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Glycoprotein B Subtyping of Cytomegalovirus (CMV) in the Vitreous of Patients with AIDS and CMV Retinitis

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A 550-bp region of the cytomegalovirus (CMV) glycoprotein B (gB) gene was amplified by polymerase chain reaction (PCR) from 141 vitreous specimens of 120 patients with AIDS and CMV retinitis from three different metropolitan centers. The distribution of gB subtypes I, II, III, and IV were 19%, 43%, 12%, and 21%, respectively, based on restriction enzyme digestion patterns of PCR-amplified DNA. Two patients had simultaneous infection with two different gB subtypes. The ratio of gB subtypes was similar among the three geographically distinct patient populations. Two of 14 patients with bilateral vitreous specimens had different viral subtypes in each eye. In addition, different gB subtypes were observed in 1 of 6 patients with serial specimens. The ratio of different gB subtypes in the vitreous of patients with CMV retinitis is similar to that previously reported in the peripheral blood of patients with advanced AIDS.

Cytomegalovirus (CMV) retinitis is one of the most common opportunistic infections of patients with AIDS, but little is known about the pathogenesis of this disease. A number of recent studies have focused on the possible role of virus-encoded glycoprotein B (gB) in this process. CMV gB has long been known to be the target of virus-neutralizing antisera [1] and more recently has been shown to mediate virus entry, cell-to-cell spread, and syncytium formation in vitro [2, 3]. The herpes simplex virus type 1 homolog of CMV gB plays a key role in viral neuroinvasiveness in vivo [4].

Genetic studies of the gB coding region of clinical CMV isolates have led to the identification of four CMV gB genotypes (gB subtypes I–IV) readily distinguished by restriction enzyme digest analysis. It has been suggested that viral pathogenicity may correlate with gB genotype. Particular attention has focused on the value of genotyping the gB coding region of CMV in the blood to predict which patients will develop serious end-organ disease. Fries et al. [5] studied CMV gB genotypes in the blood of renal transplant patients and found that patients infected with gB subtype I had a lower incidence of fatal CMV disease than did patients infected with other gB subtypes. More recently, Shepp et al. [6] studied CMV gB genotypes in the blood of patients with AIDS who were at risk...
for CMV retinitis and found that patients infected with CMV gB subtype II were more likely to develop retinitis than were those infected with other CMV gB subtypes. Furthermore, 70% of the patients who developed CMV retinitis in that study had a prior CMV gB subtype II viremia.

To further investigate the possible role of CMV gB subtypes in the pathogenesis of CMV retinitis, we have analyzed the gB subtypes of CMV DNA in the eyes of patients with AIDS-related CMV retinitis. This was accomplished by restriction digest analysis of polymerase chain reaction (PCR)–amplified CMV DNA from the vitreous of eyes with retinitis.

Methods

Study patients. We studied 203 undiluted vitreous specimens from 163 patients with AIDS and CMV retinitis. Vitreous from the eyes of 28 immunocompetent patients and 24 AIDS patients with non-CMV ocular inflammatory disease served as negative controls. All vitreous specimens were obtained in the course of otherwise scheduled vitreoretinal surgery.

CMV gB subtyping. CMV gB subtyping by MaeIII digest patterns was done as previously described by Shepp et al. [6]. Specimens that did not correspond to reported MaeIII restriction digest patterns were analyzed as described by Chou and Dennison [7] using restriction enzymes Rsal and HinII.

Sequencing. Specimens whose CMV DNA restriction digest patterns did not match the four patterns described by Shepp et al. [6] or Chou and Dennison [7] were analyzed by double-stranded DNA sequencing using standard dideoxy methods on an automated DNA sequencer (ABI 377 Prism Sequencer; Applied Biosystems, Foster City, CA).

