMOG35-55-immunized hFcγR mice

Having established that IgGs derived from MS patients recognize both hMOG and mMOG, the ability of IgG samples derived from two patients (MS-3 and MS-6) to exacerbate EAE was analyzed by transferring 250 μg polyclonal IgG into hMOG35-55-immunized hFcγR mice on day 15 post-immunization. Polyclonal IgG from patients MS-3 and MS-6 were used in these studies due to their relatively high reactivity to both hMOG and mMOG (Fig. 2A and D). Importantly, IgG isolates from these patients had significantly higher activity in exacerbating disease compared with the effects of healthy control (HC-1) IgG (Fig. 3A), with effects that are comparable to those induced by encephalitogenic chimeric antibodies, c1005 and c1011 (Fig. 1). By comparison with treatment with the monoclonal HER2-specific antibody, trastuzumab, delivery of HC-1 IgG resulted in slight exacerbation of the disease (Fig. 3A). This could be due to the very low levels of mMOG-specific antibodies observed in binding assays (Fig. 2D) and/or the presence of IgGs that recognize other mouse central nervous system (CNS) antigens.

Although the above studies indicate that MS patient-derived IgG samples recognize mMOG and hMOG and exacerbate disease in a mouse EAE model, they do not directly show that amongst the injected polyclonal IgG samples, MOG-specific antibodies contribute to this exacerbation. Therefore, to evaluate the role of MOG-specific IgG in disease exacerbation, the antigen-specific IgG was depleted from the polyclonal IgG sample (MS-3) by incubation with EL4-MOG cells. The efficiency of MOG-specific antibody depletion was determined by measuring the binding of these cell-treated samples to hMOG using ELISA. Following treatment with EL4-MOG cells, IgG derived from MS-3 exhibited close to background levels of binding to hMOG which was similar to that observed with IgG from a healthy control, HC-1 (Fig. 3B). By contrast, incubation of MS-3 IgG with EL4 cells resulted in only a slight reduction in MOG binding by comparison with untreated MS-3 IgG (Fig. 3B), demonstrating specific depletion of MOG-specific antibodies by EL4-MOG cells. 250 μg EL4-MOG-treated MS-3-derived IgG was delivered into hMOG35-55-immunized hFcγR mice on day 15 post-immunization. Control groups of mice were injected with 250 μg untreated MS-3 IgG, EL4-treated MS-3 IgG or healthy control (HC-1) IgG. Importantly, the delivery of EL4-MOG-treated IgGs did not exacerbate EAE and the disease in this group was similar to that observed in mice administered with healthy control IgG (Fig. 3C). In addition, the disease exacerbation induced by EL4-treated MS-3 IgG was comparable to the effect of untreated MS-3 IgG (Fig. 3C). These observations indicate that MOG-specific IgG in the patient serum is responsible for disease exacerbation. In addition, consistent with the increased disease activity, immunohistochemical analyses of spinal cords demonstrated higher levels of inflammatory infiltrates following the delivery of MS-3 IgG (or c1011) compared with those for control IgG (Fig. 4).

3.4. Exacerbation of EAE by chimeric anti-MOG antibodies requires Fc-FcγR interactions

To delineate the role of FcγRs in antibody-mediated disease exacerbation, variants of c1011 and c1005 with substantially reduced binding to hFcγRs were generated by mutating Asp265 in the Fc region to alanine (D265A; Table 1) [40,54]. By contrast with wild type (WT) chimeric antibodies ($c1011^{WT}$ and $c1005^{WT}$), D265A variants of c1011 and c1005 ($c1011^{D265A}$ and $c1005^{D265A}$, respectively) did not exacerbate disease in hMOG35-55-immunized hFcγR mice (Fig. 5A and B), indicating a critical role for FcγRs in the pathophysiology of antibody-induced EAE exacerbation. However, the D265A mutation also reduces binding to complement C1q [54] and it was therefore also important to exclude the possibility that complement activation via the classical pathway is involved in EAE exacerbation. The K322A mutation, which is known to reduce binding to mouse C1q and ablate CDC towards target cells in the presence of mouse serum [55,56], was therefore inserted into the Fc

