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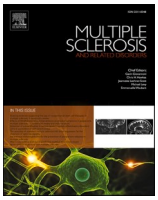

Humoral response to Epstein-Barr virus in patients with multiple sclerosis treated with B cell depletion therapy

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ABSTRACT

Background: B cell depletion therapy is highly effective in relapsing-remitting multiple sclerosis (RRMS). However, the precise underlying mechanisms of action for its biological effects in MS have still not been clarified. Epstein-Barr virus (EBV) is a known risk factor for MS and seems to be a prerequisite for disease development. EBV resides latently in the memory B cells, and may not only increase the risk of developing MS, but also contribute to disease activity and disability progression. Therefore, the effects of B cell depletion in MS could be associated with the depletion of EBV-infected cells and the altered immune response to the virus. In this study, we investigate the impact of B cell depletion on the humoral immune response specific to EBV in patients with MS.

Methods: Newly diagnosed, treatment-naïve patients with RRMS were followed up to 18 months after initiation of B-cell depletion therapy in the Overlord-MS study, a phase III trial (NCT04578639). We analyzed serum sampled before treatment and after 3, 6, 12 and 18 months for immunoglobulin γ (IgG) against Epstein-Barr nuclear antigen 1 (EBNA1) and Epstein-Barr viral capsid antigen (VCA). We analyzed antibodies to cytomegalovirus (CMV) and total IgG in serum, as controls for viral and overall humoral immunity. The risk allele, *HLA-DRB1*15:01,* and the protective allele, *HLA-A*02:01,* were determined in all participants. In addition, polymerase chain reaction (PCR) for circulating EBV-DNA was performed in the first 156 samples drawn. The associations between time on B cell-depletion therapy and serum anti-EBV antibody levels were estimated using linear mixed-effects models.

Results: A total of 290 serum samples from 99 patients were available for analysis. After 6, 12 and 18 months, the EBNA1 IgG levels decreased by 12.7 % (95 % CI -18.8 to -6.60, $p < 0.001$), 12.1 % (95 % CI -19.8 to -3.7, $p = 0.006$) and 14.6 % (95 % CI to -25.3 to -2.4, $p = 0.02$) respectively, compared to baseline level. Carriers of the *HLA-DRB1*15:01* allele had higher EBNA1 IgG levels at baseline ($p = 0.02$). The VCA IgG levels significantly increased by 13.7 % (95 % CI 9.4 to 18.1, $p < 0.001$) after 3 months, compared to baseline, and persisted at this level throughout the follow-up. CMV IgG levels decreased, but to a lesser extent than the decrease of EBNA1 IgG, and total IgG levels decreased during therapy. Circulating EBV-DNA was found in only three of 156 samples from 64 patients.

Conclusions: EBNA1 IgG levels decreased, while VCA IgG levels increased, during B cell depletion therapy. This supports the hypothesis that the mechanism of action for B cell depletion therapy might be mediated by effects on EBV infection, which, in turn, mitigate immune cross-reactivity and disease perpetuation.

Abbreviation: EBV, Epstein-Barr virus; RRMS, relapsing-remitting multiple sclerosis; EBNA1, Epstein-Barr nuclear antigen-1; VCA, EBV viral capsid antigen; CMV, cytomegalovirus; Ig, immunoglobulins; MRI, magnetic resonance imaging; PCR, polymerase chain reaction.

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1. Introduction

Epstein-Barr virus (EBV) is ubiquitous and one of the most common virus infections in humans (Young et al., 2016). EBV is able to reside latent in the memory B cells (Houen and Trier, 2020) and more than 90 % of the adult population are carriers of the virus (Young et al., 2016). Mainly two immunoglobulins (Ig) are used as markers of EBV infection in diagnostics: anti-Epstein-Barr nuclear antigen 1 (EBNA1) IgG and anti-Epstein-Barr viral capsid antigen (VCA) IgG. EBNA1 is a protein essential for the virus' persistence and replication, and VCA encapsidates the virus' genome and is expressed mainly during the lytic phase.

Infection with EBV is the strongest risk factor for multiple sclerosis (MS) and seems to be a prerequisite for disease development (Bjornevik et al., 2022). Especially high levels of IgG against EBNA1 are associated with increased MS-risk (Ascherio et al., 2001; Sundström et al., 2004; Levin et al., 2005). One of the suggested mechanisms for the association is molecular mimicry, where antibodies to EBNA1 cross-react with CNS proteins, such as GlialCAM, alpha-crystallin B and Anoctamin 2 (Lanz et al., 2022; Thomas et al., 2023; Tengvall et al., 2019). The association between high VCA IgG levels and MS-risk is less certain, as some have found an increased risk (Ascherio et al., 2001) (Levin et al., 2005) and others a reduced risk (Sundström et al., 2004). Some speculate that EBV is also a mediator of disease activity (Sollid, 2022), however, results from studies are conflicting on whether elevated EBNA1 and VCA IgG levels are associated with MRI and clinical markers of MS progression and disability (Ascherio et al., 2001) (Levin et al., 2005) (Munger et al., 2015) (Farrell et al., 2009) (Kvistad et al., 2014) (Horakova et al., 2013) (Lünemann et al., 2010) (Gieß et al., 2017).

