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Antibody response to common viruses and human leukocyte antigen-DRB1 in pediatric multiple sclerosis

Emmanuelle Waubant[1,2], **Ellen M Mowry**[2], **Lauren Krupp**[3], **Tanuja Chitnis**[4], **E Ann Yeh**[5], **Nancy Kuntz**[6], **Jayne Ness**[1,7], **Anita Belman**[3], **Maria Milazzo**[3], **Mark Gorman**[4], **Bianca Weinstock-Guttman**[5], **Moses Rodriguez**[6], and **Judith A James**[8]

[1] Regional Pediatric Multiple Sclerosis Center, University of California San Francisco, USA.

[2]Department of Neurology, University of California San Francisco, USA.

[3] National Pediatric Multiple Sclerosis Center, Stony Brook, USA.

[4] Partners Pediatric Multiple Sclerosis Center, Boston, USA.

[5] Pediatric Multiple Sclerosis Center of the *Jacobs Neurological Institute*, State University of New York, USA.

[6]Mayo Clinic Pediatric Multiple Sclerosis Center, Rochester, USA.

[7]Alabama Pediatric Multiple Sclerosis Center, USA.

[8] Oklahoma Medical Research Foundation, USA; University of Oklahoma Health Sciences Center, USA

Abstract

Background—As remote infections with common herpes viruses are associated with modulation of the risk of multiple sclerosis (MS), we hypothesized that antibody concentrations against these viruses may further modify risk. As many common viruses are first encountered during childhood, pediatric MS offer a unique opportunity to investigate more closely their influence on susceptibility. Our aim was to determine if MS patients who were positive for these viruses had higher levels of antibodies to these viruses. We also assessed whether human leukocyte antigen (HLA)-DRB1*1501 genotype influenced viral antibody levels.

Methods—Antibody response levels toward Epstein Barr virus (EBV), cytomegalovirus (CMV), and herpes simplex virus (HSV)-1, and HLA-DRB1*1501 status were determined in pediatric MS patients (n=189) and controls (n=38). Multivariate analyses were used, adjusted for age, gender, race, ethnicity and use of disease-modifying therapies.

Results—The antibody concentrations against EBV (Epstein-Barr nuclear antigen 1 (EBNA-1), viral capsid antigen (VCA) and early antigen (EA)), CMV and HSV-1 were similar between pediatric MS patients and controls positive for seroconversion against the virus of interest. EBNA-1 humoral responses were higher in HLA-DRB1 positive individuals (p=0.005) whereas other viral humoral responses were similar in HLA-DRB1 positive and negative individuals.

Corresponding author: Emmanuelle Waubant, University of California San Francisco Regional Pediatric Multiple Sclerosis Center, Neurosciences Research Building, 675 Nelson Rising Lane, Room 215B, Box 3206, San Francisco, CA 94158, USA. Emmanuelle.waubant@ucsf.edu.

Conflict of interest
None declared.

Conclusion—Among those positive for EBNA-1, MS patients did not have higher levels of antibody response to EBNA-1: however, titers for EBNA-1 were higher in those who were HLA-DRB1 positive. This suggests that genotype might influence the humoral response to EBV. Whether other genotypes influence antibody response to other viruses remains to be determined.

Keywords

Multiple sclerosis susceptibility; Epstein Barr virus; cytomegalovirus; herpes simplex virus-1; DRB1; pediatric multiple sclerosis; risk factors; gene-environment interaction

Introduction

Multiple observations suggest the presence of multifaceted gene-environment interactions as triggers of multiple sclerosis (MS).[1–8] Although the strongest genetic risk factor for MS, human leukocyte antigen (HLA)-DRB1, codes for a co-receptor for Epstein Barr virus (EBV) entry into B cells,[9] only one study suggested a possible interaction between these two risk factors.[6] We have shown that cytomegalovirus (CMV) remote infection was associated with a lesser risk of pediatric MS, while herpes simplex virus (HSV)-1 remote infection had a more complex effect suggesting a strong gene-environment interaction.[8] Specifically, HSV-1 seroconversion was associated with a lesser risk of MS in individuals carrying a HLA-DRB1*1501/1503 allele, while those who did not carry this allele were associated with a substantially stronger risk.[8] As remote infections with common herpes viruses (i.e. being positive or negative for immunoglobulin G (IgG)) are associated with modulation of risk of MS, we hypothesized that antibody concentrations against these viruses may further modify risk. As many common viruses are first encountered during childhood, pediatric MS offers a unique opportunity to more closely investigate their influence on susceptibility.

