Effects of Natalizumab Therapy on Intrathecal Immunoglobulin G Production Indicate Targeting of Plasmablasts

Miriam Schlüter, MD, Eva Oswald, BSc, Stephan Winklmeier, MSc, Ingrid Meinl, MD, Joachim Havla, MD, Peter Eichhorn, MD, Edgar Meinl, MD,* and Tania Kümpfel, MD*

Correspondence
Dr. Kümpfel
tania.kuempfel@
med.uni-muenchen.de

Abstract

Objectives

To evaluate the long-term effects of natalizumab (NTZ) on different features of intrathecal immunoglobulin (Ig) synthesis in patients with multiple sclerosis (MS) and to quantify the expression of α4-integrin in stages of B-cell maturation.

Methods

We combined a cross-sectional (49 NTZ-treated MS patients, mean treatment duration 5.1 years, and 47 untreated MS patients) and a longitudinal study (33 patients with MS before and during NTZ, mean treatment duration: 4.8 years), analyzing paired serum and CSF samples for IgG, IgA, and IgM levels, reactivity against selected viruses (measles virus, rubella virus, and varicella zoster virus [MRZ] reaction), and oligoclonal bands (OCBs). Banding patterns before and after therapy were directly compared by isoelectric focusing in 1 patient. In addition, we determined the expression of α4-integrin by FACS analysis on blood-derived B-cell subsets (plasmablasts, memory B cells, and naive B cells) of healthy controls.

Results

In serum, NTZ decreased IgM and IgG, but not IgA, levels. IgM hypogammaglobulinemia occurred in 28% of NTZ-treated patients. In CSF, NTZ treatment resulted in a strong reduction of intrathecally produced IgG and, to a lesser extent, IgA, whereas IgM indices [(Ig CSF/Serum)/(Albumin CSF/Serum)] remained largely unchanged. Reduction of the IgG index correlated with NTZ treatment duration, as did serum IgM and IgA levels. MRZ reaction was unchanged and OCB persisted. Direct comparison of OCB pattern before and after NTZ revealed the persistence of individual bands. α4-Integrin expression was highest on plasmablasts (CD19$^+$CD38$^+$CD27$^+$).

Conclusion

Our data indicate that NTZ reduces short-lived plasmablasts in the CNS compartment but has little effect on locally persisting long-lived plasma cells.

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*These authors contributed equally to this work.

From the Institute of Clinical Neuroimmunology (M.S., E.O., S.W., I.M., J.H., E.M., T.K.), Biomedical Center and LMU Klinikum; and Institute of Laboratory Medicine (P.E.), LMU Klinikum, Munich, Germany.

Glossary

DMT = disease-modifying therapy; **EID** = extended interval dosing; **HC** = healthy control; **Ig** = immunoglobulin; **LP** = lumbar puncture; **MFI** = mean fluorescence intensity; **MRZ** = measles virus, rubella virus, and varicella zoster virus; **NTZ** = natalizumab; **OCBs** = oligoclonal bands; **PML** = progressive multifocal leukoencephalopathy; **RRMS** = relapsing-remitting MS.

A hallmark of multiple sclerosis (MS) is intrathecal immuno-globulin (Ig) production,[1,2] which has different features. First, oligoclonal bands (OCBs), mainly formed by IgG1[1]; second, local IgG production against different pathogens such as measles, rubella, and varicella zoster virus (MRZ reaction)[3,4]; third, a large part of the intrathecal IgG production exceeds OCB and MRZ reaction.[5] Principally, there are 2 types of Ig-producing cells: short-lived plasmablasts and long-lived plasma cells.[6] The latter persist in survival niches, physiologically in the bone marrow, but also in the inflamed CNS.[5,7] Intrathecal IgG production strongly correlates with the number of plasmablasts in the CSF.[8,9]

Although the bidirectional migration of B cells between blood and CNS has been elaborated,[10] the role of adhesion molecules in maintenance of plasmablasts and plasma cells in the CNS is poorly understood. Natalizumab (NTZ) is a highly effective therapy for relapsing-remitting MS (RRMS) and prevents migration of immune cells into the CNS by targeting α4-integrin. NTZ has strong effects on the B-cell compartment in the periphery[11-14] and in the CNS.[15-19] Here, we analyzed the CSF of 49 patients with MS who were treated with NTZ for a mean of more than 5 years and underwent another lumbar puncture (LP) to exclude progressive multifocal leukoencephalopathy (PML) before switching to a different therapy. We analyzed effects of NTZ treatment on the different features of intrathecal Ig production and additionally determined α4-integrin expression on blood-derived B-cell subsets of healthy controls (HCs).

Methods

Patients and Samples

In this monocentric study, we investigated 2 groups of patients: group 1 consisted of 49 patients with RRMS treated with NTZ; group 2 consisted of 47 consecutive, age-matched, untreated patients with RRMS. Six of 49 NTZ-treated patients (12%) received NTZ at an extended interval dosing (EID) of 6 weeks, 3 of them started EID after up to 3.5 years (mean total time of EID: 1.4 years), and 3 patients only after more than 9 years of NTZ treatment (mean total time of EID: 0.9 years); all 6 continued EID until cessation of NTZ treatment. Clinical features of all patients including disease duration, disease severity (Expanded Disability Status Scale), NTZ therapy duration, and disease-modifying treatments (DMTs) before NTZ are summarized in table 1.