Anti-CD20 monoclonal antibodies, which depletes circulating B cells, are one of the most effective disease modifying therapies in MS (Granqvist et al., 2018) (Torgauten et al., 2021). The underlying mechanisms by which B cell depletion therapy reduces disease activity are not clear, but it has been suggested that removal of EBV-infected cells alters immune responses to the virus, which in turn, mitigates antibody cross-reactivity and MS disease perpetuation (Berger and Kakara, 2022).

The aim of our study was to investigate the changes in humoral immune response to EBV after initiation of B cell depletion therapy in MS, using prospectively collected serum samples from treatment-naïve patients, followed for up to 18 months.

2. Materials and methods

2.1. Design, study population and procedure

The study population consists of a subgroup of participants in an ongoing randomized double-blinded non-inferiority trial of ocrelizumab vs. rituximab for RRMS (Overlord-MS study, NCT04578639). All patients recruited in the trial at Haukeland University Hospital, Bergen, Norway between November 2, 2020, and October 20, 2022, were included. Overlord-MS inclusion criteria are: a diagnosis of relapsing-remitting MS according to the 2017 McDonald criteria (Thompson et al., 2018); being treatment-naïve; aged 18 - 60 years; disease activity within the latest 12 months, defined as $\geq$ 1 relapse or $\geq$ 1 new MRI lesion during the last 12 months; expanded disability status scale (EDSS) score $\leq$ 4.0; absence of comorbidity precluding study participation. Patients were randomized to receive either rituximab or ocrelizumab, at doses of 500 mg and 300 mg, respectively, initially starting with 1000 mg and 600 mg. As the therapy in both treatment arms was anti-CD20, and as this is an ongoing double-blinded trial, they were pooled in one cohort for analysis. Both treatment arms received therapy at baseline, and at month 6, 12, 18 and 24. Serum and plasma were collected pre-infusion on the same days, in addition to 3 months after the first infusion (Fig. 1).

2.2. Outcome measures

2.21. EBV serology

We assessed the presence of immunoglobulins (Ig) G antibodies to Epstein-Barr virus nuclear antigen 1 (EBNA1), viral capsid antigen (VCA)-p18 and cytomegalovirus (CMV) using the Liaison XL ® quantitative chemiluminesce assay (DiaSorin, Saluggia Italy) according to the instructions by the manufacturer. The results were expressed as relative light units (RLU) with corresponding concentration of antibodies (U/mL). For 48 % of the samples, the test results were above the upper reference range, and these were therefore diluted, 1:20. To assess the reliability of the analyses, the first 24 serum samples were analysed twice. Intra-assay coefficients of variation ranged from 3.8 % (EBNA1 IgG) to 6.0 % (VCA IgG) in the samples measured in duplicate. All sera were analysed in batches, at the Department of Microbiology, Haukeland University Hospital. Measurement of total IgG in the sera was performed simultaneously, but analysed on a nephelometer (Athellica NEPH 360, Siemens) as part of routine diagnostics at the Department of Immunology and Transfusion Medicine, Haukeland University Hospital.

2.2.2. HLA-analysis

High-resolution sequencing-based typing of the major histocompatibility complex (MHC) class I allele, *HLA-A*02,* and the MHC class II allele, *HLA-DRB1*15,* was performed at the Department of Transplantation Immunology, Oslo University Hospital. NGSgo®-AmpX v2 and NGSgo® Library Full Kit from Gendx (Utrecht, The Netherlands) on an Illumina Miseq sequencer with a MiSeq Reagent Kit v2 (300-cycles) were used. HLA genotypes were obtained using the NGSengine software from Gendx. Patients carrying at least one *HLA-A*02:01* allele were classified "*HLA-A*02:01* positive" and those carrying at least one *HLA-DRB1*15:01* were classified "*HLA-DRB1*15:01* positive".

2.2.3. EBV-DNA

EBV-DNA was measured in the plasma samples with a quantitative nucleic acid amplification test on Cobas 6800 Systems (Roche, by Mannheim Germany). The system is a fully automated molecular analyzer, all steps, including primary sample handling, DNA extraction and PCR amplification were performed in the same system without further manual intervention. The results were reported as units per milliliter (IU/mL), as recommended by the world health organization (Fryer et al., 2016). The limit of detection was 19 IU/mL, with a linear range between 35 and 100 000 000 IU/mL. All PCR analyses were run at the same laboratory and in one batch.

2.3. Statistical analyses

Demographic characteristics at baseline are presented as mean values with standard deviations (SD). All statistical data analyses were performed using R for Windows, version 4.2.1. Antibody levels that were lower than the quantification limit of the assay were replaced with the value half of the limit, before being included in the statistical analysis. In all analyses, immunoglobulin levels were log-transformed to improve normality. Where the baseline levels of immunoglobulins were normally distributed within their groups for each analysis, the levels were compared using the two-sample *t*-test. Where they were not normally distributed, the non-parametric Wilcoxon rank sum test was used. Baseline levels of immunoglobulins by age were compared by linear regression. Mixed-effects linear regression with random intercept for individuals was used to compare measurements of serum antibody levels at different time points after initiation of B cell depletion therapy, using the r-package "lme4". EBV antibody levels were included as the dependent variable, while time categorized as baseline, 3-month's visit, 6-month's visit, 12-month's visit and 18-month's visit, sex and age were included as independent variables. The regression coefficients were back-transformed to the original scale. All tests are 2-tailed. The α-level was set at 0.05.

