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# Identification of circulating MOG-specific B cells in patients with MOG antibodies

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## Abstract

### Objective

To identify circulating myelin oligodendrocyte glycoprotein (MOG)-specific B cells in the blood of patients with MOG antibodies (Abs) and to determine whether circulating MOG-specific B cells are linked to levels and epitope specificity of serum anti-MOG-Abs.

### Methods

We compared peripheral blood from 21 patients with MOG-Abs and 26 controls for the presence of MOG-specific B cells. We differentiated blood-derived B cells in vitro in separate culture wells to Ab-producing cells via engagement of Toll-like receptors 7 and 8. We quantified the anti-MOG reactivity with a live cell–based assay by flow cytometry. We determined the recognition of MOG epitopes with a panel of mutated variants of MOG.

### Results

MOG-Ab–positive patients had a higher frequency of MOG-specific B cells in blood than controls, but MOG-specific B cells were only detected in about 60% of these patients. MOG-specific B cells in blood showed no correlation with anti-MOG Ab levels in serum, neither in the whole group nor in the untreated patients. Epitope analysis of MOG-Abs secreted from MOG-specific B cells cultured in different wells revealed an intraindividual heterogeneity of the anti-MOG autoimmunity.

### Conclusions

This study shows that patients with MOG-Abs greatly differ in the abundance of circulating MOG-specific B cells, which are not linked to levels of MOG-Abs in serum suggesting different sources of MOG-Abs. Identification of MOG-specific B cells in blood could be of future relevance for selecting patients with MOG-Abs for B cell–directed therapy.

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## Glossary

**Ab** = antibody; **ADEM** = acute disseminated encephalomyelitis; **EGFP** = enhanced green fluorescent protein; **Ig** = immunoglobulin; **IL** = interleukin; **MFI** = mean fluorescence intensity; **MOG** = myelin oligodendrocyte glycoprotein; **PBMCs** = peripheral blood mononuclear cells; **TLR** = Toll-like receptor; **TT** = tetanus toxoid.

Antibodies (Abs) against myelin oligodendrocyte glycoprotein (MOG) are detected in a proportion of patients with inflammatory CNS diseases,[1–4] and there is growing consensus that these patients constitute a separate disease entity.[5–8] Abs against MOG are assumed to be pathogenic, based on in vitro experiments showing oligodendrocyte damage[9] and demyelination in slice cultures[10] and on in vivo transfer experiments with affinity-purified MOG-Abs from patients.[11]

The source of MOG-Abs is largely unexplored. Studies in animal models and human subjects have elaborated different ways to generate long-lasting immunoglobulin (Ig) G production. First, memory B cells could continuously generate short-lived plasma cells on antigen stimulation or via cytokines and Toll-like receptor (TLR) ligands.[12,13] Second, plasma cells might persist for many years in survival niches, e.g., in the bone marrow and continuously release Abs without further stimulation.[14] The optimal therapy for patients with anti-MOG disease is unknown. Current evidence indicates that only a proportion of anti–MOG-positive patients benefits from rituximab.[15–17] This might indicate different pathogenic mechanisms and different sources of MOG-Abs in these patients.

Here, we set out to identify MOG-specific B cells in blood of patients with MOG-Abs and controls by differentiating them ex vivo into Ig-producing cells and quantifying the MOG recognition of the produced IgG. Thereby, we aimed to analyze the abundance of circulating MOG-specific B cells in individual patients and to test whether there is a linkage to serum levels of MOG-Abs. Furthermore, our approach combining in vitro differentiation of B cells in separate wells with determination of epitope recognition allowed identifying intraindividual heterogeneity of anti-MOG autoimmunity.

## Methods

### Population

We analyzed 21 MOG-Ab–positive patients (52% female; mean age ±SD = 40 ± 12 years, range 15–60 years; table) and 26 age- and sex-matched healthy donors (62% female; mean age ±SD = 35 ± 13 years, range 20–61 years).

### Differentiation of PBMCs into Ig-secreting cells

Briefly, $6 \times 10^5$ peripheral blood mononuclear cells (PBMCs) were seeded in 24-well plates in 1 mL/well RPMI medium containing 10% fetal bovine serum. TLR7/8 ligand R848 (2.5 μg/mL; Sigma-Aldrich, St Louis, MO) and interleukin (IL)-2 (1,000 IU/mL; R&D Systems, Minneapolis, MN) were added, and cells were cultured for 7–11 days. This combination of TLR7/8 ligation and IL-2 differentiates $CD19^+CD27^+$ memory B cells into Ig-producing cells, which have different requirements for activation and differentiation than naive B cells.[18] The in vitro stimulation we use in this study induces the production of IgG, IgA, and IgM.[18,19] For limiting dilution assays, PBMCs were distributed from $10^3$ to $10^5$ cells/well in 200 μL and stimulated for 11 days. The frequency of antigen-specific B cells was calculated according to the Poisson distribution.[18,19] Total B-cell frequency was determined by flow cytometry using the anti-human CD19-PerCP-Cy5.5 Ab (SJ25C1; eBioscience, San Diego, CA).

### Flow cytometry for B-cell differentiation markers

Cells were stained using anti-human CD3-Alexa Fluor 700 (OKT3; eBioscience), CD19-APC/Fire 750 (HIB19; BioLegend, San Diego, CA), CD27-Brilliant Violet 605 (O323; BioLegend), CD38-eFluor 450 (HB7; eBioscience), CD138-PE (Mi15; STEMCELL Technologies, Vancouver, Canada), FcR blocking reagent (Miltenyi Biotec, Bergisch Gladbach, Germany), and TO-PRO-3 (Invitrogen, Eugene, OR).