Results

We successfully amplified the targeted portion of the CMV gB coding region from 141 of 203 vitreous specimens obtained from eyes with CMV retinitis. Restriction enzyme digestion of PCR amplification products from 133 specimens (114 patients) produced gel electrophoresis patterns typical for gB subtypes I–IV. A more detailed analysis of the gB genotype of the remaining 8 specimens (7 patients) is described below. Of the 62 clinical specimens that failed to yield amplifiable CMV DNA, 4 were from eyes with newly diagnosed CMV retinitis, 20 were from eyes with inactive CMV retinitis, and 38 were from eyes with reactivated CMV retinitis.

On the basis of restriction enzyme digest analysis of amplified viral DNA, 19% of the vitreous samples from patients with AIDS and CMV retinitis were infected with CMV gB subtype I, 43% with gB subtype II, 12% with gB subtype III, and 21% with gB subtype IV. A similar ratio of gB subtypes was found in each of the three geographically distinct study populations (table 1). By χ² analysis, there was no statistically significant difference in these ratios (P = .79, χ² test, df = 6).

Our study included serial vitreous specimens from 6 eyes obtained 5–9 months apart. In 5 of these eyes, gB subtyping of the second specimen was identical to that of the original specimen. In the 1 remaining eye, we initially detected CMV gB subtype II in the vitreous, but 8 months later we detected CMV gB subtype IV.

Our study also included 14 pairs of vitreous specimens from patients with bilateral retinitis. The same gB subtype was identified in both eyes in 12 of these patients. In 1 patient, we detected gB subtype I in the right eye and 2 weeks later gB subtype III in the left eye. In a second patient, we detected gB subtype IV in the right eye and 10 weeks later a mixture of gB subtypes II and IV in the left eye.

The MaeIII digest patterns of amplified CMV gB DNA from 8 vitreous specimens (7 eyes) did not match any of the four patterns previously described [6]. The digest pattern of 6 of these specimens (5 eyes) was identical and consisted of two bands of ~138 bp and 362 bp (figure 1A). The gB DNA amplified from these 6 specimens was further analyzed using the HinII and Rsal digest scheme described by Chou and Dennison [7]. For all 6 specimens, the HinII digest pattern was identical to that of CMV gB subtype III, but the Rsal digest pattern did not match any of the established gB subtypes. Double-stranded DNA sequencing of the gB fragments amplified from 5 of these samples (we chose not to sequence a repeat sample from 1 eye) revealed >98% DNA sequence homology with CMV gB subtype III (GenBank accession numbers AF062417, AF062418, AF062419, AF062420, AF062421). The predicted amino acid sequences from these specimens are presented in figure 1B.

The atypical MaeIII digest patterns found in gB DNA from the remaining 2 vitreous specimens were consistent with mixed infections. This was confirmed by restriction digest analysis of at least 10 clones of amplified DNA from each specimen. In 1 vitreous specimen, we found a mixture of CMV gB subtypes I and III; in the other, we found a mixture of gB subtypes II and IV.

We next attempted to determine whether the prevalence of infection with the four gB subtypes differed among patients with different patterns of clinical disease. By χ² analysis, we found no statistically significant difference in the ratio of gB subtypes found in the vitreous of patients with newly diagnosed retinitis (37 patients), reactivated disease (76 patients), and clinically resistant retinitis (15 patients) (P = .24, χ² test, df = 6).

To control for contamination of vitreous samples from nonocular sources, we attempted to amplify CMV gB DNA from the vitreous of 52 patients with and without AIDS and ocular inflammation not caused by CMV. We were unable to amplify CMV DNA from any of these 52 vitreous samples.

Discussion

In this study, we have analyzed the gB coding region of CMV in the vitreous of patients with AIDS to determine the viral subtypes responsible for CMV retinitis. CMV gB subtype
Table 1. Distribution of CMV gB subtypes from the vitreous of patients with AIDS and CMV retinitis from the three geographic locations.

| Location     | gB 1 | gB 2 | gB 3 | gB 4 | Atypical | Mixed | Total |
|--------------|------|------|------|------|----------|-------|-------|
| San Francisco| 7 (22)| 13 (41)| 3 (9) | 8 (25)| 1 (3)    | 0     | 32    |
| Orange County| 9 (16)| 22 (39)| 9 (16)| 13 (23)| 2 (4)    | 2 (4) | 57    |
| Atlanta      | 9 (20)| 23 (50)| 4 (9) | 8 (17)| 2 (4)    | 0     | 46    |
| Total        | 25 (19)| 58 (43)| 16 (12)| 29 (21)| 5 (4)    | 2 (1) | 135   |

NOTE. In 6 eyes with serial specimens, only first specimen was tabulated. Data are no. of specimens (%).

