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ORIGINAL ARTICLE

Glycoprotein B Genotype of Human Cytomegalovirus: Distribution in HIV-infected Patients

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Glycoprotein B (gB) is involved in cell to cell transmission of human cytomegalovirus (HCMV) and may be a critical factor in tissue tropism and viral pathogenesis. We analyzed the distribution of the four known gB genotypes of HCMV in 99 HIV-positive patients. 29 patients had HCMV retinitis, and 70 patients had asymptomatic HCMV infection. DNA was isolated from blood, urine, and aqueous humor, and gB genotypes were determined by PCR and restriction analysis. Infections with gB type 1 were less frequent in patients with retinitis than in patients with asymptomatic HCMV infection (17% versus 37%; $p = 0.05$). Furthermore, the gB type was correlated with dissemination of infection. In patients with HCMV detected in only one compartment (blood or urine) the gB type 1 was found more frequently than in patients with HCMV detected in at least two compartments ($p = 0.01$). The data show that gB genotypes differ in their association with clinical disease, and indicate that the gB genotype may contribute to the course of HCMV infection.

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INTRODUCTION

Human cytomegalovirus (HCMV) is one of the most important opportunistic infectious agents in HIV-infected patients. Although host factors clearly influence the course of HCMV infection, little is known about the role of viral strain variations. It has been shown that variations of the glycoprotein B (gB) of clinical HCMV isolates are clustered and adopt 1 of 4 gB types (1, 2). In a recent study performed on bone-marrow-transplant recipients, HCMV infection with gB type 1 was associated with a lower mortality compared to infection with gB types 2–4 (3). In the present study, we investigated whether the gB type correlates with the clinical course of HCMV infection in HIV-infected patients.

MATERIALS AND METHODS

The gB type of 151 samples (79 blood, 65 urine, 7 aqueous humor) was determined. The samples were obtained from 99 HIV-infected patients in all stages of disease. If the same material was obtained several times from one patient, only one sample was included (123 samples were included in the study). Patients were attending the clinical departments in Freiburg, Hamburg, or Bern. HCMV retinitis was diagnosed in 29 patients by indirect ophthalmoscopy. Asymptomatic HCMV infection was found in 70 patients by the detection of HCMV DNA either in peripheral blood leukocytes (PBLs) or urine. PBLs were separated for polymerase chain reaction (PCR) as described previously (4, 5). 2×10^5 PBL or 25 µl of aqueous humor was mixed with 25 µl of a PCR-compatible lysis buffer supplemented with proteinase K as described previously (5). The DNA of 200 µl of urine was extracted using the Qiamp Blood Kit (Quiagen, Chatsworth, USA) according to the manufacturer's instructions. A region of high peptide variability in the gB gene was amplified as described previously (3). A first round of amplification was added to improve sensitivity. To overcome sequence variation between strains, a mixture of three 5′ primers was used (primers of additional first round of amplification of the nested PCR: 5′ gBout: 5′-GTTCCGAAGCCGAAGACTCG. 5′ gBout2: 5′-GCAGCACCTGGCTCTATCG. 5′ gBout3: 5′ GCCAGCTCACCTTCTGGG. 3′ gBout: 5′-GCACCTTGACGCTGGTTTGG). Amplification products were subjected to restriction analysis using Hinf I and Rsa I (New England Biolabs, Beverly, USA) as described previously (3). Digested DNA was analyzed on 10% polyacrylamide gels. Four distinct gB genotypes were identified by different patterns of fragment length (36–339 bp). Statistical analysis was performed using the χ^2 test.

RESULTS AND DISCUSSION

In this study, the distribution of the 4 known gB genotypes of HCMV was determined using sequential samples (blood, urine, aqueous humor) of 99 HIV-infected subjects with and without symptomatic HCMV disease. 84/99 patients were infected by only 1 of the 4 gB types. The total number (108 samples) of urine, blood, and aqueous humor findings are given in Table I. All these samples could be assigned to 1 of the 4 gB types. A mixture of gB types was found in 15/99 patients and was classified as follows: different gB types were found in (i) the same sample of 1 patient (6/15 patients), or (ii) multiple sites of 1 patient (9/15 patients). The pathogenic role of multiple infections, however, needs to be investigated further. The distribution of gB types in HIV-infected patients when only 1 sample from each patient was included is shown in Table II. The gB types 1 and

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Table I. *Distribution of gB types in different materials*