### Enzyme-linked immunosorbent assays

IgG was measured with the human IgG ELISA development kit (Mabtech, Nacka Strand, Sweden). Abs against tetanus toxoid (TT) were determined by coating TT (1 μg/mL; Merck Millipore, Burlington, MA) or bovine serum albumin (BSA, 1 μg/mL; Sigma-Aldrich) and detected by anti-human IgG horseradish peroxidase (Jackson ImmunoResearch, West Grove, PA; 109-036-003).

### Detection of MOG-Abs

MOG-Abs were detected in a live cell assay, as described.[11,20] Briefly, HeLa cells were transiently transfected with human full-length MOG fused C-terminally to enhanced green fluorescent protein (EGFP)-N1 (Clontech Laboratories, Mountain View, CA) or with EGFP alone (control cells). As secondary reagents, biotin-SP-conjugated goat anti-human IgG (Jackson ImmunoResearch, West Grove, PA) and Alexa Fluor 647–conjugated streptavidin (Jackson ImmunoResearch, West Grove, PA) were applied. For the determination of anti-MOG reactivity, we gated on cells with an FITC fluorescence intensity above 500 and determined their mean fluorescence intensity (MFI) in the allophycocyanin channel. For serum (diluted 1:50), we calculated the MFI ratio between MOG-EGFP–transfected cells and cells transfected with EGFP alone. For cell culture supernatants

**Table** Features of patients with anti-MOG reactivity

| ID | Initial diagnosis | Sex | Age at sampling (y) | Reactivity to MOG in serum (MFI ratio)[a] | Treatment at sampling (y) | Duration of disease (y) | Duration of last treatment (y) |
|---|---|---|---|---|---|---|---|
| **4** | MS | F | 57 | 3.4 | Glatiramer acetate | 20 | 13 |
| **7a** | CRION | M | 47 | 47.4 | Azathioprine | 2.5 | 1 |
| **7b** | | | 50 | 32.9 | Azathioprine | 5.3 | 3.8 |
| **13** | ON | M | 38 | 20.5 | None | 4.8 | — |
| **14** | Relapsing bilateral ON | M | 54 | 45.4 | Azathioprine | 28 | 0.5 |
| **16** | NMOSD | M | 30 | 58.0 | Cortisone | 0.2 | 0.1 |
| **17** | Relapsing bilateral ON | F | 33 | 54.4 | Azathioprine | 6.1 | 0.8 |
| **22a** | ON | M | 37 | 7.4 | Cortisone | 0.1 | 0.1 |
| **22b** | | | 38 | 6.2 | Azathioprine | 1.2 | 0.3 |
| **23** | Relapsing bilateral ON | M | 15 | 111.0 | None | 6 | — |
| **24** | ADEM | F | 20 | 3.5 | None | 0.3 | — |
| **25** | MS | F | 59 | 4.5 | Teriflunomide | 4 | 0.8 |
| **26a** | MS | F | 47 | 66.3 | Teriflunomide | 16 | 5 |
| **26b** | | | 47 | 63.9 | Rituximab | 16.2 | 0 |
| **26c** | | | 47 | 62.0 | Rituximab | 16.5 | 0.3 |
| **26d** | | | 47 | 73.1 | Rituximab | 16.8 | 0.7 |
| **28a** | ADEM | F | 34 | 25.5 | None | 0.3 | — |
| **28b** | | | 34 | 19.4 | None | 0.6 | — |
| **31a** | Autoimmune encephalitis | F | 44 | 38.0 | None | 0.5 | — |
| **31b** | | | 44 | 40.1 | None | 0.7 | — |
| **37** | ON | F | 60 | 21.0 | None | 0.1 | 0.1 |
| **38** | Relapsing ON | F | 34 | 199.7 | Rituximab | 9 | 0.1 |
| **39** | Relapsing ON | M | 43 | 213.9 | Rituximab | 8 | 1.3 |
| **40** | NMOSD | F | 41 | 7.5 | Eculizumab | 4 | 3 |
| **41** | ON | M | 37 | 24.8 | Azathioprine | 3.3 | 3.2 |
| **42** | NMOSD | M | 35 | 27.1 | Azathioprine | 20 | 2.2 |
| **43** | Bilateral ON | F | 35 | 32.5 | Azathioprine | 3 | 0.7 |
| **44** | NMOSD | M | 32 | 26.1 | Cyclophosphamide | 0.1 | 0.1 |

Abbreviations: ADEM = acute disseminated encephalomyelitis; CRION = chronic relapsing inflammatory optic neuropathy; MFI = mean fluorescence intensity; MOG = myelin oligodendrocyte glycoprotein; NMOSD = neuromyelitis optica spectrum disorder; ON = optic neuritis.
[a] The cutoff for recognition of human MOG was 2.27 (mean +3 SD of controls).[11,20] The MFI ratio was calculated as the mean of 2–4 experiments.

(used undiluted), the MOG reactivity was determined as delta MFI (reactivity to MOG-transfected cells—reactivity to control transfected cells) because the reactivity to control cells of the cell culture supernatant was close to zero. Negative delta MFI was considered as zero. Threshold was set to mean +3 SD of the values from controls. Values beyond mean +5 SDs were not included in the threshold calculation. The recognition of epitopes on MOG was determined with a panel of mutated variants of MOG essentially as described.[21]

## Statistical analysis

For Mann-Whitney *U* test, the nonparametric, unpaired, and 2-tailed test statistics were performed using GraphPad Prism 7 (GraphPad Software Inc., La Jolla, CA).

## Data availability

Data presented in this study are available upon reasonable request.

## Ethics statement

This study was approved by ethical committees of the Ludwig-Maximilians-Universität Munich and Hacettepe University Ankara. Informed consent was obtained from each donor according to the Declaration of Helsinki.

