Analysis of Candidate-Host Immunogenetic Determinants in Herpes Simplex Virus–Associated Mollaret’s Meningitis

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Infection due to herpes simplex virus (HSV) is associated with recurrent aseptic meningitis (Mollaret’s meningitis); however, the neuropathogenesis of this disease remains unknown. We collected 20 cerebrospinal fluid (CSF) specimens that were positive for HSV DNA by using polymerase chain reaction (PCR) assay from patients with a clinical diagnosis of Mollaret’s meningitis. Patients were predominantly female (female : male, 22 : 1), with an average age of 32.8 years (range, 18–46 years). Using direct sequence analysis of HSV PCR products obtained from the CSF, we determined that all of the patients were infected with HSV type 2. In addition, we evaluated polymorphisms in 2 human genomic loci, which are associated with either severe or recurrent microbial infections (interferon-γ receptor [IFN-γR] and mannose binding lectin [MBL]); these host genes were also amplified directly from the CSF specimens. No mutations were found in exons 2 or 3 of the IFN-γR gene (n = 20). In contrast, there were 4 (20%), 4 (20%), and 0 mutations found in codons 52, 54, and 57, respectively, in exon 1 of MBL (n = 20). A significantly higher frequency of codon 52 mutations (P = .04) was observed, compared with racially matched control patients.

Mollaret’s meningitis is an unusual but perhaps underappreciated syndrome of benign, recurrent, aseptic meningitis. The disease is characterized by repeated episodes of fever and signs of meningeal inflammation that usually subside within 2–5 days after onset [1–3]. Until recently, this syndrome had been considered a disease of unknown etiology. However, in 1991, the isolation of herpes simplex virus type 1 (HSV-1) and the detection of both HSV-1 DNA and HSV-1 antibodies in the CSF suggested an association of Mollaret’s meningitis with HSV infection [1]. Picard et al. [2] described 3 patients who experienced 3 episodes each of aseptic meningitis; HSV-2 DNA was amplified by PCR assay of CSF specimens from these 3 patients during the third attacks of meningitis [2]. Tedder et al. [3] detected HSV-2 DNA in CSF specimens from 10 of 13 patients who had benign recurrent lymphocytic meningitis and HSV-1 DNA in the CSF from one of those patients [3]. Thus, the association of HSV infection, especially HSV-2, with recurrent benign meningitis has expanded significantly [2–4].

Since September 1993, the molecular microbiology laboratory at the Mayo Clinic has identified >400 cases of CNS disease by the detection of HSV DNA in CSF [4]. From a subset of these cases, we identified 23 patients who presented with a clinical diagnosis of Mollaret’s meningitis. To determine whether 2 candidate-host susceptibility factors may play a role in the development of CNS disease in documented cases of HSV infection, we performed sequence analysis for genomic targets that encode the IFN-γ receptor (IFN-γR) and the mannose binding lectin (MBL). Mutations in the IFN-γR predispose patients to severe disseminated atypical mycobacterial infections [5]. MBL is a calcium-dependent binding protein that plays an important role in innate immunity. Point mutations in the gene encoding this product are apparently associated with immunodeficiency in patients that is caused by an opsonic defect [6]. Because of the potential involvement of these 2 immunogenetic loci in the development of antiviral responses [7] or in the cytotoxic T cell response [5], we studied these loci in patients who presented with Mollaret’s meningitis or necrotizing encephalitis, both of which represent HSV CNS infection.

Materials and Methods

Patients and samples. The medical histories of patients at the Mayo Medical Laboratories during 1996 and 1997 whose CSF specimens had been found positive for HSV DNA by the Mayo Clinic Molecular Microbiology Laboratory were obtained from the primary physician either by telephone or by written questionnaire.
Patients with a clinical diagnosis of Mollaret’s meningitis were identified as having repeated attacks (>3 episodes) of fever and meningeal irritation, and predominantly mononuclear cells in the CSF. CSF specimens were stored at -20°C until DNA was extracted for further study.