The primary objectives of this study were to determine whether the antibody response to EBV, CMV, or HSV-1 differs in children with MS compared to those children without MS, whether the associations persist when restricted to only those children who demonstrate an antibody response to the viruses, and whether HLA-DRB1*1501 or 1503 influences, either independently or in an interactive fashion, these antiviral responses.

Methods

Cases and controls

This study was approved by the local Institutional Review Board (IRB) of all participating centers. Patients under 18 years of age at the onset of their first MS symptoms were recruited at six Regional Pediatric MS Centers sponsored by the National MS Society (NMSS).[10] Pediatric controls included pediatric patients seen at the same clinics during the same period for whom clinically isolated syndrome (CIS) or MS was excluded.[8] Race and ethnicity were defined according to the self-reported standards as defined by National Institutes of Health (NIH).

Viral studies

Batched EBV-viral capsid antigen (VCA), EBV-early antigen (EA), CMV and HSV-1 assays (IgG) were performed blindly at the Oklahoma Medical Research Foundation with normalized enzyme-linked immunosorbant assays (ELISAs) (Wampole Laboratories, Princeton, New Jersey, USA) and are presented as international standardized ratios (ISRs).[8] The ISR is calculated based upon manufacturer recommendations and is considered semiquantitative as the relationship may not be linear with higher optical density (OD) values above the top of the linear part of the binding curve, allowing some higher levels of

antibody to potentially be relatively underestimated. Using an ELISA with full-length Epstein-Barr nuclear antigen 1 (EBNA-1) as an antigen (Biodesign, Carmel, New York, USA), samples were tested for anti-EBNA-1 seropositivity and relative concentration reported as previously described.[11,12] Briefly, EBNA-1 was diluted in carbonate coating buffer and coated at 1 mcg per well of a 96-well plate. Serum was diluted at 1:100 and 1:1000 and incubated with the bound antigen. Specific antibodies were detected using alkaline phosphatase-conjugated anti-human IgG (Jackson Immunoresearch, West Grove, Pennsylvania, USA). All ELISAs included positive and negative controls which met set quality control measures to include data for analysis. In addition, a positive control was used to standardize assays between plates. Positive anti-EBNA-1 responses were greater than or equal to four standard deviations (SDs) above a panel of controls known to be EBV seronegative.

HLA-DRB1*1501 and 1503 genotyping

All DNA samples of pediatric-onset MS patients and controls were genotyped by TaqMan polymerase chain reaction (PCR) for the presence of HLA-DRB1*1501/1503 as previously reported.[8]

Statistical analysis

Calculations and statistical analyses were performed using Stata 10.0 statistical software (StataCorp, College Station, Texas, USA) and SAS version 9.2 software (SAS Institute, Cary, North Carolina, USA). Means (±SD) or medians (with ranges) were used to summarize demographic and clinical data. Among those who were seropositive for responses against each virus, multivariate linear regression models were generated in which the outcomes were the viral antibody responses. In these models, we explored the contributions of and interactions between MS/CIS status and DRB1 status, adjusting for age, race and ethnicity.

Interactions between MS/CIS status and DRB1 status were generated by creating an interaction term. We also explored whether adding disease-modifying therapy status at the time of the blood sample to the full models changed the results. As the sample size was small, we performed within-DRB1 (positive or negative) group analyses on the effect of viral status when the p value for the interaction term was <0.2.