# Results

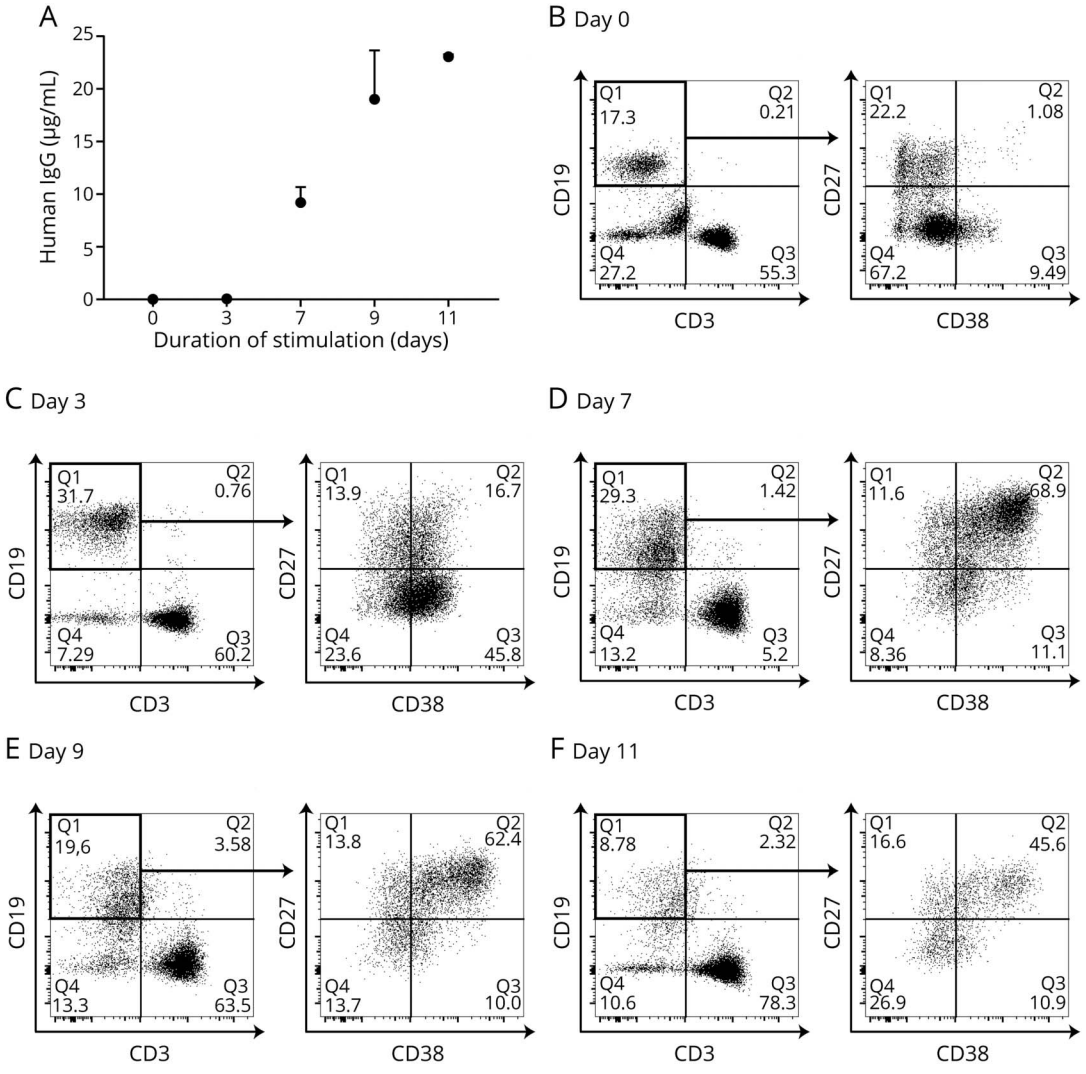
## Differentiation of human B cells in vitro into Ig-secreting cells

We differentiated B cells into antibody-secreting cells and noted a strong IgG production at day 7, which further increased until day 11 (figure 1A), accompanied by development of plasmablasts ($CD3^{-}CD19^{+}CD27^{++}CD38^{++}$) (figure 1, B–F) that made up about 20% of all cells at day 7. At later time points, plasmablasts declined, whereas $CD3^{+}$ T cells prevailed (figure 1F and data not shown). About 10% of the plasmablasts (day 7) coexpressed CD138 (data not shown).

