Alemtuzumab therapy changes immunoglobulin levels in peripheral blood and CSF

Nora Möhn, MD,* Steffen Pfeuffer, MD,* Tobias Ruck, MD, Catharina C. Gross, PhD, Thomas Skripuletz, MD, Luisa Klotz, MD, Heinz Wiendl, MD, Martin Stangel, MD,* and Sven G. Meuth, MD, PhD*

Correspondence
Dr. Stangel
Stangel.Martin@mh-hannover.de

Abstract

Objective

The use of alemtuzumab, a humanized monoclonal anti-CD52 antibody has changed the therapy of highly active relapsing-remitting MS (RRMS). Alemtuzumab infusion depletes most lymphocytes in peripheral blood, whereas differential recovery of immune cells, probably those with a less CNS-autoreactive phenotype, is supposed to underlie its long-lasting effects. To determine whether alemtuzumab significantly reduces immunoglobulin levels in blood and CSF of treated patients, we analyzed blood and CSF samples of 38 patients with MS treated with alemtuzumab regarding changes in immunoglobulin levels.

Methods

Blood and CSF samples of patients were collected at the beginning of alemtuzumab treatment and at 12, 24, and 36 months after the first administration of the drug. Specimens were analyzed regarding immunoglobulin concentrations in blood and CSF.

Results

We observed significant and dose-dependent reductions of immunoglobulin levels (IgG, IgM, and IgA) in serum and CSF 12 and 24 months following 2 courses of alemtuzumab. Patients with persistent or returning disease activity who were treated with a third course of alemtuzumab exhibited even further decrease in IgG levels compared with matched controls treated twice. Here, alemtuzumab-treated patients with IgG levels below the lower limits of normal were more susceptible to pneumonia, sinusitis, and otitis, whereas upper respiratory tract and urinary tract infections were not associated therewith.

Conclusions

Our results suggest to monitor IgG levels for safety reasons in patients treated with alemtuzumab—in particular when additional treatment courses are required—and to consider preventive action in critical cases.

Classification of evidence

This study provides Class IV evidence that for patients with RRMS alemtuzumab reduces immunoglobulin levels.

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*These authors contributed equally as first and senior authors, respectively.

From the Department of Neurology and Clinical Neuroimmunology and Neurochemistry (N.M., T.S., M.S.), Hannover Medical School, Hannover, Germany; and Neurology Clinic with Institute of Translational Neurology (S.P., T.R., C.C.G., L.K., H.W., S.G.M.), University of Münster, Münster, Germany.

Glossary

DMT = disease-modifying therapy; **OCB** = oligoclonal band; **RA** = rheumatoid arthritis; **RRMS** = relapsing-remitting MS.

Alemtuzumab was approved for therapy of active relapsing-remitting MS (RRMS) in Europe in November 2014.[1–3] The humanized monoclonal antibody selectively binds CD52, a human glycosylphosphatidylinositol-anchored protein. CD52 is highly expressed on the surface of T and B lymphocytes and, at lower levels, on cells of the innate immune system, namely monocytes and macrophages.[4–6] Consequently, alemtuzumab administration leads to rapid depletion of circulatory CD52-positive cells through antibody-dependent cell-mediated cytolysis and complement-dependent cytolysis.[7] Subsequent to depletion, immune cells derived from hematopoietic stem cells slowly repopulate. The repopulation dynamics are distinct for different immune cell types with B lymphocytes recovering faster than T cells. B lymphocyte numbers return to the baseline level after approximately 3 months; after 12 months, they can even exceed baseline levels.[8,9]

The trade-off for clinical efficiency of alemtuzumab is the occurrence of frequent and sometimes serious adverse events.[10] The most interesting and not yet fully understood adverse events are secondary autoimmune phenomena.[11]

Several case reports also described either viral or complex bacterial or fungal infections, highlighting consequences of T-cell depletion.[12] Common and usually noncomplicated infections, such as pneumonia or bacterial upper respiratory tract infections fostered by hypogammaglobulinemia, have received much less attention to date. Because of new reports of immune-mediated and partly fatal cardiovascular adverse events, the European Medicines Agency (EMA) restricted the use in April 2019.

