ABSTRACT

Selected molecular markers of congenital cytomegalovirus infection: the role of polymorphism of genes encoding the gCIII viral complex and expression of RIG-I-like receptors

The human cytomegalovirus (hCMV) belongs to the *Herpesviridae* family and is the leading cause of congenital viral infections worldwide. The hCMV genome encodes about 65 glycoproteins, including more than 20 belonging to the viral envelope. The major hCMV envelope glycoproteins form glycoprotein complexes (gC): gCI (gB), gCII (gM/gN), and gCIII complex. The gCIII complex exists in two forms of which trimer gH/gL/gO is required for the induction of fusion and the pentamer gH/gL/pUL128-pUL131A mediates entry into epithelial and endothelial cells. The gCIII complex is involved in the process of hCMV entry into host cells, the release of progeny virions, cell-to-cell spread, and specific antibody activation. The overall prevalence at birth of congenital hCMV infection is 0.64% of live births in industrialized countries. In approximately 11.0% of congenitally infected newborns, signs of infection are detected immediately after birth. Effects of the virus include neurological disorders, central nervous system damage (e.g., microcephaly, intracranial calcification, and cystic lesions), growth restriction, and sensorineural hearing loss. The innate immune system is the first line of host defense against pathogen invasion, in which pattern recognition receptors (PRRs) including RIG-I-like receptors (RLRs) play an important role. RLRs are a family of cytoplasmic DExD/H-box RNA helicases that recognize viral nucleic acids and activate the antiviral response.

The main goal of this study was to determine the selected molecular markers in congenital hCMV infection. The frequency and role of genetic variability in hCMV genes encoding the gCIII complex in newborns with congenital hCMV infection and in infants with postnatal or unproven congenital hCMV infection was investigated, and RLR expression in third-trimester human placentas and its contribution in the identification of hCMV genetic material were determined.

The hCMV genotyping studies were performed in whole blood and urine samples from 30 newborns with congenital hCMV infection and 100 infants with postnatal or unproven congenital hCMV infection. Amplification of genes encoding viral proteins of the hCMV gCIII complex was performed by polymerase chain reaction (PCR). Trimer gH/gL/gO genotypes were identified by the analysis of restriction fragment length polymorphism (RFLP), and protein from the UL128 *locus* was identified by PCR products sequencing. To determine the hCMV DNA copy number and to evaluate the relative *DDX58*, *IFIH1*, *DHX58*, and *YWHAZ* expression at the mRNA level, real-time PCR (RT-PCR) was used. Third-trimester human placentas (38-42 weeks of gestation)

were obtained immediately after spontaneous vaginal delivery or cesarean section. The experimental hCMV, herpes simplex virus type 1 (HSV-1) and vesicular stomatitis Indiana virus (VSIV) *ex vivo* infections were carried out in a decidual and chorionic villi organ culture model. Protein expression in tissue lysates was quantified using enzyme-linked immunosorbent assay (ELISA) and Western blot methods. The production level of selected cytokines was quantified using ELISA or by cytometric beads array (CBA).

All genotypes of the hCMV gCIII complex were found to be vertically transmitted from the mother to the fetus in the examined infants. The frequency of hCMV genotypes of the trimer and pentamer gCIII complex in newborns and infants were determined. No significant differences in the genotype distributions of the examined genes were found. A higher frequency of gL3 genotypes in the group of newborns with congenital hCMV infection than in infants with postnatal or unproven congenital hCMV infection was found (P = 0.008). Mixed infections were detected in 46.9% of the examined infants. Symptomatic infections in newborns were most often associated with the gL4 genotype of the viral strains in the gCIII and G3 *UL128* and G6 *UL130* in the case of the UL128 hCMV *locus*. In the infant group, the gH1 and gL3 genotypes were associated with symptomatic infection, as well as the G1 and G4 genotypes of the *UL131A*. Some of the hCMV genotypes may be associated with the risk of some cytomegalovirus symptoms in infants, e.g., the G1 genotype of *UL131A* hCMV was associated with the increased risk of central nervous system damage.

In the human third-trimester placenta, constitutive expression of all RLR genes and proteins was found. An increase in RLR expression in the experimental viral infections was observed, including enhanced RIG-I expression in response to hCMV, HSV-1 and VSIV infection, and MDA5 in the case of VSIV infection. The increase in RLR expression was demonstrated in decidua and chorionic villi in the case of VSIV infection, whose genetic material is ssRNA, as well as for hCMV and HSV-1, which are DNA viruses. Based on the data obtained in this study, we can speculate that LGP2 can regulate the transmission of viral infection in the placenta.

The obtained results show that genetic variation in hCMV strains does not affect the transmission of intrauterine infections. Polymorphism of genes encoding proteins of the gCIII virus complex may, however, determine the pathogenicity of hCMV strains and increase the risk of some cytomegalovirus symptoms. RIG-I-like receptors in the placenta are likely to play an important role in the recognition of viral infections and the activation of signaling pathways leading to an antiviral response.