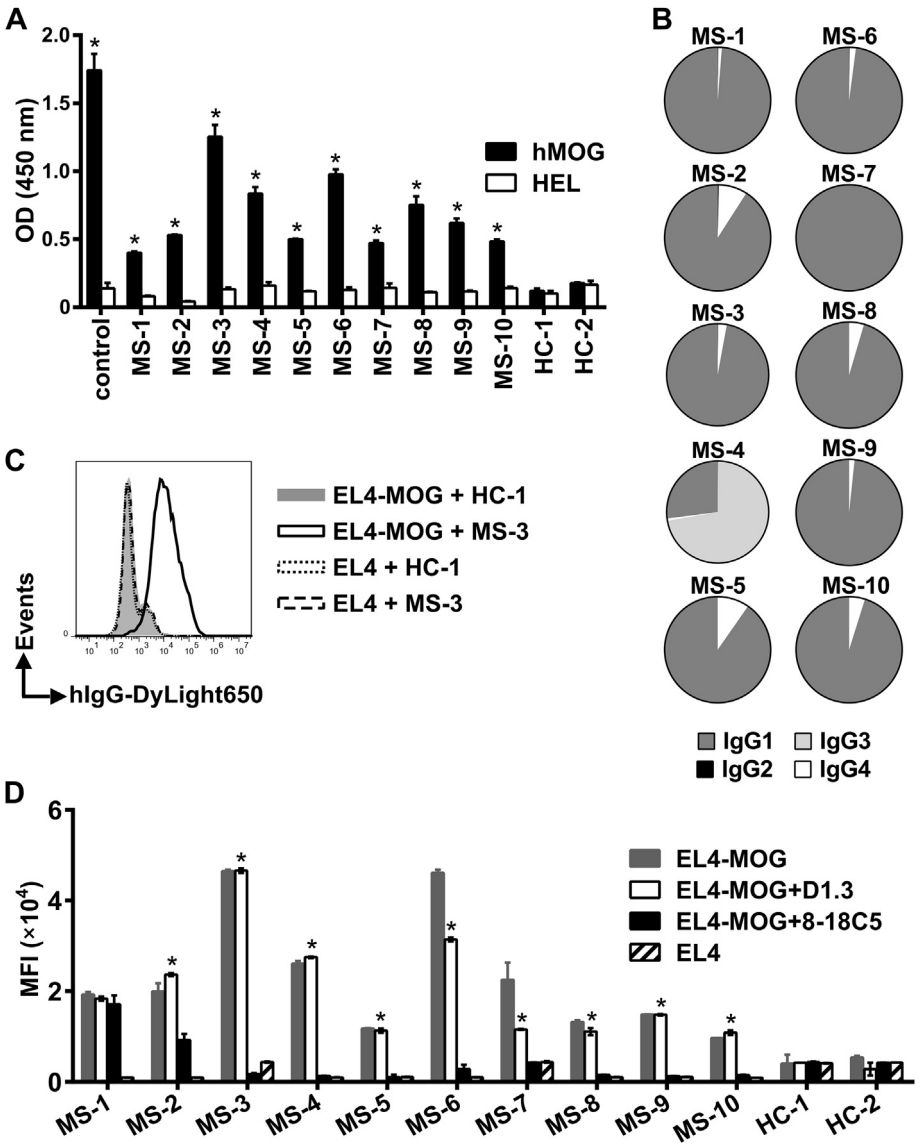

Fig. 2. MS patient-derived polyclonal IgG isolates recognize native MOG and are primarily of the IgG1 isotype. (A) Binding of MS patient- and HC-derived IgG to recombinant hMOG and an irrelevant antigen (hen egg lysozyme, HEL) was determined by direct ELISA. The mouse (IgG1) monoclonal MOG-specific antibody, 8−18C5, was used as a control. (B) Pie charts showing the relative levels of hMOG-specific IgG1, IgG2, IgG3 and IgG4 antibodies in polyclonal IgG isolated from MS patient sera, determined by direct ELISA. (C, D) EL4 cells or EL4-MOG (transfected to express full-length mMOG) cells were incubated with polyclonal IgG from MS patients or HCs and analyzed by flow cytometry. Binding of the human antibodies to the cells was detected using DyLight 650−labeled anti-human IgG (H + L) conjugate. (C) Representative histogram plot showing flow cytometry analyses for EL4 and EL4-MOG cells treated with MS-3 and HC-1 IgG. (D) Binding analyses of patient- or HC-derived IgG to EL4 or EL4-MOG cells in the presence or absence of a 5-fold excess of 8-18C5 or control mouse IgG1 (D1.3, mouse monoclonal HEL-specific antibody). Averages of mean fluorescence intensities (MFIs) are shown for triplicate samples with error bars indicating SEM. Significant differences ($p < 0.05$; two-tailed Student's *t*-test) between the binding to hMOG and HEL (A) and between the co-incubations with 8-18C5 and D1.3 (D) for each IgG sample are indicated by *. Data are representative of two independent experiments.

region of c1011 (c1011^{K322A}; Table 1). Importantly, c1011^{K322A} was as effective as its wild type parent antibody in exacerbating EAE (Fig. 5C).

hFcγRs include both activating (FcγRI, FcγRIIA and FcγRIIIA/B) and inhibitory (FcγRIIB) receptors and among the activating receptors, FcγRIIA and FcγRIIIA play an important role in IgG-mediated effector functions [35,57]. An increase in the ratio of activating FcγR binding affinity to inhibitory FcγR binding affinity (A/I ratio) of an IgG molecule translates into increased FcγR-dependent effector function [27]. One set of hIgG1 mutations, ADE (G236A/S239D/I332E; Table 1), leads to an increase in the A/I ratio from 2.5 (hFcγRIIA/hFcγRIIB) and 2.3 (hFcγRIIIA/hFcγRIIB) to 13 and 5.3, respectively [37]. ADE variants of c1005 and c1011 (c1005^{ADE} and c1011^{ADE}, respectively) were generated and administered to hMOG35-55-immunized hFcγR mice. Significantly, disease exacerbation following the delivery of c1005^{ADE} and c1011^{ADE} was greater than that induced by the corresponding WT chimeric antibodies (Fig. 5D and E), confirming an important role for FcγRIIA- and/or FcγRIIIA-dependent effector functions in antibody-mediated disease.