Autoantibody production leading to consecutive autoimmune phenomena after alemtuzumab treatment has already been reported; however, physiologic immunoglobulin production following alemtuzumab administration has not yet been analyzed in greater depth.

Methods

Study design and setting

Between January 2015 and December 2016, 38 patients with diagnosed active RRMS were treated with at least 2 cycles of alemtuzumab at the Neurology Clinic of the University Hospital Münster. Serum (n = 38) and CSF (n = 24) samples were collected at treatment start and at 12, 24, and 36 months after the first alemtuzumab administration. None of the patients had received previous immunosuppressive or B cell–depleting therapy, had any history of chronic (autoimmune or infective) disease other than RRMS, or experienced a clinical relapse within the 4 weeks before sample collection. Patients were interviewed and clinically examined in regular 3-monthly follow-up visits (table 1).

This study provides Class IV evidence that long-term treatment with alemtuzumab can reduce immunoglobulin levels in patients with RRMS.

Participants

Demographic data collected included age, sex, duration of MS before alemtuzumab therapy, baseline EDSS score, number of previous disease-modifying therapies (DMTs), and number of MS relapses within the 2 years before alemtuzumab therapy. Diagnostic data included analysis of CSF cell count, CSF protein levels, CSF lactate, serum/CSF albumin ratio, CSF and serum analysis of immunoglobulin concentrations (IgG, IgA, and IgM) including serum/CSF immunoglobulin ratios as an indicator for intrathecal synthesis of IgG, IgA, and IgM, and oligoclonal bands (OCB) as an indicator for intrathecal IgG synthesis. During alemtuzumab treatment cycles, patients were examined for opportunistic infections, and data on disease activity and MS relapses were gathered.

Standard protocol approvals, registrations, and patients consents

Patients gave written informed consent for data publication, and the study was approved by the institutional review boards at both centers (University of Muenster, 2014-398-f-S; Hannover Medical School, 3142-2016).

Table 1 Baseline characteristics of the study cohort

Patients, no.	38
Blood samples available, no. (%)	38 (100)
CSF samples available, no. (%)	24 (63.2)
Age, yr, median (range)	33 (18–58)
Male sex, no. (%)	18 (47.3)
MS duration, yr, median (range)	
Since manifestation	4 (0–27)
Since diagnosis	4 (0–17)
Previous DMTs, no., median (range)	2 (0–6)
Baseline EDSS score, median (range)	2.5 (0–6)
Relapses within past 2 years, median (range)	3 (0–9)
OCBs positive at baseline (%)	22 (79)

Abbreviations: DMT = disease-modifying therapy; EDSS = Expanded Disability Status Scale; OCB = oligoclonal band.

CSF and serum analysis

CSF and serum were analyzed using routine methods.[13] Immediately after CSF sampling via lumbar puncture, CSF cell count, total protein, and lactate were analyzed. CSF cells were counted manually with a Fuchs-Rosenthal counting chamber. For further analyses, the residual CSF was centrifuged (145g for 15 minutes), and the supernatant was frozen at −80°C. The corresponding serum was also frozen at −80°C. CSF total protein was determined by a Bradford dye-binding procedure. Albumin, IgG, IgA, and IgM were examined in serum and CSF in the same latex-enhanced assay by kinetic nephelometry (Siemens BN ProSpec, Münster; Beckman Coulter IMMAGE, Hannover) according to the guidelines by the manufacturer. The function of the blood-CSF barrier was estimated as CSF-serum albumin quotient (QAlbumin). The age-adjusted upper reference limit of QAlbumin (QAlb) was calculated using the formula QAlb = 4 + (age in years/15). Intrathecal synthesis of immunoglobulins (IgG, IgA, and IgM) was calculated based on the method of Reiber-Felgenhauer, referring IgG, IgA, and IgM quotients to the albumin quotient.[14] CSF and serum OCBs were determined by isoelectric focusing (Pharmacia Biotech) in polyacrylamide gels (EDC) with consecutive silver staining (GE Healthcare) according to the manufactures' recommendations. Five patterns of OCBs were distinguished following the recommendations of the first European consensus on CSF analysis in MS, whereas the type 2 and 3 pattern is an indicator for intrathecal IgG synthesis.[15]