2.4. Ethics

The study protocol was approved by the Regional Committee for Medical and Health Research Ethics in Western Norway (66,391) and the Norwegian Medicines Agency. All patients provided written informed consent. The Overlord-MS trial is registered at ClinicalTrials.gov, ID NCT04578639.

3. Results

3.1. Descriptive statistics

We collected a total of 290 serum samples from 99 patients. The patients were followed up to 18 months, but as the trial is still ongoing, the median follow-up time after the start of therapy was 6 months. For one patient, the HLA-status was missing for both *HLA-A*02*- and *HLA-DRB1*15*-status, and for 7 patients only the *HLA-DRBB1*15*-status was missing. Demographic characteristics are shown in Table 1.

Table 1
Sample characteristics.

Characteristic	Number (%)
Sex	
Female	70 (70.7)
Male	29 (29.3)
Age at baseline, median years (SD)	37.7 (9.7)
HLA-status/available sample results	
*HLA-DRB1*15:01*-positive	41 /91 (45.0)
*HLA-A*02:01*-positive	47 / 98 (48.0)
Both *HLA-A*02:01* and *HLA -DRB1*15:01*-positive	22 / 91 (24.2)
Number of patients at visits, n	
Visit 1 (baseline)	99
Visit 2 (3 months after baseline)	80
Visit 3 (6 months after baseline)	62
Visit 4 (12 months after baseline)	35
Visit 5 (18 months after baseline)	14

Abbreviations: HLA, human leukocyte antigen; n, number; SD, standard deviations.

3.2. Baseline levels of antibodies, by HLA-status, sex and age

All the patients were seropositive for EBV at baseline. Only one patient had low levels of VCA IgG, but this patient had high levels of EBNA1 IgG, and was therefore considered EBV seropositive. 94 of 99 patients were positive for EBNA1 IgG, two were negative and three intermediate. 56 of 99 patients were CMV IgG positive, the rest were negative. The reference ranges for the Liaison XL ® quantitative chemiluminesce assays are found in the supplemental Table 1.

*HLA-DRB1*15:01* positive patients had significantly higher EBNA1 IgG levels at baseline, compared to those *HLA-DRB1*15:01* negative (Fig. 2; $p = 0.014$). Baseline levels of VCA IgG, CMV IgG and total IgG were not affected by *HLA-DRB1*15*-status. *HLA-A*02*-status and sex did not affect any of the immunoglobulin levels at baseline (Fig. 2, 3 and supplemental Fig. 1). Higher age at baseline was associated with lower total IgG levels (supplemental Fig. 1; $p = 0.023$).

3.3. Changes in EBNA1 IgG levels

EBNA1 IgG levels decreased with time since initiation of B cell depletion therapy. At the visit 3 months after baseline, there was no statistically significant change compared to baseline. At the visits 6, 12 and 18 months after baseline, there was a decrease of 12.7 % (95 % CI −18.8 to −6.60, $p < 0.001$), 12.1 % (95 % CI −19.8 to −3.7, $p = 0.006$) and 14.6 % (95 % CI to −25.3 to −2.4, $p = 0.021$) respectively, compared to baseline (Fig. 4A and supplemental Fig. 2A).

When *HLA-DRB1*15*- and *HLA-A*02*-status were included in the model, the estimates remained significant, except 18 months after treatment initiation. When we stratified the cohort based on *HLA-DRB1*15:01*-positivity before analysis, both subgroups showed decreasing levels of EBNA1 IgG during therapy, but the estimates were not statistically significant (Fig. 5). When stratified for *DRB1*15:01*-positivity and sex, the response to B cell depletion therapy did not seem to be affected in any of the immunoglobulin levels analyzed (supplemental Fig. 3 and supplemental Fig. 4).

3.4. Changes in VCA IgG levels

VCA IgG levels increased with time since initiation of B cell depletion therapy, by 13.7 % (95 % CI 9.4 to 18.1, $p < 0.001$), 12.2 % (95 % CI 7.6 to 17.0, $p < 0.001$), 13.4 % (95 % CI 7.6 to 19.6, $p < 0.001$) and 12.0 % (95 % CI 3.7 to 20.9, $p = 0.004$) respectively, at 3, 6, 12 and 18 months compared to baseline (Fig. 4B and supplemental Fig. 2B). The ratio of EBNA1 IgG/VCA IgG decreased with 0.59 ($p = 0.020$) and 0.83 ($p = 0.004$) 3 and 6 months after therapy, compared to the ratio at baseline (supplemental Fig. 5), though individuals seronegative for any of the two immunoglobulins at baseline were excluded in this analysis.

