Cytomegalovirus Glycoprotein B Genotypes and Central Nervous System Disease in AIDS Patients

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To investigate any association between cytomegalovirus glycoprotein B (CMV gB) subtypes and central nervous system (CNS) disease in AIDS patients, proportions of different gB genotypes detected in AIDS patients with CNS disease were compared with the gB genotypes detected in AIDS patients with no neurological disorder. The patients were matched by CD4+ cell counts. CMV was detected by PCR in cerebrospinal fluid (CSF) samples obtained from AIDS patients with CNS disease and from urine and saliva samples obtained from AIDS patients without CNS disease. CMV strains obtained were digested by restriction enzymes Hinfl and RsaI to classify the genotypes. The CMV gB genotype was determined in 26 CSF samples. Of these, 11/26 (42.3%) typed as gB group 1, seven (26.9%) as gB2, four (15.4%) as gB3, and four (15.4%) as gB4. The CMV gB genotype frequency distribution in the 42 AIDS patients without CNS disease showed that 18/42 (42.8%) were classified as gB group 1, 10 (23.8%) as gB2, seven (16.6%) as gB3, and seven (16.6%) as gB4. In the present study, no association was found between CMV gB genotypes and CMV-related central nervous system disease.

KEY WORDS: cytomegalovirus; PCR; genotypes; gB; CNS disease; AIDS

INTRODUCTION

The glycoprotein B (gB) of the cytomegalovirus (CMV) constitutes a major component of the virion envelope. Experiments in vitro have shown that CMV gB promotes virion penetration into the cell, transmission of infection from cell to cell, and fusion of infected cells [Navarro et al., 1993; Tugizov et al., 1994, 1995; Pietropaolo and Compton, 1997]. The gB protein elicits strong neutralizing antibody and cytotoxic T-cell immune responses in infected individuals [Rasmussen et al., 1988; Kniess et al., 1991; Liu et al., 1991; Marshall et al., 1992; Schoppel et al., 1997, Speckner et al., 1999]. Levels of glycoprotein-specific antibodies and detection of viral DNA in peripheral blood are inversely correlated [Schoppel et al., 1997]. In recipients of allogeneic bone marrow transplants, high titers of glycoprotein-specific neutralizing antibodies are correlated with the absence of viral DNA in the blood [Schoppel et al., 1998]. Although strains of CMV propagated in the laboratory are genetically distinct from the wild type [Prichard et al., 2001], this variation does not affect the gB region. Restriction enzyme analyses have revealed genetic variation in the gB region in clinical strains of CMV, and clinical CMV isolates have been found to assume one of four gB sequence configurations [Chou and Dennison, 1991]. The gB genotype has been shown to correlate with cell tropism in vivo and may influence the virulence of CMV [Meyer-Konig et al., 1998b]. The finding that CMV envelope proteins activate inflammatory cytokine responses via CD14 and Toll-like receptor 2 (TLR2) [Compton et al., 2003] provides another rationale for studying a possible association between gB and disease outcome.

A number of studies have attempted to correlate the distribution of the four gB CMV genotypes with the clinical outcome of CMV infection [Fries et al., 1994; Bongarts et al., 1996; Shepp et al., 1996; Vogelberg et al., 1996; Torok-Storb et al., 1997; Chern et al., 1998; Peek et al., 1998; Rosen et al., 1998; Gilbert et al., 1999;...
The aim of the present study was to investigate a possible association between CMV gB subtypes and central nervous system disease in AIDS patients.

**MATERIALS AND METHODS**

**Patients**

From February 1995 to December 2000, cerebrospinal fluid (CSF) samples from 203 AIDS patients exhibiting various central nervous system diseases were tested for CMV-DNA by PCR. As controls, we examined CMV samples obtained from the urine and saliva of AIDS patients with asymptomatic CMV infection. Asymptomatic CMV infection was defined as the isolation of CMV in a patient with no clinical, neurological, or laboratory signs of infection. All control patients were submitted to physical and neurological examination, fundoscopy and determination of CMV antigenemia, and were matched with CSF-CMV positive cases by CD4+ cell counts.