All patients underwent LP for clinical and diagnostic purposes. OCB status was available for 33 patients before NTZ

treatment. Immunoglobulin data from a LP before NTZ therapy were available for 27 patients with MS in the NTZ-treated group. All 49 NTZ-treated patients switched therapy because of the risk of PML and underwent another LP to exclude PML before timely start of subsequent cell-depleting therapies. CSF and blood sampling at second LP was performed around the time point of patients' last NTZ application (mean time from the last NTZ infusion to lumbar puncture +11 days; range −30 to 113 days). Untreated MS patients had a diagnostic LP usually at first diagnosis. CSF and serum were analyzed by routine methods for cell count, total protein and albumin levels, OCB, IgG, IgM, and IgA. Whenever possible, residual CSF/serum samples were immediately centrifuged and supernatants stored at −80°C. None of the patients, NTZ treated or untreated, underwent plasma exchange therapy in the 30 days before LP.

Cross-Sectional Study

We compared serum and CSF IgG, IgA, and IgM levels, OCB status, and MRZ reaction of the NTZ-treated patient cohort (49 patients) with corresponding data of 47 untreated RRMS patients.

Longitudinal Study

Within the NTZ-treated cohort, we performed paired analyses of serum and CSF IgG, IgA, and IgM levels. In addition, percentage of OCB-positive patients with MS before and during NTZ therapy was determined and compared. Complete data sets before NTZ were not available for all patients of group 1; hence, patient number varied for the individual analyses (for details, see figure legends).

Determination of IgG, IgA, IgM, and Albumin Levels

The quantitative determination of immunoglobulins IgG, IgA, and IgM and albumin in serum as well as in the CSF was performed by means of immunonephelometry on an Atellica NEPH 630 analyzer using the diagnostic reagents supplied from Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany, in accordance with the manufacturer's instructions. For CSF IgM values below the quantification limit of the assay reaching from 0.12 to 0.15 mg/L, these values were used for further calculations. Ig indices were calculated as $\left(\frac{Ig\ ratio\ \frac{CSF}{serum}}{Albumin\ ratio\ \frac{CSF}{serum}}\right)$ and used as measures of intrathecal Ig synthesis.

MRZ Reaction

MRZ reaction was detected by the calculation of the respective antibody indices (AIs) as described earlier.[20] ELISAs (Euro-immun, Lübeck, Germany) were measured on an automated

Table 1 Patient Characteristics

	Group 1 NTZ-treated cohort	Group 2 untreated cohort
Patient number	49	47
Diagnosis	49 RRMS	47 RRMS
Mean age at sampling (y)	41 (range 21–65)	39 (range 19–62)
Sex	30 females, 19 males (ratio 1.6:1)	28 females, 19 males (ratio 1.5:1)
Mean time disease onset—first lumbar puncture (y)	2 (range 0–10)	3 (range 0–22)
Mean time disease onset—first NTZ infusion (y)	8 (range 0–21)	n/a
Mean time first lumbar puncture—first NTZ infusion (y)	5 (range 0–14)	n/a
Mean time disease onset—lumbar puncture during NTZ (y)	14 (range 2–30)	n/a
Median EDSS	3.0 (range 0–7)	2.0 (range 1–5.5)
Mean NTZ treatment duration (y)	5.1 (range 1.2–11.8)	n/a
Mean number of DMT before NTZ	2 (range 0–7)	n/a
Mean time from last NTZ application to lumbar puncture (wk)	+1.5 (−4.3–16.1)	n/a

Abbreviations: DMT = disease-modifying therapy; EDSS = Expanded Disability Status Scale; NTZ = natalizumab; RRMS = relapsing-remitting MS.

ELISA processing system (Analyzer I, Euroimmun). Virus-specific AI ≥1.5 was considered indicative of intrathecal antibody production against the respective virus. We analyzed MRZ reaction status of untreated and NTZ-treated patients as one-fold positive (MRZ-1: reactivity against 1 of the 3 viruses) and 2-fold positive (MRZ-2: reactivity against 2 viruses) MRZ reaction.

Determination of Oligoclonal Bands

Detection of OCBs was performed as part of routine CSF analysis using a high-sensitive isoelectric focusing technique on agarose gel followed by immunofixation (Hydrasis Focusing instrument using the Hydragel 9 CSF Isofocusing kit, both Sebia, France). The presence of OCB was assumed if there was >1 OCB exclusively in the CSF. A sample stored at −80°C in the biobank of the Institute of Clinical Neuroimmunology (LMU Klinikum, Munich) was used to analyze Ig band patterns of a patient in direct comparison before and during NTZ treatment.

Flow Cytometric Analysis of α4-Integrin Expression

We analyzed the intensity of α4-integrin expression on B-cell subsets using blood samples of HCs. Human peripheral blood mononuclear cells (PBMCs) of 8 HCs (mean age 30.5 years, male:female 5:3) were isolated using Pancoll solution (PAN-Biotech Pancoll human, density: 1.077 g/mL) and stored in liquid nitrogen until further use. After thawing, cells were incubated with the FcR blocking reagent (Miltenyi Biotec, Bergisch Gladbach, Germany) and stained using anti-human CD3-Alexa Fluor 700 (OKT3; eBioscience, San Diego, CA), CD14-Alexa Fluor 700 (M5E2, BioLegend, San Diego, CA), CD20-PerCP (2H7 BioLegend), IgD-PerCP (IA6-2, BioLegend), CD19-APC/Fire 750 (HIB19, BioLegend), CD27-Brilliant Violet 605 (O323; BioLegend), CD38-eFluor 450 (HB7; eBioscience), α4-integrin—(CD49d) FITC (9F10, BioLegend), and FITC Mouse IgG1, κ Isotype Control (MOPC-21, BioLegend). B-Cell subpopulations were analyzed within the CD3−CD14−, CD19+ population. In this article, naive B cells were defined as CD38− CD27−; memory B cells were defined as CD38−CD27+ and plasmablasts as CD38+CD27+. Expression levels of α4-integrin were analyzed as delta-mean fluorescence intensity (ΔMFI) (MFI of VLA-4-FITC-staining − MFI of fluorescence minus one control without α4-integrin—FITC). Flow cytometry (FACS) data were analyzed using FlowJo 10.6.1 software (Ashland, OR).