To analyze immunoglobulin changes after 3 courses of alemtuzumab, we thawed serum samples obtained immediately before additional courses. Those samples were processed differently to samples from the previous time point and were eventually not comparable to previous values. We therefore decided to also analyze matched samples from patients with MS with ongoing natalizumab therapy and treatment-naive patients with MS, which underwent a similar thawing process to exclude process-related effects on serum immunoglobulin concentrations. To compare between groups, patients were matched for age, sex, disease duration since manifestation, and storage duration of samples. In accordance with the inclusion criteria for alemtuzumab-treated patients, none of the controls had received previous immunosuppressive or B cell–depleting therapy, and samples were obtained minimally 4 weeks after the last clinical relapse.

Statistical analysis

To compare between the groups at treatment start and at 24 months, the Mann-Whitney rank-sum test was used. To compare samples from 36 months after treatment start with respective controls, the Kruskal-Wallis test for multiple-group comparison including the Dunn post-test was used. Continuous variables (e.g., quantitative fraction of intrathecal IgG synthesis over time) were analyzed using the Wilcoxon paired test. To test categorical variables (proportion of patients with positive OCBs at different time points), The McNemar test with Edwards correction was applied. Statistical analysis was performed using SPSS 25 (IBM, CA). p Values below 0.05 were considered significant.

Data availability

All authors have full access to all data sets and take full responsibility for the integrity of the data and accuracy of the data analysis. Data will be shared on request from any qualified investigator.

Results

Patient characteristics

In total, we analyzed blood samples of 38 patients treated with alemtuzumab. For 24 patients, additional CSF samples were available. The patient characteristics at baseline are summarized in table 1.

Serum IgG, IgM, and IgA concentrations of alemtuzumab-treated patients are reduced after 12 and 24 months

We measured serum immunoglobulin before the first administration of alemtuzumab and at 12 and 24 months. All patients received at least 2 cycles of alemtuzumab therapy according to SmPC. Eight patients required a third course 24 months after the first infusion due to ongoing disease activity. We measured immunoglobulin concentrations before start of each therapy course, respectively. We found a statistically significant decrease of immunoglobulin concentrations compared with baseline values for each subgroup (IgG, IgM, and IgA; figure 1, A–C). In all examined immunoglobulin subgroups, we found a stronger reduction after 24 months than after 12 months. All median values and p values are listed in an additional table (table e-1, links.lww.com/NXI/A172). Within the IgA group, 3 outliers with noticeably increased values could be identified before and 12 months after the first administration of alemtuzumab. At 24 months, no outliers remained. Notably, the majority of patients showed IgG levels within the normal range. At 24 months after therapy start, only 6 patients presented with IgG levels under 7 g/L (normal value >7 g/L).

Intrathecal IgG synthesis decreases during alemtuzumab therapy

For the majority of cases, we observed a significant decrease of the quantitative fraction of intrathecal IgG synthesis at 12 and 24 months after the first alemtuzumab administration. Except for 1 case, the decrease was progressive over time. We identified 3 patients with rising intrathecal IgG synthesis between therapy initiation and 12 months. For these 3 patients, synthesis significantly decreased thereafter. Furthermore, median CSF IgG, IgM, and IgA concentrations significantly decreased over time. All median values and p values are listed in an additional table (table e-2, links.lww.com/NXI/A172). At baseline, 79% of all patients exhibited positive OCBs in the CSF. At 12 and 24 months after therapy start, their percentage amounted to 75% (p = 1.000) and 71% (p = 0.0662), respectively. In fact, for 2 patients, OCBs were no longer detectable at 24 months (figure e-1, links.lww.com/NXI/A172).

