Broadened and elevated humoral immune response to EBNA1 in pediatric multiple sclerosis

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**BROADENED AND ELEVATED HUMORAL IMMUNE RESPONSE TO EBNA1 IN PEDIATRIC MULTIPLE SCLEROSIS**

Epidemiologic studies suggest that childhood viral exposures are important determinants of multiple sclerosis (MS) risk in both adults and children and, so far, Epstein-Barr virus (EBV) stands out as the infectious agent for which there is the most compelling evidence for an association with the disease.1-3

**Patients, healthy donors, and methods.** Twenty-three children with a diagnosis of definite MS and 17 demographically matched healthy peers were enrolled at the Department of Pediatrics and Pediatric Neurology, University of Göttingen, Germany (table e-1 on the Neurology® Web site at www.neurology.org). Virus-specific antibody responses were assessed using standardized ELISA kits (Bio-Quant, San Diego, CA) for IgG antibodies directed against the EBV-encoded viral capsid antigen (EBV-VCA), the HCMV-derived early antigens (HCMV-EA), the immunogenic C-terminus of the latent EBV nuclear antigen-1 (EBNA1, aa 458-641), and toward EBV-infected B cell lysates.4 In order to assess the specificity of EBNA1-targeting IgG, 211 dodecamer peptides, overlapping by nine amino acids and covering the entire sequence of EBNA1 (aa 1-641), were covalently linked to cellulose membranes as described previously.5 Positive spots were identified as blue or purple. Individual membranes were processed and evaluated blinded to the clinical diagnosis.

**Results.** A total of 91% and 87% of MS cases compared to 64% and 58% of controls showed IgG responses toward EBV-VCA and EBNA1, respectively. The frequency of HCMV-EA seropositivity did not differ between both cohorts. IgG titers to EBV-VCA, EBV-infected B cell lysates which predominantly contain lytic EBV antigens,6 and to HCMV-EA did not significantly differ between the cohorts (figure, A). By contrast, children with MS showed a moderate but significant increase of EBNA1-specific IgG1 antibody titers (p = 0.02) (figure, B). The difference in EBNA1 recognition between patients and controls was preserved after eliminating the samples derived from treated patients (8/23) from our analysis (p = 0.03). These data suggest a primary deregulation of EBNA1-, but not generally of EBV-specific immune responses in pediatric MS.

We next determined target epitopes of EBNA1-specific IgG in 10 patients and 10 controls, in whom IgG responses to EBNA1 were detectable by ELISA (figure, C). The overall titer of EBNA1-specific IgG antibodies, including all IgG isotypes, did not statistically differ between these subgroups (mean IgG titer ± SEM in MS vs HD: 1,024 ± 152 vs 711 ± 106, p = 0.15). Antibody specificities were primarily directed toward the glycine-alanine repeat domain of EBNA1 (aa 88-323). In addition, pediatric MS sera bound to distinct epitopes which were not recognized by any of the control sera. However, the broadened recognition of EBNA1-specific IgG was not restricted to a distinct part of the antigen. As shown in the figure, D, patients recognized a higher number of epitopes within all three domains of the protein (p < 0.0001). Although we cannot completely exclude that the broadening of the antibody response resulted from the elevated IgG1 titers, we consider this explanation unlikely, because the overall immunoglobulin levels for EBNA1 were not increased in this subgroup of patients. Instead, our data point toward an upregulated and qualitatively distinct immune recognition of EBNA1 in children with MS.

**Discussion.** EBNA1 is the only EBV antigen consistently expressed in proliferating EBV-infected B cells of healthy virus carriers. Increased titers of EBNA1-specific antibodies were found to be associated with various autoimmune diseases including SLE and MS7 as well as more recently with pediatric SLE and pediatric MS.2 Cepok et al.7 reported that the two most frequent MS-specific and high affinity epitopes, recognized by CSF-derived oligoclonal IgG in MS, are both derived from EBV, i.e., EBNA1 and BRRF2, suggesting that EBNA1-specific antibodies are not only systemically elevated in MS, but also enriched in the CSF.

EBNA1 represents a key target antigen for CD4+ T cell-mediated immune control of latent EBV infection and we suggest that the observed quantitatively and qualitatively altered IgG1 responses to EBNA1 in pediatric patients with MS reflect the...
finding that adult patients show increased frequencies and broadened specificities of EBNA1-specific CD4+ T helper 1 cells. Increased availability of EBNA1 protein due to higher frequencies of EBV-infected and potentially autoreactive B cells in MS or continuous cross-stimulation of EBNA1-specific T cells by a disease-relevant autoantigen could trigger a selective increase of EBNA1-targeting immune responses in pediatric and adult MS.

We conclude that the increase and broadened recognition of EBNA1-specific IgG suggests an early dysregulation of EBV specific immune control in pediatric MS development. Further studies are necessary to elucidate the mechanisms by which altered EBV and in particular EBNA1-specific immune responses could potentially contribute to the early pathobiology of MS.