Statistical Analysis

Data were analyzed using Graphpad Prism 7 software (GraphPad Software Inc., La Jolla, CA). Differences in Ig levels were evaluated using the Mann-Whitney test for cross-sectional and Wilcoxon matched-pairs signed-rank tests for longitudinal comparisons. OCB and MRZ data were analyzed using the Fisher exact test. Changes in antibody levels and indices with NTZ therapy duration were evaluated using Spearman nonparametric correlation. Potential differences in α4-integrin expression levels were analyzed using the Quade all-pairs test. Differences were considered statistically significant for p values of *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$ and marked accordingly in the figures.
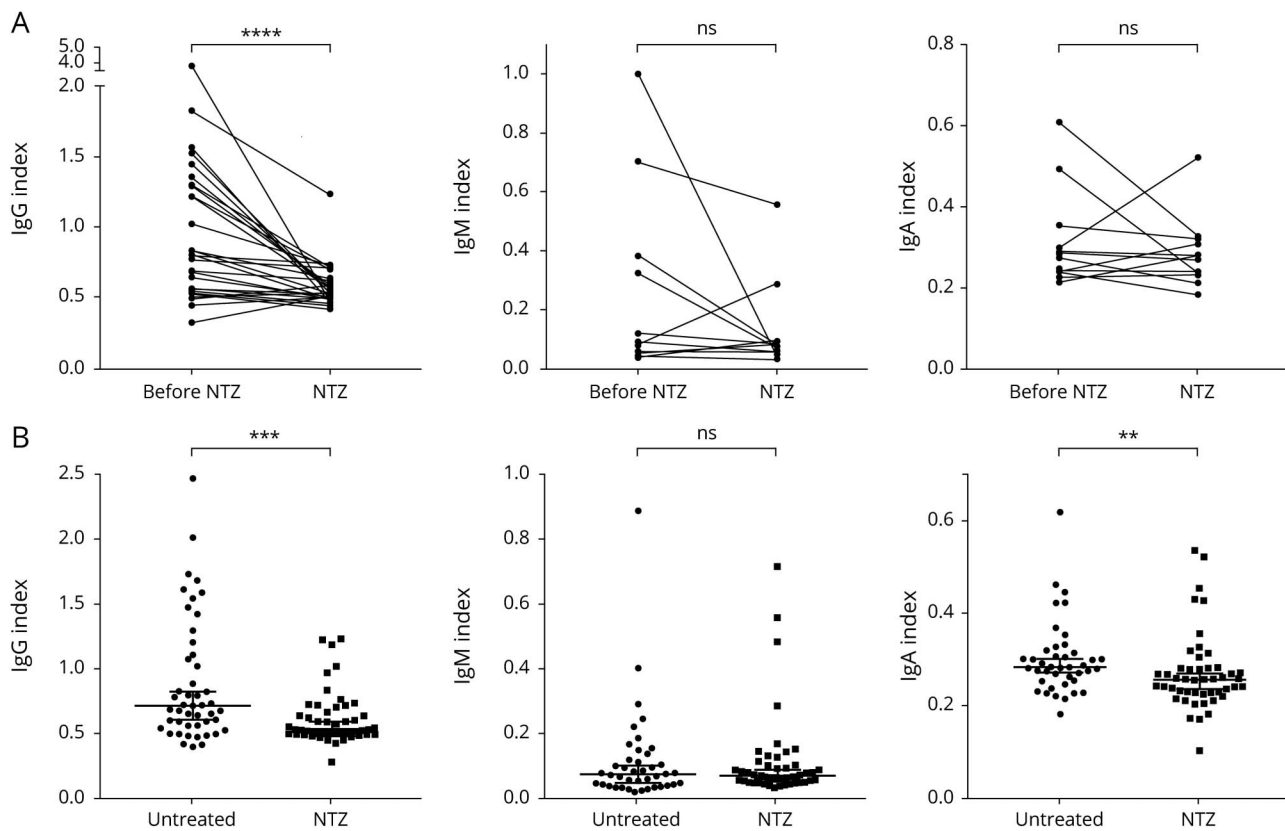
Data Availability

After publication, anonymized data will be made available on reasonable request to the corresponding author.

Standard Protocol Approvals, Registrations, and Patient Consents

The local ethics committee (Ethik-Kommission der Medizinischen Fakultät der LMU, protocol number 159-03 and

The Ig indices as a measure of intrathecal Ig production were calculated as described under Methods. Ig indices for IgG (left), IgM (middle), and IgA (right) are shown. (A) Longitudinal analysis: Paired data on Ig indices of patients before and during NTZ therapy were available for 26 patients for IgG, 13 patients for IgM, and 12 patients for IgA. IgG index (mean 1.0 before vs 0.6 during NTZ) was significantly reduced during NTZ therapy ($p < 0.0001$). Four patients with high IgM indices before NTZ showed a stronger reduction of IgM indices during NTZ treatment, but when the whole group was considered, the decrease in IgM indices (mean 0.2 before vs 0.1 during NTZ) did not reach statistical significance ($p = 0.3054$). IgA indices (mean before 0.3 vs 0.3 during NTZ) remained stable ($p = 0.4697$). Wilcoxon matched-pairs signed-rank test. (B) Cross-sectional analysis: Each dot represents 1 patient. Patient numbers: IgG index: untreated n = 47, NTZ n = 49; IgM index: untreated n = 41, NTZ n = 47; IgA index untreated n = 41, NTZ = 47. IgG and, to a lesser extent, IgA indices were lower in NTZ-treated compared with untreated MS patients ($p = 0.0007$ and $p = 0.0076$). Mann-Whitney U test. ns = non significant; NTZ = natalizumab. ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$.