Figure 1 Measurement of serum immunoglobulin (IgG, IgA, and IgM) levels in alemtuzumab-treated patients
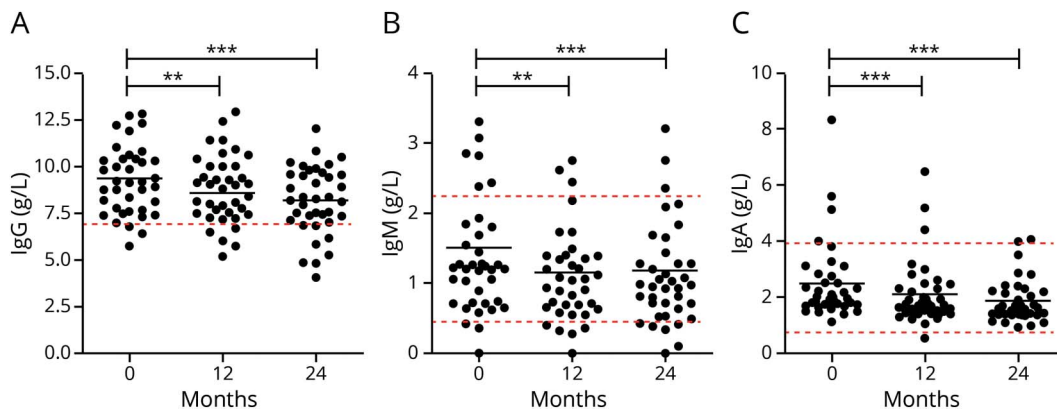
Serum IgG (A), IgM (B), and IgA (C) levels (in g/L) were analyzed before the first alemtuzumab course and at 12 and 24 months. Stars represent levels of significance (**$p < 0.01$; ***$p < 0.001$).

Patients exhibit reduced serum IgG levels after the third course of alemtuzumab

Eight patients required a third course of alemtuzumab due to sustained disease activity (new relapses: 4/8; EDSS progression: 1/8, cMRI with new/enlarging T2 lesions: 7/8). We measured serum immunoglobulin levels 12 months after the third course of alemtuzumab (36 months after initiation of therapy). Unlike previous measurements, those samples underwent freezing and thawing before analysis, and our quality control indicated slight differences in 4 samples. We performed a quality control measurement of 4 samples that were both freshly analyzed stored and found slight differences in IgG levels (5%). We therefore decided not to perform comparison between 36-month samples and other time points but compared our findings with similarly processed samples from patients having not received a third course. In addition, we included samples from naive patients with MS as further control.

Compared with patients treated twice and treatment-naive patients with MS (table e-3, links.lww.com/NXI/A172), patients with a third alemtuzumab course showed further, significant reduction of serum IgG levels, ranging from 5.0 to 7.5 g/L ($p = 0.006$ compared with patients with 2 courses). The majority of controls presented with serum IgG levels of about 10.0 g/L at 36 months. For the other immunoglobulin subgroups (IgM and IgA), no differences were detectable. Because specimens were thawed and serum immunoglobulin levels were measured afterward, values are comparable among each other but not with regard to reference values.

Patients with reduced immunoglobulin levels were more likely to have otitis, sinusitis, and pneumonia