Figure 1. Analysis of CMV DNA from vitreous of patients with AIDS and CMV retinitis.

A, Polymerase chain reaction–amplified fragments of CMV gB gene digested with restriction endonuclease MaeIII. Control digest patterns are shown for gB subtype I (lanes 2, 3), gB subtype II (lanes 4, 5), gB subtype III (lanes 6, 7), and gB subtype IV (lanes 9, 10). Also present are digest patterns of 2 clinical samples, DM14 and P51 (lanes 11, 12), which did not match gB subtypes I–IV. Lanes 1 and 8 contain DNA marker V (Boehringer Mannheim, Indianapolis).

B, Predicted amino acid sequence of CMV gB subtypes I–IV (variable amino acids between codon 440 and 525) and CMV DNA amplified from 5 clinical samples (DM14, DM73, V15, SSS, and P51) with atypical restriction digest patterns. Sequences for AD169 and other clinical specimens (C116, C128, C178, C194, C326, C336, C354) were previously reported in figure 3 of [7]. Sequences are shown with single amino acid code where residue is different from Towne strain, with dash where residue is identical, and with ‘X’ where amino acid has been deleted. Amino acid residues differing from predicted sequence of gB subtype III are indicated in bold.
I was present in 19% of the vitreous samples, gB subtype II in 43%, gB subtype III in 12%, and gB subtype IV in 21%.

Prevalence of infection with the different CMV gB subtypes was similar in San Francisco, Orange County (California), and Atlanta. Prevalence of infection with the different CMV gB subtypes was also similar in patients with newly diagnosed, clinically resistant, and reactivated CMV retinitis.

The distribution of CMV gB subtypes in the eyes of our study patients, while different from that reported in the blood of patients with solid organ transplants [5, 7, 8], closely resembles the distribution of gB subtypes in the blood of AIDS patients at risk for CMV retinitis [6, 9]. Thus, it is unlikely that infection with any one specific CMV gB subtype, such as gB subtype II, confers increased risk of development of retinitis. This conclusion appears to conflict with the data of Shepp et al. [6], who, in a smaller study, found that viremia with CMV gB subtype II was associated with a higher risk of CMV retinitis than viremia due to other CMV gB subtypes. Clearly, a prospective study of paired blood and vitreous samples from patients at risk for developing CMV retinitis will be needed to properly resolve this issue.

In the course of this study, we found evidence of ocular infection with more than one strain of CMV, bilateral ocular infection with different virus strains, and in serial ocular samples, strain variation over time. These results, while novel, were not unexpected, since previous studies of nonocular tissues have revealed that patients with AIDS may be infected with more than one strain of CMV, either simultaneously, sequentially, or in disparate body sites [9–13]. We were surprised, however, at the relatively high rate of CMV genotypic variation in bilateral and sequential vitreous samples, given the small region of the viral genome that we assayed. Isolation of different CMV genotypes from different body sites in patients with AIDS raises serious concerns about the use of nonocular virus isolates in genetic studies of the pathogenesis of CMV retinitis.

The restriction digest patterns of CMV DNA amplified from 8 of our study eyes did not match patterns previously reported [6, 7, 9]. In 2 eyes, this was a consequence of ocular infection with more than one virus strain. In the remaining 6 eyes, sequence analysis revealed that the CMV gB coding region had a high degree of homology with gB subtype III. Thus, despite the novel restriction digest pattern, we believe that the CMV DNA in these vitreous samples should be classified as gB subtype III. This observation highlights the need for restraint in establishing new categories of CMV gB subtypes based on restriction digest patterns alone. We were unable to amplify the gB coding region from 31% of the vitreous specimens that were assayed. This was not surprising, since 94% of these specimens were from patients who had received aggressive antiviral therapy, and as previously described [14], detection of CMV DNA in the eyes of these patients is significantly reduced. Failure to detect CMV in some of the study specimens may have also been due to polymorphisms at primer-binding sites. The use of multiple primer pairs would have probably increased our ability to amplify the gB coding region from the vitreous [14, 15] but was not incorporated into the assay used in the current study so that we might more directly compare our results with the published results of other investigations.