Results

Case and control characteristics

One hundred and eighty-nine patients with pediatric-onset MS (n=161) or CIS (n=28) and 38 pediatric neurological controls provided blood samples for this study. Details pertaining to the neurological controls, demographics, and viral and HLA-DRB1*1501 or 1503 status (i.e. positive or negative) were previously reported.[8]

Anti-viral response relative concentrations

Viral antibody relative concentrations in seropositive patients and controls were similar (Table 1). In multivariate models, viral antibody responses for EBNA-1, VCA, EA, HSV-1, and CMV were similar in MS/CIS patients and controls who demonstrated seroconversion against the viruses (data not shown). No meaningful confounding of the association of MS/CIS status and viral antibody responses by the presence of HLA-DRB1*1501 or 1503 was identified in any model. However, EBV positive HLADRB1*1501 or 1503 positive individuals had higher EBNA-1 antibody levels (coefficient 0.12, 95% confidence interval (CI) 0.04 to 0.20, p=0.005), when controlled for MS/CIS status, age at sampling, race and ethnicity. This was not found for VCA antibody levels (coefficient -0.10, 95% CI –0.47 to

0.27, p=0.59) or EA (coefficient 0.06, 95% CI –0.23 to 0.35, p=0.67). These results were unchanged when models were adjusted for use of disease-modifying therapy at the time of sampling (data not shown).

Interactions between HLA-DRB1 and MS/CIS status in models of viral antibody response

We found no statistical interaction between MS/CIS status and HLA-DRB1*1501/1503 status influence on viral antibody responses for VCA, CMV, and HSV-1 antibody responses (i.e. outcomes) (Table 1). In contrast, a trend for an interaction was found between MS/CIS status and HLA-DRB1*1501/1503 status in models in which viral antibody responses for EBNA-1 or EA was the outcome (Table 2). Among controls, DRB1 was associated with 0.30 higher EBNA-1 antibody response (95% CI 0.03 to 0.57; p=0.031) while among MS patients, it was associated with a 0.10 higher EBNA-1 antibody response (95% CI 0.02 to 0.19; p=0.022). Among those positive for EA, DRB1 positivity in controls tended to be associated a lower EA titer (–0.44, 95% CI –1.23 to 0.35, p=0.27) while in MS subjects, it tended to be associated with a higher titer to EA (0.13, 95% CI –0.17 to 0.44, p=0.38).

Discussion

We report that, while the proportion of EBNA-1 positive subjects is not affected by DRB1 status,[8] DRB1 positivity is associated with higher EBNA-1 antibody response among those who are EBNA-1 positive (p=0.005) after adjusting for MS/CIS status, age at sampling, race and ethnicity. In contrast, DRB1 positivity is not associated with higher VCA, EA, CMV or HSV-1 antibody response among those who are positive for seroconversion against the virus. This is an intriguing finding as VCA IgG seroconversion represents remote EBV infection whereas EBNA-1 IgG seroconversion oftentimes represents evidence of EBV latency. This may be related to DRB1 being a co-receptor for EBV entry in B-cells, but it is then unclear why this association is not found for VCA and EA. Whether the interactions between MS status and DRB1 status in the EBNA-1 and possibly EA models can be confirmed in larger studies or are clinically relevant remains to be determined.

Similar findings of higher EBNA-1 antibody response in DRB1 positive individuals were reported in adult controls but were not statistically significant in adult MS cases although this latter group was smaller and thus, the 95% CI did not exclude 1.0 (OR 1.47, 95% CI 0.74 to 2.93).[6] An association of HLA class I polymorphisms respectively with EBV titers, number of EBV copies and risk of infectious mononucleosis has been reported in healthy individuals,[13] suggesting a role for genetic background as a regulator of viral infection rate and clinical expression. Another study recently reported that three gene variants, HLA-DR15, HLA-A and CTLA4 altered the association between higher anti-EBNA response and risk of first demyelinating event in adults.[14] That HLA-DRB1 is associated with EBNA-1 antibody response regardless of MS status suggests that DRB1 status or the status for a nearby gene (or a gene in linkage disequilibrium) influences the humoral response to EBNA-1, but not to VCA or EA. The reasons for this selectivity are elusive. It is also unclear whether and how the effect of DRB1 on the humoral response to EBNA-1 contributes to MS pathogenesis. Processes such as cross-reactivity between EBV and myelin protein,[15] EBV activation of superantigens, and EBV activation of autoreactive B cells have been proposed as potentially underlying these results. Higher EBNA-1 titers were recently reported in smokers, and the risk of adult MS associated with high EBNA-1 titers was stronger in smokers.[16] In this study, little modification by HLA-DR15 was observed.

