MOG-reactive B cells exacerbate the severity of CD4$^+$ T cell-driven CNS autoimmunity.

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Abstract

BACKGROUND: Multiple sclerosis (MS) is an autoimmune disorder of the central nervous system (CNS) that has traditionally been considered T cell-mediated. However, accumulating evidence points to a crucial role for B cells in disease processes. Experimental autoimmune encephalomyelitis (EAE) is a well-established model to study the immune aspects of CNS autoimmunity.

METHODS: In order to examine the collaboration of B cells and T cells in EAE, we studied non-obese diabetic (NOD)-background IgH[MOG] mice, whose B cells express a transgenic IgH chain derived from a myelin oligodendrocyte glycoprotein (MOG)-specific antibody. We immunized these and NOD WT controls with the MHC class II-restricted peptide $MOG_{[35-55]}$, which induces a $CD4^+$ T cell-driven response. CNS tissue inflammation and demyelination were assessed histopathologically, and the phenotype of CNS-infiltrating mononuclear cells was studied by flow cytometry. The capacity of IgH[MOG] B cells to present antigen to $CD4^+$ T cells was assessed using *in vitro* priming assays with $MOG_{[35-55]}$ as the antigen.

RESULTS: $MOG_{[35-55]}$-immunized IgH[MOG] mice rapidly developed severe EAE characterized by leukocytic infiltration and demyelination in the brain, spinal cord and optic nerve. Notably, while the frequency of $CD4^+$ T cells was increased in the CNS of IgH[MOG] with severe disease relative to controls, no differences were observed with respect to the frequency of B cells. Further, IgH[MOG] CNS-

infiltrating $CD4^+$ T cells produced significantly higher levels of Th17-associated cytokines GM-CSF and IL-17 compared to those from controls. Mechanistically, IgH[MOG] B cells were better able than WT B cells to elicit inflammatory cytokine production from $MOG_{[35-55]}$-specific $CD4^+$ T cells in *in vitro* priming assays.

CONCLUSION: These data show that MOG-specific B cells contribute to $CD4^+$ T cell-driven EAE by promoting $CD4^+$ T cell inflammation and recruitment to the CNS.

Keywords: experimental autoimmune encephalomyelitis, B cell, $CD4^+$ T cell, myelin oligodendrocyte glycoprotein (MOG), IgH[MOG], 1C6, non-obese diabetic (NOD)

Background

Multiple sclerosis (MS) is a chronic neurodegenerative disease in which the adaptive immune system launched an attack against central nervous system (CNS) proteins, such as myelin, ultimately resulting in neurological dysfunction and death. MS affects more than 2 million people worldwide [1]. Approximately 80% of patients present an initially relapsing-remitting (RR) disease course for which there are now more than 10 disease-modifying therapies available. However, 30-60% of these (RR) patients will eventually transition to a chronically worsening secondary progressive (SP) phase, for which treatment options are limited [2]. Pathophysiological mechanisms in progressive MS are thus of intense current interest [3].

T cells, and $CD4^+$ T cells in particular, have been the most intensively studied players in the immune pathogenesis of MS. However, it has become increasingly clear that B cells additionally play important roles in MS pathogenesis. Clonally expanded B cells are present in the cerebrospinal fluid (CSF) and MS plaques [4-6], and the presence of meningeal follicles adjacent to cortical lesions is associated with disease progression [7,8]. Further, antibodies against myelin oligodendrocyte glycoprotein (MOG), a key component of myelin, were found in active MS lesions [9]. Crucially, the B cell targeting reagent ocrelizumab (anti-CD20) results in striking improvements in RR-MS, and is the only FDA approved drug for primary progressive MS [10,11]. Intriguingly, antibody-secreting B cells do not express CD20 [12]. This suggests that the principal pathogenic role of B

77 cells in MS might be unrelated to the generation of autoantibodies, and rather to
78 their capacity to interact with other immune cell types, such as T cells.

79 Experimental autoimmune encephalomyelitis (EAE) is an animal disease
80 that models many of the immune aspects of MS pathogenesis. Use of this model
81 has helped us to understand the role of T cells, and $CD4^+$ T cells in particular, in
82 the initiation and maintenance of autoreactive inflammation in the CNS [13].
83 However, studies using the transgenic IgH[MOG] mouse strain have indicated
84 that B cells may also play a crucial role in EAE pathology. These mice express a
85 knock-in transgenic IgH chain derived from a MOG-specific antibody; thus,
86 around 30% of their B cells are therefore specific for MOG protein [14]. IgH[MOG]
87 animals develop severe EAE when immunized with either whole MOG protein
88 [14] or with its extracellular domain ($MOG_{[1-125]}$)[15], indicating an important role
89 for MOG-reactive B cells in neuroimmune processes. However the potential
90 mechanisms by which MOG-reactive B cells facilitate T cell-driven pathogenicity,
91 such as in class II-restricted peptide immunization models of EAE, remain
92 incompletely understood.

93 Here, we studied the co-operative role of B cells and T cells in CNS
94 autoimmunity using IgH[MOG] mice on the non-obese diabetic (NOD) genetic
95 background. We immunized these, and wildtype (WT) NOD mice, with $MOG_{[35-55]}$,
96 a MHC class II-restricted peptide that obligatorily drives a $CD4^+$ T cell response.
97 While WT NOD mice gradually develop a relapsing chronic form of EAE over the
98 course of 80-100 days when immunized with $MOG_{[35-55]}$ [16], we observed that
99 IgH[MOG] mice developed a severe form of EAE within 14 days that was

characterized by demyelination and inflammation of the CNS. Disease in these animals was accompanied by an increase in CNS-infiltrating T cells that expressed higher levels of the inflammatory cytokines IL-17 and GM-CSF as compared to wildtype controls. Importantly, IgH[MOG] B cells could prime $MOG_{[35-55]}$-reactive T cells to produce increased inflammatory cytokines *in vitro*. Hence, we demonstrate that the collaborative role of B cells and T cells are important for severe CNS autoimmunity.

Methods

Animals

IgH[MOG] mice on the NOD background were a gift from Dr. Hartmurt Wekerle and 1C6 mice were a gift from Dr. Vijay Kuchroo. NOD/ShiLtJ mice were purchased from Jackson Laboratories. The sex of the mice used is indicated in each figure legend.

EAE induction and scoring

NOD and IgH[MOG] mice were immunized subcutaneously with 200µg $MOG_{[35-55]}$ (Feldan), emulsified in incomplete Freund's adjuvant (BD Difco) that was supplemented with 500 µg *M. tuberculosis* extract (BD Difco). On day 0 and day 2 post-immunization, mice received 200 ng pertussis toxin (List Biological Laboratories) intraperitoneally. Mice were monitored daily for signs of EAE, which were assessed using a semi-quantitative 0-5 scale: 0; no disease, 0.5; ruffled fur,

1; limp tail, 1.5; mild impairment in gait, 2; severe impairment in gait, 2.5; partial hind limb paralysis, 3; hind limb paralysis, 4; forelimb paralysis, 5; moribund [17]. Pre-onset analyses were conducted a minimum of 5 days post-immunization but before the onset of symptoms. Endpoint analyses were conducted at d14.