163-16) approved the study. All patients and controls gave written informed consent.

Results

Patient Characteristics

The mean time of NTZ treatment duration in group 1 was 5.1 years. Patients had been on a mean of 2 DMT before starting NTZ. Most (n = 31) had been treated with basic DMT (interferon beta, glatiramer acetate, dimethyl fumarate, and teriflunomide) before starting NTZ, 10 patients with fingolimod, and 6 with other immunotherapies. Two patients were untreated before commencing NTZ treatment. None of the patients underwent cell-depleting MS therapies before NTZ. Clinical characteristics of all patients are shown in table 1.

Results of routine CSF parameters (cell count, total protein, and blood-brain barrier dysfunction) of both groups before and after NTZ can be seen in table e-1 links.lww.com/NXI/A500.

Isotype-Specific Effect of NTZ on Systemic and Intrathecal Ig Production

NTZ strongly reduced the IgG index reflecting total intrathecal IgG production but had no effect on the IgM index as seen in both the cross-sectional and longitudinal part of our study (figure 1A, B). We noted a reduction of the IgA index in the cross-sectional study (figure 1B). In serum, NTZ therapy reduced IgM levels in both the longitudinal and cross-sectional comparison; furthermore, we noted a slight reduction of IgG in only the longitudinal part of the study. IgA levels remained unchanged (figure e-1 links.lww.com/NXI/A501). The reduction in serum IgM was substantial, from a mean of 1,307 to 653 mg/L (50% reduction) after a mean of 3.7 years of NTZ treatment. In 3 patients, the serum IgM level decreased below the reference value during NTZ treatment (figure e-1 links.lww.com/NXI/A501). IgG reduction was much less pronounced with a mean of 10.9 g/L before and 9.8 g/L during NTZ treatment (10% reduction, 4.7 years of treatment). In the cross-sectional part of the study, 13 patients (28%) in the NTZ group showed serum IgM levels below the

Figure 2 Differential Effects of NTZ Treatment Duration on Peripheral and Intrathecal Immunoglobulin (Ig) Levels
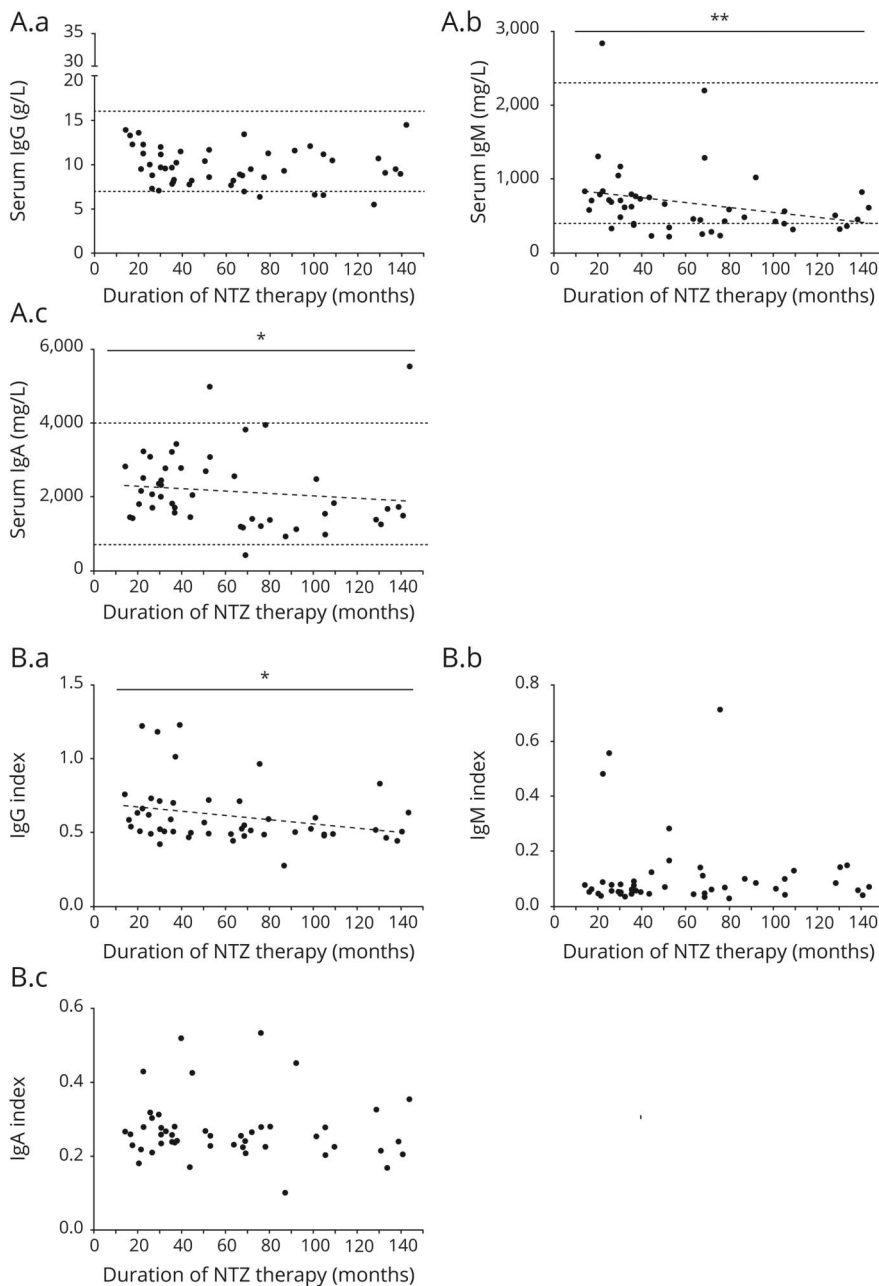
Levels of IgG (left), IgM (middle) and IgA (right) in serum (A.a–A.c) and CSF (B.a–B.c) are shown along with NTZ therapy duration (months). Dotted horizontal lines represent upper and lower reference values. Linear regression analysis and Spearman nonparametric correlation analysis were performed. Regression lines (dashed) are shown for significant correlations. (A.b) Duration of NTZ therapy correlated strongly with a decrease of serum IgM ($p = 0.0031$, n = 47, Spearman r = −0.4227) and (A.c) weakly with a decrease of serum IgA ($p = 0.0354$, n = 49, Spearman r = −0.3077), while (A.a) serum IgG levels remained stable over time. (B.a) Analysis of Ig indices showed a reduction of intrathecal IgG production over time ($p = 0.0198$, n = 51, Spearman r = −0.3318). NTZ = natalizumab. *$p < 0.05$ and **$p < 0.01$.