We also report that pediatric MS patients have similar antibody concentrations against EBV (EBNA-1, VCA, EA), CMV and HSV-1 compared with seropositive neurologic disease controls. This finding is in contrast with previous studies which have reported higher antibody response to EBNA-1 in pediatric MS patients.[17,18] However, differences exists

between these studies and ours possibly explaining the discrepancy. One study used healthy and non demyelinating controls while the other averaged the response for all individuals including those EBV negative.[19]

Limitations of this study include the small number and type of controls who had other neurological conditions, some of which are inflammatory in nature such as acute disseminated encephalomyelitis, neurosarcoid or neuromyelitis optica. This could have biased our findings toward the null hypothesis. Although a few patients were on disease-modifying therapy at the time of blood sampling, our results based on analyses adjusted for the use of such therapies were very similar. We have not measured total IgG serum levels but considering the fact that antibody responses were similar for VCA, EA, CMV and HSV-1, we doubt that differences in IgG levels between controls and cases explain our findings. We have only studied HLA-DRB1*1501/1503 status. Future studies including healthy controls will have to confirm these observation and study full, high resolution HLA genotyping and how it relates to antibody response to various viruses associated with MS risk. Furthermore, other relevant viruses such as HHV-6 (Human Herpes Virus) should also be studied.

The role of EBV in MS pathogenesis is still overall poorly understood. It is unclear whether the virus plays a direct (molecular interaction) or indirect (promotion of smoldering inflammation) role in processes leading to disease onset, and whether this role is specific to MS or also applies to other autoimmune disorders. Although the two strongest risk factors for MS include EBV and DRB1, and HLA-type II molecules are necessary for EBV infection, very few reports are available that suggest gene-environment interactions between these two factors in MS. Our data suggest that the effect of DRB1, although restricted to EBNA-1, and not other viruses under study, may in fact not be disease specific, as found in both cases and controls.

Understanding factors that regulate antibody response to specific viral antigens may prove helpful to understand the multiple processes at play in MS onset.

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Table 1

Mean viral antibody response in individuals positive for remote infection.

	Pediatric MS/CIS	Pediatric neurologic controls	*p* values (*t* test)
EBNA-I[a]	0.95±0.26	0.89±0.34	0.47
VCA[b]	3.06±1.18	3.28±1.01	0.33
CMV[b]	2.04±0.88	1.96±0.72	0.79
HSV-1[b]	3.69±2.02	4.46±2.86	0.33

CMV: cytomegalovirus; EBNA-1: Epstein-Barr nuclear antigen 1; HSV: herpes simplex virus;VCA: viral capsid antigen.

[a]Optical density.

[b]International standardized ratio.

Table 2

Presence of interactions between multiple sclerosis status and DRBI status in the various viral models.

	Estimate (interaction term)	95% CI	*p* value
EBNA-1 model	–0.19	–0.47, 0.08	0.17
VCA model	–0.73	–2.03, 0.57	0.27
EA model	0.57	–0.27, 1.42	0.18
CMV model	–0.05	–1.47, 1.36	0.94
HSV-1 model	0.27	–3.80, 4.34	0.89

CI: confidence interval; CMV: cytomegalovirus; EA: early antigen; EBNA-1: Epstein-Barr nuclear antigen 1; HSV: herpes simplex virus;VCA: viral capsid antigen.