Overall, gB subtype testing of vitreous specimens as we have done in this study was unable to provide additional prognostic or predictive information about which patients may have resistant or recurrent CMV retinitis. Furthermore, using historic controls, we were unable to find statistically significant differences between the distribution of gB subtypes in the vitreous and blood of AIDS patients. Analysis of other regions of the CMV genome, including other regions of the gB coding region, may prove valuable in this regard.

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Effect of High-Dose Acyclovir on Survival in Allogeneic Marrow Transplant Recipients Who Received Ganciclovir at Engraftment or for Cytomegalovirus pp65 Antigenemia

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This study sought to determine whether high-dose acyclovir improves posttransplant survival in cytomegalovirus (CMV)-seropositive patients when ganciclovir is given for prophylaxis or as early therapy. Three groups were studied: Group 1 (n = 112) received ganciclovir from engraftment without prior acyclovir treatment, group 2 (n = 114) was given ganciclovir for CMV pp65 antigenemia without prior acyclovir, and group 3 (n = 133) received ganciclovir at engraftment with prior intravenous acyclovir (500 mg/m² every 8 h) from day 5 before transplant until engraftment. In a multivariable Cox model, there was no significant difference in the adjusted risk of transplant survival between the groups during the first 2 years after transplant (relative risk for mortality: group 1, 1.0; group 2, 0.75 (95% confidence interval [CI], 0.52–1.1); group 3, 1.04 (95% CI, 0.74–1.47). The incidence of CMV disease and CMV-related mortality was not significantly different between the groups. Thus, high-dose acyclovir does not appear to improve survival when ganciclovir is given either at engraftment or for CMV pp65 antigenemia.

Two studies have shown improved survival in patients given high-dose acyclovir (500 mg/m² every 8 h) intravenously (iv) from day 5 before transplant (−5) until day 30 after transplant alone or with subsequent oral acyclovir (800 mg 4 times/day until day 210) [1–3]. Despite these positive results, the issue of acyclovir for prevention of cytomegalovirus (CMV) disease remains controversial among stem cell transplant centers [4, 5]. The major reason for this controversy is a poor understanding of the mechanisms of the observed survival benefit. The differences in survival in these studies could not clearly be attributed to a reduction of CMV-associated mortality [1–3]. It has been hypothesized that early suppression of CMV infection or other herpesviruses, such as human herpesvirus 6, during the first month after transplant by iv high-dose acyclovir may be responsible for the observed survival benefit [2, 6]. Other reasons for the controversy include the availability of more potent anti-CMV compounds, such as ganciclovir and foscarnet, the high cost of the proposed acyclovir regimen, especially when added to oral acyclovir (800 mg 4 times/day until day 210) [1–3]. Despite these positive results, the issue of acyclovir for prevention of CMV in allogeneic marrow transplant recipients is still controversial [12].

It is unclear whether high-dose acyclovir has additional benefit if ganciclovir is given at engraftment or for early treatment based on CMV pp65 antigenemia or polymerase chain reaction (PCR) detection of CMV DNA, the two most common strategies at this time. Because there are no published reports or ongoing controlled studies addressing the issue and because the cost of the proposed acyclovir regimen is substantial, we examined the impact of high-dose iv acyclovir treatment as described by Meyers et al. [1] on transplant survival, CMV-related mortality, and CMV disease in seropositive allogeneic marrow transplant recipients who received either ganciclovir at engraftment or for pp65 antigenemia.

Methods

Patients. Consecutive CMV-seropositive patients of all ages undergoing allogeneic marrow transplantation at the Fred Hutchinson Cancer Research Center between July 1991 and March 1994...