Histopathology

EAE mice were euthanized, and CNS tissue and optic nerves were immediately fixed in 4% paraformaldehyde solution. After 24 hours, the tissues were transferred into PBS for paraffin embedding. Paraffin embedded sections were made at 4 µm thickness and stained with Hematoxylin & Eosin (H&E) to detect the infiltration of immune cells, or Luxol fast blue (LFB) to detect demyelination. The images were taken at 10X and 20X magnifications with Nikon Eclipse 80i microscope and were analysed using ImageJ software (NIH).

Measurement of serum immunoglobulin

Blood samples were collected from NOD and IgH[MOG] unimmunized mice, as well as from immunized mice at pre-onset and experimental endpoint, in an EDTA-coated Microvette (Sarstedt). They were centrifuged at 2000g for 20 minutes at 4°C to obtain plasma. The concentration of plasma IgM was analysed using the HRP C57BL/6J Mouse Clonotyping System (Southern Biotech) according to the manufacturer's instructions.

146 *Isolation of CNS-infiltrating mononuclear cells*

147 Mice were euthanized and perfused intracardially with PBS. Brain and spinal cord
148 were dissected from the skull and vertebral column respectively and were
149 prepared as previously described [17]. Briefly, CNS tissues were digested with
150 liberase (Roche) and DNAse I (Sigma) and cells were enriched using a 35%
151 Percoll (GE Healthcare) gradient.

152

153 *Flow cytometry*

154 Single cell suspensions were obtained from spleens, lymph nodes and CNS of
155 EAE mice. For detection of surface antigens, cells were stained with Fixable
156 Viability Dye (eBioscience) and incubated with Fc Block (Biolegend) prior to
157 staining with antibodies against surface antigens (CD45, CD4, CD8, CD19,
158 CD11b, CD11c, Ly6G; details in following section). For detection of intracellular
159 cytokines, cells were first stimulated with 50ng ml^{-1} PMA (Sigma), 1µM ionomycin
160 (Sigma) and 1µL mL^{-1} GolgiStop (BD) for 4 hours at 37°C, prior to being labeled
161 with viability indicator, Fc Block and relevant surface antigens as above. They
162 were then fixed and permeabilized (Fixation Buffer and Intracellular Staining
163 Perm Wash Buffer, both Biolegend) and stained for intracellular cytokines (IFN-γ,
164 TNF-α, IL-17A, GM-CSF; details in following section). Samples were analyzed on
165 a FACS Aria (BD) and data were analyzed using FlowJo software (Treestar).

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Flow cytometry antibodies

The following monoclonal antibodies against mouse antigens were used: CD45, clone A20 (Biolegend); CD11b, clone M1/70 (eBioscience); CD11c, clone N418 (Biolegend); Ly6G, clone 1A8 (BD Biosciences); CD4, clone RM4-5 (eBioscience); CD8, clone 53-6.7 (Biolegend); CD19, clone 1D3 (eBioscience); IFN-γ, clone XMG1.2 (eBioscience); TNF-α, clone MP6-XT22 (eBioscience); IL-17a, clone TC11-18H10.1 (Biolegend); GM-CSF, clone MP1-22E9 (eBioscience); CD40, clone 3/23 (Biolegend); CD80, clone 1610A1 (Biolegend).

Antigen presentation assay

Single cell suspensions were obtained from the spleens of unimmunized NOD and IgH[MOG] mice. Cells were labeled with CD43 (Ly-48) Microbeads (Miltenyi), and $CD43^+$ leukocytes (all leukocytes except resting B cells) were depleted on a magnetic MACS column (Miltyeni). Unlabelled $CD43^-$ cells were collected and were subsequently stained with anti-mouse CD19. $CD19^+$ B cells were purified using high-speed cell sorting. In parallel, $CD4^+$ T cells were purified from the spleens of 1C6 mice using mouse CD4 MicroBeads (Miltenyi) and labeled with CellTrace Violet (CTV; Thermo Fisher Scientific). CTV-labeled 1C6 $CD4^+$ T cells were cultured with B cells at a ratio of 1 CD4: 1 B, with 0, 1 or 10µg ml^{-1} $MOG_{[35-55]}$ for 72 hours. CTV dilution and intracellular cytokine production were assessed by flow cytometry.

Statistical analysis

For comparison of EAE scores on individual days, Mann-Whitney *U* test was used. Fisher's exact test was used to measure the frequency of mice attaining ethical endpoints. For immunoglobulin analyses and *ex vivo* cytokine profiling, unpaired Student *t*-tests were used. For cytokine production in the *in vitro* priming experiment, two way ANOVA was used. Two-tailed analyses were used in all instances. All statistical analyses were conducted using Prism software (GraphPad).

Results

IgH[MOG] mice develop severe EAE upon active immunization with $MOG_{[35-55]}$

When immunized with $MOG_{[35-55]}$, wildtype (WT) NOD strain gradually develop an initial relapse remitting pattern that ultimately transitions to a chronically worsening phase over the course of 60-100 days [16]. NOD-EAE has thus been considered a possible model of SPMS [18,19]. To assess whether the presence of substantial numbers of MOG-reactive B cells could alter this disease pattern, we immunized IgH[MOG] and NOD controls with $MOG_{[35-55]}$. Strikingly, immunized IgH[MOG] mice rapidly developed severe EAE within 14 days (Figure 1), while WT NOD mice either were disease-free or had only mild symptoms at this timepoint (mean maximal severity, 3.1±0.2 for IgH[MOG], n=27; 0.07±0.07 for WT NOD, n=19; $p<0.0001$). Strikingly, 55% of immunized IgH[MOG] mice (15/27) attained ethical endpoints and had to be euthanized by d14; no WT NOD

attained disease of this severity (0/19; $p<0.0001$). For the remainder of the study, d14 was therefore treated as the endpoint of immunization protocols.

We next examined CNS tissue damage in actively immunized mice. Immunized IgH[MOG] mice displayed immune infiltration the cerebellum (Figure 2A) and pons (Figure 2B), as well as the spinal cord (Figure 2C) and optic nerve (Figure 2D). Demyelination was also observed in these tissues (Figures 2E-H). By contrast, there was little to no infiltration or demyelination of CNS tissue observed in immunized WT NOD mice sacrificed in parallel. Our data thus show that the presence of myelin-reactive B cells can exacerbate CNS autoimmunity and tissue damage when EAE is induced in a $CD4^+$ T cell dependent manner.

