Virology 462-463 (2014) 207–217

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Cytomegalovirus alpha-chemokine genotypes are associated with clinical manifestations in children with congenital or postnatal infections ☆

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ARTICLE INFO

Article history:
Received 12 February 2014
Returned to author for revisions
10 June 2014
Accepted 17 June 2014
Available online 5 July 2014

Keywords:
Human cytomegalovirus
α-chemokine
Virus genotype
Congenital infection
Postnatal infection

ABSTRACT

Human cytomegalovirus (HCMV) is the leading cause of congenital infections. The aim of our study was to determine the prevalence of genotypes based on the highly polymorphic UL146 and UL147 HCMV genes and the relationship between the genotype and symptoms or viral load.

We analyzed samples from 121 infants with symptomatic HCMV infection, including 32 congenitally infected newborns. The G7 and G5 genotypes were predominant in postnatal infection, whereas the G1 genotype was prevalent in congenital infection. Central nervous system (CNS) damage and hepatomegaly were detected more frequently among children infected with the G1 genotype than in those infected by other genotypes. An association between the viral genotype and viruria level was found. There was a strong correlation between HCMV genotypes determined through the UL146 and UL147 sequences (κ=0.794).

In conclusion, we found that certain vCXCL genotypes are associated with clinical sequelae following HCMV infection.

Introduction

Human cytomegalovirus (HCMV) is an important and widespread pathogen, congenitally infecting 0.3–2.3% of all newborns (Dollard et al., 2007; Kenneson and Cannon, 2007). Approximately 10–15% of infected newborns are symptomatic and have multisystem or fatal disease. Symptomatic newborns exhibit central nervous system (CNS) damage, hepatosplenomegaly, jaundice, petechiae, hepatitis, intrauterine growth retardation (IUGR), and thrombocytopenia. The remaining 85–90% of children show no clinical evidence of infection during the neonatal period, but they can develop negative clinical outcomes, including hearing loss, motor deficits, and ocular abnormalities, in subsequent years (Stagno et al., 1982). Both symptomatic and asymptomatic infants excrete the virus in their urine and saliva for several years after birth. The virulence determinants causing congenital or postnatal HCMV infection and symptomatic disease have yet to be unambiguously identified.

HCMV has a large and complex genome containing more than 200 open reading frames (ORFs), but the genetic variability of HCMV is limited to distinct parts of the genome (Arav-Boger et al., 2005, 2006a; Bradley et al., 2008; He et al., 2006; Heo et al., 2008; Lurain et al., 2006; Pignatelli et al., 2004, 2010). "Key genes" encoding the envelope glycoproteins (*e.g.*, gB, gN, and gH) and homologs of cytokine/chemokine receptors, including UL144 and US28, have been studied due to their potential relevance to cell tropism and virulence. Although specific genotypes were detected in some symptomatic congenital infections, these associations were unconfirmed in functional studies (Barbi et al., 2001; Pignatelli et al., 2010; Paradowska et al., 2013; Paradowska et al., 2014; Pati et al., 2013). HCMV also possesses genes that encode

☆This research was presented in part at the 36th Annual International Herpesvirus Workshop, Poland, Gdansk, 24–26 July 2011, abstract 8.17.

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http://dx.doi.org/10.1016/j.virol.2014.06.020
0042-6822/

factors involved in immune evasion, which could function to increase viral spread and survival (Mocarski, 2002). It encodes a *bona fide* CXC chemokine UL146 (vCXCL-1) (Penfold et al., 1999), CXC chemokine homolog UL147 (vCXCL-2) (Penfold et al., 1999), CC chemokine homolog UL128 (Akter et al., 2003), and chemokine receptors. Both the UL146 and UL147 genes are located at the UL/b' boundary and in 19 ORFs between UL133 and UL151; these genes are lost after extensive serial passaging of the Towne and AD169 strains in tissue culture (Cha et al., 1996; Prichard et al., 2001). However, the Towne strain is known to contain a mixture of two variants, one of which is intact in UL/b′ region (Hahn et al., 2003; Bradley et al., 2009). UL146 encodes vCXCL-1, an IL-8-like chemokine that is secreted late during infection and induces calcium mobilization, chemotaxis, and the degranulation of neutrophils (Penfold et al., 1999). Furthermore, vCXCL-1 appears to be a determinant of host neutrophil behavior in response to HCMV infection (Penfold et al., 1999). This glycoprotein functions as a selective agonist for CXCR1 and CXCR2, although with differing affinities and potencies (Lüttichau, 2010). vCXCL-2 is not known to be functional in any assay (Penfold et al., 1999). Although these genes are not essential for virus replication *in vitro*, it has been suggested that they are maintained for infectivity *in vivo* (Lurain et al., 2006). Both genes show a high degree of variability in clinical isolates (Arav-Boger et al., 2005; Bradley et al., 2008; Dolan et al., 2004; Hassan-Walker et al., 2004; Lurain et al., 2006; Prichard et al., 2001; Stanton et al., 2005). Moreover, the UL146 gene is one of the most variable genes in the HCMV genome, and 14 genotypes have been cataloged (Arav-Boger et al., 2005; Dolan et al., 2004; Penfold et al., 1999; Prichard et al., 2001). More recently, a fifteenth vCXCL-1 genotype was described (Heo et al., 2008). It has been suggested that HCMV could abate antiviral immunity by manipulating the host chemokine system and suppressing the immune system (Bradley et al., 2008). It was recently found that the UL146 ORF is transcribed with 4 downstream ORFs, from UL147 to UL132 (He et al., 2012).