reference value compared with only 2 patients (5%) in the untreated group ($p = 0.0048$, Fisher exact test). We further analyzed the effect of duration of NTZ application. In the CSF, we found a significant negative correlation between the duration of NTZ therapy and IgG index; in serum, IgA and IgM levels also decreased significantly with therapy duration (figure 2A, B). We observed a stronger decrease of IgM indices in the 4 patients with the highest IgM indices, but overall IgM indices in the CSF did not decline significantly after NTZ treatment (figure 1A). The total amount of IgM in the CSF declined significantly ($p = 0.0012$, data not shown). This indicates that reduced overall IgM in the CSF is due to reduced

intrathecal IgM production in a few patients with a high IgM index and due to reduction of IgM in the serum. Taken together, NTZ strongly reduced systemic IgM levels and intrathecal IgG production.

OCB Status and Individual OCBs Remain During NTZ Therapy

The cross-sectional part of our study revealed no significant differences in OCB status between the untreated (OCB positive: 41/47, 87%) and NTZ-treated cohort (OCB positive: 37/49, 76%; figure 3A). A comparison of OCB in patients with MS before NTZ (OCB positive: 28/33, 85%) and

Figure 3 Persistence of OCB and MRZ Reaction During NTZ Therapy
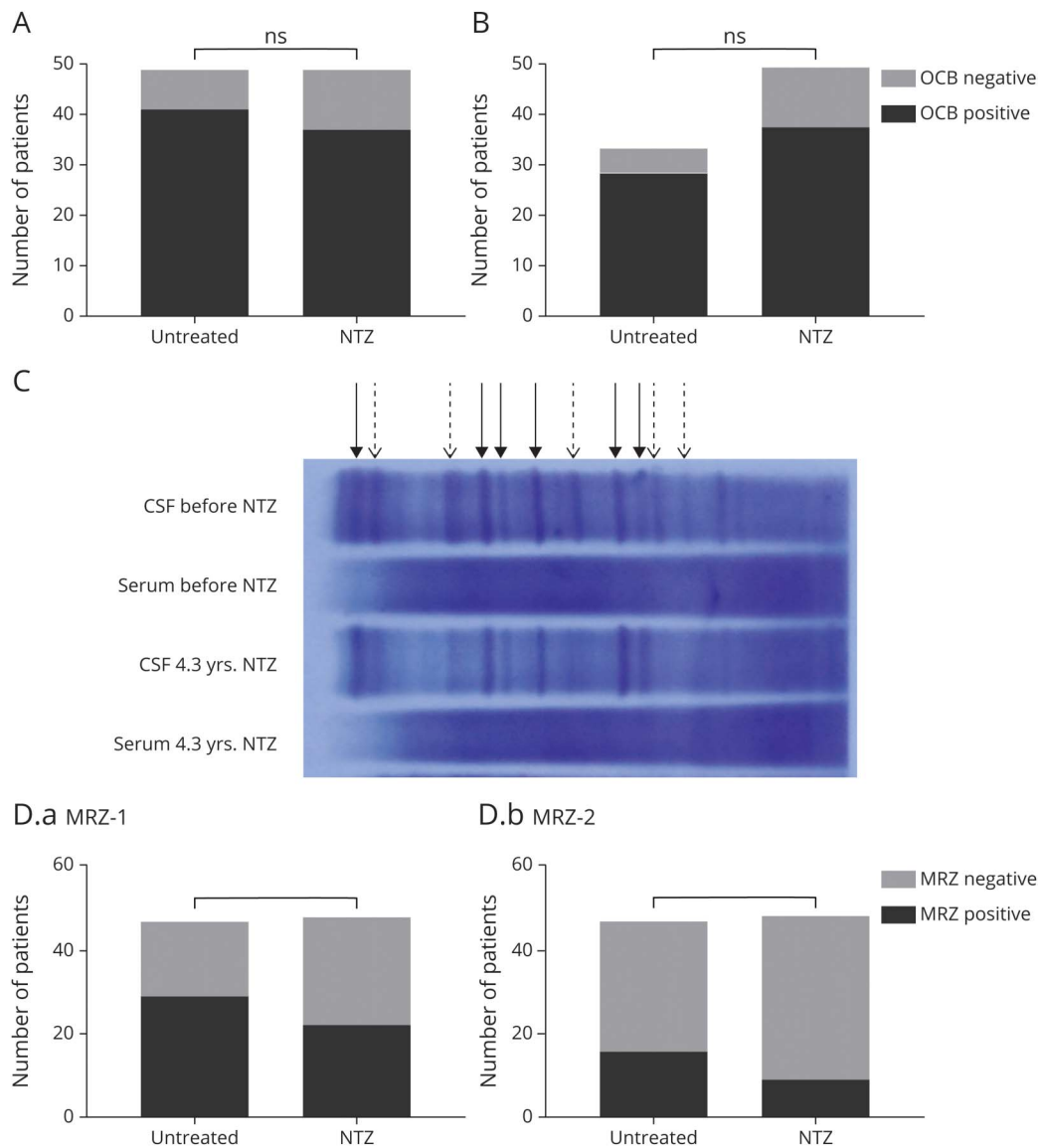
(A) Cross-sectional analysis of CSF-specific OCB: No differences in OCB status could be detected between untreated and NTZ-treated MS patients ($p = 0.1923$, untreated patients n = 47, NTZ-treated patients n = 49; OCB positive: 87% [n = 41] of untreated patients vs 76% of NTZ patients [n = 37]). Fisher exact test. (B) Longitudinal analysis of CSF-specific OCB: OCB status was similar in patients before and during NTZ treatment ($p = 0.4082$, patients before NTZ n = 33, patients during NTZ n = 49; OCB positive: 86% of patients before [28 patients] vs 76% of patients during NTZ [n = 37]). Fisher exact test. (C) Comparison of serum-CSF pairs of 1 patient with MS before NTZ therapy and after 4.3 years of NTZ treatment. The majority of bands persist during treatment (solid arrows), whereas some become weaker or disappear entirely (dashed arrows). (D) Cross-sectional analysis: MRZ reaction status of untreated and NTZ-treated patients. Positive MRZ reaction was defined as an intrathecal antibody reaction against 1 (D.a, MRZ-1) or 2 (D.b, MRZ-2) viruses (measles, rubella, or varicella zoster virus). No clear differences could be detected between untreated and NTZ-treated patients for MRZ-1 ($p = 0.1513$, MRZ-1 positive: 61% and 46%, respectively) or MRZ-2 ($p = 0.1069$, MRZ-2 positive: 34% and 19%, respectively). Fisher exact test. MRZ = measles virus, rubella virus, and varicella zoster virus; ns = non significant; NTZ = natalizumab; OCB = oligoclonal band.