Decrease in plasma IgM in immunized IgH[MOG] mice

Antigen-specific antibody (Ab) secretion is the primary function of B cells. Further, oligoclonal immunoglobulin (Ig) banding in cerebrospinal fluid (CSF) is an important diagnostic marker for MS [20]. Upon initial activation, B cells first secrete antigen-specific Abs of the IgM isotype [21]. The Ab secreted by a given B cell clone can later “switch” to a different isotype based on the presence of differentiation cues in the local milieu, such as cytokines secreted by T cells [22]. We therefore wanted to examine whether changes in total serum IgM might reflect the rapid nature of the disease observed in IgH[MOG] mice. We collected sera from immunized NOD versus IgH[MOG] at pre-onset (PO) and at disease endpoint (EP), and additionally from unimmunized (UI) mice of both strains. We then analyzed these sera for the presence of IgM. We found that the

concentration of plasma IgM levels was significantly reduced in IgH[MOG] mice at endpoint relative to NOD controls, indicative of increased switching to secondary isotype subclasses in the context of severe disease (Figure 3). These data indicated that exacerbated disease in IgH[MOG] mice is accompanied by isotype class switching and therefore pointed towards the potential collaboration of MOG-reactive B cells with other immune cell types.

Increased presence of immune cells in the CNS of immunized IgH[MOG] mice.

We next wanted to examine whether there were differences between NOD and IgH[MOG] mice in the composition of the immune cells infiltrating the CNS. We first sacrificed mice at the pre-onset stage (d5-post immunization) and analyzed the percentage of immune cells ($CD19^+$ B cells, $CD4^+$ T cells, $CD8^+$ T cells, $CD11b^+CD11c^+$ dendritic cells, $CD11b^+CD11c^-Ly6G^-$ macrophages and $CD11b^+Ly6G^+$ neutrophils) in both CNS and spleen. At this early timepoint, differences in the frequency of such cells were modest in both CNS (Figure 4A) and spleen (Figure 4B), although we did observe an increase in the relative proportion of macrophages infiltrating the CNS of IgH[MOG] mice (Figure 4A).

We then assessed the frequency of immune cell proportions at disease endpoint (d14). Strikingly, the proportion of $CD4^+$ T cells was significantly increased in the CNS of IgH[MOG] mice relative to NOD (Figure 4C). $CD8^+$ T cells, dendritic cells and neutrophils were also more prevalent in the CNS of IgH[MOG] (Figure 4C), though overall frequency of these cells were low in all mice studied (<5%). The frequency of $CD4^+$ T cells was also higher in the

spleens of IgH[MOG] mice at endpoint, suggesting their expansion in these animals (Figure 4D). Interestingly, despite having an antigenic repertoire heavily skewed towards MOG reactivity, immunized IgH[MOG] mice did not display an increased frequency of B cells in either the CNS (Figure 4C) or spleen (Figure 4D) at endpoint. These findings suggested that while IgH[MOG] B cells might be important in driving T cell expansion and infiltration into the CNS, they might themselves be of secondary importance to the development of severe EAE in IgH[MOG] mice.

CNS-infiltrating IgH[MOG] CD4$^+$ T cells produce increased levels of IL-17 and GM-CSF.

As we had observed an elevated frequency of CD4$^+$ T cells in the CNS of sick IgH[MOG] mice, we next examined the capacity of these cells to produce inflammatory Th1 and Th17 cytokines by flow cytometry, due to the well-established role of these CD4$^+$ effector T cell subsets in EAE [23]. We observed no differences between IgH[MOG] and WT CD4$^+$ T cells in their production of the Th1 signature cytokine IFN-γ in the CNS (Figure 5A). By contrast we saw a striking upregulation of IL-17 production from CNS-infiltrating CD4$^+$ T cells from IgH[MOG] at disease endpoint (Figure 5B). Notably, production of GM-CSF, a key pathogenic cytokine implicated in Th17-driven tissue inflammation, was also augmented in IgH[MOG] CD4$^+$ T cells (Figure 5C), though no differences were noted in the production of TNFα (Figure 5D). In sum, our data showed that Th17

cells were preferentially recruited to the CNS of IgH[MOG] mice with severe EAE.

IgH[MOG] B cells have an augmented capacity to prime inflammatory $MOG_{[35-55]}$-specific $CD4^+$ T cells

Thus far, we had found evidence of increased expansion and cytokine production from $CD4^+$ T cells in IgH[MOG] mice. As B cells can express MHC class II and are thus capable of presenting antigen to $CD4^+$ T cells [24], we wanted to assess whether IgH[MOG] B cells intrinsically possess an enhanced capacity to prime $MOG_{[35-55]}$-specific T cell responses. We purified splenic B cells from unimmunized IgH[MOG] or WT NOD mice and, in the presence or absence of $MOG_{[35-55]}$, co-cultured these cells with antigen-inexperienced $CD4^+$ T cells from transgenic 1C6 mice. 1C6 mice are on the NOD background and have class II-restricted T cell receptor specificity to $MOG_{[35-55]}$ [25]. IgH[MOG] and WT B cells did not differ in their capacity to induce $CD4^+$ T cell proliferation in response to $MOG_{[35-55]}$ (Figure 6A). However, IgH[MOG] B cells elicited a greater frequency of $CD4^+$ T cells that expressed IFN-γ, IL-17, and the autocrine T cell growth factor IL-2 (Figure 6B). Altogether, our findings indicated that IgH[MOG] B cells may shape the initial Ag-specific activation of T cells by promoting their generation of inflammatory cytokines.

Discussion

The role of $CD4^+$ T cells in the autoimmune pathogenesis of MS is well-established. Notably, genome-wide association studies (GWAS) have revealed that polymorphisms in the human leukocyte antigen class II region, which restricts the $CD4^+$ T cell repertoire, are strongly associated with MS susceptibility [26]. Further, many of the current treatments of MS are believed to target T cell responses [27-29]. However, the success of the B cell-depleting drug ocrelizumab in both RRMS and PPMS has intensified recent interest in the contribution of B cells to disease processes [30].

IgH[MOG] mice were initially described on the C57BL/6 (B6) and SJL/J genetic backgrounds. On the B6 background, IgH[MOG] mice showed an increased incidence of EAE, relative to WT, when immunized with whole MOG protein [14]. Intriguingly, IgH[MOG] SJL/J mice developed EAE of greater severity than controls when immunized with the myelin-derived epitope proteolipid protein $(PLP)_{[139-154]}$ [14], which induces a relapsing/remitting disease pattern in SJL/J background mice [31]. This suggested that the presence of MOG-reactive B cells could contribute to EAE pathology that was driven by a class II-restricted peptide; however, the nature of a putative collaboration between B cells and T cells in disease processes remained incompletely defined.

In our study, we actively immunized IgH[MOG] mice on NOD background with the class II restricted peptide $MOG_{[35-55]}$. This permitted us to study the contribution of MOG-reactive B cells in a model of EAE that is initiated by $CD4^+$ T

cells. While WT NOD mice developed a gradual, chronic $MOG_{[35-55]}$-driven disease course, with severe symptoms appearing only many weeks after immunization, we found that 80% of IgH[MOG] NOD mice develop severe and frequently lethal disease within 14 days, that was characterized by inflammation and demyelination in brain, optic nerve and spinal cord. Cellular characterization of mononuclear infiltrate showed an increased frequency of $CD4^+$ T cells, but not B cells, in the CNS of IgH[MOG] relative to controls. It was previously shown that IgH[MOG] x 1C6 double-transgenic mice on the NOD background develop spontaneous EAE, while single-transgenic IgH[MOG] or 1C6 mice do not [25]. These findings indicated that the collaboration of both myelin-specific B and T cells in the same animal could induce CNS autoimmunity; however, it was difficult to determine whether B or T cell-driven responses were initially responsible for disease induction in this model. Here, by using a myelin-derived, class II-restricted, immunogen, we show that B cells augment EAE even when $CD4^+$ T cells initiate disease.