3.5. Changes in CMV IgG and total IgG levels

CMV IgG levels in patients who were seropositive at baseline ($n = 56$) did not change significantly during follow-up, except for a decrease of 7.2 % (95 % CI −13.2 to −0.8, $p = 0.027$) at 6 months and 16.4 % (95 % CI – 27.4 to −3.97, $p = 0.012$) at 18 months after initiation of B cell depletion therapy, compared to baseline (Fig. 4C and supplemental Fig. 2C). Total IgG levels decreased significantly by 12.4 % (95 % CI −14.7 to −10.0, $p < 0.0001$), 12.5 % (95 % CI -15.3 to −9.6, $p < 0.0001$), 12.6 % (95 % CI −16.0 to −9.0, $p < 0.0001$) and 17.3 % (95 % CI −22.5 to −11.8, $p < 0.0001$) at the 3, 6, 12 and 18 month's visits, compared to baseline (Fig. 4D and supplemental Fig. 2D).

3.6. EBV-DNA

Plasma for analysis of EBV-DNA was available from 156 samples of 64 patients. Of these samples, 64 were drawn at baseline, 49 after 3 months, 31 after 6 months and 12 after 12 months after baseline. All, except three samples, were negative. The three positive samples had low concentrations, between 38–42 IU/ml. Two of the positive samples were

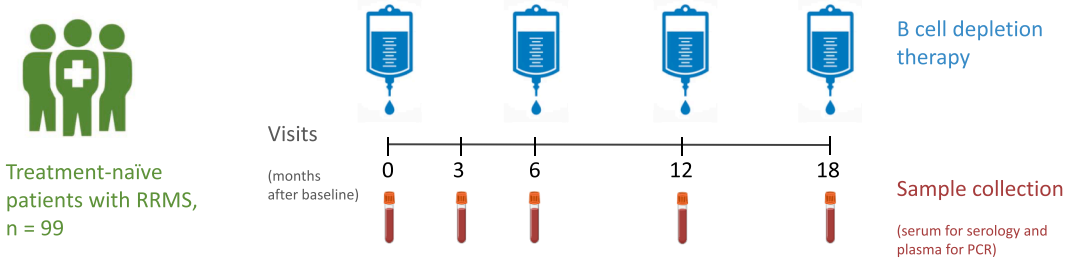

Fig. 1. Trial procedure.