Figure Broadened and elevated humoral immune response to EBNA1 in pediatric MS

Children with MS were more frequently infected with EBV than age-matched healthy individuals, but they had similar titers of EBV-VCA, EBV-infected cell lysate, and HCMV-EA binding IgG antibodies (A). In contrast, pediatric MS patients showed an increase of EBNA1-specific IgG1, but not of IgG2, IgG3, or IgG4 EBNA1-specific antibody titers (p = 0.02; Mann Whitney test) (B). Neither patients nor controls showed EBNA1-specific IgG3 seropositivity. IgG2 and IgG4 responses to EBNA1 were detected in a minor subgroup of pediatric MS cases and controls with no statistically significant difference between both cohorts. To explore qualitative differences in the immune recognition of EBNA1 between untreated children with and without MS, we determined target epitopes of EBNA1-specific IgG by using membranes carrying spots of 211 covalently linked overlapping dodecamer peptides which cover the entire sequence of EBNA1 (aa 1–641) (C). The three main domains of EBNA1 (N-terminal, GA-repeat, and C-terminal) are outlined in the primary protein sequence (figure e-1), and the number of peptides recognized in these different domains by patients with MS and healthy controls is displayed in D. Percentages below bars in panel D represent the frequency of peptides recognized compared to all EBNA1-derived peptides. MS = multiple sclerosis; EBV = Epstein-Barr virus; EBNA1 = EBV nuclear antigen-1; EBV-VCA = EBV-encoded viral capsid antigen; HCMV-EA = human cytomegalovirus-derived early antigens; Ig = immunoglobulin.
The etiology of spontaneous cervical artery dissection (sCAD) is largely unknown.1–2 Approximately 15% of patients with sCAD develop multiple sCAD either simultaneously or in short succession.3 Therefore, we hypothesized that patients with sCAD are generally susceptible to dissection and that this predisposition manifests itself in the arterial wall. Previously, we demonstrated signs of a generalized arteriopathy in superficial temporal artery (STA) biopsies of patients with sCAD but not of controls.4 In this study we investigated STA and skin biopsies of two pairs of identical twins in whom one twin of each pair had had a sCAD.

**Case reports.** Twin 1.1 was 56 years old at the time of the dissection. He experienced severe dizziness, nausea, and vomiting preceding admission. He presented with left sided Horner syndrome only. MRI showed no brain infarct but a long dissection reaching from close to the bifurcation of the left internal carotid artery to the base of the skull. The skin and STA biopsies were performed 9 months after the dissection.

Twin 1.2 is the healthy brother of 1.1. Ultrasound examination showed no dissection. Twin 1.2 was 49 years old at the time of the dissection. He experienced right-sided facial pain during the night preceding admission. The following morning he noticed a left-sided facial weakness and clumsiness of his left hand. He presented with right-sided Horner syndrome and minimal left-sided brachiofacial hemiparesis. MRI showed a partial middle cerebral artery infarct on the right side and revealed a mural hematoma of the right internal carotid artery. The skin and STA biopsies were performed 8 months after the dissection. A symptomatic aneurysm (2.8 × 3.0 cm) of his left common iliac artery was detected by ultrasound.

Twin 2.1 was 59 years old at the time of the dissection. He had clinical signs of a generalized neurovascular syndrome and minimal left-sided brachiofacial hemiparesis. No dissection occurred. Twin 2.2 is the healthy brother of 2.1. Ultrasound examination of the cervical arteries did not show a dissection.

None of the four brothers showed clinical signs of a connective tissue disorder.

**Laboratory investigations.** DNA analysis proved that both twin pairs were monozygous. The skin biopsies of all four twin brothers did not show any severe connective tissue abnormalities.5 The figure, A, shows the structurally intact arterial wall of the STA of a control subject (control C2). Vascular smooth muscle cells (SMC) were of the physiologic contractile phenotype and neither fresh nor degraded erythrocytes or other cellular debris were found. In contrast, light microscopy of the STA biopsies of the two index twins with sCAD (twins 1.1 and 2.1) showed a zone of structural weakening along the medial-adventitial border (figure, F and H) and breakdown of SMC. The media and adventitia of companion twins without sCAD (twins 1.2 and 2.2) also showed, unlike the previously described unrelated controls,4 structural aberrations along the medial-adventitial border (figure, G and I, encircled). Erythrocytes located outside of vasa vasorum were found in all four twin brothers (figure, H, I). The pathologic findings on electron microscopy of all four twin brothers were comparable to our previous findings in patients with sCAD. SMC often showed a synthetic phenotype and signs of cellular breakdown. Scattered erythrocytes at various stages of degradation and cellular remnants were found (figure, I). An overt microhematoma at the medial-adventitial border coming close but not penetrating the intima was present in index twin 2.1 (figure, B and C). Immune cells along the border of the hematoma had already phagocytosed erythrocytes demon-