Intriguingly, CNS-infiltrating IgH[MOG] $CD4^+$ T cells generated significant amounts of the type-17 cytokines IL-17 and GM-CSF. Several lines of evidence indicate that Th17 cells can shape B cell responses *in vivo*. Adoptive transfer of myelin-specific Th17 cells induced both B cell isotype class switching and germinal center formation in an IL-17-dependent manner [32]. Further, Th17 cells are crucial for the development of B cell-rich ectopic lymphoid structures [33,34], which are associated with rapid acceleration of the disease. Here, our data

suggest that the inverse may also be true and that B cells may reinforce and augment Th17-mediated pathogenicity in the CNS.

A key antibody independent function of B cells is the antigen presentation to T cells, and B cells are particularly efficient at presenting their cognate antigen [35]. Interestingly, we found that in the presence of $MOG_{[35-55]}$, IgH[MOG] B cells were better able than WT B cells to induce the production of inflammatory cytokines from $MOG_{[35-55]}$-reactive $CD4^+$ T cells. These findings are broadly in line with previous observations that splenocytes from 1C6 x IgH[MOG] mice showed enhanced responses to $MOG_{[35-55]}$ relative to splenocytes from 1C6 single-transgenic animals [25], and they demonstrate that MOG-specific B cells have a greater intrinsic capacity to induce the differentiation of cognate antigen specific T cells. B cell antigen presentation plays a crucial role in autoimmune responses to whole MOG protein, as mice specifically lacking MHC class II expression on B cells (B-MHC-$II^{-/-}$) are resistant to human MOG-induced EAE [24]. Notably, however, B-MHC-$II^{-/-}$ mice develop EAE in response to $MOG_{[35-55]}$. Thus, in mice with an unbiased B cell repertoire, B cell-dependent antigen presentation to T cells may play an important role in the processing and presentation of secondary MOG-derived epitopes. However, when a large frequency of MOG-reactive B cells are initially present, as is the case in IgH[MOG] mice, antigen presentation of $MOG_{[35-55]}$ itself by B cells may exacerbate disease severity.

Conclusion

In this study, we provide evidence that in the presence of a B cell repertoire that is skewed towards MOG, NOD background mice develop unusually severe EAE upon immunization with the classic class II-restricted peptide $MOG_{[35-55]}$. Disease is characterized by an influx of highly inflammatory $CD4^+$ T cells into the CNS. Our findings support a role for myelin-reactive B cells in augmenting T cell-driven CNS autoimmunity.

Abbreviations

Ab, antibody; CNS, central nervous system; CSF, cerebrospinal fluid; CTV, CellTrace Violet; EAE, experimental autoimmune encephalomyelitis; EP, endpoint; GM-CSF, granulocyte and macrophage colony stimulating factor; H&E, hematoxylin & eosin; IFN, interferon; Ig, immunoglobulin; IL, interleukin; LFB, Luxol fast blue; MHC, major histocompatibility complex; MOG, myelin oligodendrocyte glycoprotein; MS, multiple sclerosis; NOD, non-obese diabetic; PLP, proteolipid protein; preonset, PO; RR, relapsing/remitting; SP, secondary progressive; TNF, tumor necrosis factor; UI, unimmunized; WT, wildtype

Declarations

Ethics approval

All mouse breedings and experiments are approved by the Animal Protection Committee of the Centre de recherche du CHU de Quebec - Université Laval (protocols 13-070-2 and 17-090-2).

Consent for publication

Not applicable

Availability of data and materials

Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

Competing interests

The authors declare no competing interests.

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Authors' contributions

PMIAD directed the project, conducted experiments and wrote the manuscript. APY and JB conducted experiments. BM and SL conducted histological analyses. PG and NB assisted with serum detection of immunoglobulins. MR supervised the project and wrote the manuscript. All authors read and approved the final manuscript.

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Figure legends

Figure 1. Active immunization with $MOG_{[35-55]}$ induces severe EAE in IgH[MOG] mice. Female IgH[MOG] (n=10) and WT NOD (n=8) were immunized with $MOG_{[35-55]}$ and were monitored for the development of EAE. IgH[MOG],n=10; NOD, n=8. ** p<0.01, Mann-Whitney *U* test. Representative of 3 experiments and a total of 27 IgH[MOG] and 19 NOD.

Figure 2. Immune cells infiltrated into the CNS of IgH[MOG] mice and caused demyelination. CNS tissues from an immunized female IgH[MOG] recipient were isolated once it reached experimental endpoints, and H&E (**A-D**) and hematoxylin and Luxol fast blue (**E-H**) stainings were conducted. Cerebellum (**A, E**) and pons (**B, F)**, 10 X magnification; spinal cord (**C, G**) and optic nerve (**D, H**), 20X magnification. Scale bar: 100µm. Representative of 5 mice.

Figure 3. Decrease in plasma IgM in immunized IgH[MOG] mice. Plasma were collected from the blood of female NOD or IgH[MOG] mice that were unimmunized (UI) NOD or from $MOG_{[35-55]}$-immunized NOD or IgH[MOG] mice at pre-onset (PO) or disease endpoint (EP). Plasma IgM levels were measured. * $p<0.05$, *t*-test. IgH[MOG], n=4; NOD, n=5.

Figure 4. Increased infiltration of peripheral immune cells in the CNS of immunized IgH[MOG] mice. A, B. IgH[MOG] (n=5) and WT NOD (n=5) female mice were immunized with $MOG_{[35-55]}$, sacrificed at d5, and CNS (**A**) and Splenic (**B**) immune populations ($CD4^+$ T cells, $CD8^+$ T cells, $CD11c^+CD11b^+$ dendritic cells, $CD11c^-Ly6G^-CD11b^+$ macrophages and $Ly6G^+CD11b^+$ neutrophils) were enumerated. **C, D.** IgH[MOG] (n=13) and WT NOD (n=8) female mice were immunized with $MOG_{[35-55]}$ and sacrificed at d14. CNS-infiltrating (**C**) and splenic (**D**) immune cells were enumerated. * $p<0.05$, ** $p<0.001$, *** $p<0.0005$, **** $p<0.0001$, *t*-test n.s., not significant.

Figure 5. CNS-infiltrating IgH[MOG] CD4$^+$ T cells produce increased levels of IL-17 and GM-CSF. IgH[MOG] (n=5) and WT NOD (n=5) female mice were immunized with $MOG_{[35-55]}$ and sacrificed at disease endpoints. Production of IFNγ (**A**), IL-17 (**B**), GM-CSF (**C**) and TNFα (**D**) was assessed by intracellular flow cytometry from CNS-infiltrating CD4$^+$ T cells. * p<0.05, ** p<0.005, *t*-test.