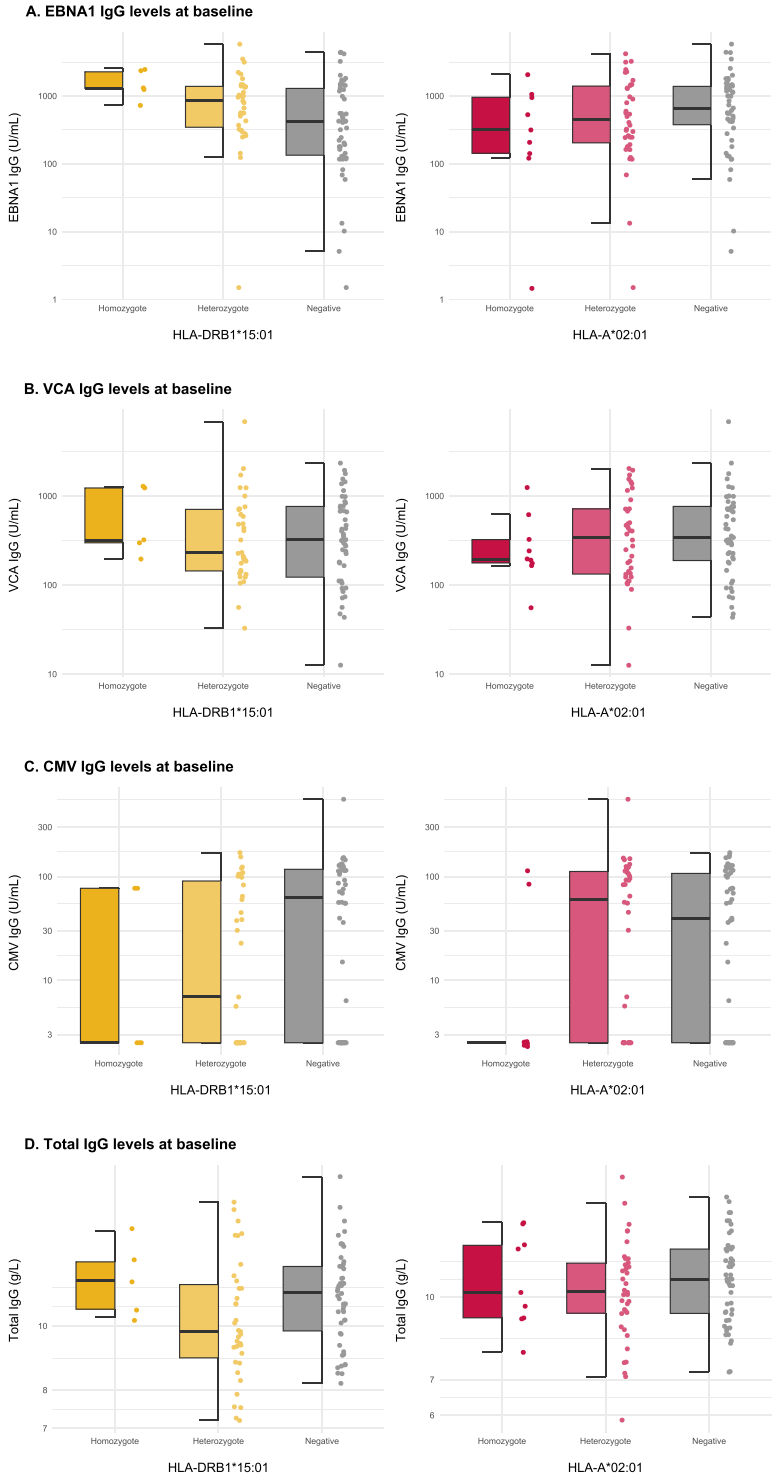

Fig. 2. ***Immunoglobulin levels at baseline by HLA-DRB1*15- and HLA-A*02-status.*** The figure shows box plots of immunoglobulin levels at baseline in patients with multiple sclerosis, by HLA-status. Yellow indicates patients who are *HLA-DRB1*15:01*-positive (41 of 91) and red *HLA-A*02:01*-positive (47 of 98). Each dot represents one serum sample from one patient. Log(EBNA1 IgG) and log(CMV IgG) were not normally distributed, and the non-parametric Wilcoxon rank sum test was used. Log(total IgG) and log(VCA IgG) were normally distributed within each group, and compared using the two-sample *t*-test. *HLA-DRB1*15:01* positive patients had higher EBNA1 IgG levels at baseline, compared to those *HLA-DRB1*15:01* negative ($p = 0.014$). Baseline levels of VCA IgG, CMV IgG and total IgG were not affected by *HLA -DRB1*15*-status. *HLA-A*02*-status was not associated with either of the immunoglobulin levels. For the analysis of CMV IgG by HLA-status, those CMV seronegative were not included, but they are included in the figure, Fig. 2C.