In adults, the lower limit of normal for serum IgG levels is about 7 g/L. For lower values, IgG deficiency is diagnosed. For the 38
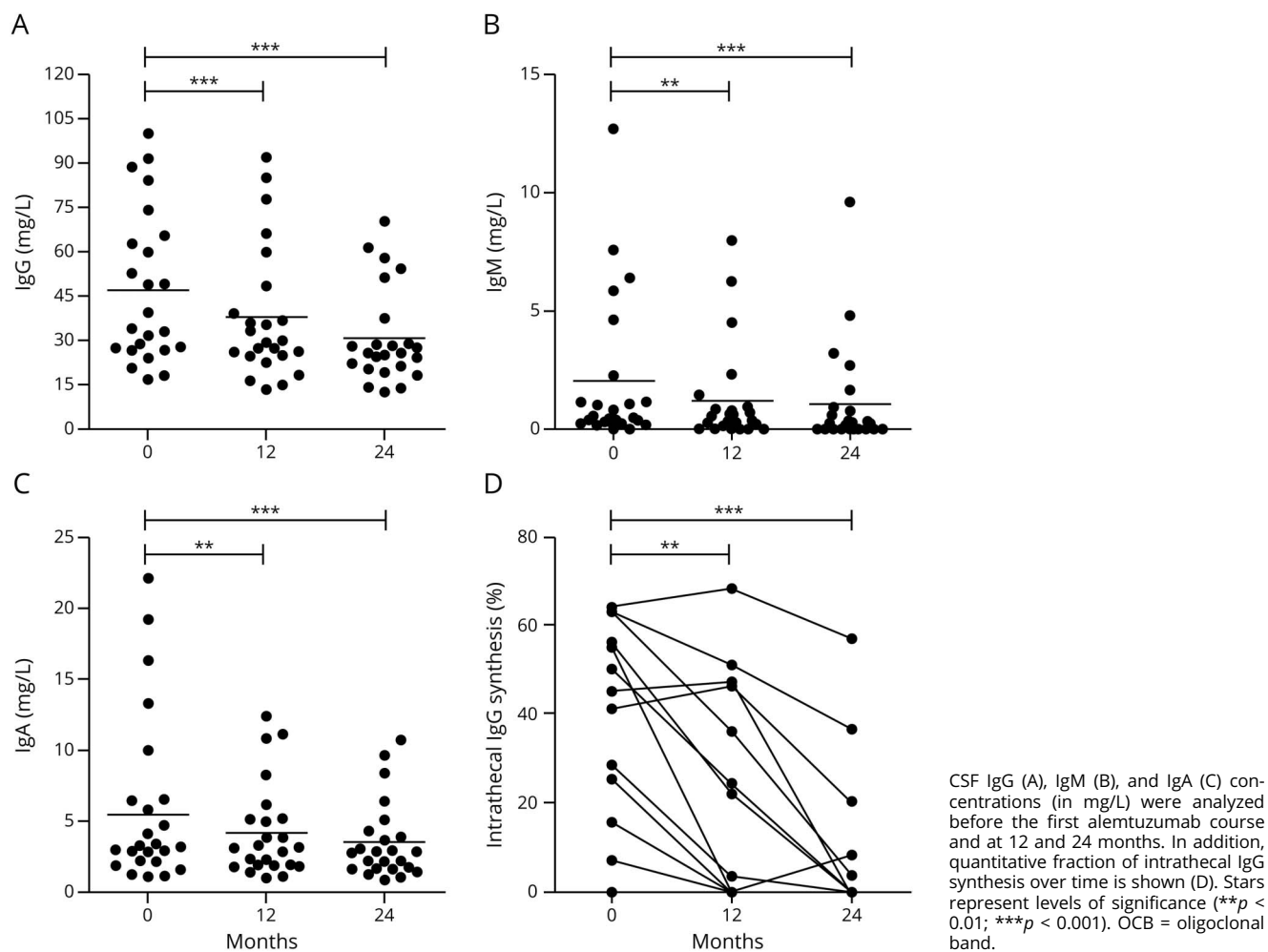
Table 2 Frequency of different infections during alemtuzumab treatment

	Total events with qualified IgG measurement	Serum IgG >7 g/L in closest proximity to event	Serum IgG <7 g/L in closest proximity to event	Time from last course of ALEM, range (months)
Upper respiratory tract infection	57	50	7	3–35
Otitis media	9	3	**6**	13–32
Sinusitis	10	2	**8**	11–26
Pneumonia	3	0	**3**	14–26
Skin infection	2	2	0	5–12
Urinary tract infection	48	42	6	2–30

Abbreviation: ALEM = alemtuzumab.
Patients with serum IgG levels >7 g/L were compared with patients with serum IgG levels <7 g/L. Numbers in columns 1-3 represent observed events.
Bold numbers indicate infections associated with hypogammaglobulinemia.

Figure 2 Measurement of CSF immunoglobulin (IgG, IgA, and IgM) levels in alemtuzumab-treated patients


CSF IgG (A), IgM (B), and IgA (C) concentrations (in mg/L) were analyzed before the first alemtuzumab course and at 12 and 24 months. In addition, quantitative fraction of intrathecal IgG synthesis over time is shown (D). Stars represent levels of significance (**p < 0.01; ***p < 0.001). OCB = oligoclonal band.

alemtuzumab-treated patients, we documented any infections as events in a 3-year follow-up. In 36/38 patients (95%) of patients, we observed infections as adverse events. Mainly, patients had upper respiratory tract or urinary tract infections (44% and 37% of all events, respectively). Several cases of otitis and sinusitis were also observed. Three individuals had pneumonia, and 2 had soft tissue infections. We analyzed IgG levels when patients presented to the neurologic department for their regular 3-monthly visit after alemtuzumab treatment. Some patients required repeated measurement. We evaluated all events for which serum IgG levels were available within a 3-month period before reported infections. We checked whether preexisting IgG deficiency was associated with an increased susceptibility toward different types of infections. In case of multiple measurements within the specified time, we included the measurement closest to onset of infection.