Figure 6. B cells from IgH[MOG] are better antigen presenting cells to CD4$^+$ T cells. B cells from male IgH[MOG] or NOD mice were co-cultured with CellTrace Violet-labeled CD4$^+$ T cells from male 1C6 mice, and with 0,1 or 10µg/ml $MOG_{[35-55]}$. Cell proliferation (**A**) and the production of IFNγ, IL-17 and IL-2 (**B**) by CD4$^+$ T cells were assessed using flow cytometry. Gating percentage in (**A**) corresponds to the percentage of cells that underwent at least one division. **B**, * p<0.05, ** p<0.01, *** p<0.001, two way ANOVA measuring the effect of mouse strain (NOD vs. IgH[MOG]) as a variable. Triplicate cultures, representative of three experiments.

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610

Figure 1

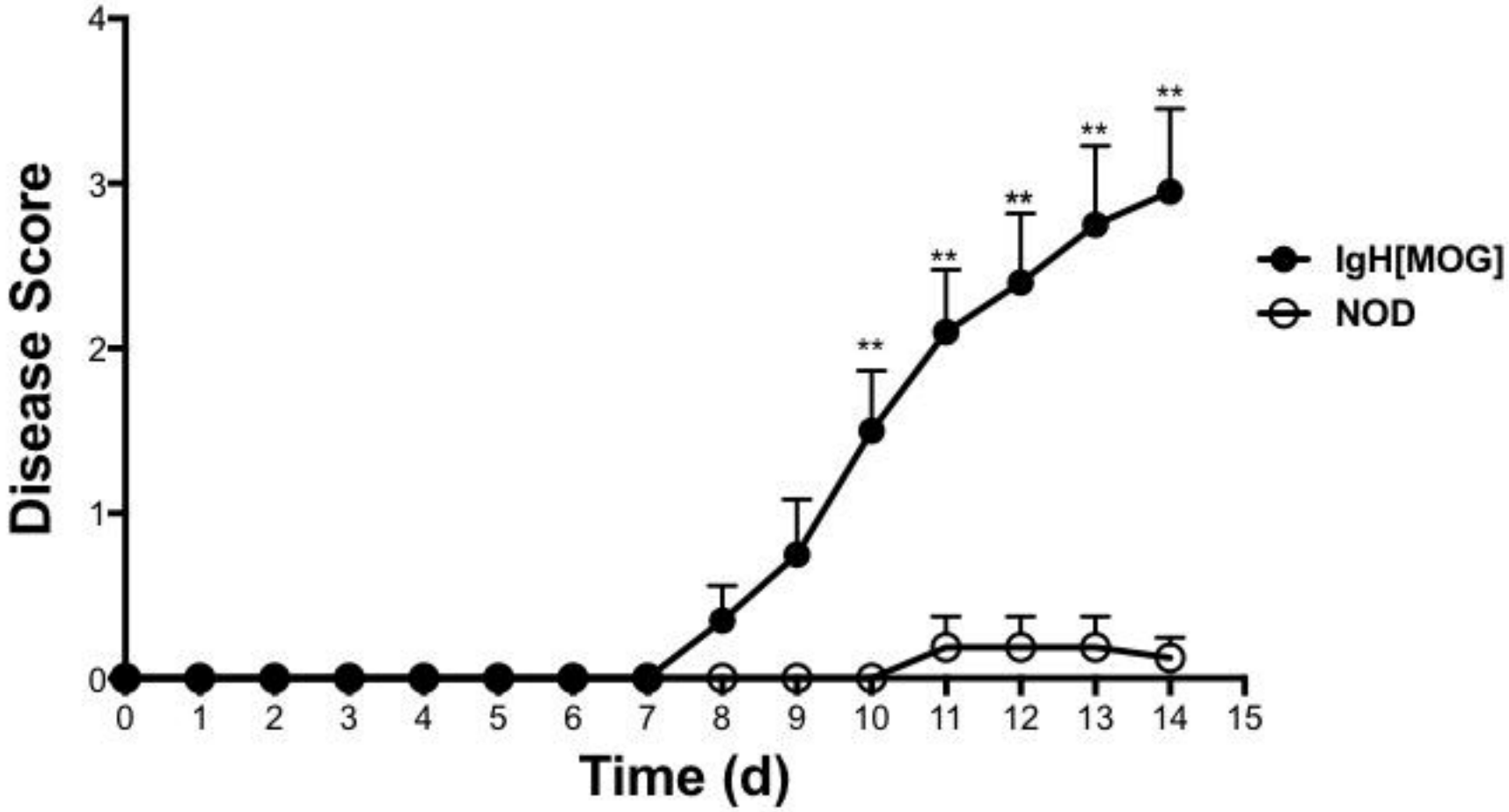
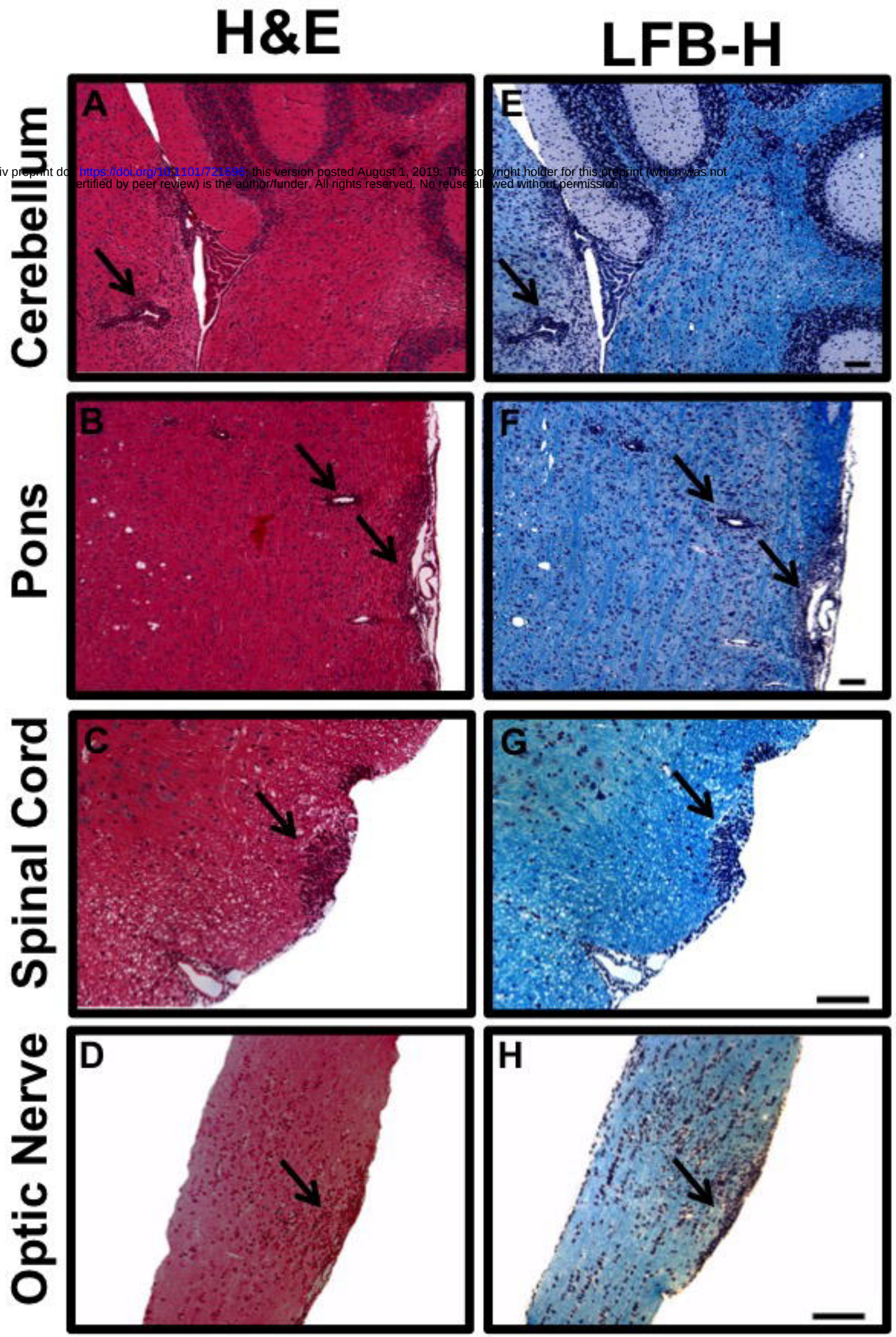