during NTZ treatment (OCB positive: 37/49, 76%) also revealed similar proportions of OCB-positive patients (figure 3B). Overall, only 5 patients (18%) lost their OCB during NTZ therapy. Taken together, long-term NTZ treatment does not have an effect on OCB status in most individuals.

To analyze the persistence or disappearance of individual OCB, we analyzed CSF/serum pairs of 1 patient, stored before the beginning of NTZ therapy and obtained after 4.3

years of treatment, in parallel on the same gel. This revealed the persistence of individual OCB. However, some of the OCB lost intensity or disappeared entirely (figure 3C).

MRZ Reaction Status Remains Largely Unchanged During NTZ Treatment

MRZ-1 reaction status was similar between untreated (MRZ-1 positive: 29/47, 62%) and NTZ-treated patients (22/48, 46%). Likewise, 16 of 47 (34%) untreated MS patients were

Figure 4 α4-Integrin Expression on B-Cell Subsets
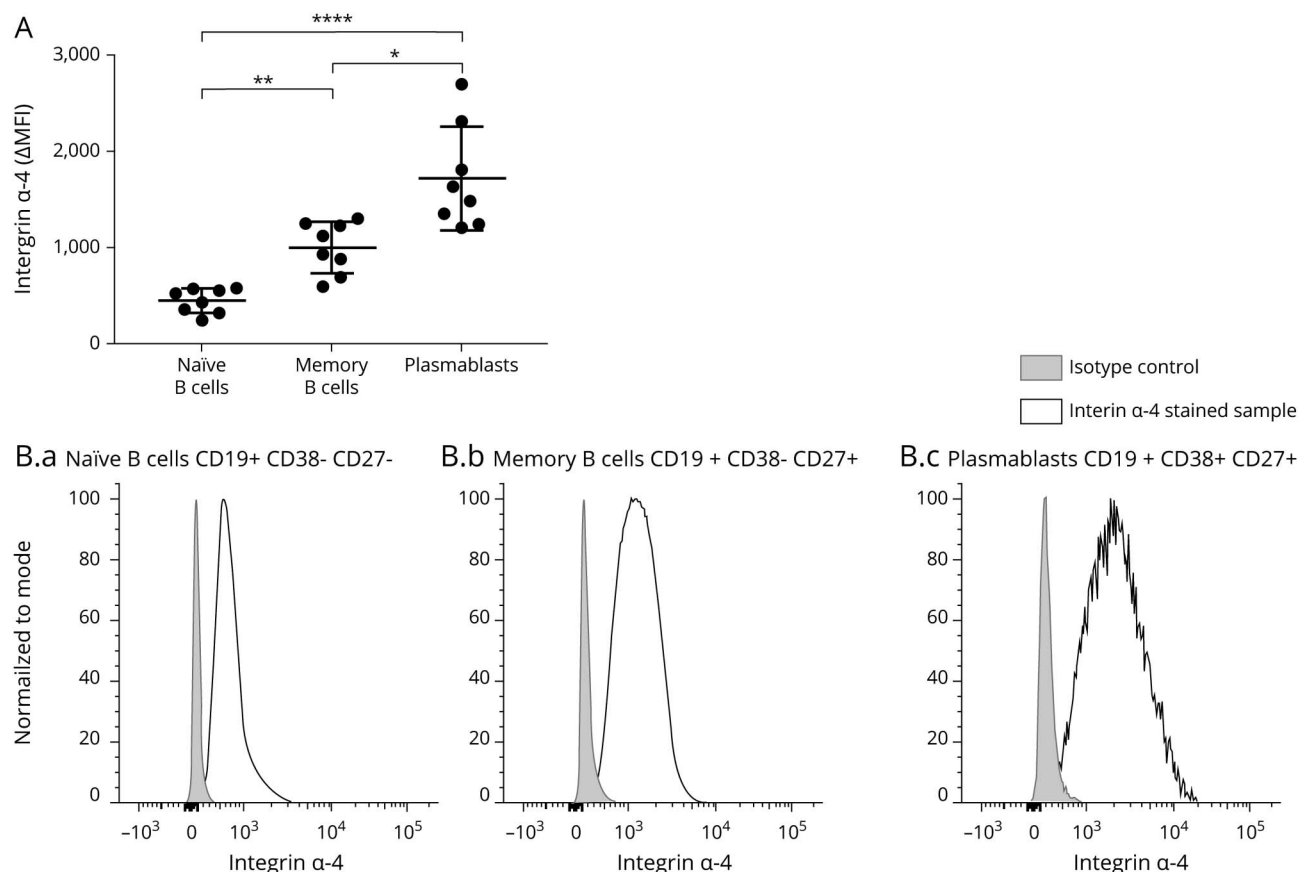
PBMCs of healthy donors (n = 8) were stained for α4-integrin (CD49d) and markers for flow cytometric differentiation of lymphocyte subsets. α4-Integrin expression on naive B cells (CD3⁻ CD19⁺ CD38⁻ CD27⁻), memory B cells (CD3⁻ CD19⁺ CD38⁻ CD27⁺), and plasmablasts (CD3⁻ CD19⁺ CD38⁺ CD27⁺) was analyzed. (A) Each dot represents ΔMFI (MFI[stained sample] − MFI[FMO control]) of 1 donor. α4-Integrin expression levels of memory cells and plasmablasts were significantly higher than those of naive B cells ($p = 0.0071$ and $p < 0.00001$, respectively). α4-Integrin expression was higher on plasmablasts than on memory B cells ($p = 0.0176$). Quade all-pairs test. (B) Representative α4-integrin staining of 1 donor. Light gray curves represent isotype controls, and black curves represent α4-integrin staining on naive B cells (B.a), memory B cells (B.b), and plasmablasts (B.c). MFI = mean fluorescence intensity. * $p < 0.05$, ** $p < 0.01$, and **** $p < 0.0001$.

MRZ-2 positive compared with 9 of 48 (19%) NTZ-treated patients (figure 3D). NTZ treatment does not appear to affect the intrathecal IgG response to MRZ.

Highest α4-Integrin Expression on Plasmablasts

Analysis of α4-integrin expression on B-cell subsets in the blood of HCs revealed a different intensity level of α4-integrin expression on various B-cell subsets (figure 4). We observed the highest levels of α4-integrin on plasmablasts (CD19⁺ CD38⁺ CD27⁺), followed by memory B cells (CD19⁺ CD38⁻ CD27⁺) and naive B cells (CD19⁺ CD38⁻ CD27⁻).

Discussion

Details of the intrathecal Ig production in MS are useful for distinguishing MS from other neuroinflammatory diseases[1,4] and are of prognostic relevance: intrathecal IgM production is linked to conversion of clinically isolated syndrome to MS[21]

