Journal of Neuroimmunology 285 (2015) 156–160

Contents lists available at ScienceDirect

Journal of Neuroimmunology

journal homepage: www.elsevier.com/locate/jneuroim

Association of serum Epstein–Barr nuclear antigen-1 antibodies and intrathecal immunoglobulin synthesis in early multiple sclerosis

Catherina Pfuhl [a,b,c,1], Johanna Oechtering [a,c,1], Ludwig Rasche [a,b,c,1], René M. Gieß [a,b,c], Janina R. Behrens [b,c], Katharina Wakonig [a,b,c], Erik Freitag [a,b,c], Florence C. Pache [a,b,c], Carolin Otto [d], Jörg Hofmann [e,f], Bettina Eberspächer [f], Judith Bellmann-Strobl [a,b,c,g], Friedemann Paul [a,b,c,g], Klemens Ruprecht [a,c,*]

[a] Department of Neurology, Charité - Universitätsmedizin Berlin, Germany
[b] NeuroCure Clinical Research Center, Charité - Universitätsmedizin Berlin, Germany
[c] Clinical and Experimental Multiple Sclerosis Research Center, Charité - Universitätsmedizin Berlin, Germany
[d] St. Josefs-Krankenhaus Potsdam, Germany
[e] Institute of Medical Virology, Charité – Universitätsmedizin Berlin, Germany
[f] Labor Berlin Charité-Vivantes GmbH, Berlin, Germany
[g] Experimental and Clinical Research Center, Charité – Universitätsmedizin Berlin, Max Delbrück Center for Molecular Medicine, Berlin-Buch, Germany

ARTICLE INFO

Article history:
Received 2 April 2015
Received in revised form 30 May 2015
Accepted 19 June 2015

Keywords:
Multiple sclerosis
Epstein–Barr virus
Intrathecal immunoglobulin G
Oligoclonal bands
Epstein–Barr nuclear antigen-1
Antibodies

ABSTRACT

Multiple sclerosis (MS) is associated with Epstein–Barr virus (EBV) infection. A characteristic feature of MS is an intrathecal synthesis of immunoglobulin (Ig)G. In 90 patients with clinically isolated syndromes/early relapsing–remitting MS, serum antibodies to Epstein–Barr nuclear antigen-1, but not to EBV viral capsid antigen, rubella, or varicella zoster virus, were higher ($p = 0.03$) in those with than those without a calculated intrathecal IgG synthesis >0% and correlated with the percentage ($r = 0.27$, $p = 0.009$) and concentration ($r = 0.27$, $p = 0.012$) of intrathecally produced IgG. These findings suggest a link between EBV infection and the events leading to intrathecal IgG synthesis in patients with MS.

1. Introduction

Epstein–Barr virus (EBV) infection is the strongest currently known risk factor for multiple sclerosis (MS) (Ascherio, 2013). Among individuals with EBV infection in childhood, the hazard of developing MS is about 15-fold and among individuals with EBV infection in adulthood about 30-fold higher than in EBV seronegative persons (Ascherio, 2013). However, despite the strong and consistent evidence for an association of MS and EBV, the mechanisms underlying this association remain unclear (Ascherio and Munger, 2007; Ascherio et al., 2012; Ascherio, 2013).

A highly characteristic, yet poorly understood, feature of MS is an intrathecal synthesis of immunoglobulin (Ig)G. Intrathecal IgG synthesis can either be qualitatively proven by the detection of cerebrospinal fluid (CSF)-specific oligoclonal IgG bands (OCB) or quantitatively evaluated by calculating the percentage of the intrathecally produced IgG within the total blood-derived intrathecal IgG with reference to the statistically defined upper limit of the reference range (Q_{lim} = 0% intrathecal synthesis) (Reiber, 1998). Of note, an intrathecally produced fraction as low as 0.5–1% of the total intrathecal IgG can be detectable as OCB, whereas in the quantitative evaluation a higher increase of the intrathecally produced IgG fraction is necessary to obtain a value >0% with reference to the upper limit of the reference range (Q_{lim}) (Reiber et al., 2009).

To investigate a possible link between EBV infection and intrathecal IgG synthesis in patients with early MS, we herein analyzed the association of serum antibody levels to Epstein–Barr nuclear antigen-1 (EBNA-1), EBV viral capsid antigen (VCA), rubella virus, and varicella zoster virus (VZV) with intrathecal IgG synthesis in a total of 96 patients with clinically isolated syndromes (CIS) or early relapsing–remitting MS (RRMS).