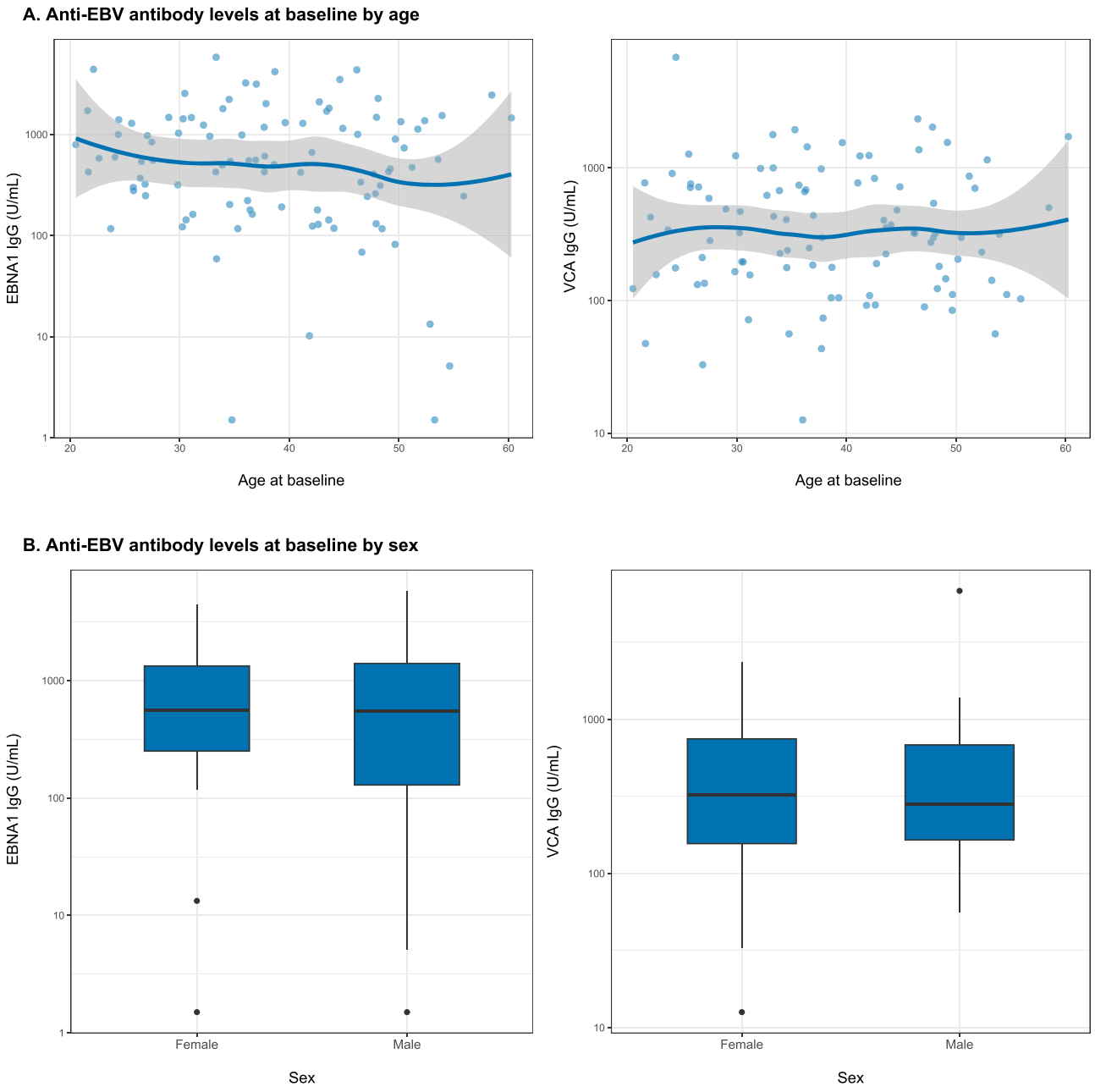

Fig. 3. ***Anti-Epstein-Barr virus-immunoglobulin levels at baseline by age and sex.*** In the scatter-plots of anti-EBV immunoglobulin levels at baseline by age, each dot represents one serum sample. The blue lines are smooth trend lines with their corresponding 95 % confidence intervals. The box plots for anti-Epstein-Barr virus-immunoglobulin levels at baseline by sex represent 50 % of the samples and the middle line is the median. The whiskers extend out 1.5 times the interquartile range. 70 patients were female and 29 were male ($n = 99$). Sex and age were not significantly associated with the log of EBNA1 IgG or VCA IgG at baseline, when Wilcoxon rank sum test, two-sample *t*-test and linear regression were used, respectively.

baseline samples, and one was from a visit 12 months after treatment initiation.

4. Discussion

In this study, we found that the EBNA1 IgG levels in the serum of treatment-naïve patients with RRMS had decreased by 13 % six months after initiation of B cell depletion therapy, and persisted at this level throughout the follow-up of 18 months. These findings suggest that the underlying mechanisms by which B cell depletion therapy affects disease activity involves regulation of EBV activity.

Our findings are consistent with two smaller studies, reporting a decline in EBNA1 IgG concentration after 12 and 24 months of B cell depletion therapy (Pham et al., 2023) (Zivadinov et al., 2022). Reduced EBNA1 IgG after initiation of therapy could be due to reduced expression of the antigen, EBNA1, as there are fewer EBV-infected cells after B cell depletion. Still, it can also be a result of a reduced number of immunoglobulin-producing cells, as one would expect, removing all circulating precursors for the plasma cells. In our study, the CMV IgG levels decreased during therapy, but not to the same extent as the decrease in EBNA1 IgG levels, in line with the hypothesis of reduced available EBV. However, there was also a general reduction of total immunoglobulin levels, by 12 % three months after therapy initiation. This implies that the altered humoral immune response during therapy is not entirely specific for EBV, but indicates that both mechanisms, less available antigen and a reduced number of IgG-producing cells, are