## Identification of MOG-specific B cells in blood in a proportion of patients

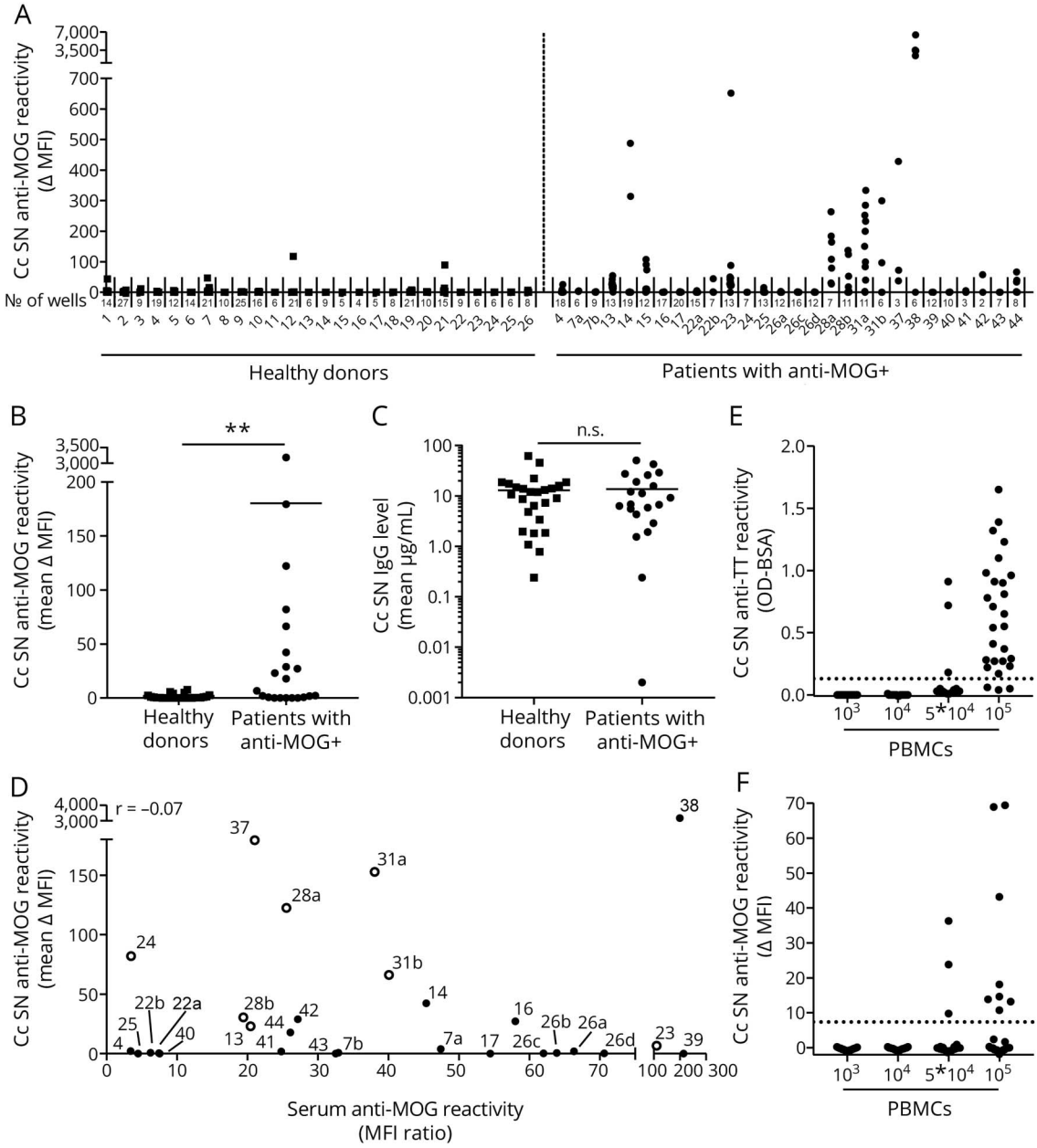
We determined the anti-MOG reactivity of IgG secreted from in vitro differentiated B cells and thereby obtained information on the presence and frequency of MOG-specific B cells in blood. We compared 21 anti–MOG-positive patients with 26 controls (figure 2, A–C). Patient versus control group showed a highly significant difference in anti-MOG reactivity of the in

**Figure 1** Differentiation of human B cells in vitro into Ig-secreting cells




PBMCs of healthy controls were stimulated with IL-2 and R848 for the indicated periods. (A) IgG levels of cell culture supernatants were measured by ELISA. Each dot represents the mean of in total 2 stimulated wells from 2 different individuals. Error bars represent SEM. (B–F) Flow cytometry panels are displayed from 1 representative donor. For each time point, PBMCs were pregated on live and singlet cells. Gates Q1 ($CD3^{-}CD19^{+}$; black rectangles) of left panels were used for further gating on CD27 and CD38 in right panels. Plasmablast formation ($CD3^{-}CD19^{+}CD27^{++}CD38^{++}$) is shown in Q2 of right panels and peaked at day 7 (D). Ig = immunoglobulin; IL = interleukin; PBMCs = peripheral blood mononuclear cells.

**Figure 2** Identification of MOG-specific B cells in blood of patients with MOG-Abs in serum




(A and B) PBMCs from MOG-Ab–positive patients (n = 21) and healthy donors (n = 26) were stimulated with IL-2 and R848. Anti-MOG reactivity in cc SNs was determined. Each dot represents 1 stimulated well. The number of stimulated wells is enclosed directly under the x-axis. (B and C) Each symbol represents the mean of all stimulated wells in 1 donor. Horizontal lines indicate the mean of all donors. (B) MOG-Ab production was significantly higher in patients than in controls (Mann-Whitney *U* test). (C) IgG levels of cc SNs were not significantly different between the 2 groups (Mann-Whitney *U* test). (D) Comparison of MOG-Ab levels in serum of patients and cc SNs of stimulated PBMCs. The mean anti-MOG reactivity of the stimulated PBMCs did not correlate with MOG-Ab serum levels in the respective patients (Spearman correlation; $r_{all} = -0.07$). Open circles indicate samples from patients with no treatment at time point of blood withdrawal ($r_{untreated} = -0.12$). (E–F) Limiting dilution analysis with PBMCs from anti–MOG-positive patient 24. PBMCs were seeded at concentrations of $10^3$ (17 wells), $10^4$ (17 wells), $5 \times 10^4$ (17 wells), and $10^5$ (27 wells) cells/well and cultured for 11 days in the presence of IL-2 and R848. (E) TT production was assessed by ELISA. Dotted line indicates applied cutoff calculated as mean + 3 SD of negative wells. (F) MOG-Abs in cell culture supernatants were analyzed by flow cytometry with transfected cells. Dotted line indicates applied cutoff calculated as mean +4 SD of negative wells. According to the Poisson distribution in whole PBMCs, the frequency of MOG-specific B cells in patient 24 is calculated as 1:224,000 and for TT 1:68,000. $^{**}p \leq 0.01$. Ab = antibody; BSA = bovine serum albumin; cc SN = cell culture supernatant; Ig = immunoglobulin; IL = interleukin; MFI = mean fluorescence intensity; MOG = myelin oligodendrocyte glycoprotein; ns = not significant; OD = optical density; PBMCs = peripheral blood mononuclear cells; TT = tetanus toxoid.

vitro differentiated B cells (figure 2B), while similar amounts of total IgG were produced (figure 2C).