We next dissected the relative importance of hFcγRIIA and hFcγRIIIA in disease exacerbation induced by the chimeric antibodies by generating additional c1011 mutants harboring G236A [37] (c1011^{G236A}) or A330L/I332E [38] (c1011^{LE}). G236A and LE mutations have been shown to selectively increase the affinity of hIgG1 towards hFcγRIIA (9.3-fold) and hFcγRIIIA (5.4-fold), respectively (Table 1) [38]. Following transfer into hMOG35-55-immunized hFcγR mice, c1011^{G236A} induced greater exacerbation

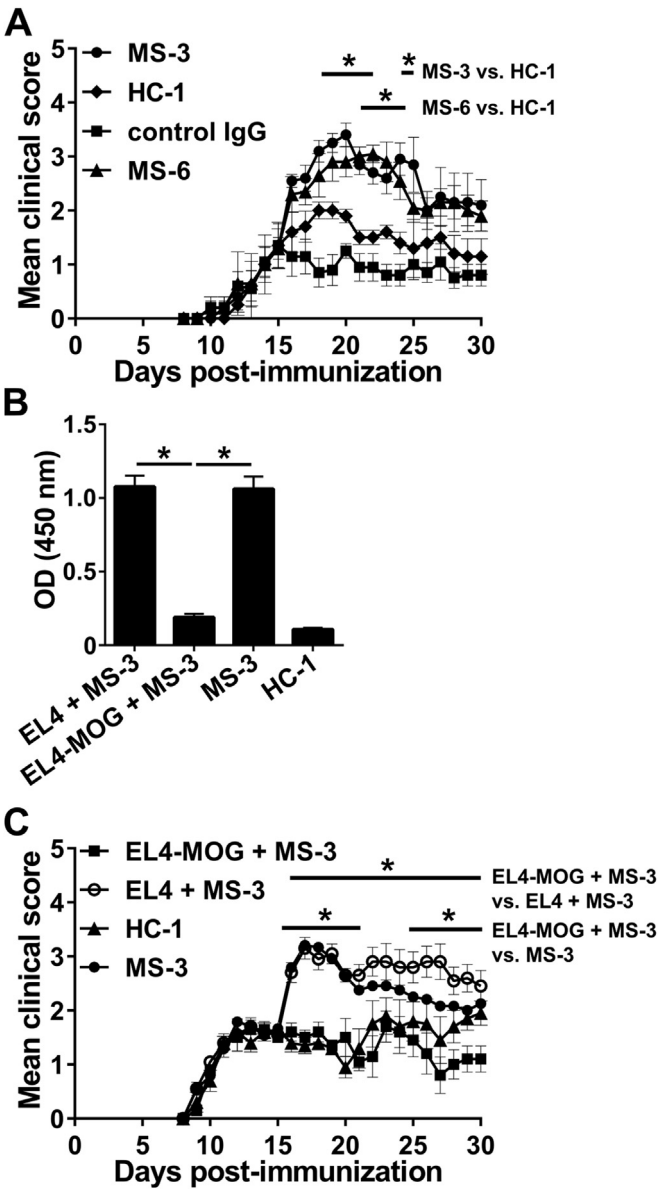

Fig. 3. Polyclonal MOG-specific IgG from MS patients exacerbates EAE in hFcγR mice. (A) hFcγR mice (n = 5–6 mice/group) were immunized as in Fig. 1. 15 days post-immunization, mice were sorted into equivalent groups (mean disease score of ~1) and intravenously injected with 250 μg IgG purified from the serum of MS patients MS-3 or MS-6 or from a healthy control (HC-1). Control mice were injected with trastuzumab. Mice were scored daily for disease activity. (B) MS patient (MS-3)-derived IgG was incubated with EL4 or EL4-MOG cells at 4 °C. The binding of the IgG recovered following incubation with cells to recombinant hMOG was determined by direct ELISA. (C) hFcγR mice (n = 5–6 mice/group) were immunized as in panel A and 250 μg untreated, EL4 or EL4-MOG-treated IgG intravenously transferred into mice on day 15 post-immunization. As a control, mice were injected with IgG derived from HC-1. Mice were scored daily for disease activity. Error bars indicate SEM. Significant differences between the groups in panels A and C ($p < 0.05$; two-way ANOVA and Tukey's multiple comparison test) or in panel B ($p < 0.05$; one-way ANOVA and Tukey's multiple comparison test) are indicated by *. Data are representative of two independent experiments.

than c1011^{WT} that was analogous to the activity of c1011^{ADE} (Fig. 5E). By contrast, the effect of c1011^{LE} was similar to that of c1011^{WT}, except that the disease exacerbation lasted for a shorter time period in c1011^{LE}-treated mice (Fig. 5E).