**DNA extraction and PCR.** DNA extraction from CSF specimens and PCR assays were performed as described elsewhere [4]. CSF (200 μL) was used for nucleic acid extraction, and extracted DNA was resuspended in 50 μL of water. The specific primers were designed to amplify 335-bp thymidine kinase (TK) genome fragment (5'-GACMAGCGCCAGATAACAA-3' and 5'-MCAGCATRGGCGGTCAACG-3'). M = A or C, R = A or G). IFN-γR exons 2 (5'-GTGCCATACCACTAACTATG-3' and 5'-ACC-CATAGTTCTTTAACCCTC-3') and 3 (5'-GTGTTAAAGATTCAG-AATG-3' and 5'-CACCCTTGTCCGGTCACAGC-3'), and MBL exon 1 genome (5'-CTGTAACCGTGGAGGTGC-3' and 5'-CC-AACACGTCCCTGGTTC-3'), according to the sequence information published elsewhere [4–7].

**Sequence analysis.** Amplification products were detected by use of agarose gel electrophoresis (3%; 1.5% NuSieve/1.5% SeaKem, FMC Bioproducts, Rockland, ME). Primers for sequencing were the same as those for PCR. Target DNA, amplified by PCR, was purified by the QIAquick Purification Kits (Qiagen, Chatsworth, CA). A reaction mixture including approximately 200 ng of purified DNA fragment and 2-3 pmol of primer was used for cycle sequencing with an automated instrument (Model 377, Applied Biosystems, Foster City, CA). Multiple-sequence alignment was carried out by use of the software program Pileup/Gap included in the Wisconsin GCG Package (Madison, WI).

**Results**

Of 5033 CSF specimens that were processed during 1996 and 1997, 256 (5.1%) were positive for HSV DNA. Of the HSV-positive specimens, 23 (9%) were from patients who were clinically diagnosed with Mollaret’s meningitis; 22 of these patients were female, with a mean age of 32.8 years (range, 18–46 years). One of the 23 patients had HIV-1 infection, 2 were pregnant, and 9 (39.1%) had a clinical history of genital herpes lesions. Of the CSF specimens from the 23 patients with a clinical diagnosis of Mollaret’s meningitis, 20 had sufficient volume for further study.

Target DNA sequence from the HSV viral TK gene was amplified directly by PCR assay of DNA extracted from CSF specimens. Direct sequence analysis of a polymorphic 335-bp viral TK gene fragment revealed all 20 strains to be HSV-2; this region of the TK gene contains >20 phylogenetically informative sites relative to HSV-1. Nonconsensus polymorphisms corresponding to the wild type HSV-2 strain [8] were not found in any of the CSF specimens analyzed by direct sequencing, suggesting that our analytical procedure did not produce sequencing artifacts.

For analysis of immunogenetic determinants, we used the same DNA extracted from the CSF, because we assumed that sufficient human genomic DNA would be present for analysis. Previous studies have shown that HSV infections are often accompanied by pleocytosis, which further justified this approach [3]. The 118- and 177-bp regions spanning exons 2 and 3 of the IFN-γR gene, respectively, were recovered from all 20 CSF specimens; no heterozygous or homozygous polymorphisms previously described in association with primary immunodeficiency were identified, and no other nonconsensus polymorphisms were found. Exon 1 of the MBL gene was also amplified and sequenced to determine the frequency of known polymorphisms at codons 52, 54, and 57. Of 20 CSF specimens studied, 4 (20%), 4 (20%), and 0 mutations were detected at codons 52 (R52C), 54 (G54D), and 57 (G57E), respectively, in this exon.

**Table 1.** Genetic polymorphisms in exon 1 of the mannose binding lectin gene in patients with Mollaret’s meningitis compared with those in other populations.