A closer look at the patient group revealed a striking heterogeneity. In some patients, MOG-specific B cells were present in each well, in others in the majority of wells, and yet in others, no anti-MOG reactivity was detected in the secreted IgG. In 13/21 (about 60%) of anti–MOG-positive patients, we noted anti-MOG reactivity in at least 1 cultured well (figure 2A). The total amount of IgG produced

in vitro was similar in the samples from patients with (mean IgG = 6.82 μg/mL, n = 13) or without MOG-specific B cells (mean IgG = 8.82 μg/mL, n = 8) in their blood (data not shown).

From 5 patients, we could analyze samples obtained at different time points, and this showed the stability of our approach: From patient 7, 2 samples with a time interval of 3 years were negative. Likewise, both samples of patient 22 obtained with an interval of 1 year were negative. For patients 28a/b (interval of 4 months) and 31a/b (interval of 1 month), we could detect a positive signal for both time points. Patient 26 (no treatment for a/b; rituximab for c/d; all within 1 year) only showed a marginal positive signal in 1 well for the first blood sampling and was completely negative for samples b-d (figure 2A and table). We noted that in 4/26 healthy donors, a reactivity toward MOG was seen in at least 1 well (figure 2A).

We set out to determine the frequency of MOG-specific B cells in those patients where our first round of analysis indicated the presence of circulating MOG-specific B cells and where further samples were available. We performed a limiting dilution assay with samples from patients 24, 28, and 31. We calculated a frequency of about 1 MOG-specific B cell in $4.5 \times 10^4$ B cells and about 1 TT-specific B cell in $1.4 \times 10^4$ B cells for patient 24 (figure 2, E, F). Patient 28 had about 1 MOG-specific B cell in $1.4 \times 10^5$ B cells and about 1 TT-specific B cell in $8.3 \times 10^4$ B cells; patient 31 had about 1 MOG-specific B cell in $8.8 \times 10^4$ B cells and about 1 TT-specific B cell in $3.9 \times 10^3$ B cells.

### MOG-specific B cells in blood and anti-MOG levels in serum did not correlate

Within the patient group, the amount of anti-MOG IgG produced after in vitro stimulation was not linked to the level of anti-MOG reactivity in serum (r = −0.07; figure 2D). We selectively analyzed the 8 samples we obtained from 6 patients who were untreated at the time of blood sampling. Also, in these samples, no correlation between circulating anti-MOG B cells and serum anti-MOG level was observed (open circles in figure 2D; r = −0.12).

### Intraindividual heterogeneity of the anti-MOG response

We combined the B-cell differentiation in separate wells with the analysis of epitope reactivity. This was performed with samples from 6 patients. We show original data from selected wells of 2 patients (figure 3A) and the summary of all analyzed wells (figure 3B). The in vitro differentiated B-cell cultures reflected the fine specificity of the serum in 27/37 wells. Looking at individuals, this analysis revealed an intraindividual heterogeneity of the anti-MOG response in 4 of 6 patients that was not detectable when analyzing only serum.