	Asymptomatic HCMV infection		HCMV retinitis			
gB type	Blood	Urine	Blood	Urine	Aqueous humor	Total
1	16 (41%)	15 (41%)	4 (22%)	1 (12.5%)	1 (17%)	37 (34%)
2	19 (49%)	13 (35%)	9 (50%)	4 (50%)	3 (50%)	48 (44%)
3	3 (8%)	3 (8%)	3 (17%)	1 (12.5%)	1 (17%)	11 (10%)
4	1 (3%)	6 (16%)	2 (11%)	2 (25%)	1 (17%)	12 (11%)
Total	39	37	18	8	6	108

2 were found most frequently, namely in 31/99 (31%) and 35/99 (35%) patients, respectively. Types 3 and 4 were found in 9/99 (9%) patients each. We found a different pattern in renal-transplant recipients where the gB types 1, 2, and 3 were almost equally distributed (14/60 patients with type 1 (23%), 18/60 patients with type 2 and type 3 each (30%), 4/60 patients with type 4 (10%), and 6/60 patients with mixed types (10%)) (6). A recently published study showed that virus isolates from bone-marrow-transplant recipients were predominantly gB type 1 viruses (60/112 patients with type 1 (54%), 11/112 patients with type 2 (10%), 24/112 patients with type 3 (21%), 10/112 patients with type 4 (9%), and 7/112 patients with mixed types (6%)) (3). These differences are difficult to explain. Possibly, gB strains differ in their site of latency and reactivate at different sites in each of these groups of patients. A study is under way to analyze the distribution of gB types in different tissues of immunosuppressed hosts. Alternatively, the type and severity of the immune defect, which differs between these patient groups, could determine the prevalence of the gB types.

Infections with the gB type 1 were found less frequently in retinitis patients (5/29 patients (17%)) as compared to HIV-positive patients with asymptomatic HCMV infection (26/70 patients (37%); $p = 0.05$). Furthermore, gB types 1 and 2 were correlated with dissemination of infection. In patients with HCMV detected in only 1 compartment (blood or urine), the gB type 1 was found more frequently than in patients with HCMV detected in at least 2 compartments ($p = 0.01$) (Table III). Thus, our results indicate that gB type 1 infections in AIDS patients may be less severe, leading less frequently to retinitis. The results are in agreement with findings in bone-marrow-transplant recipients, in whom gB type 1 infections correlated with a more favorable clinical outcome (3). However, the complete lack of gB type 1 infection in AIDS patients with retinitis, as shown previously, was not confirmed (7). Thus, these data provide further evidence that the gB genotype might be a virulence marker for HCMV. It is, however, unclear whether the gB variation alone or in combination with other factors influences the virulence of HCMV strains. The gB protein is important for viral transmission from cell to cell and is highly conserved among herpesviruses (8). It has been shown that a single amino acid exchange in the gB protein of HSV-1 conferred increased neuroinvasiveness to mice (9). Moreover, recent data provide evidence that HCMV gB types differ in their tropism for T-cells in vivo (10). The gB genotype of HCMV is known to be linked to the genotype of another membrane protein, the gH protein. However, the gH genotype was not found to correlate with the clinical outcome (3). No linkage was found between the gB type and the genotypes of the integral membrane protein and the immediate early gene products (11). Further in vitro studies are necessary to clarify the impact of the gB variation on the virulence of HCMV strains.

Table II. *Distribution of gB types in HIV-infected patients: absolute and relative numbers*

	Asymptomatic HCMV infection				
		CD4 count ($\times 10^6$/l)		HCMV	
gB type		>50	<50	retinitis	Total
1	26 (37%)	18 (40%)	7 (29%)	5 (17%)	31 (31%)
2	23 (32%)	14 (31%)	9 (38%)	12 (41%)	35 (35%)
3	5 (7%)	1 (2%)	4 (17%)	4 (14%)	9 (9%)
4	6 (9%)	5 (11%)	1 (4%)	3 (10%)	9 (9%)
Mix	10 (15%)	7 (16%)	3 (13%)	5 (17%)	15 (15%)
Total	70			29	99

Table III. *gB types in patients with the detection of HCMV in only 1 site (blood or urine) and in more than 1 site (blood and urine)*

	HCMV detection		
gB type	1 site	>1 site	Total
1	13	6	19
2	5	13	18
Total	18	19	37

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