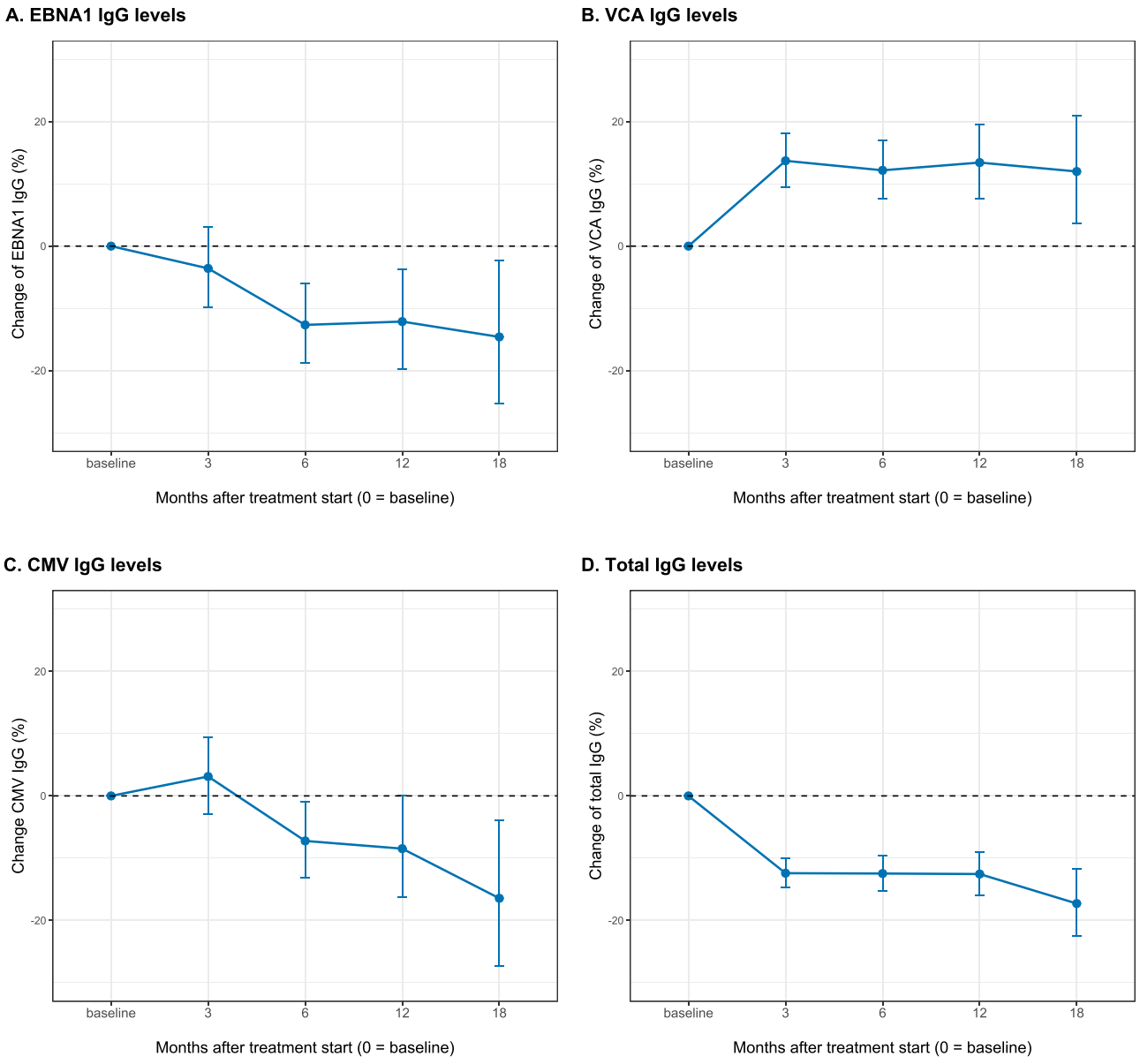

Fig. 4. ***Within-person change of immunoglobulin levels during B cell depletion therapy.*** The graphs represent the percentage change of the geometric means of the immunoglobulin levels from the baseline levels, with their 95 % confidence intervals. The graphs are visualisations of the estimates from the mixed-effects linear regression with random intercept for individuals, adjusted for sex and age. EBNA1 IgG levels of treatment-naïve patients with RRMS decreased by 13 % six months after initiation of B cell depletion therapy ($p < 0.001$) and persisted at this level throughout the follow-up of 18 months. VCA IgG levels increased by 14 % three months after therapy initiation ($p < 0.001$). CMV IgG levels of the seropositive patients at baseline did not change significantly during follow-up, except for a decrease of 7.2 % ($p = 0.03$) after 6 months and of 16.4 % ($p = 0.01$) 18 months after initiation of B cell depletion therapy, compared to baseline. Total IgG levels decreased by 12 % after three months ($p < 0.0001$).

involved.

*HLA-DRB1*15:01*-positivity was associated with higher EBNA1 IgG serum levels in the baseline samples, as previously reported (Jacobs et al., 2020), supporting that the increased MS-risk due to the HLA genotype involves regulation of EBV activity. *HLA-DRB1*15:01*-positivity has earlier been linked to increased MS disease activity, measured by new MS-lesions detected on MRI (Kvistad et al., 2014) (Brownlee et al., 2022). A more inflammatory MS phenotype may correlate with a better response to immunomodulatory therapies (Brownlee et al., 2022). In our study, regardless of *HLA-DRB1*15*-status, the EBNA1 IgG levels seemed to decrease at the same rate during therapy, although change during follow-up was not statistically significant. These trends may indicate that the *HLA-DRB1*15*-status does not have a considerable influence on the change in EBNA1 IgG levels during B cell depletion therapy.

A novel finding in our study was that the VCA IgG levels increased by 13 % three months after treatment initiation compared to baseline, and persisted at this level. This happened despite a decrease in the levels of EBNA1 IgG, CMV IgG and total IgG. The underlying mechanism for the increase in VCA IgG levels is currently not known, and should be addressed in further studies. It might have been caused by a temporary release of VCA-proteins to the circulation from the EBV-infected cells before lysed during B cell depletion, giving a responsive raise in the VCA IgG levels. The antibody levels remained elevated throughout the study period, perhaps because of repeated B cell depletion every six months with associated release of immunostimulating VCA-proteins. Our findings deviate from those of two smaller studies on patients on ocrelizumab treatment. One study reported no significant change in VCA IgG levels among 20 patients during 24 months (Zivadinov et al., 2022),