Figure 2



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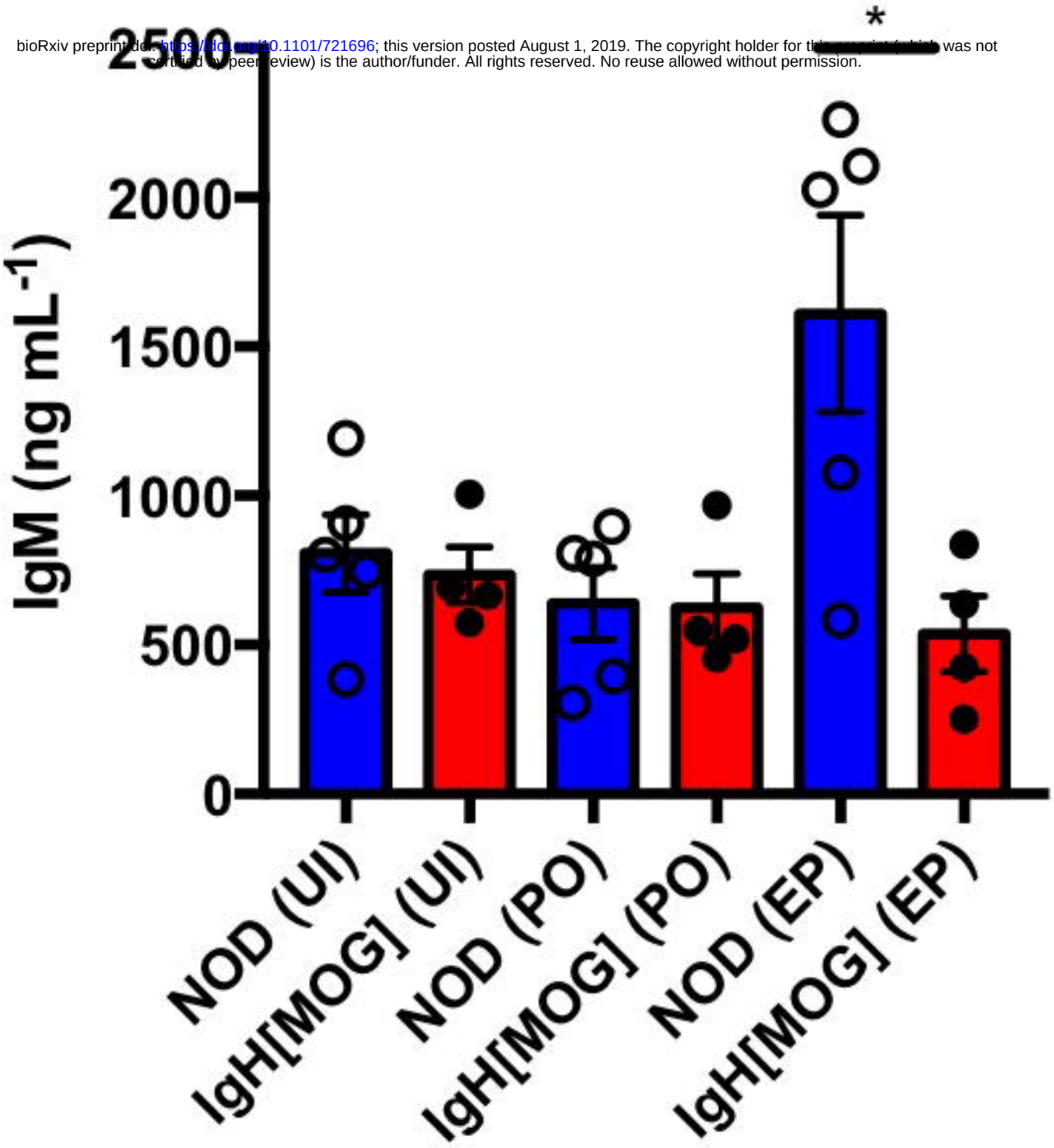