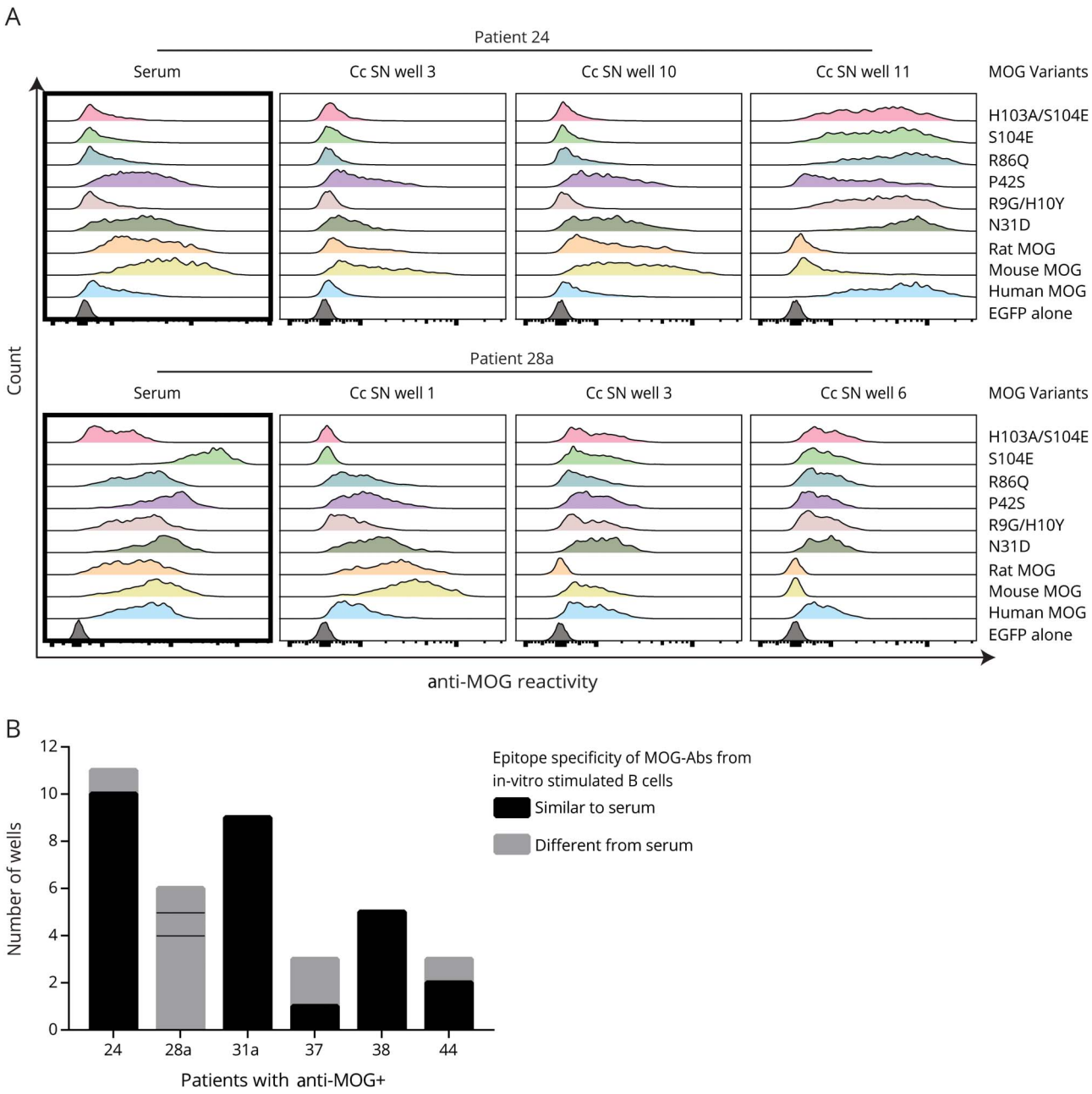
## Discussion

Here, we describe circulating MOG-specific B cells in a proportion of patients with MOG-Abs. Although it is frequently a challenge to identify antigen-specific autoreactive T cells in patients with autoimmune diseases, the method we apply here is useful to quantify not only highly abundant antigen-specific B cells after infection and vaccination[18] but also autoreactive B cells such as MOG-specific B cells, which occur at much lower frequency. We have identified MOG-specific B cells by differentiating them into plasmablasts and then determining the in vitro development of MOG-Abs with a cell-based assay. An alternative method to enumerate antigen-specific B cells is the usage of a purified and labeled antigen.[22] The extracellular domain of recombinant MOG, however, does not completely mirror MOG in transfected cells.[11] We had used the recombinant extracellular part of MOG to form a tetramer, sorted B cells binding this MOG and produced their Ig in a recombinant way; we then found that these recombinant MOG-Abs bound MOG by ELISA, but did not bind to MOG on transfected cells (unpublished observation). Thus, the method we applied in this article is the first choice to identify and quantify MOG-specific B cells. The quantity of MOG-specific B cells was much lower than for the recall antigen TT. This reveals a difference to GAD65 autoimmunity, where GAD65-specific B cells were as abundant as B cells specific for recall antigens.[19]

The differentiation of B cells into antibody-secreting cells after TLR stimulation is a general feature of human memory B cells.[18] TLR7/8 stimulation, as applied in this study, induced MOG-Ab production provided the patient had preexisting MOG-specific B cells. TLR7/8 recognize single-stranded RNA viruses such as influenza virus; TLR9, which recognizes unmethylated CpG dinucleotide motifs located in bacterial and viral DNA, also mediates plasma cell differentiation.[18] Children with acute disseminated encephalomyelitis (ADEM)[1] and adult patients with optic neuritis and MOG-Abs frequently had an infectious prodrome.[23] The development of MOG-Abs after genital herpes has been described.[24] Attacks were preceded by infection in about 40% of anti–MOG-positive patients as seen in a multicentre study with 50 patients.[15] These clinical observations and our in vitro studies suggest that MOG-Abs can be induced on TLR stimulation. We noted that in 4/26 control donors, B cells could also be differentiated into MOG-Ab–producing cells in vitro. This is in line with the concept that autoreactive immune cells are part of the normal repertoire.[25] This is not necessarily linked to autoimmune pathology, but may reflect the susceptibility to develop autoantibodies, in the context of infections.

The extent of diversity of the individual anti-MOG response has been unknown. Our previous work with mutated variants of MOG has shown that individual patients respond to mutations at different loops of MOG; but this does not allow for conclusions about the heterogeneity of the anti-MOG response because MOG is so small that the maximal dimensions of a single Ab epitope (2.1 × 2.8 nm)[26] span a great area of the surface of MOG.[27] The approach we use here—differentiating B cells in separate wells and combining this with epitope analysis—allows identifying intraindividual heterogeneity of the anti-MOG autoimmunity.

**Figure 3** Analysis of the intraindividual heterogeneity of the B-cell response to MOG




The cc SNs of individual wells with anti-MOG reactivity and the serum were further analyzed for recognition of mutants of MOG. (A) Flow cytometry histograms of selected cc SNs and serum from 2 patients. The MOG reactivities of the serum samples are framed. From patient 24, serum, cc SN well 3, and cc SN well 10 had the same pattern of reactivity to the MOG variants, whereas cc SN well 11 was different. From patient 28, serum and all cc SN samples showed a different reactivity to at least 1 MOG variant. (B) Summary of anti-MOG heterogeneity from 37 cc SNs from 6 patients. Cultured wells with the same reactivity pattern as found in serum of the respective patient are shown in black; those which differ from the pattern found in serum are shown in gray. In the blood sample 28a, 3 different patterns of anti-MOG reactivity could be dissected, indicated by the black lines, details in (A). cc SN = cell culture supernatant; MOG = myelin oligodendrocyte glycoprotein.

We found a highly significant difference in the frequency of MOG-specific B cells between patients and controls; but a closer look at the group with MOG-Abs revealed 2 subsets; in our study, about 60% of patients with MOG-Abs in serum had MOG-specific B cells in blood. This stratification of patients with MOG-Abs is not related to the intensity of the anti-MOG response in serum. In this respect, the autoimmunity against MOG is different to autoimmunity against AQP4 and NMDA-R, where a close correlation between serum levels of autoantibodies and circulating autoreactive B cells has been described.[28,29]

One limitation of our study is that some patients were under immunosuppressive treatment at the time of blood withdrawal; also, the number of patients with the same clinical phenotype and the same therapy is limited. However, despite

immunosuppressive treatment, patients had circulating MOG-Abs and also MOG-specific B cells in blood, consistent with other studies examining B cells of treated patients with other autoantibodies.[19,28,29] Furthermore, we had the chance to analyze blood cells from 6 patients with MOG-Abs before the onset of treatment, and these patients are very similar to the total cohort of patients in terms of abundance of MOG-specific B cells and lack of correlation between serum anti-MOG and circulating MOG-specific B cells.