Within the group of patients with upper respiratory tract infections and urinary tract infections, IgG levels were above 7 g/L in the majority of cases. Patients having otitis, pneumonia, or sinusitis frequently exhibited reduced serum IgG levels during their previous examination.

Discussion

Administering alemtuzumab leads to rapid depletion of circulating CD52–positive immune cells. However, immune cells in lymphoid organs are less affected.[16] T cells recover within 11–12 months, whereas the B-lymphocyte count typically normalizes within 6 months posttreatment. However, Jones and colleagues observed early T-cell recovery after alemtuzumab treatment, largely driven by homeostatic expansion of cells that escaped depletion. Furthermore, they demonstrated that homeostatic proliferation increased the risk of secondary autoimmunity.[17] Between 30% and 41% of alemtuzumab-treated patients develop thyroid autoimmune disease. Less common, but potentially more critical, are immune thrombocytopenia and glomerulonephritis.[11] A possible mechanism behind secondary autoimmunity is the faster repopulation of B cells in the absence of effective T-cell regulation in individuals with genetic susceptibility for autoimmunity.[18] However, T cell–mediated secondary autoimmune phenomena have also been described[19] and the mechanisms of reeducation of immune regulatory networks after CD52 depletion remain largely elusive.[20]

Figure 3 Serum immunoglobulin levels of patients with third alemtuzumab course compared with controls and treatment-naive patients with MS
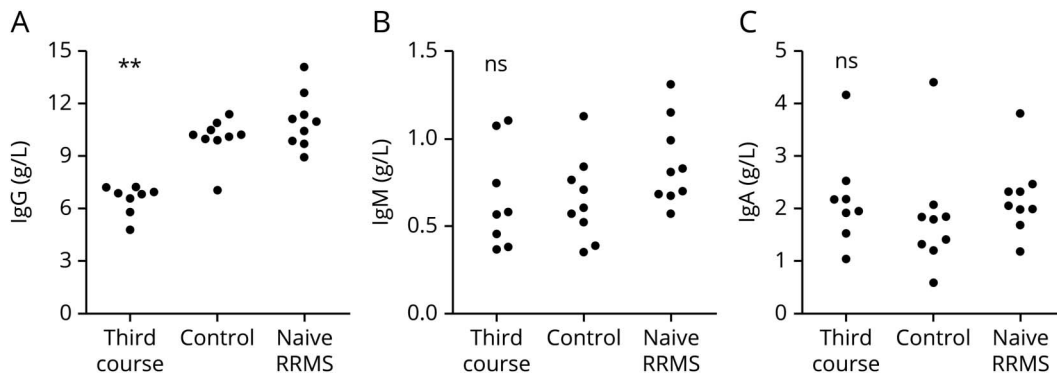
Samples were collected 36 months after initiation of therapy; serum IgG (A), IgM (B), and IgA (C) levels (in g/L) were measured. Controls had received 2 courses of alemtuzumab treatment and were matched to third-course patients. Samples taken from third-course patients and treatment-naive patients with MS were analyzed after thawing. Stars represent levels of significance (**$p < 0.01$ vs control).

Despite the faster recovery of B cells, our results demonstrate significantly decreased immunoglobulin levels in alemtuzumab-treated patients 12 and 24 months after administering the first dose (figure 1). The levels even further decrease after a third therapeutic cycle. In patients without a third course, IgG but not IgM and IgA concentrations increased 36 months after therapy initiation (figure 3). The ongoing reduction of immunoglobulin levels despite normalized B-cell counts indicates a decrease of immunoglobulin concentrations in serum independent from B-lymphocyte repopulation. Recently, it has been shown that B-cell distribution shifts toward a naive phenotype following alemtuzumab treatment. CD19+CD24hiCD38hi and CD19+PD-L1hi cells, which are deficient in peripheral blood of patients with RRMS, increase after alemtuzumab administration.[21] These cells are known for supporting an anti-inflammatory environment by limiting Th1 and Th17 differentiation and maintaining regulatory T cells.[22] This may serve as an explanation for continuously low immunoglobulin concentrations. Wray and colleagues found that patients in phase 2 (CAMMS223) and phase 3 (CARE-MS I and CARE-MS II) studies exhibited similar absolute counts of CD4+ T-cell, CD8+ T-cell, and CD19+ B-cell subsets whether they developed an infection or not. This finding was common to all time points examined.[23] The authors hypothesized that unchanged concentrations of immunoglobulins to common viruses, as shown by Clark and colleagues,[24] result from CD52-negative antibody-secreting long-lived plasma cells.[25] While we did not measure specific antibodies, the total immunoglobulin concentration decreased in our patient cohort.