and correlates with disease activity[22]; in addition, intrathecal IgG production is associated with worsening.[23] Here, we present a comprehensive analysis of the effects of long-term NTZ therapy on different features of intrathecal Ig production and show that α4-integrin is expressed more highly on plasmablasts than on naive and memory B cells. NTZ reduced total intrathecal IgG production, correlating with treatment duration, but the intrathecal polyspecific IgG response (MRZ reaction) and OCB (consisting of IgG) remained. This was seen in both the longitudinal and the cross-sectional part of our analysis. Moreover, we found that individual OCBs persisted in a patient despite 4.3 years of NTZ treatment.

In the MS brain, Ig-producing cells are found in parenchymal, perivascular, and meningeal areas[7,24] as well as in the spinal fluid.[1,8,25] Both short-lived plasmablasts[8] and long-lived plasma cells[7] are considered to contribute to intrathecal Ig production in MS. In patients with MS, OCBs in the CSF remain stable for many years,[26] indicating that individual long-lived plasma cells migrate to a survival niche in the CNS and remain there.[5] The

presence of OCB after a shorter period of NTZ treatment than in this study has been analyzed before with mixed results.[15,17-19] A more recent study revealed the persistence of OCB in more than 90% of patients with MS after 1 year of NTZ therapy.[15] Both the longitudinal and cross-sectional part of our study showed that the OCB status remained largely unchanged even after long-term NTZ therapy. We could further directly compare individual OCB in CSF samples obtained before and more than 4 years after NTZ treatment on the same gel. This revealed that not only OCB status but also individual bands remained, indicating that NTZ did not remove the corresponding plasma cells from their niche. We also noted that a few bands became weaker or disappeared. It is unclear whether this is due to NTZ or reflects a natural disease process because changes in OCB banding patterns have been described in some patients with MS.[27,28]

A limitation of this study is the relatively long time span between the first LP and the beginning of NTZ therapy; hence, effects of other disease-modifying drugs before NTZ or even disease course itself on the development of the analyzed parameters over time cannot be entirely excluded. Furthermore, we were only able to analyze the persistence of individual OCBs after long-term treatment in 1 individual patient.