It was also important to investigate whether the FcγR-enhancing mutations affected their pharmacokinetic behavior in hFcγR mice. Although c1011^{WT}, c1011^{K322A}, c1011^{G236A} and c1011^{D265A} showed similar behavior to that described previously for human IgG1 in mice [58], c1011^{LE} and c1011^{ADE} were cleared more rapidly than their wild type parent (Fig. 6A). Further, the highly biphasic clearance curves for c1011^{LE} and c1011^{ADE} indicated target-mediated clearance by hFcγRs, suggesting that different clearance models would need to be used to determine the half-lives of these antibodies compared with the models required for c1011^{WT}, c1011^{K322A}, c1011^{G236A} and c1011^{D265A}. As an alternative to half-life determination, we therefore assessed the whole body exposure to the antibodies following delivery by calculating the areas under the curves (AUCs) for clearance curves obtained for c1011^{WT} and mutated variants (Fig. 6B). This revealed that the AUCs for c1011^{D265A}, c1011^{K322A} and c1011^{G236A} were similar to that of c1011^{WT}, whereas for c1011^{LE} and c1011^{ADE}, were significantly lower. Importantly, the variant with the highest clearance rate, c1011^{ADE}, has the greatest activity in exacerbating EAE (Figs. 5E and 6), indicating that despite this behavior, FcγR-enhancement can increase disease severity. Further, the observation that the AUC for c1011^{LE} is greater than that for c1011^{ADE} suggests that, by contrast with FcγRIIA, FcγRIIIA interactions play a lesser role in exacerbating EAE.

3.5. EAE exacerbation is associated with increased levels of infiltrating, activated FcγRIIA^{+} macrophages

We next used flow cytometry to characterize the hFcγRIIA^{+} cells present in the spinal cords of mice following the transfer of MS patient-derived IgG. These analyses demonstrated elevated levels of CD86 expression on macrophages (CD11b^{+}CD45^{hi}F4/80^{+}I-A^{b+}FcγRIIA^{+}) for mice treated with MS-3 IgG relative to control IgG (Fig. 7A and B). Although a trend towards higher levels of CD86 was observed for microglia (CD11b^{+}CD45^{lo}F4/80^{+}I-A^{b+}FcγRIIA^{+}) in MS-3 IgG-treated mice relative to mice in the control group, this difference was not statistically significant (Fig. 7A and B). In addition, no difference in CD86 levels between groups was observed for granulocytes (CD11b^{+}CD45^{hi}F4/80^{-}FcγRIIA^{+}) (data not shown). The numbers of macrophages and microglia in the spinal cords of mice treated with MS-3 IgG were also greater than the numbers observed for the control mice (Fig. 7C and D). Increased disease activity is therefore accompanied by higher levels of CD86 on infiltrating macrophages combined with elevated numbers of macrophages and microglia in the spinal cord.

4. Discussion

The contribution of MOG-specific antibodies to pathogenesis and the mechanism of action of such antibodies in MS represent matters of controversy [10–13,30,31,59–62]. In the current study we have demonstrated that human MS patients have circulating antibodies specific for both human and mouse MOG. Further, purified antibodies from two of these patients exacerbate EAE in a transfer model involving mice that transgenically express human FcγRs [34], and exacerbation is inhibited by antigen-specific removal of MOG-specific antibodies. By engineering the Fc region of MOG-specific antibodies to modulate their FcγR and complement binding activity, we also demonstrate that FcγR interactions, particularly those involving FcγRIIA, are necessary and sufficient for EAE exacerbation.

In earlier studies, IgGs derived from a MS patient have been demonstrated to exacerbate EAE and increase demyelination and inflammation in the CNS of proteolipid protein (PLP)139-151-immunized mice [61]. In addition, although transfer of concentrated MS patient sera into MBP72-85-immunized rats did not affect the EAE score, increased demyelination and axonal loss was

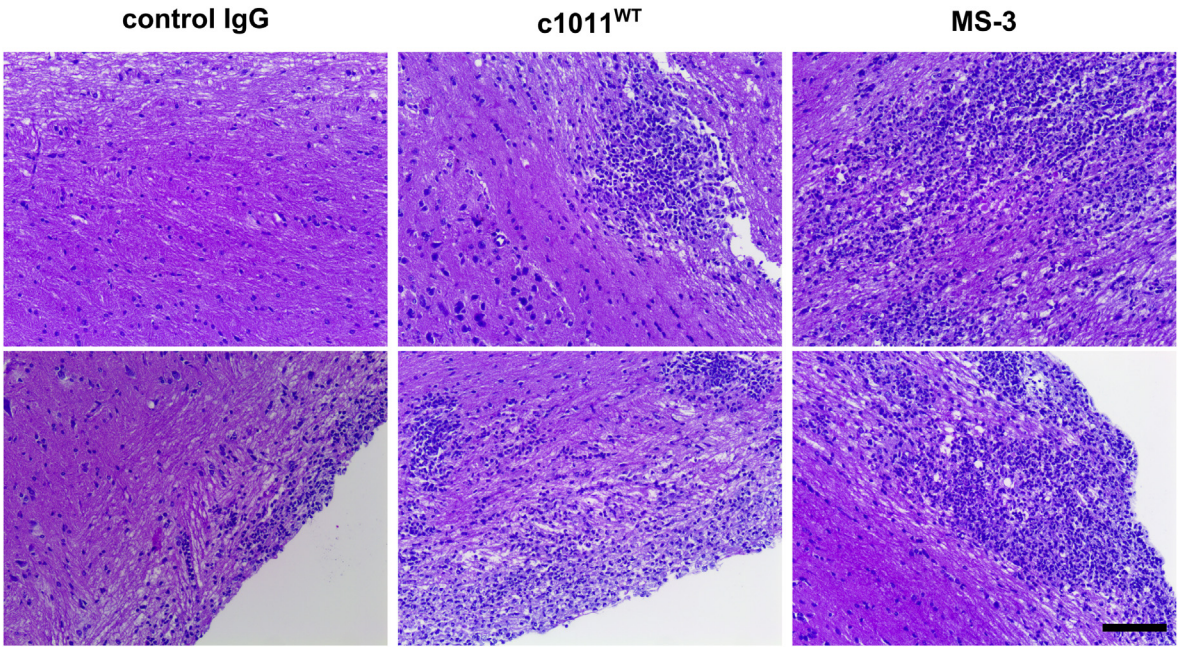

Fig. 4. Immunohistochemical analyses demonstrate that EAE exacerbation is accompanied by increased inflammatory infiltrates. hFcγR mice (n = 4 mice/group) were immunized and treated with 200 μg control IgG (human IgG1), 200 μg c1011 or 250 μg MS-3 IgG as described in Fig. 1 (control, c1011) or Fig. 3 (MS-3). Three days following the delivery of IgG, spinal cords were isolated, sectioned, stained with H&E and imaged. Two representative images from each group are shown. Bar = 100 μm.