The lack of linkage between autoantibodies to MOG and circulating MOG-specific B cells indicates different sources of the anti-MOG-Abs. Two sources have to be considered: long-lived plasma cells, which are negative for CD20, and $CD20^+$ memory B cells that are readily differentiated into anti–MOG-secreting cells.[12–14] MOG-Abs are transient in patients with an ADEM-like phenotype, whereas they persist for many years in others.[11,20,27,30]

The function of B cells extends beyond antibody production. B cells are extremely potent presenters of antigens that bind to their surface Igs; they selectively internalize their antigen and present it to T cells at concentrations $10^3$- to $10^4$-fold lower than required for presentation by nonspecific B cells or monocytes.[31] In animal models, MOG-specific B cells were essential as antigen-presenting cells to drive activation of MOG-specific T cells and encephalitis,[32] and in addition, MOG-specific Abs enhanced activation of cognate MOG-specific T cells.[11,33,34] Furthermore, B cells produce proinflammatory cytokines such as GM-CSF.[35]

The rationale for anti-CD20 therapy in patients with MOG-Abs is twofold: reduction of autoantibodies and elimination of B cells as central drivers of the immune response. The effect of rituximab on autoantibody levels is particularly strong in autoimmune diseases driven by IgG4 autoantibodies.[36] MOG-Abs are typically IgG1,[37] and previous results obtained with small cohorts showed that MOG-Abs may persist after rituximab,[27,38] but larger longitudinal studies are still pending. Clinically, only a proportion of patients with MOG-Abs respond to B-cell depletion,[15–17] and there is no biomarker for predicting the therapeutic response to anti-CD20: Treatment with the B cell–depleting Ab rituximab led to a decrease in the relapse rate in only 3/9 patients.[15] An international consortium analyzed the response to rituximab in 98 patients and reported that the overall response was weaker than in anti–AQP4-positive patients, and only a proportion of anti–MOG-positive patients benefited from rituximab.[16] In an Austral-Asian study, 1/6 patients failed to respond to rituximab.[17] The different responses to anti-CD20 might indicate different pathogenic mechanisms and different sources of MOG-Abs in these patients. Our study shows that MOG-Ab positive differ in the abundance of circulating MOG-specific B cells. Whether anti–MOG-positive patients with MOG-specific B cells in blood are preferred candidates for B cell depleting therapy needs to be assessed in future studies. Longitudinal observations from a decent number of patients are needed to analyze effects of therapies on circulating MOG-specific B cells. Our study shows that such examinations could be performed with frozen PBMCs, so a central analysis could be performed of PBMCs collected within a consortium.

Together, we show that circulating MOG-specific B cells are present in a proportion of patients with MOG-Abs and that their abundance is not linked to anti-MOG levels in serum. Our approach of differentiating B cells in separate wells and testing then the epitope specificity of the MOG-specific B cells gives insight into the intraindividual heterogeneity of the anti-MOG autoimmunity.

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## Appendix Authors

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## References


1. Reindl M, Waters P. Myelin oligodendrocyte glycoprotein antibodies in neurological disease. Nat Rev Neurol 2019;15:89–102.
2. Ramanathan S, Dale RC, Brilot F. Anti-MOG antibody: the history, clinical phenotype, and pathogenicity of a serum biomarker for demyelination. Autoimmun Rev 2016;15:307–324.
3. Hohlfeld R, Dornmair K, Meinl E, Wekerle H. The search for the target antigens of multiple sclerosis, part 2: CD8+ T cells, B cells, and antibodies in the focus of reverse-translational research. Lancet Neurol 2016;15:317–331.
4. Ogawa R, Nakashima I, Takahashi T, et al. MOG antibody-positive, benign, unilateral, cerebral cortical encephalitis with epilepsy. Neurol Neuroimmunol Neuroinflamm 2017;4:e322. doi: 10.1212/NXI.0000000000000322.
5. Zamvil SS, Slavin AJ. Does MOG Ig-positive AQP4-seronegative opticospinal inflammatory disease justify a diagnosis of NMO spectrum disorder? Neurol Neuroimmunol Neuroinflamm 2015;2:e62. doi: 10.1212/NXI.0000000000000062.
6. Weber MS, Derfuss T, Metz I, Bruck W. Defining distinct features of anti-MOG antibody associated central nervous system demyelination. Ther Adv Neurol Disord 2018;11:1756286418762083.
7. Jurynczyk M, Jacob A, Fujihara K, Palace J. Myelin oligodendrocyte glycoprotein (MOG) antibody-associated disease: practical considerations. Pract Neurol 2019;19:187–195.
8. Jarius S, Paul F, Aktas O, et al. MOG encephalomyelitis: international recommendations on diagnosis and antibody testing. J Neuroinflammation 2018;15:134.
9. Dale RC, Tantsis EM, Merheb V, et al. Antibodies to MOG have a demyelination phenotype and affect oligodendrocyte cytoskeleton. Neurol Neuroimmunol Neuroinflamm 2014;1:e12. doi: 10.1212/NXI.0000000000000012.
10. Peschl P, Schanda K, Zeka B, et al. Human antibodies against the myelin oligodendrocyte glycoprotein can cause complement-dependent demyelination. J Neuroinflammation 2017;14:208.
11. Spadaro M, Winklmeier S, Beltran E, et al. Pathogenicity of human antibodies against myelin oligodendrocyte glycoprotein. Ann Neurol 2018;84:315–328.
12. Bernasconi NL, Traggiai E, Lanzavecchia A. Maintenance of serological memory by polyclonal activation of human memory B cells. Science 2002;298:2199–2202.
13. Traggiai E, Puzone R, Lanzavecchia A. Antigen dependent and independent mechanisms that sustain serum antibody levels. Vaccine 2003;21(2 suppl):S35–S37.
14. Manz RA, Thiel A, Radbruch A. Lifetime of plasma cells in the bone marrow. Nature 1997;388:133–134.
15. Jarius S, Ruprecht K, Kleiter I, et al. MOG-IgG in NMO and related disorders: a multicenter study of 50 patients. Part 2: epidemiology, clinical presentation, radiological and laboratory features, treatment responses, and long-term outcome. J Neuroinflammation 2016;13:280.
16. Whittam D, Cobo-Calvo A, Lopez-Chiriboga AS, et al. Treatment of MOG-IgG-associated demyelination with Rituximab: a multinational study of 98 patients (S13.003). Neurology 2018;90.
17. Ramanathan S, Mohammad S, Tantsis E, et al. Clinical course, therapeutic responses and outcomes in relapsing MOG antibody-associated demyelination. J Neurol Neurosurg Psychiatry 2018;89:127–137.
18. Pinna D, Corti D, Jarrossay D, Sallusto F, Lanzavecchia A. Clonal dissection of the human memory B-cell repertoire following infection and vaccination. Eur J Immunol 2009;39:1260–1270.
19. Thaler FS, Thaller AL, Biljecki M, et al. Abundant glutamic acid decarboxylase (GAD)-reactive B cells in gad-antibody-associated neurological disorders. Ann Neurol 2019;85:448–454.
20. Spadaro M, Gerdes LA, Krumbholz M, et al. Autoantibodies to MOG in a distinct subgroup of adult multiple sclerosis. Neurol Neuroimmuno Neuroinflamm 2016;3:e257. doi: 10.1212/NXI.0000000000000257.
21. Mayer MC, Breithaupt C, Reindl M, 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 2013;191:3594–3604.