<table>
<thead>
<tr>
<th>Race or ethnicity</th>
<th>Mutation detected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R52C</td>
</tr>
<tr>
<td>Mollaret’s meningitis (n = 20)</td>
<td>4 (20)</td>
</tr>
<tr>
<td>HSV encephalitis (n = 20)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Control CSF specimens (n = 20)*</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Local population (n = 148)b</td>
<td>Predominantly white</td>
</tr>
</tbody>
</table>

NOTE. Data are no. (%) of patients. Row 1 vs. row 4: \( \chi^2 = 4.03, P = .04 \); Row 1 vs. rows 2 and 3: \( \chi^2 = 2.06, P > .05 \)

* HSV-negative with leukocyte count <50/mm³ and protein level <45 mg/dL.

b D. Babovic-Vukanovic et al., unpublished data.

Discussion

Since we began genotypic analysis of HSV amplification products obtained from the CSF in 1997, we have observed a
prevalence of HSV-1 in patients with CNS diseases [4]. Most of the latter consist of patients with HSV encephalitis. However, we have also observed an increasing frequency of HSV-2 detection, often in female patients who exhibit signs of meningitis. In our study, only 39.1% of patients with benign, recurrent meningitis had previous local lesions. Although patients usually have their own distinct recurrence patterns, stress and pregnancy have been reported to be associated with recurrence [1, 10]. Taken together, these findings suggest that the interplay of both viral and host factors may determine the likelihood of developing this presumably rare but perhaps underappreciated manifestation of HSV infection.

Individual variation in host immunogenetic factors may influence the risk and/or the outcome of infection by several microbial pathogens. Several examples suggest a pivotal role for genetic predisposition of the host, including: (1) deficiency in the IL-12 receptor gene has been associated with severe tuberculosis and Salmonella infection [11]; (2) resistance to HIV-1 infection, which has been associated with an internal 32-bp deletion in the human chemokine receptor CCR-5 gene [12]; (3) absence of the erythrocyte P antigen among inbred human subpopulations, which appears to confer absolute resistance to parvovirus B19 infection [13]; and (4) IL-10 promoter polymorphism predicts initial response of chronic hepatitis C to IFN-α [14].

Our study focused on the sequence analysis of 2 host genes, IFN-γR and MBL. Mutations in the IFN-γR locus have been associated with a predisposition for development of severe disseminated mycobacterial infections [5] and presumably reduce the intensity of the human Th1-type response. Similarly, mutations in 3 codons of exon 1 of the MBL gene, via a less understood mechanism, have been associated with several recurrent infections, as well as the persistence of hepatitis B virus infection [6, 7]. Several studies have demonstrated an ~ 5% frequency of G→A polymorphisms at codon 52 in control populations of whites and blacks [6, 9]. This mutation in codon R52C was significantly overrepresented relative to ethnically matched local population control patients ($P = .04$); our own ethnically matched control patients showed a similar allele frequency compared with the local population control patients. However, further analysis of additional patients and control patients are needed to strengthen this conclusion. For this purpose, we are developing a microtiter-based system for MBL allele discrimination that eliminates the need for DNA sequencing.

Previous studies have suggested an association between pregnancy and HSV-associated Mollaret’s meningitis [10]; our own study showed a heavy female predominance, consistent with previous findings, and 2 women were known to be pregnant at the time of the illness. However, although most of the women with Mollaret’s meningitis were of childbearing age (mean age, 32.8 years), data regarding parity status, inapparent pregnancy, illness, or oral contraceptive use were not available. Ultimately, it is likely that multiple factors, including viral, host, and other yet unknown environmental determinants, play a role in the pathogenesis of this curious viral syndrome.

Acknowledgments

We thank Drs. Neville Bernnett, Juraj Braun, Bill Guelig, John Gullett, Livia Hantos, Alfred Harney, Yvorne Jurik, Charles Linehan, Thomas Martin, Charles Miley, Joel Morgenlander, Paul Mumma, Tim Podhalsky, Christine Schroek, Richard Silberman, Mike Stadiem, Sala Subramanian, John Tune, and Joseph Yao for providing clinical information.

References