Table 1
Properties of Fc mutations.

Mutation	Effect on FcγR/Clq binding
D265A	↓↓ FcγRs[a]
G236A/S239D/I332E (ADE)	↑ FcγRs
G236A	↑ FcγRIIA
A330L/I332E (LE)	↑ FcγRIIIA
K322A	↓↓ C1q

[a] hIgG1 variants harboring D265A mutation have substantially reduced binding to all hFcγRs [40].

observed [13]. MS patient-derived IgGs have also been shown to lead to demyelination (and axonal loss) and cell death in *in vitro* cultures of oligodendrocytes and a MOG-expressing glioblastoma cell line, respectively [13,62]. However, by contrast with our results, antibodies isolated from MS patients in two of the above studies did not recognize MOG [61,62]. The reason for these apparent discrepancies is not clear, although they could relate to the differences in binding assays (e.g. antigen form, threshold used to score positives etc.) and rodent models, and/or the use of serum rather than purified IgG in transfer [13,61,63]. An earlier study, employing a cell-based assay and sera from MS patients, demonstrated that non-specific binding of anti-human IgG (heavy and light chain-specific) antibody to human IgM can lead to detection of false positives during screening for MOG-specific IgG in patients [53]. Significantly, in the current study we used protein G-Sepharose, which can only bind to human IgG and not other human immunoglobulin classes, to purify IgG from the patient sera. In addition, whilst all 10 MS patients included in this study met 2011 revised McDonald Diagnostic Criteria for MS, it is worth noting that the two patients with the highest anti-MOG titers (patients MS-3 and MS-6) had clinical histories that suggest possible neuromyelitis optica spectrum disorder. One patient had severe optic neuritis and myelitis with only mild brain involvement, whereas the other patient had optic neuritis and significant brainstem involvement. Both patients failed initial therapies and had to progress to more immunosuppressive regimens. These findings suggest that screening for MOG-specific antibodies that exacerbate EAE in mouse models could be helpful in identifying MS patients that may have predominantly antibody-mediated pathology.

Our results also demonstrate that for most (8/10) of the patient IgGs analyzed, the well characterized MOG-specific antibody, 8-18C5 [42], effectively competes with binding of patient-derived antibodies to mMOG expressed on the surface of transfected EL4 cells. This competition is similar to that observed for two reports in which antibodies in pediatric MS patients were analyzed, and also for one adult MS patient in a distinct study [13,64,65]. These observations indicate that the patient-derived antibodies recognize an epitope(s) encompassing FG-loop amino acids of MOG, since residues 103 and 104 in this loop have been demonstrated to be core residues of the 8-18C5 epitope [9,52]. Alternatively, as shown in earlier studies [25,63], MOG-specific antibodies that are not dependent on residues 103 and 104 for recognition can still compete with 8-18C5 for binding due to steric effects.

In the current study, Fc engineering of MOG-specific antibodies has been used to delineate the roles of complement and FcγRs in disease exacerbation. Ablation of binding of the MOG-specific, human IgG1 chimeric antibody (K322A mutation) to mouse complement does not affect the ability of this antibody to increase disease activity, whereas reduced FcγR binding (D265A mutation) eliminates the ability of MOG-specific antibodies to exacerbate EAE. Although the D265A mutation also decreases complement binding, based on the results obtained using an antibody variant harboring the K322A mutation, we have excluded a role for complement activation via the classical pathway. Autoantibodies can also function through the alternative complement pathway in animal models of autoimmunity and humans [66], but it is currently unknown whether the K322A or D265A mutations affect this arm of the complement pathway. In addition, despite substantial reductions in FcγR interactions for IgGs containing the D265A mutation, recent studies have demonstrated that the presence of such antibodies in multivalent form i.e. on opsonized cells, is sufficient to