The ongoing reduction of IgG levels in peripheral blood seems to be of clinical relevance. Among our 38 patients, those with IgG values below 7 g/L showed a higher susceptibility to develop infections such as pneumonia, sinusitis, and otitis (table 2), whereas the more common infections such as upper respiratory tract and urinary tract infections were not related to reduced serum IgG levels. It has to be noted that we did not differentiate between mild hypogammaglobulinemia (defined as IgG <7 g/L) and severe hypogammaglobulinemia (defined as IgG <4 g/L). In a study of 389 patients with secondary hypogammaglobulinemia, Blot and colleagues detected no significant difference between patients with mild and severe hypogammaglobulinemia regarding their infectious risk,[26] which indicates that hypogammaglobulinemia generally increases the risk of infection. However, Furst stated that very low levels of IgG are indeed associated with a heightened risk of infections, but he also demonstrated that less severe hypogammaglobulinemia (>5 g/L) appears to be tolerated in most subjects.[27]

Within our cohort, pneumonia, sinusitis, and otitis were observed much later following alemtuzumab compared with urinary tract infections and upper respiratory tract infections, which occurred early after infusions. This could indicate differences in underlying pathophysiology. Hypogammaglobulinemia is also known as a relevant risk factor for infections with cytomegalovirus (CMV). Recently, few case reports have described CMV infections in alemtuzumab patients.[12,28] Although an increased risk of infections is clearly acknowledged in phases around infusions, a clear signal for an increased risk of infections has not been reported to date. Other depleting antibodies used to treat autoimmune diseases, such as the anti-CD20 antibody rituximab, also lead to a significant decrease of blood immunoglobulin levels during therapy.[29] However, several studies on patients with rheumatoid arthritis (RA) treated with rituximab could demonstrate that patients with below normal immunoglobulin levels did not have more serious infections than patients with normal immunoglobulin levels.[29–32]

Our results appear to be in contrast to the findings of McCarthy et al.[33] who performed a pilot study regarding immunologic memory to common viruses and responses to vaccinations in 24 patients with prior alemtuzumab treatment. Their patients exhibited a normal humoral response to diphtheria, tetanus, and poliomyelitis vaccine, haemophilus influenzae type b and meningococcal group C conjugate vaccine, and pneumococcal

polysaccharide vaccine with normal IgG titers. One possible explanation might be that in contrast to the McCarthy cohort, most of our patients had received multiple immunomodulatory therapies before alemtuzumab administration. Being a real-world cohort with patients including complex history of MS treatment despite exclusion of patients with a history of mitoxantrone, azathioprine or anti-CD20 therapy might also explain why the observation of developing hypo-gammaglobulinemia has not been made in the phase 3 trials and their respective extension studies.[23] Age-dependent effects in our cohort are rather unlikely as the median even undercuts the median age of patients from CARE-MS II. However, real-world cohorts have shown different frequencies or even entities of adverse events following immunomodulatory treatment with progressive multifocal leukoencephalopathy following natali-zumab treatment being rapidly present in one's mind.[34]

If immunoglobulin levels would be monitored during and following alemtuzumab therapy, especially in patients with multiple previous DMTs, patients with markedly reduced IgG levels could be identified, and possibly, a prophylactical antibiotic treatment or immunoglobulin substitution therapy could be initiated to prevent serious infections in those patients. Besides McCarthy et al., another group assessed antigenic responses following influenza, polyvalent pneumococcus vaccine (PPV23) and combined diphtheria/tetanus/poliovirus vaccines in patients with RA who had been treated with alemtuzumab 20 years ago. Similar levels of seroprotection following poliovirus (P1-P3), tetanus, and diphtheria vaccination were observed for alemtuzumab-treated patients and controls.[35] As the nature of the observed infections in our cohort (pneumonia, otitis, and sinusitis) indicates that encapsulated bacteria such as *Streptococcus pneumoniae* might be responsible, PPV23 vaccination before alemtuzumab induction seems reasonable.