The study was approved by the Ethics and Research Committee of the University of São Paulo School of Medicine (CAPPesq).

**Sample Preparation**

CSF was boiled for 10 min and spun in a microfuge for 15 min at 4°C. Urine samples were diluted 1:4 in MILLIQ water, while saliva samples were diluted 1:1 in a lysis buffer consisting of 10 mM Tris-HCl (pH 8.3) 0.9% NP 40 and 0.9% Tween 20, with proteinase K (100 μg/ml). The mixture was then incubated at 55°C for 1 hr, followed by denaturation of the enzyme at 95°C for 10 min.

**gB PCR and Genotyping**

gB gene amplification and subsequent genotyping were performed directly on patients’ specimens using previously described methods [Chou and Dennison, 1991]. Primers gB 1319 and gB 1605 amplified a region of high variability in the gB gene. The gB PCR products were then cut in two separate reactions with HinfI and RsaI, and were classified into gB types 1-4.

**Statistical Analysis**

Proportions of CMV genotypes detected in CSF and saliva or urine were compared by chi-square or Fisher’s exact test using Epi-Info 6 (version 6.04d). For continuous variables that were normally distributed (CD4+ cell counts), Student’s t test was used. P values ≤ 0.05 were considered significant.

**RESULTS**

**Cytomegalovirus Detection in Patients With and Without Central Nervous System (CNS) Disease**

Cytomegalovirus DNA was successfully amplified in 30 samples from 203 AIDS patients exhibiting various CNS diseases. Of the 129 AIDS patients without CNS disease, CMV was detected in 39 (26 from urine samples and 16 from saliva samples). From three patients of this group, CMV was amplified from both urine and saliva. Of these, two yielded the same gB genotype in urine and saliva (gB1 or gB2) and the remaining one yielded CMV gB1 in the urine and CMV gB3 in the saliva.

**CD4+ Cell Counts**

There was no difference between the CD4+ cell counts in the group of patients exhibiting CMV-related CNS disease (range, 1–106 cells/mm³, median = 13, mean = 25.1 ± 28.0 cells/mm³) and AIDS patients without CNS disease (range, 5–86 cells/mm³, median = 37, mean = 36.8 ± 24.6 cells/mm³) (P = 0.14).

**Distribution of gB Genotypes in CSF Samples**

The gB genotype was determined in 26 out of the 30 CMV samples. Of these, 11/26 (42%) typed as gB group 1, 7 (26%) as gB2, 4 (15%) as gB3, and 4 (15%) as gB4.

**Distribution of gB Genotypes in AIDS Patients Without CNS Disease**

All CMV strains detected in the clinical specimens of saliva and urine could be assigned to gB genotypes 1–4; no additional gB types were found in this study. The gB genotype frequency distribution in the 42 AIDS patients without CNS disease showed that 18/42 (42.8%) belonged to gB group 1, 10/42 (23.8%) to gB2, 7/42 (16.6%) to gB3, and 7 (16.6%) to gB4.

There was no difference between the gB genotype frequency distribution of CMV samples from the CSF of AIDS patients exhibiting central nervous system diseases and those from the urine or saliva of AIDS patients without CNS disease (Table I).

<table>
<thead>
<tr>
<th>gB type</th>
<th>CSF (n = 26)</th>
<th>Urine or saliva (n = 42)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11 (42.3%)</td>
<td>18 (42.8%)</td>
<td>0.96</td>
</tr>
<tr>
<td>2</td>
<td>7 (26.9%)</td>
<td>10 (23.8%)</td>
<td>0.77</td>
</tr>
<tr>
<td>3</td>
<td>4 (15.4%)</td>
<td>7 (16.7%)</td>
<td>0.84</td>
</tr>
<tr>
<td>4</td>
<td>4 (15.4%)</td>
<td>7 (16.7%)</td>
<td>0.84</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Correlation between gB genotype and the risk for CMV disease has been evaluated in various patient groups. In bone marrow transplant recipients, patients who survived CMV infection more frequently shed CMV
of the gB type 1 genotype than those who died [Fries et al., 1994]. These findings have been confirmed by other studies showing that disease and death due to CMV are significantly lower in narrow transplant recipients infected with the gB1 genotype compared to patients infected with other gB subtypes [Hebart et al., 1997; Torok-Storb et al., 1997; Woo et al., 1997]. However, in renal [Vogelberg et al., 1996; Woo et al., 1997; Aquino and Figueiredo, 2000] and liver [Rosen et al., 1998] transplant recipients, gB type did not correlate with the development of symptomatic or tissue-invasive CMV disease.