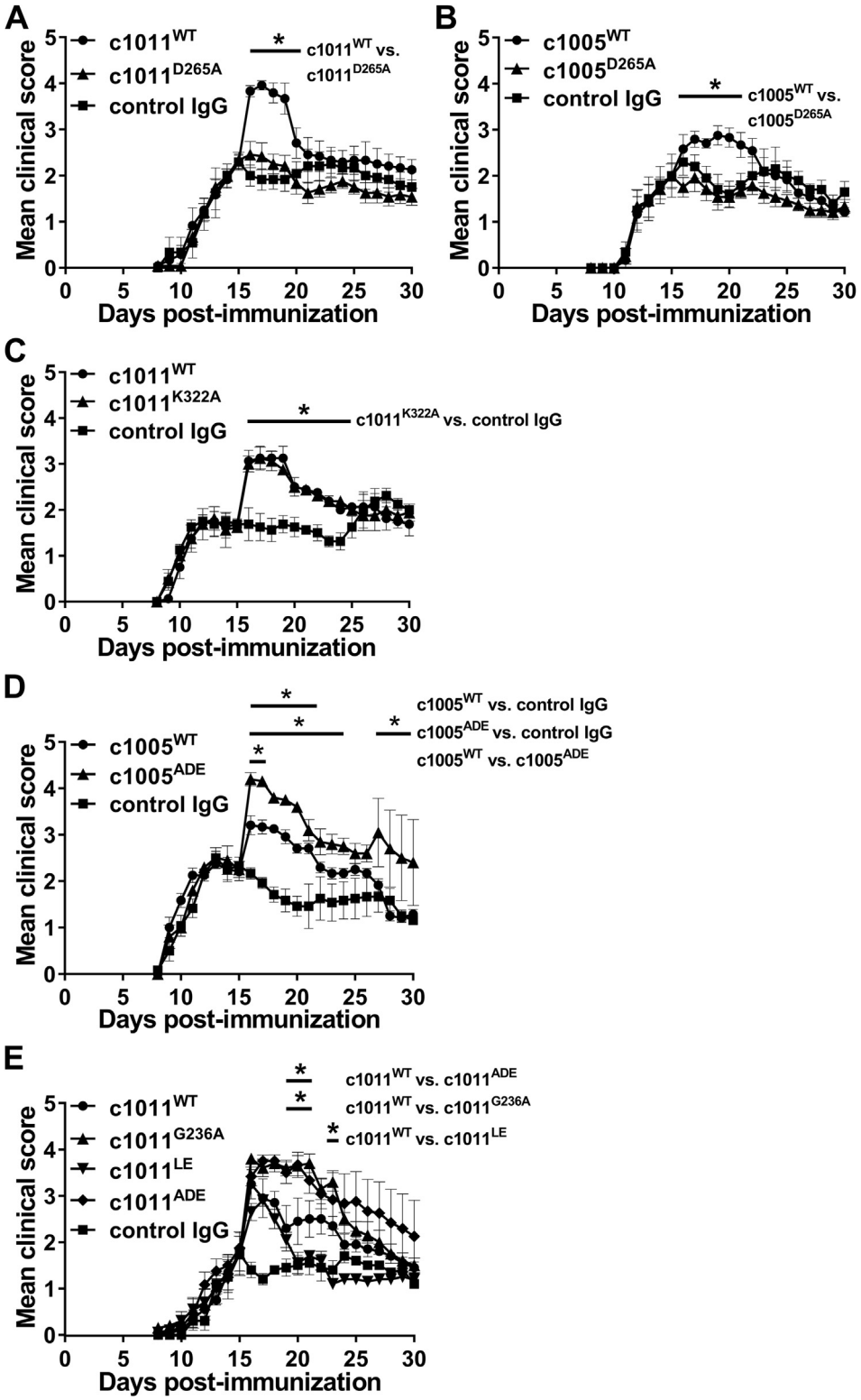

Fig. 5. Fc-FcγR interactions are required for antibody-mediated EAE exacerbation and FcγR enhancement can increase disease activity in hFcγR mice. hFcγR mice (n = 4–6 mice/group) were immunized as in Fig. 1. On day 15 post-immunization, mice were sorted into equivalent groups (mean disease score of ~1–2) and were intravenously administered with 200 μg $c1011^{D265A}$ (A), $c1005^{D265A}$ (B), $c1011^{K322A}$ (C), $c1005^{ADE}$ (D), $c1011^{ADE}$, $c1011^{G236A}$ or $c1011^{LE}$ (E). Mice injected with $c1011^{WT}$ or $c1005^{WT}$ were included in the experiments shown in panels A–E as comparators, and trastuzumab was used as a negative control. Mice were scored daily for disease activity. Error bars indicate SEM and significant differences ($p < 0.05$; two-way ANOVA and Tukey's multiple comparison test) between the groups are indicated by *. Data are representative of two independent experiments.

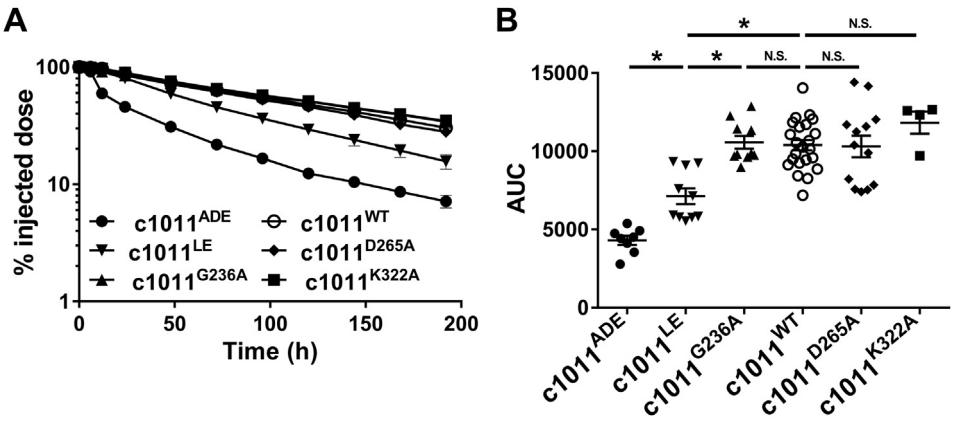

Fig. 6. Increased binding to hFcγRs results in reduced persistence in mice. (A) hFcγR mice (n = 4–5 mice/group) were injected with ^{125}I-labeled c1011 variants and remaining radioactivity levels in the whole body assessed at the indicated times. (B) Areas under the curves (AUCs) for whole body (μCi × h) for wild type and mutated c1011 variants in hFcγR mice (n = 4–23 mice/group) were calculated. Error bars indicate SEM and significant differences ($p < 0.05$; One-way ANOVA and Tukey's multiple comparison test) between the groups are indicated by *. N.S., no significant difference. Data shown in (A) and (B) are representative of, or combined from, at least two independent experiments, respectively, except for c1011^{K322A}.

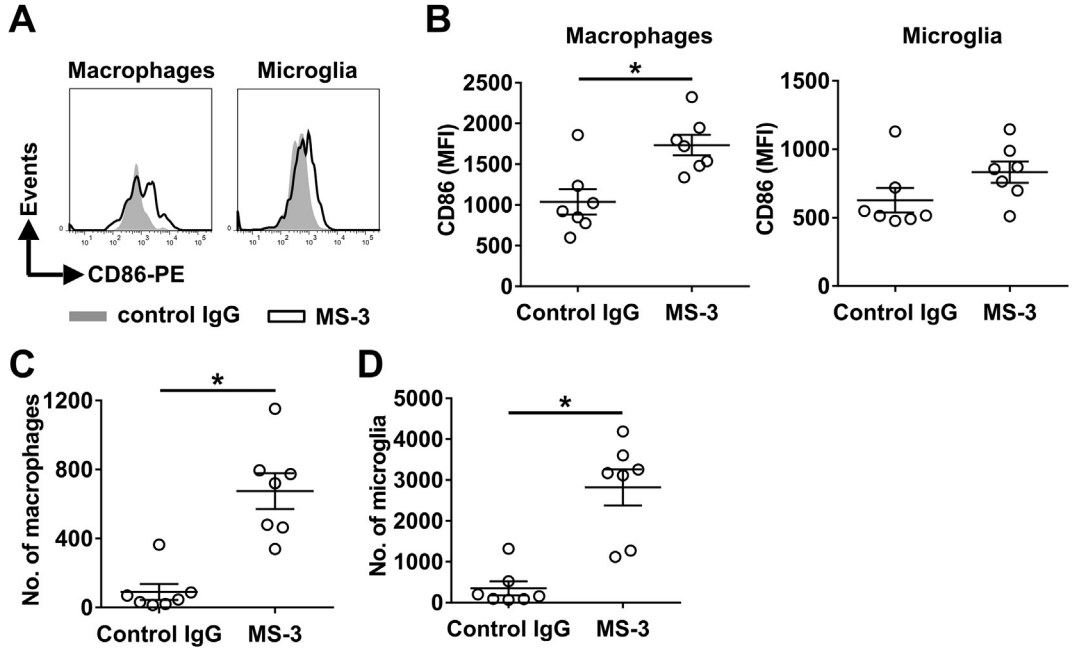

Fig. 7. EAE exacerbation is accompanied by increased numbers of hFcγRIIA-expressing cells in the CNS. (A–D) hFcγR mice (n = 7 mice/group) were immunized and treated with 250 μg control (human IgG1, trastuzumab) or patient-derived IgG (MS-3) as described in Fig. 3. Three days following the delivery of IgG, spinal cords were isolated and single cell suspensions stained with antibodies specific for CD45, CD11b, F4/80, I-A^b, CD86 and hFcγRIIA. Stained cells were analyzed by flow cytometry. (A) Representative histogram plots for individual mice showing flow cytometry analyses of CD86 levels for CD11b$^+$CD45^{hi}F4/80$^+$I-A^{b+}FcγRIIA$^+$ macrophages or CD11b$^+$CD45^{lo}F4/80$^+$I-A^{b+}FcγRIIA$^+$ microglia. (B) Averages of mean fluorescence intensities (MFIs) showing CD86 levels on macrophages or microglia for each treatment group. (C, D) Numbers of infiltrating macrophages (C) or microglia (D). For panels B–D, error bars indicate SEM and statistically significant differences are shown by * ($p < 0.05$; two-tailed Student's *t*-test).

induce FcγR-mediated effector functions, albeit at lower levels than for the corresponding wild type antibodies [67]. Our observations therefore indicate the need to achieve a threshold level of FcγR engagement for disease exacerbation.