An interesting aspect of our study is the parallel measurement of CSF immunoglobulins, in addition to the periphery. Because of the decrease of serum IgG concentrations, reduction of IgG levels measured in CSF is to be expected. Of interest, the percentage of intrathecal IgG synthesis itself is lowered in nearly all patients (figure 2). This indicates that reduction of CSF IgG is not merely a consequence of decreased serum IgG concentrations and could suggest that alemtuzumab inhibits the autoimmune process within the CNS. This is also reflected by the disappearance of OCBs in 2 patients (figure 2).

Within our cohort, 8 patients required a third treatment cycle because of ongoing/returning clinical or paraclinical disease activity. They exhibited significantly reduced serum IgG concentrations compared with matched controls who received only 2 treatment cycles and also with treatment-naive patients with MS (figure 3). Therefore, the decrease in serum IgG concentrations is not merely a consequence of thawing the specimens. The fact that patients with 3 treatment courses presented with lower IgG concentrations is not surprising, as they received a higher overall dose of alemtuzumab and their last treatment took place only 12 months ago. Of interest,

other immunoglobulin subgroups were not affected. In addition, it can be observed that serum IgG levels of alemtuzumab patients without a third treatment course obviously normalized over time, as they do not differ from IgG concentrations of untreated patients with MS (figure 3).

Our study has several limitations. First, this work is based on a retrospective analysis of clinical data. Because of the nature of this investigation, no causal relations can be interpreted. Moreover, the number of cases especially of patients with a third alemtuzumab course is limited, and their samples have been thawed before the analysis of immunoglobulin levels. As a result, the findings need to be verified in larger samples. In addition, real-world cohorts are needed to determine the exact temporal expansion of hypo-gammaglobulinemia following alemtuzumab treatment.

Conclusion

We could demonstrate reduced concentrations for all immunoglobulin subgroups at 12, 24, and 36 months after initiation of alemtuzumab therapy. Patients requiring a third treatment cycle due to ongoing disease activity had the most severe drop in IgG concentrations. Reduced IgG concentrations were associated with an increase in pneumonia, otitis, and sinusitis. We therefore suggest that serum IgG levels should be monitored at least in those patients receiving more than 2 treatment courses of alemtuzumab. In addition, we recommend a pretreatment pneumococci vaccination. The decreased intrathecal IgG production in the CSF suggests that alemtuzumab effectively suppresses the autoimmune process within the CNS.

Publication history

Appendix Authors

Name	Location	Role	Contribution
Nora Möhn, MD	Hannover Medical School, Hannover, Germany	Author	Acquisition and interpretation of data and writing the manuscript
Steffen Pfeuffer, MD	University of Münster, Münster, Germany	Author	Acquisition and interpretation of data and critical revision of the manuscript for intellectual content
Tobias Ruck, MD	University of Münster, Münster, Germany	Author	Critical revision of the manuscript for intellectual content
Catharina C. Gross, MD, Prof	University of Münster, Münster, Germany	Author	Supervision of acquisition, analysis, and interpretation of CSF routine parameters and critical revision of the manuscript for intellectual content

Appendix *(continued)*

Name	Location	Role	Contribution
Thomas Skripuletz, MD, Prof	Hannover Medical School, Hannover, Germany	Author	Critical revision of the manuscript for intellectual content
Luisa Klotz, MD, Prof	University of Münster, Münster, Germany	Author	Critical revision of the manuscript for intellectual content
Heinz Wiendl, MD, Prof	University of Münster, Münster, Germany	Author	Critical revision of the manuscript for intellectual content
Martin Stangel, MD, Prof	Hannover Medical School, Hannover, Germany	Author	Study concept and design and critical revision of the manuscript for intellectual content
Sven G Meuth, MD, Prof	University of Münster, Münster, Germany	Author	Study concept and design and critical revision of the manuscript for intellectual content

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