22. Doucett VP, Gerhard W, Owler K, Curry D, Brown L, Baumgarth N. Enumeration and characterization of virus-specific B cells by multicolor flow cytometry. J Immunol Methods 2005;303:40–52.
23. Ramanathan S, Reddel SW, Henderson A, et al. Antibodies to myelin oligodendrocyte glycoprotein in bilateral and recurrent optic neuritis. Neurol Neuroimmunol Neuroinflamm 2014;1:e40. doi: 10.1212/NXI.0000000000000040.
24. Nakamura M, Iwasaki Y, Takahashi T, et al. A case of MOG antibody-positive bilateral optic neuritis and meningoganglionitis following a genital herpes simplex virus infection. Mult Scler Relat Disord 2017;17:148–150.
25. Cohen IR. Activation of benign autoimmunity as both tumor and autoimmune disease immunotherapy: a comprehensive review. J Autoimmun 2014;54:112–117.
26. Ramaraj T, Angel T, Dratz EA, Jesaitis AJ, Mumey B. Antigen-antibody interface properties: composition, residue interactions, and features of 53 non-redundant structures. Biochim Biophys Acta 2012;1824:520–532.
27. Spadaro M, Gerdes LA, Mayer MC, et al. Histopathology and clinical course of MOG-antibody associated encephalomyelitis. Ann Clin translational Neurol 2015;2:295–301.
28. Wilson R, Makuch M, Kienzler AK, et al. Condition-dependent generation of aquaporin-4 antibodies from circulating B cells in neuromyelitis optica. Brain 2018;141:1063–1074.
29. Makuch M, Wilson R, Al-Diwani A, et al. N-methyl-D-aspartate receptor antibody production from germinal center reactions: therapeutic implications. Ann Neurol 2018;83:553–561.
30. Pröbstel AK, Dornmair K, Bittner R, et al. Antibodies to MOG are transient in childhood acute disseminated encephalomyelitis. Neurology 2011;77:580–588.
31. Lanzavecchia A. Antigen-specific interaction between T and B cells. Nature 1985;314:537–539.
32. Molnarfi N, Schulze-Topphoff U, Weber MS, 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 2013;210:2921–2937.
33. Kinzel S, Lehmann-Horn K, Torke S, et al. Myelin-reactive antibodies initiate T cell-mediated CNS autoimmune disease by opsonization of endogenous antigen. Acta Neuropatho 2016;132:43–58.
34. Flach AC, Litke T, Strauss J, et al. Autoantibody-boosted T-cell reactivation in the target organ triggers manifestation of autoimmune CNS disease. Proc Natl Acad Sci U S A 2016;113:3323–3328.
35. Li R, Rezk A, Miyazaki Y, et al. Proinflammatory GM-CSF-producing B cells in multiple sclerosis and B cell depletion therapy. Sci Transl Med 2015;7:310ra166.
36. Huijbers MG, Plomp JJ, van der Maarel SM, Verschuuren JJ. IgG4-mediated autoimmune diseases: a niche of antibody-mediated disorders. Ann N Y Acad Sci 2018;1413:92–103.
37. Waters P, Woodhall M, O'Connor KC, et al. MOG cell-based assay detects non-MS patients with inflammatory neurologic disease. Neurol Neuroimmunol Neuroinflamm 2015;2:e89. doi:10.1212/NXI.0000000000000089.
38. Choi SJ, Kim B, Lee HJ, Kim SJ, Kim SM, Sung JJ. Rebound of relapses after discontinuation of rituximab in a patient with MOG-IgG1 positive highly relapsing optic neuritis: a case report. BMC Neurol 2018;18:216.