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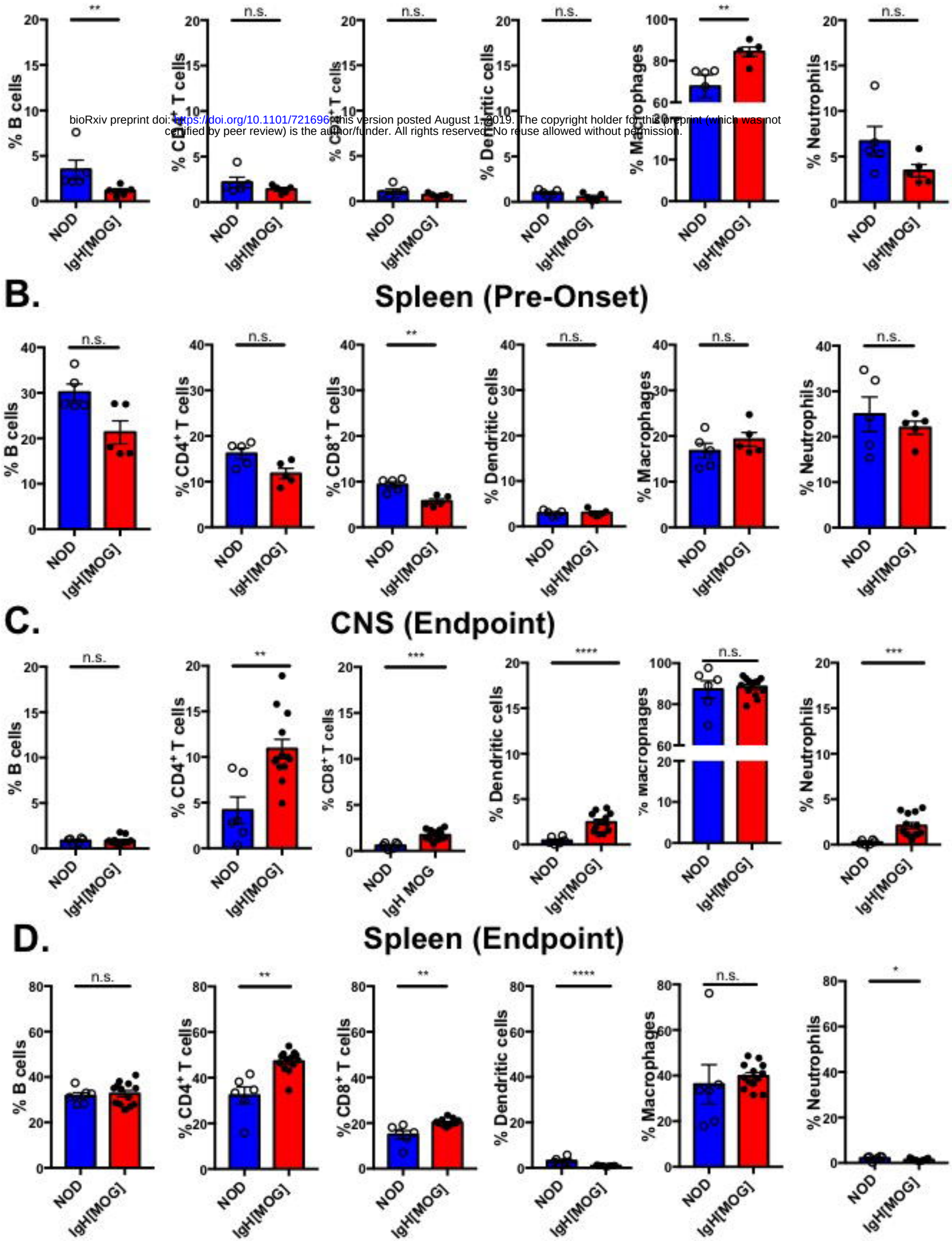
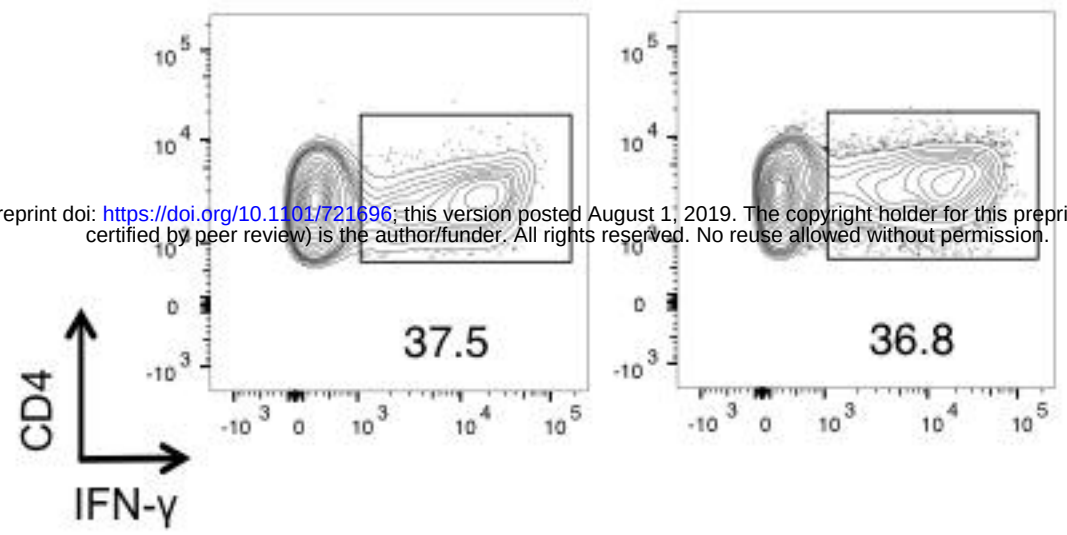


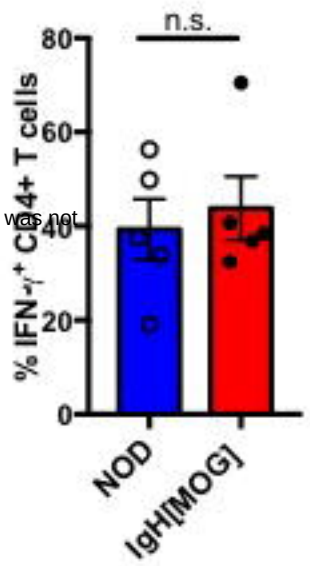
Figure 5

CNS

NOD **IgH[MOG]**

A.





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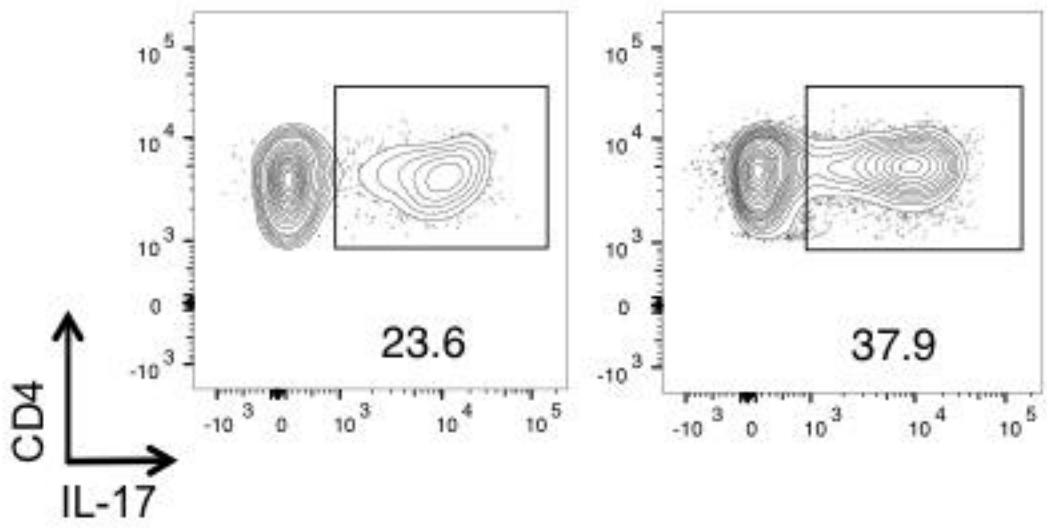