Although an initial study in AIDS patients reported a higher risk of CMV retinitis in those presenting viremia caused by gB group 2 CMV strains [Shepp et al., 1996], subsequent studies found no association of glycoprotein B genotypes with the viral DNA load or the presence of retinitis [Chern et al., 1998; Peek et al., 1998; Gilbert et al., 1999]. In the present study, the ratio among gB subtypes in the CSF of patients manifesting different CMV-related neurological diseases was similar to that of subtypes found in the urine or saliva of AIDS patients with asymptomatic CMV infection. In AIDS patients, progression to invasive disease is associated with a low CD4+ T-lymphocyte count [Gallant et al., 1992; Monforte et al., 1993; Monforte et al., 1997]. The fact that in the present study both patients with or without CNS disease were matched by CD4+ cell counts decreased possible interference of the patients' immune status with the invasive capacity of the different CMV strains.

In previous studies, the gB2 genotype was found more often in AIDS patients than was gB1 [Fidouh-Houhou et al., 2001]. In the present study, the gB types 1 and 2 were the CMV genotypes most frequently found, both in the CSF of AIDS patients with CMV-related neurological disease (42.3% and 26.9%, respectively) and in the urine and saliva of AIDS patients without CNS disease (42.8% and 23.8%, respectively). However, the difference in the prevalence of CMV gB1 and CMV gB2 was not statistically significant in our data, neither in the CSF samples \( (P = 0.24) \) nor in the urine and saliva samples \( (P = 0.06) \). The present study is relatively limited both in patient numbers and in geographical distribution and before this type of information can be extrapolated to the population at large, more samples are needed, and more patients from a wider geographical distribution are required. In previous reports, geographical and demographic differences in patients were shown to affect the frequency of CMV gB genotypes 1–4 in immunocompromised patients [Rasmussen et al., 1997; Wada et al., 1997; Woo et al., 1997; Zipeto et al., 1998]. Further, gB genotypes may exhibit different cell tropisms in vivo, as demonstrated by the lower capacity of gB type 1 to infect T-lymphocytes in vivo in BMT and solid organ recipients [Meyer-König et al., 1998b], and by the observation that gB4 was found more frequently in the semen than in leukocytes of HIV-infected patients with CD4 cell counts <100/μl [Rasmussen et al., 1997]. It is thus important to consider that the frequency of a specific CMV gB genotype may be a function of the body compartment under evaluation. Different results could have been obtained in the present study if CMV plasma/leukocytes samples were examined in the control group. Furthermore, prospective analysis of sequential specimens from the same patient have shown that usually only a single gB genotype is detected [Woo et al., 1997], although simultaneous infection with multiple gB genotypes has been demonstrated in AIDS and allograft recipient patients [Chern et al., 1998; Gilbert et al., 1999; Aquino and Figueiredo, 2000] and in healthy people [Meyer-Konig et al., 1998a; Numazaki et al., 1998]. The demonstration that extensive recombination takes place between strains of CMV [Haberland et al., 1999; Rasmussen et al., 2003] and the lack of genetic linkage between gB and other loci within the CMV genome [Rasmussen et al., 2003] also limit the use of the small loci in the gB gene to genotype CMV strains. These findings underline that molecular epidemiologic studies must consider various clinical, laboratorial, and genetic aspects before attempting to establish an association between CMV gB genotypes and the pathogenesis or virulence of CMV.

REFERENCES