In further support of the involvement of FcγRs, engineered antibodies with increased affinity for activating FcγRs induce higher disease severity. Interestingly, selective enhancement for FcγRIIA binding (G236A mutation) exacerbates EAE more effectively than the wild type parent antibody, whereas this effect was not observed following the delivery of a MOG-specific antibody specifically enhanced for FcγRIIIA interactions. In earlier studies, FcγRIIA on macrophages has been shown to be involved in antibody-dependent cell-mediated phagocytosis (ADCP) of opsonized tumor cells [37]. Our data indicate that in the presence of MOG-specific antibodies, FcγRIIA on infiltrating macrophages that are known to be crucial for EAE pathogenesis may play a similar role [51]. Consistent with this possibility, we observe increased activation and numbers of FcγRIIA$^+$ macrophages in the spinal cords of mice following the delivery of MS patient-derived IgG. In addition, neutrophils can be important in EAE pathogenesis [68], and therefore FcγRIIA engagement on this cell type could contribute to the MOG-specific antibody-induced disease exacerbation observed in our studies.

An important outcome of our studies, that has broad

implications for the engineering of antibodies for increased binding to FcγRs, is that the *in vivo* persistence of FcγR-enhanced antibodies is reduced compared with that of their parent, wild type antibody. This effect is most likely due to the propensity of such enhanced antibodies to bind in monomeric form to the low affinity FcγRs on cells *in vivo*, whereas wild type antibodies typically require aggregation to bind [35,69]. However, despite the decreased persistence, and consistent with our observations in the current study, increased potency of FcγR-enhanced antibodies in FcγR-mediated functions can counteract their relatively short half-lives [38].

In summary, we have shown that MOG-specific antibodies from MS patients can contribute to EAE exacerbation in a transfer model, and that antibody-mediated disease is dependent on FcγR engagement. These studies not only describe an instructive model for assessing MS patient-derived antibodies, but also suggest that the targeting of antigen-specific B cells, their corresponding antibodies and FcγR-mediated effector functions may provide an effective strategy for the treatment of MS cases where antibody involvement is indicated.

Author contributions

P.K., D.K.C. and E.S.W. designed the experiments; P.K., D.K.C. and S.C.D. performed the experiments; P.K., D.K.C., S.C.D., R.V., B.M.G., R.J.O. and E.S.W. analyzed the data; S.H. provided human material; P.K., D.K.C., B.M.G., R.J.O. and E.S.W. wrote the manuscript.

Competing financial interests

The authors declare no competing financial interests.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jaut.2017.09.002.

References

[1] D.A. Hafler, J.M. Slavik, D.E. Anderson, K.C. O'Connor, P. De Jager, C. Baecher-Allan, Multiple sclerosis, Immunol. Rev. 204 (2005) 208–231.
[2] B.F. Popescu, C.F. Lucchinetti, Pathology of demyelinating diseases, Annu. Rev. Pathol. 7 (2012) 185–217.
[3] C.S. Raine, B. Cannella, S.L. Hauser, C.P. Genain, Demyelination in primate autoimmune encephalomyelitis and acute multiple sclerosis lesions: a case for antigen-specific antibody mediation, Ann. Neurol. 46 (1999) 144–160.
[4] S.L. Hauser, E. Waubant, D.L. Arnold, T. Vollmer, J. Antel, R.J. Fox, et al., B-cell depletion with rituximab in relapsing-remitting multiple sclerosis, N. Engl. J. Med. 358 (2008) 676–688.
[5] C. Brunner, H. Lassmann, T.V. Waehneldt, J.M. Matthieu, C. Linington, Differential ultrastructural localization of myelin basic protein, myelin/oligodendroglial glycoprotein, and 2',3'-cyclic nucleotide 3'-phosphodiesterase in the CNS of adult rats, J. Neurochem. 52 (1989) 296–304.
[6] T.G. Johns, C.C. Bernard, The structure and function of myelin oligodendrocyte glycoprotein, J. Neurochem. 72 (1999) 1–9.
[7] H.-C. von Büdingen, S.L. Hauser, A. Fuhrmann, C.B. Nabavi, J.I. Lee, C.P. Genain, Molecular characterization of antibody specificities against myelin/oligodendrocyte glycoprotein in autoimmune demyelination, Proc. Natl. Acad. Sci. U.S.A. 99 (2002) 8207–8212.
[8] K.C. O'Connor, H. Appel, L. Bregoli, M.E. Call, I. Catz, J.A. Chan, et al., Antibodies from inflamed central nervous system tissue recognize myelin oligodendrocyte glycoprotein, J. Immunol. 175 (2005) 1974–1982.
[9] C. Breithaupt, B. Schafer, H. Pellkofer, R. Huber, C. Linington, U. Jacob, Demyelinating myelin oligodendrocyte glycoprotein-specific autoantibody response is focused on one dominant conformational epitope region in rodents, J. Immunol. 181 (2008) 1255–1263.
[10] T. Menge, P.H. Lalive, H.C. von Büdingen, C.P. Genain, Conformational epitopes of myelin oligodendrocyte glycoprotein are targets of potentially pathogenic antibody responses in multiple sclerosis, J. Neuroinflamm. 8 (2011) 161.
[11] M. Khalil, M. Reindl, A. Lutterotti, B. Kuenz, R. Ehling, C. Gneiss, et al., Epitope specificity of serum antibodies directed against the extracellular domain of myelin oligodendrocyte glycoprotein: influence of relapses and immunomodulatory treatments, J. Neuroimmunol. 174 (2006) 147–156.
[12] S. Ramanathan, R.C. Dale, F. Brilot, Anti-MOG antibody: the history, clinical phenotype, and pathogenicity of a serum biomarker for demyelination, Autoimmun. Rev. 15 (2016) 307–324.
[13] D. Zhou, R. Srivastava, S. Nessler, V. Grummel, N. Sommer, W. Brueck, et al., Identification of a pathogenic antibody response to native myelin oligodendrocyte glycoprotein in multiple sclerosis, Proc. Natl. Acad. Sci. U.S.A. 103 (2006) 19057–19062.
[14] B. Pollinger, G. Krishnamoorthy, K. Berer, H. Lassmann, M.R. Bosl, R. Dunn, et al., Spontaneous relapsing-remitting EAE in the SJL/J mouse: MOG-reactive transgenic T cells recruit endogenous MOG-specific B cells, J. Exp. Med. 206 (2009) 1303–1316.
[15] C.B. Marta, A.R. Oliver, R.A. Sweet, S.E. Pfeiffer, N.H. Ruddle, Pathogenic myelin oligodendrocyte glycoprotein antibodies recognize glycosylated epitopes and perturb oligodendrocyte physiology, Proc. Natl. Acad. Sci. U.S.A. 102 (2005) 13992–13997.
[16] S.S. Zamvil, L. Steinman, The T lymphocyte in experimental allergic encephalomyelitis, Annu. Rev. Immunol. 8 (1990) 579–621.
[17] V.K. Kuchroo, A.C. Anderson, H. Waldner, M. Munder, E. Bettelli, L.B. Nicholson, T cell response in experimental autoimmune encephalomyelitis (EAE): role of self and cross-reactive antigens in shaping, tuning, and regulating the autopathogenic T cell repertoire, Annu. Rev. Immunol. 20 (2002) 101–123.
[18] S.D. Wolf, B.N. Dittel, F. Hardardottir, C.A. Janeway Jr., Experimental autoimmune encephalomyelitis induction in genetically B cell-deficient mice, J. Exp. Med. 184 (1996) 2271–2278.
[19] B.N. Dittel, T.H. Urbania, C.A. Janeway Jr., Relapsing and remitting experimental autoimmune encephalomyelitis in B cell deficient mice, J. Autoimmun. 14 (2000) 311–318.
[20] A.R. Oliver, G.M. Lyon, N.H. Ruddle, Rat and human myelin oligodendrocyte glycoproteins induce experimental autoimmune encephalomyelitis by different mechanisms in C57BL/6 mice, J. Immunol. 171 (2003) 462–468.
[21] J.A. Lyons, M.J. Ramsbottom, A.H. Cross, Critical role of antigen-specific antibody in experimental autoimmune encephalomyelitis induced by recombinant myelin oligodendrocyte glycoprotein, Eur. J. Immunol. 32 (2002) 1905–1913.
[22] M.S. Weber, T. Prod'homme, J.C. Patarroyo, N. Molnarfi, T. Karnezis, K. Lehmann-Horn, et al., B-cell activation influences T-cell polarization and outcome of anti-CD20 B-cell depletion in central nervous system autoimmunity, Ann. Neurol. 68 (2010) 369–383.
[23] J.A. Lyons, M. San, M.P. Happ, A.H. Cross, B cells are critical to induction of experimental allergic encephalomyelitis by protein but not by a short encephalitogenic peptide, Eur. J. Immunol. 29 (1999) 3432–3439.
[24] N. Molnarfi, U. Schulze-Topphoff, M.S. Weber, J.C. Patarroyo, T. Prod'homme, M. Varrin-Doyer, et al., MHC class II-dependent B cell APC function is required for induction of CNS autoimmunity independent of myelin-specific antibodies, J. Exp. Med. 210 (2013) 2921–2937.
[25] P. Bansal, T. Khan, U. Bussmeyer, D.K. Challa, R. Swiercz, R. Velmurugan, et al., The encephalitogenic, human myelin oligodendrocyte glycoprotein-induced antibody repertoire is directed toward multiple epitopes in C57BL/6-immunized mice, J. Immunol. 191 (2013) 1091–1101.
[26] S.J. Piddlesden, H. Lassmann, F. Zimprich, B.P. Morgan, C. Linington, The demyelinating potential of antibodies to myelin oligodendrocyte glycoprotein is related to their ability to fix complement, Am. J. Pathol. 143 (1993) 555–564.
[27] F. Nimmerjahn, J.V. Ravetch, Divergent immunoglobulin G subclass activity through selective Fc receptor binding, Science 310 (2005) 1510–1512.
[28] F. Nimmerjahn, J.V. Ravetch, Fcγ receptors: old friends and new family members, Immunity 24 (2006) 19–28.
[29] L. Fossati-Jimack, A. Ioan-Facsinay, L. Reininger, Y. Chicheportiche, N. Watanabe, T. Saito, et al., Markedly different pathogenicity of four immunoglobulin G isotype-switch variants of an antierythrocyte autoantibody is based on their capacity to interact in vivo with the low-affinity Fcγ receptor