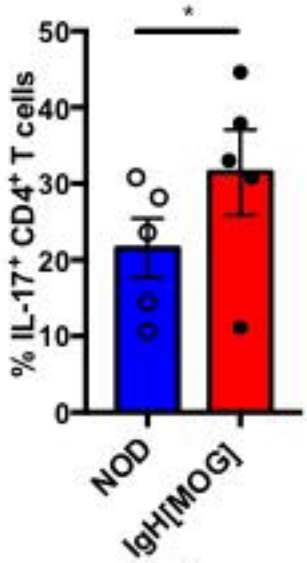

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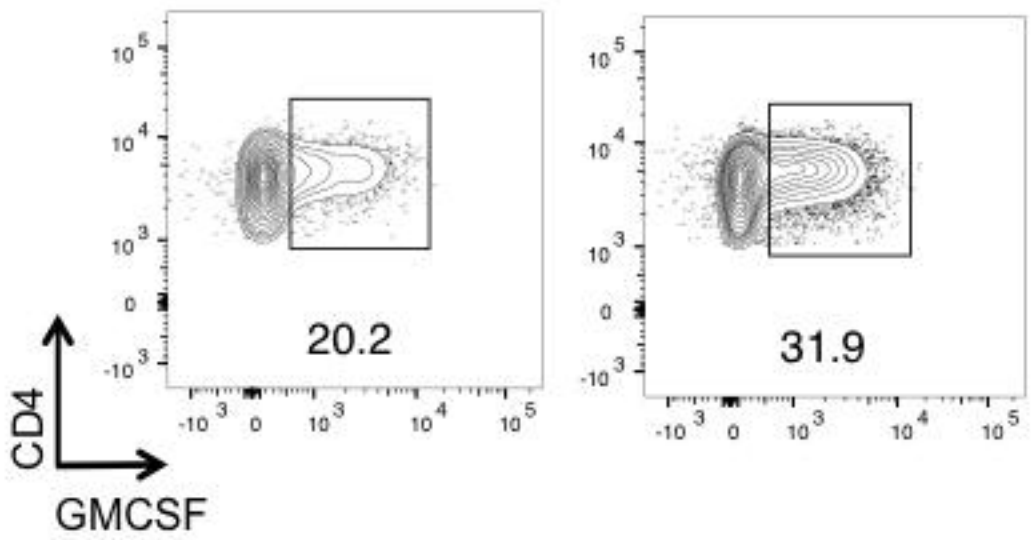
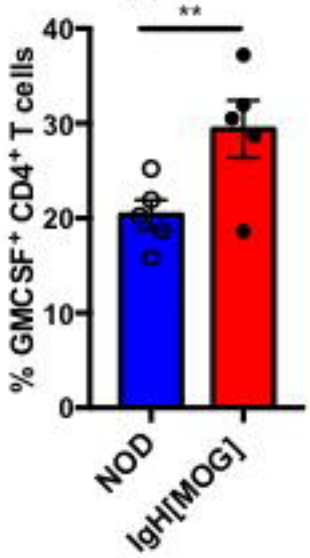
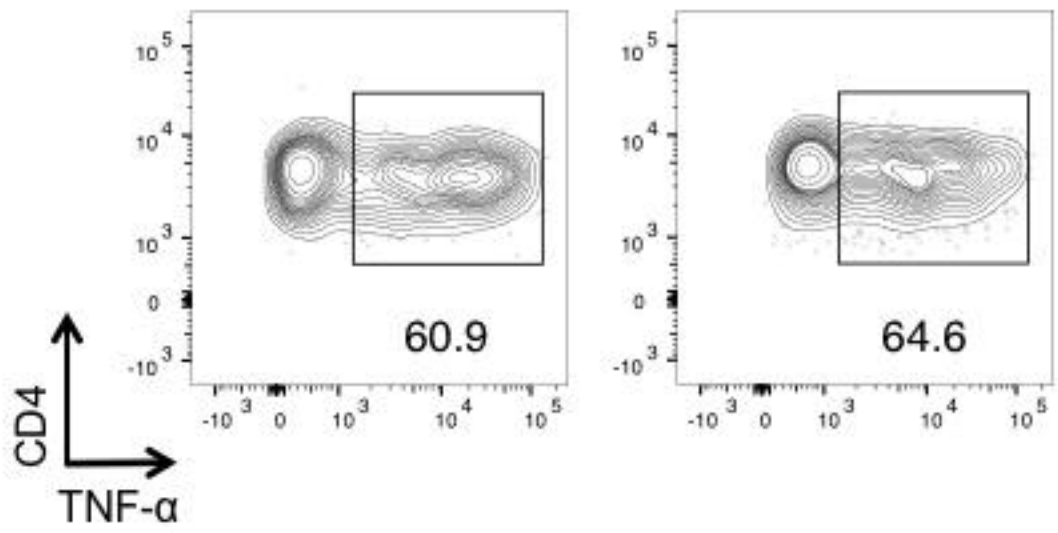




D.



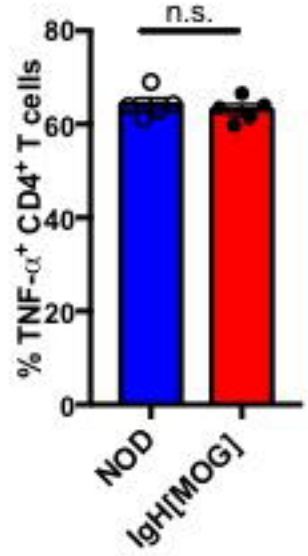
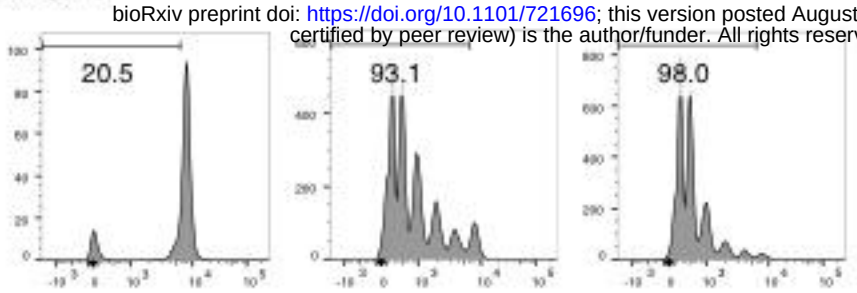


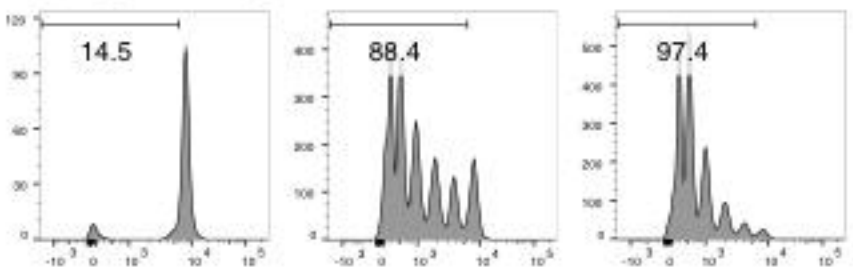
Figure 6

A.

NOD



IgH[MOG]



0 µg/ml 1 10

$MOG_{[35-55]}$

B.