Intrathecal IgG production in patients with MS often involves a polyspecific response against different pathogens (MRZ reaction), which in some countries is used to support the diagnosis. MRZ reaction is typically present at the beginning of the disease and relatively specific for MS, whereas OCB can also be detected in other entities of CNS inflammation, e.g., neuroborreliosis.[4] Quantitatively, the MRZ reaction represents only a minor part of intrathecally produced IgG, estimated around 2%.[3] The MRZ reaction may indicate an enhanced B-cell–promoting environment long before the onset of clinical disease and may also reflect the individual's history of infections.[5] In the absence of treatment, MRZ reaction persists,[5] presumably reflecting—like persisting OCB—that long-lived plasma cells have found a survival niche in the brain. In our study, the MRZ reaction was not abrogated by 5.1 years of treatment with NTZ. A previous study reported the persistence of MRZ reaction after 1 year of NTZ therapy but differential effects on other antiviral IgG responses.[13] It is tempting to speculate that this is due to a differential formation of long-lived plasma cells by these different antiviral responses. Both the persisting OCB and the persisting MRZ reaction indicate that α4-integrin is not a major component of the survival niche of plasma cells in the CNS. In the bone marrow, laminin β1 has been identified as an important adhesion molecule for IgG-secreting plasma cells.[29] The role of laminin β1 in maintenance of plasma cells in the CNS remains to be identified.

The magnitude of intrathecal IgG production was reported to correlate with the number of short-lived plasmablasts in the CSF.[8] We assume that the reduction of intrathecal IgG production by NTZ is based on its effect on plasmablasts and comprises 2 mechanisms: First, NTZ might reduce the new formation of plasmablasts because B-cell activation is facilitated by VLA-4 (integrin α4β1) interacting with VCAM-1.[30] Second, NTZ could reduce homing of plasmablasts to the CNS. Plasmablasts are circulating cells,[31] and B cells exchange between the CNS and peripheral compartment.[10] Our finding of a high expression of integrin α4 on plasmablasts suggests that NTZ also reduces the migration of plasmablasts to the CNS. Animal models underscore the importance of integrin α4–dependent migration of B cells for disease development and show different effects depending on the particular model.[32-35]

In serum, we found that NTZ strongly reduced IgM, with little or no effect on IgG and IgA levels, in accordance with previous reports.[12,15] This differential effect of NTZ on systemic IgG and IgM levels can also be explained by an inhibition of plasmablasts but not long-lived plasma cells. Serum IgG levels are largely maintained by long-lived plasma cells,[6] whereas IgM is produced to a greater extent by short-lived plasmablasts, although also long-lived IgM plasma cells have been described.[36] These may contribute to IgM remaining in the presence of NTZ.[30] Whether patients who switch from NTZ to B-cell–depleting therapies might subsequently develop earlier and more pronounced hypogammaglobulinemias has not been investigated in detail. Although this has to be evaluated in larger studies, we would recommend determination of serum immunoglobulin levels in patients with infections during NTZ therapy and close monitoring in patients before and after switching to cell-depleting therapies.

In summary, we show that long-term treatment with NTZ has differential effects on features of intrathecal IgG production: NTZ time dependently reduces the intrathecal IgG index and serum IgM levels, but OCB status, MRZ reaction, and individual OCB remain largely unchanged. This indicates that NTZ targets short-lived plasmablasts, but not long-lived plasma cells in their survival niche in the brain.

Disclosure
M. Schlüter, E. Oswald, and S. Winklmeier report no disclosures relevant to the manuscript. I. Meinl received travel

Publication History

Appendix Authors

Name	Location	Contribution
Miriam Schlüter, MD	Institute of Clinical Neuroimmunology, Biomedical Center and LMU Klinikum, Munich, Germany	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; and analysis or interpretation of data
Eva Oswald, BSc	Institute of Clinical Neuroimmunology, Biomedical Center and LMU Klinikum, Munich, Germany	Major role in the acquisition of data and analysis or interpretation of data
Stephan Winklmeier, MSc	Institute of Clinical Neuroimmunology, Biomedical Center and LMU Klinikum, Munich, Germany	Major role in the acquisition of data
Ingrid Meinl, MD	Institute of Clinical Neuroimmunology, Biomedical Center and LMU Klinikum, Munich, Germany	Major role in the acquisition of data
Joachim Havla, MD	Institute of Clinical Neuroimmunology, Biomedical Center and LMU Klinikum, Munich, Germany	Major role in the acquisition of data
Peter Eichhorn, MD	Institute of Laboratory Medicine, LMU Klinikum, Munich, Germany	Major role in the acquisition of data
Edgar Meinl, MD	Institute of Clinical Neuroimmunology, Biomedical Center and LMU Klinikum, Munich, Germany	Drafting/revision of the manuscript for content, including medical writing for content; study concept or design; and analysis or interpretation of data

Appendix (continued)

Name	Location	Contribution
Tania Kümpfel, MD	Institute of Clinical Neuroimmunology, Biomedical Center and LMU Klinikum, Munich, Germany	Drafting/revision of the manuscript for content, including medical writing for content; study concept or design; and analysis or interpretation of data

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