In this study, we determine the prevalence of genotypes based on the highly polymorphic UL146 and UL147 HCMV genes in congenitally or postnatally infected infants with symptomatic infection. The study also addresses whether there is an association between specific vCXCL genotypes, viral load, and the presence of specific symptoms in HCMV-infected children. All of the examined infants demonstrated symptomatic HCMV infection at birth or in the next months of infancy. We studied the UL146 and UL147 genes from the DNA isolates of 32 newborns and 89 infants. While the gene products were detected in all newborns, we were unable to amplify them in one-third of the infants. Genotypic analysis of both genes showed a strong linkage between the vCXCL-1 and vCXCL-2 genotypes. Moreover, we documented that the HCMV G7 and G5 genotypes were the most prevalent in symptomatic infants with postnatal or uncharacterized infection, whereas different genotypes were transmitted from mother to fetus. Our results suggest that the clinical manifestations of prenatal HCMV transmission are likely to be associated with a high viral load in urine samples. The certain vCXCL genotypes are associated with clinical sequelae following HCMV infection during childhood.

Results

Study population and clinical outcome

One hundred twenty-one infants with HCMV infection were enrolled in the study. The results showed that 32 of them had congenital HCMV infection that was confirmed by HCMV DNA detection within the first 2–3 weeks of life. Cytomegalovirus serology assays were performed for 114 of the 121 patients (94.2%), including 29 newborns with congenital HCMV infection and 85 postnatally infected infants. The anti-HCMV IgG serology results were positive for all newborns, whereas 15 of them were positive for IgG and IgM. Sera from 43 infants were positive for IgG and IgM, 40 were positive for IgG only, and 2 were IgG and IgM negative. HCMV DNA was detected in all examined children. The UL146 and UL147 gene products were successfully amplified in all of the examined newborns with congenital infection and in 56 of 89 (62.9%) infants, and the remainder of the analysis was performed using the patients with successful amplification. Additionally, both genes were detected in all adult patients. In all subjects, the HCMV UL55 gene was quantified by real-time PCR.

Symptoms of HCMV infection were determined at birth in all newborns and when they became evident later in childhood in infants. The most common findings were as follows, 63 cases of hematological disorders, 47 cases of neurological dysfunction, 37 cases of psychomotor retardation, 30 cases of CNS damage, and 26 cases of jaundice, among others (Table 1). As expected from many studies, symptoms were detected in newborns more often than in infants: intracranial calcification, cystic lesions, IUGR, hepatomegaly, petechiae/purpura, hearing loss ($p < 0.001$); ocular abnormalities ($p = 0.001$); thrombocytopenia ($p = 0.002$); neurological dysfunction ($p = 0.026$); anemia ($p = 0.003$); microcephaly or ventriculomegaly ($p = 0.004$); and splenomegaly ($p = 0.007$). The incidence of specific symptoms was similar in infant groups with and without successful amplification.

Viral load

The number of HCMV DNA copies was determined in urine and blood samples obtained from children (Fig. 1A and B). The median HCMV DNA concentration in urine was significantly higher (7.74×10^6 copies/mL; range 0–8.73×10^8 copies/mL) in newborns with congenital infection than in infants with postnatal HCMV infection (3.32×10^4 copies/mL; range 0–2.21×10^8 copies/mL), $p < 0.001$ (Mann–Whitney U test) (Fig. 1A). The viremia levels

Table 1
Clinical and laboratory findings in infants with symptomatic HCMV infection.

Findings	No. positive infants (%)		
	Congenital infection	Postnatal infection	*p*-value
Anemia	28 (87.5)	33 (58.9)	**0.003**
Thrombocytopenia	12 (37.5)	5 (8.9)	**0.002**
Other hematological disorders[a]	1 (3.1)	9 (16.1)	0.087
Neurological dysfunction[b]	22 (68.8)	25 (44.6)	**0.026**
Microcephaly	8 (25.0)	2 (3.6)	**0.004**
Intracranial calcification	14 (43.8)	3 (5.4)	< **0.001**
Ventriculomegaly	8 (25.0)	2 (3.6)	**0.004**
Cystic lesions	16 (50.0)	6 (10.7)	< **0.001**
Psychomotor retardation	17 (53.1)	20 (35.7)	0.116
Hearing loss	14 (43.8)	6 (10.7)	< **0.001**
Ocular defects	10 (31.2)	3 (5.4)	**0.001**
IUGR	14 (43.8)	4 (7.1)	< **0.001**
Hepatomegaly	12 (37.5)	4 (7.1)	< **0.001**
Splenomegaly	9 (28.1)	3 (5.4)	**0.007**
Liver damage	2 (6.2)	8 (14.3)	0.318
Jaundice	11 (34.4)	15 (26.8)	0.469
Petechiae/purpura	10 (31.2)	2 (3.4)	< **0.001**
Pneumonia	3 (9.4)	12 (21.4)	0.237
Congenital heart disease	0 (0)	3 (5.4)	0.550
Total	32 (100)	56 (100)	

p-values were determined by the Fisher exact test.

[a] Other hematological disorders (neutropenia, leukocytosis, and thrombocytosis).

[b] Neurological dysfunction (tremor, hypotonia, and poor sucking reflex).