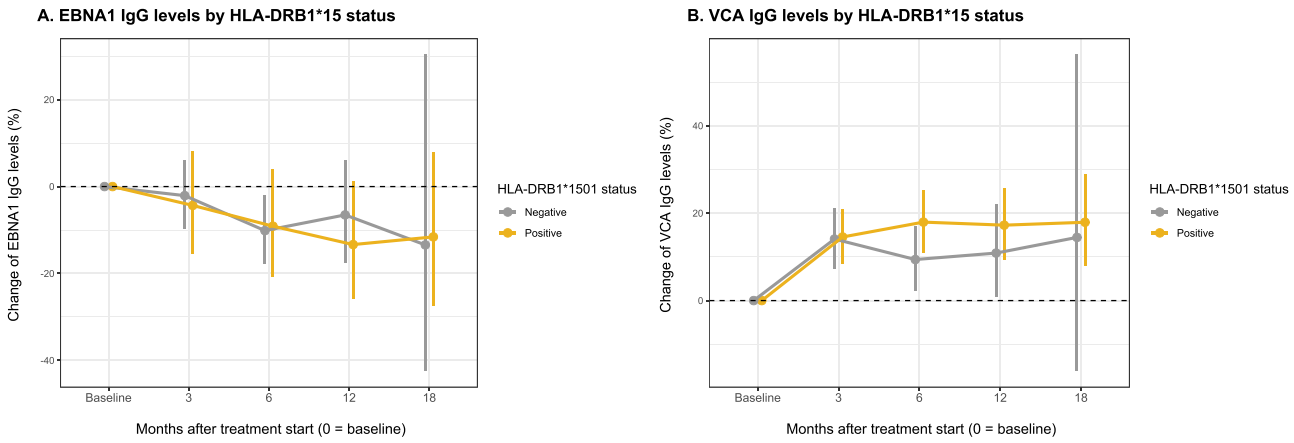

Fig. 5. ***Changes in EBV-antibody levels during B cell depletion therapy, by HLA-DRB1*15:01-positivity.*** The graphs represent the percentage change of the geometric means of the immunoglobulin levels from the baseline levels, with their 95 % confidence intervals. The graphs are visualisations of the estimates from the mixed-effects linear regression with random intercept for individuals, adjusted for sex and age. Before analysis, the cohort was stratified by HLA-DRB1*15-status. The yellow dots represent immunoglobulin levels of the *HLA-DRB1*15:01*-positive patients (n = 41) and the gray dots the immunoglobulin levels of the *HLA-DRB1*15:01*-negative patients (n = 50). The 95 % confidence intervals for the estimates of the subgroups overlap at every time point, indicating no significant difference in the specific humoral response to the treatment between the subgroups of different HLA- DRB1*15-status.

while the other reported a decline in the levels of antibodies to the small viral capsid protein, BFRF3, in 36 patients during 12 months (Pham et al., 2023).

The high levels of EBNA1 IgG in serum of patients with MS could be a result of poor elimination of the virus reservoir in these individuals. EBV DNA in plasma is mainly used as a biomarker for EBV-associated cancers, such as nasopharyngeal carcinomas (Chan et al., 2017), but is also found during the first days of infectious mononucleosis (Lupo et al., 2023). Earlier studies have found discrepant results regarding the proportion of patients with MS with detectable levels of EBV DNA in plasma (Gieß et al., 2017) (Wagner et al., 2004). We performed PCR-analysis on the samples to measure viral activity and changes during B cell depletion therapy. EBV-DNA was detected in only three of 156 plasma samples, and was therefore not a suitable biomarker of virus activity in this study population. An interesting finding was the detectable virus in plasma from a patient after initiation of B cell depletion therapy. This supports earlier findings of EBV DNA being present in the absence of circulating B cells, though earlier only reported in saliva (Hoover et al., 2008). B cells are essential for the latency of EBV (Faulkner et al., 1999). However, our finding, if confirmed, suggests that EBV can survive B cell depletion and persist in either non-circulating B cells in lymphoid tissue or in other cell types, such as natural killer-cells (Trempat et al., 2002).

The study has several strengths. It includes exclusively newly diagnosed treatment-naïve patients, has a prospective follow-up, and is the largest study reported examining patients with MS being followed for changes in the immune response to EBV during B cell depletion therapy. Furthermore it is the first to control for the second strongest risk factor, *HLA-DRB1*15*-status. The fact that our cohort is treatment-naïve at baseline is a strength when evaluating the effects of the therapy, as there seem to be longstanding effects on immune repertoires of disease-modifying drugs given before B cell depletion therapy (Mathias et al., 2023).

Nevertheless, the study has limitations. Our main outcome measure is ambiguous. The decrease in EBNA1 IgG levels during the study duration can be interpreted as a result of removal of EBV-infected cells, or as a general reduction in the number of antibody-producing cells. Additionally, the secondary outcome measure, EBV-DNA in plasma, did not prove to be a reliable biomarker, possibly because our assay lacked sufficient sensitivity. Another methodological limitation is that the Overlord-MS trial is an ongoing double-blinded clinical trial, and we could therefore not analyze for any differences between ocrelizumab and rituximab effects. However, considering that both therapies are monoclonal IgGs targeting the same protein subtype, this limitation appears minor. The fact that the trial is ongoing also means that participants have varying follow-up periods, leading to wide confidence intervals for most estimates at the later time points. Lastly, CMV may protect against MS development and may therefore not be an ideal control virus (Sundqvist et al., 2014). It has, however, not been associated with disease progression, and was chosen because it is a relatively common herpes virus that is not latent in B cells and because antibody levels are not affected by vaccination.

Alternative methods of assessing the EBV-specific immune response, such as measuring the anti-EBV cellular response, have been explored by Pham and colleagues (Pham et al., 2021). They report a decline of T cell responses to EBV during B cell depletion therapy in a cohort of 32 patients with MS, supporting the overall hypothesis that modification of the EBV-specific immune response contributes to the effect of B cell depletion in MS. However, another study followed 14 patients with MS up to 12 months after initiation of B cell depletion therapy and found no significant change of EBV-specific T cell occurrence compared to before therapy (Schneider-Hohendorf et al., 2022). T cell responses may prove to better reflect of therapeutic response to B-cell depletion therapy than humoral responses, and this should be examined in larger studies in the future. Alternative methods of assessing the EBV activity could be measuring EBV DNA in saliva or in isolated B cells from peripheral blood.

To summarize, EBNA1 IgG levels decreased, while VCA IgG levels increased, during B cell depletion therapy. Our findings support the hypothesis that the mechanism of action for B cell depletion therapy in MS involves immune responses to EBV. This may reduce immune cross-reactivity, which in turn could interrupt disease perpetuation. To further address this hypothesis, additional investigations focusing on T cell responses to EBV during B cell depletion therapies should be conducted.

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CRediT authorship contribution statement

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Declaration of Competing Interest

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.msard.2023.105037.

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