2. Patients and methods

The study was approved by the institutional review board, Charité - Universitätsmedizin Berlin (EA1/182/10). Lumbar punctures were

* Corresponding author at: Department of Neurology, Charité - Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin, Germany.
E-mail address: klemens.ruprecht@charite.de (K. Ruprecht).
[1] These authors contributed equally to this study.

http://dx.doi.org/10.1016/j.jneuroim.2015.06.012
0165-5728/

performed for diagnostic purposes only. All participants provided written informed consent.

2.1. Patients

Patients investigated in this work participate in an ongoing prospective observational study (Berlin CIS cohort; NCT01371071) of patients with a first clinical event suggestive of central nervous system inflammatory demyelination (i.e. a CIS, Montalban, 2014) or early relapsing–remitting MS (RRMS), which started recruitment in January 2011. Inclusion criteria are: age ≥18 years, a first clinical event suggestive of central nervous system inflammatory demyelination not meeting the McDonald 2010 criteria for RRMS (Polman et al., 2011) within 6 months before inclusion into the study or a diagnosis of RRMS according to the McDonald 2010 criteria within 24 months before inclusion into the study. From all participants, baseline serum samples were obtained by peripheral venipuncture, processed at the NeuroCure Clinical Research Center, Charité – Universitätsmedizin Berlin, according to standard operating procedures, and stored at −80 °C. CSF analyses were performed during routine diagnostic work-up before study inclusion and results of CSF studies were extracted retrospectively from the patients' records.

2.2. CSF analyses

Total albumin and IgG concentrations in serum and CSF samples obtained simultaneously on the same day were measured nephelometrically (BN ProSpec, Siemens Healthcare Diagnostics, Erlangen, Germany). OCB were determined by isoelectrofocusing on agarose gels with subsequent in-gel immunofixation using peroxidase-labeled anti-human IgG antibodies (Hydrasys, Sebia, Fulda, Germany). For all protein analyses, CSF and serum samples were analyzed within the same analytical series. The percentage of intrathecally produced IgG (IgG_{IF}) was calculated as previously described using the formula $IgG_{IF} = (IgG_{Loc}/IgG_{CSF}) \times 100$ [%] with IgG_{Loc} being the concentration of locally synthesized intrathecal IgG in CSF (mg/l) and IgG_{CSF} being the concentration of total IgG in CSF (mg/l). IgG_{Loc} was calculated by the formula $IgG_{Loc} = (Q_{IgG}–Q_{Lim}) \times IgG_{Serum}$ (mg/l) with Q_{IgG} being the quotient of the IgG concentrations in CSF and serum, Q_{Lim} being the upper limit of the reference range for Q_{IgG} with respect to Q_{Alb}, and IgG_{Serum} being the concentration of IgG in serum. Q_{Lim} (IgG) was calculated by the formula $Q_{Lim}\ (IgG) = (0.93 \times [Q^2_{Alb} + 6]^{0.5}–1.7) \times 10^{-3}$ with Q_{Alb} being the quotient of the albumin concentrations in CSF and serum (Reiber, 1998; Reiber et al., 2009). While Q_{Lim} represents the upper limit of the reference range for Q_{IgG} with respect to Q_{Alb}, Q_{mean} is the mean of the reference range for Q_{IgG} with respect to Q_{Alb}, with Q_{Lim} also being defined as $Q_{Lim} = Q_{mean} + 3$ standard deviations (SD), and an intrathecal IgG production being defined as $Q_{IgG} > Q_{Lim} = Q_{mean} + 3$ SD. Whereas this definition provides a high specificity, we also calculated intrathecal IgG production as defined as $Q_{IgG} > Q_{mean} + 2$ SD, which was more recently introduced as a more sensitive approach for the detection of calculated intrathecal IgG production in groups of patients (Reiber et al., 2009). Q_{mean} (IgG) was calculated by the formula $Q_{mean}\ (IgG) = (0.65 \times [Q^2_{Alb} + 8]^{0.5}–1.4) \times 10^{-3}$. $IgG_{Loc\ mean}$ was calculated by the formula $IgG_{Loc\ mean} = (Q_{IgG}–Q_{mean}) \times IgG_{Serum}$ (mg/l). The percentage of intrathecally produced IgG ($IgG_{IF\ mean}$) with reference to Q_{mean} was calculated by the formula $IgG_{IF\ mean} = (IgG_{Loc\ mean}/IgG_{CSF}) \times 100$ [%] (Reiber et al., 2009).