III, J. Exp. Med. 191 (2000) 1293–1302.
[30] K.B. Abdul-Majid, A. Stefferl, C. Bourquin, H. Lassmann, C. Linington, T. Olsson, et al., Fc receptors are critical for autoimmune inflammatory damage to the central nervous system in experimental autoimmune encephalomyelitis, Scand. J. Immunol. 55 (2002) 70–81.
[31] E. Urich, I. Gutcher, M. Prinz, B. Becher, Autoantibody-mediated demyelination depends on complement activation but not activatory Fc-receptors, Proc. Natl. Acad. Sci. U.S.A. 103 (2006) 18697–18702.
[32] E.C. Breij, P. Heijnen, R. Vloet, T. Saito, J.G. van de Winkel, C.D. Dijkstra, et al., The FcRγ chain is not essential for induction of experimental allergic encephalomyelitis (EAE) or anti-myelin antibody-mediated exacerbation of EAE, J. Neuropathol. Exp. Neurol. 64 (2005) 304–311.
[33] F. Nimmerjahn, J.V. Ravetch, Fcγ receptors as regulators of immune responses, Nat. Rev. Immunol. 8 (2008) 34–47.
[34] P. Smith, D.J. DiLillo, S. Bournazos, F. Li, J.V. Ravetch, Mouse model recapitulating human Fcγ receptor structural and functional diversity, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 6181–6186.
[35] P.M. Hogarth, G.A. Pietersz, Fc receptor-targeted therapies for the treatment of inflammation, cancer and beyond, Nat. Rev. Drug Discov. 11 (2012) 311–331.
[36] P.A. Ramsland, W. Farrugia, T.M. Bradford, C.T. Sardjono, S. Esparon, H.M. Trist, et al., Structural basis for Fc gammaRIIa recognition of human IgG and formation of inflammatory signaling complexes, J. Immunol. 187 (2011) 3208–3217.
[37] J.O. Richards, S. Karki, G.A. Lazar, H. Chen, W. Dang, J.R. Desjarlais, Optimization of antibody binding to FcγRIIa enhances macrophage phagocytosis of tumor cells, Mol. Cancer Ther. 7 (2008) 2517–2527.
[38] D.J. DiLillo, J.V. Ravetch, Differential Fc-receptor engagement drives an anti-tumor vaccinal effect, Cell 161 (2015) 1035–1045.
[39] R.L. Shields, A.K. Namenuk, K. Hong, Y.G. Meng, J. Rae, J. Briggs, et al., High resolution mapping of the binding site on human IgG1 for FcγRI, FcγRII, FcγRIII, and FcRn and design of IgG1 variants with improved binding to the FcγR, J. Biol. Chem. 276 (2001) 6591–6604.
[40] R.A. Clynes, T.L. Towers, L.G. Presta, J.V. Ravetch, Inhibitory Fc receptors modulate in vivo cytotoxicity against tumor targets, Nat. Med. 6 (2000) 443–446.
[41] M. Hezareh, A.J. Hessell, R.C. Jensen, J.G. van de Winkel, P.W. Parren, Effector function activities of a panel of mutants of a broadly neutralizing antibody against human immunodeficiency virus type 1, J. Virol. 75 (2001) 12161–12168.
[42] H.J. Schluesener, R.A. Sobel, C. Linington, H.L. Weiner, A monoclonal antibody against a myelin oligodendrocyte glycoprotein induces relapses and demyelination in central nervous system autoimmune disease, J. Immunol. 139 (1987) 4016–4021.
[43] A.G. Amit, R.A. Mariuzza, S.E. Phillips, R.J. Poljak, Three-dimensional structure of an antigen-antibody complex at 2.8 Å resolution, Science 233 (1986) 747–753.
[44] J. Foote, G. Winter, Antibody framework residues affecting the conformation of the hypervariable loops, J. Mol. Biol. 224 (1992) 487–499.
[45] B. Devaux, F. Enderlin, B. Wallner, D.E. Smilek, Induction of EAE in mice with recombinant human MOG, and treatment of EAE with a MOG peptide, J. Neuroimmunol. 75 (1997) 169–173.
[46] R.J. Ober, E.S. Ward, Compensation for loss of ligand activity in surface plasmon resonance experiments, Anal. Biochem. 306 (2002) 228–236.
[47] R.J. Ober, J. Caves, E.S. Ward, Analysis of exponential data using a noniterative technique: application to surface plasmon experiments, Anal. Biochem. 312 (2003) 57–65.
[48] A. Minguela, S. Pastor, W. Mi, J.A. Richardson, E.S. Ward, Feedback regulation of murine autoimmunity via dominant anti-inflammatory effects of interferon γ, J. Immunol. 178 (2007) 134–144.
[49] J.K. Kim, M.F. Tsen, V. Ghetie, E.S. Ward, Catabolism of the murine IgG1 molecule: evidence that both CH2-CH3 domain interfaces are required for persistence of IgG1 in the circulation of mice, Scand. J. Immunol. 40 (1994) 457–465.
[50] D.K. Challa, U. Bussmeyer, T. Khan, H.P. Montoyo, P. Bansal, R.J. Ober, et al., Autoantibody depletion ameliorates disease in murine experimental autoimmune encephalomyelitis, MAbs 5 (2013) 655–659.
[51] D.R. Huang, J. Wang, P. Kivisakk, B.J. Rollins, R.M. Ransohoff, Absence of monocyte chemoattractant protein 1 in mice leads to decreased local macrophage recruitment and antigen-specific T helper cell type 1 immune response in experimental autoimmune encephalomyelitis, J. Exp. Med. 193 (2001) 713–726.
[52] C. Breithaupt, A. Schubart, H. Zander, A. Skerra, R. Huber, C. Linington, et al., Structural insights into the antigenicity of myelin oligodendrocyte glycoprotein, Proc. Natl. Acad. Sci. U.S.A. 100 (2003) 9446–9451.
[53] P. Waters, M. Woodhall, K.C. O'Connor, M. Reindl, B. Lang, D.K. Sato, et al., MOG cell-based assay detects non-MS patients with inflammatory neurologic disease, Neurol. Neuroimmunol. Neuroinflamm. 2 (2015) e89.
[54] L. Baudino, Y. Shinohara, F. Nimmerjahn, J. Furukawa, M. Nakata, E. Martinez-Soria, et al., Crucial role of aspartic acid at position 265 in the CH2 domain for murine IgG2a and IgG2b Fc-associated effector functions, J. Immunol. 181 (2008) 6664–6669.
[55] E.E. Idusogie, L.G. Presta, H. Gazzano-Santoro, K. Totpal, P.Y. Wong, M. Ultsch, et al., Mapping of the C1q binding site on rituxan, a chimeric antibody with a human IgG1 Fc, J. Immunol. 164 (2000) 4178–4184.
[56] M.B. Overdijk, S. Verploegen, J.H. van den Brakel, J.J. Lammerts van Bueren, T. Vink, J.G. van de Winkel, et al., Epidermal growth factor receptor (EGFR) antibody-induced antibody-dependent cellular cytotoxicity plays a prominent role in inhibiting tumorigenesis, even of tumor cells insensitive to EGFR signaling inhibition, J. Immunol. 187 (2011) 3383–3390.
[57] P. Bruhns, F. Jönsson, Mouse and human FcR effector functions, Immunol. Rev. 268 (2015) 25–51.
[58] H. Perez-Montoyo, C. Vaccaro, M. Hafner, R.J. Ober, W. Mueller, E.S. Ward, Conditional deletion of the MHC Class I-related receptor, FcRn, reveals the sites of IgG homeostasis in mice, Proc. Natl. Acad. Sci. U.S.A. 106 (2009) 2788–2793.
[59] M. Wuhrer, M.H. Selman, L.A. McDonnell, T. Kumpfel, T. Derfuss, M. Khademi, et al., Pro-inflammatory pattern of IgG1 Fc glycosylation in multiple sclerosis cerebrospinal fluid, J. Neuroinflamm. 12 (2015) 235.
[60] M. Benkhoucha, N. Molnarfi, M.L. Santiago-Raber, M.S. Weber, D. Merkler, M. Collin, et al., IgG glycan hydrolysis by EndoS inhibits experimental autoimmune encephalomyelitis, J. Neuroinflamm. 9 (2012) 209.
[61] R. Pedotti, S. Musio, S. Scabeni, C. Farina, P.L. Poliani, E. Colombo, et al., Exacerbation of experimental autoimmune encephalomyelitis by passive transfer of IgG antibodies from a multiple sclerosis patient responsive to immunoadsorption, J. Neuroimmunol. 262 (2013) 19–26.
[62] C. Elliott, M. Lindner, A. Arthur, K. Brennan, S. Jarius, J. Hussey, et al., Functional identification of pathogenic autoantibody responses in patients with multiple sclerosis, Brain 135 (2012) 1819–1833.
[63] M.C. Mayer, C. Breithaupt, M. Reindl, K. Schanda, K. Rostasy, T. Berger, et al., Distinction and temporal stability of conformational epitopes on myelin oligodendrocyte glycoprotein recognized by patients with different inflammatory central nervous system diseases, J. Immunol. 191 (2013) 3594–3604.
[64] K.A. McLaughlin, T. Chitnis, J. Newcombe, B. Franz, J. Kennedy, S. McArdel, et al., Age-dependent B cell autoimmunity to a myelin surface antigen in pediatric multiple sclerosis, J. Immunol. 183 (2009) 4067–4076.
[65] A.K. Probstel, K. Dornmair, R. Bittner, P. Sperl, D. Jenne, S. Magalhaes, et al., Antibodies to MOG are transient in childhood acute disseminated encephalomyelitis, Neurology 77 (2011) 580–588.
[66] J.M. Thurman, V.M. Holers, The central role of the alternative complement pathway in human disease, J. Immunol. 176 (2006) 1305–1310.
[67] A. Lux, X. Yu, C.N. Scanlan, F. Nimmerjahn, Impact of immune complex size and glycosylation on IgG binding to human FcγRs, J. Immunol. 190 (2013) 4315–4323.
[68] K. Steinbach, M. Piedavent, S. Bauer, J.T. Neumann, M.A. Friese, Neutrophils amplify autoimmune central nervous system infiltrates by maturing local APCs, J. Immunol. 191 (2013) 4531–4539.
[69] P. Bruhns, Properties of mouse and human IgG receptors and their contribution to disease models, Blood 119 (2012) 5640–5649.