2.3. Serology

All antiviral antibodies were determined in the baseline serum samples of the patients included in our prospective observational study (see 2.1), i.e. in serum samples obtained at a later point in time than CSF/serum samples for routine CSF examinations. Serum IgG antibodies to EBNA-1 (Ref. 310520), VCA (p18, Ref. 310510), and VZV (Ref. 310850) were measured by Liaison® (DiaSorin, Saluggia, Italy) automated chemiluminescent assays according to the manufacturer's recommendations. Samples with EBNA-1 or VCA IgG values above the upper detection limit were re-measured at a dilution of 1:20, as suggested by the manufacturer. Samples with VZV IgG values above the upper detection limit were re-measured at a dilution of 1:10, as suggested by the manufacturer. Rubella virus IgG was measured using the Architect chemiluminescent microparticle immunoassay (Abbott, Wiesbaden, Germany, Ref. 6C17). Samples with rubella virus IgG values above the upper detection limit were re-measured at a dilution of 1:10, as suggested by the manufacturer.

2.4. Statistical analysis

Significance of different frequencies was assessed by Fisher's exact test. Age and EDSS of patients with CIS and RRMS were compared by Mann–Whitney test. Significance of different antibody levels in patients with and without an intrathecal IgG synthesis >0% in CSF/serum quotient diagrams and with and without OCB was assessed by Mann–Whitney test. Correlations between viral antibodies and the calculated intrathecal IgG synthesis was assessed by Spearman correlation coefficients. All statistical analyses were performed with GraphPad Prism Version 5.04. *P*-values < 0.05 were considered significant.

3. Results

Demographical and clinical characteristics as well as CSF findings of patients (n = 96) included in this study were typical of patients with CIS/early RRMS and are summarised in Table 1. Except for a higher proportion of patients with immunomodulatory therapies among the group of patients with RRMS, there were no differences between patients with CIS and RRMS with respect to gender, age, EDSS, and frequencies of calculated intrathecal IgG synthesis or OCB. The median (range) interval between CSF examinations and blood withdrawals for antiviral serologies was 2.5 (0–30) months. At the time serum was obtained for measurement of antiviral serologies patients also underwent 3 T cerebral and spinal MRIs. Of the 96 patients included in the study 84 had at least two T2-weighted hyperintense lesions and 91 had at least one T2-weighted hyperintense lesion typical of MS on cerebral and/or spinal MRI. The median (range) number of T2-weighted hyperintense lesions on cerebral MRI of the 96 patients was 8 (0–176).

An intrathecal IgG production defined as $Q_{IgG} > Q_{Lim} = Q_{mean} + 3$ SD, indicating an intrathecal IgG synthesis >0% (Q_{lim} = 0% intrathecal synthesis), was detected in 37 of 90 (41%) patients with available data. Patients with a thus calculated intrathecal IgG synthesis (IgG_{IF}) >0% had higher serum antibody levels to EBNA-1 ($p = 0.03$), but not to VCA ($p = 0.56$), rubella virus ($p = 0.76$), or VZV ($p = 0.12$) than patients without a calculated intrathecal IgG synthesis >0% (Fig. 1, top row). Consistent with this, the calculated percentage of intrathecally produced IgG correlated with levels of antibodies to EBNA-1 ($r = 0.27$, $p = 0.009$), but not to VCA ($r = 0.07$, $p = 0.54$), rubella virus ($r = 0.005$, $p = 0.96$), or VZV ($r = 0.17$, $p = 0.12$; Fig. 1, middle row). In 86 patients, we could also calculate the absolute concentrations of intrathecally synthesized IgG (IgG_{Loc} [mg/l]). In those patients, IgG_{Loc} likewise correlated with the levels of antibodies to EBNA-1 ($r = 0.27$, $p = 0.012$), but not to VCA ($r = 0.011$, $p = 0.92$), rubella virus ($r = −0.001$, $p = 0.99$), or VZV ($r = 0.15$, $p = 0.16$; data not shown). As expected, an intrathecal IgG synthesis defined as $Q_{IgG} > Q_{mean} + 2$ SD was more frequently detected (45 out of 88 [51%] patients with data available, Table 1) than an intrathecal IgG synthesis defined as $Q_{IgG} > Q_{Lim} = Q_{mean} + 3$ SD. When we correlated the calculated percentage of intrathecal IgG synthesis ($IgG_{IF\ mean}$) based on the more sensitive definition of $Q_{IgG} > Q_{mean} + 2$ SD with levels of antiviral antibodies in serum in n = 85 patients with available data we again observed a significant correlation only with serum antibody levels to EBNA-1 ($r = 0.26$, $p = 0.02$), but not with serum antibodies to VCA