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Short communication

Cytomegalovirus (CMV) glycoprotein H-based serological analysis in Japanese healthy pregnant women, and in neonates with congenital CMV infection and their mothers

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ABSTRACT

Background: Congenital cytomegalovirus (CMV) infection is caused by maternal primary infection as well as CMV reinfection or reactivation during pregnancy, although differences in the clinical impact between these modes of infection remain to be clarified.
Objectives: To investigate the latest prevalence and risk of multiple CMV infection in healthy pregnant women, as well as the types of maternal CMV infection associated with congenital CMV infection.
Study design: Seroprevalence against CMV and IgG subclasses were determined in 344 serum samples from healthy pregnant women in Japan. CMV genotype and serotype were also determined in 18 pairs of mothers and neonates with congenital CMV infection identified in our CMV screening program.
Results: Thirty-two percent of the pregnant women were seronegative, while 66% of CMV seropositive women had IgG3 antibodies against one epitope on glycoprotein H (gH) as the major subclass, and 52% had IgG1 antibodies against one epitope on glycoprotein B (gB). Only a single genotype determined by CMV gH neutralizing epitope was found in the urine from the 18 neonates with congenital CMV infection, even though one case possessed antibodies against multiple CMV strains. In that case, the antibodies against the strain not detected in the urine from the infant disappeared within one month after birth, whereas the antibodies against the infecting CMV strain continued to be detected at 12 months after birth.
Conclusions: Two (11%) of 18 cases of congenital CMV infection occurred via maternal CMV reinfection. Maternal humoral immunity did not prevent congenital CMV infection with another gH subtype.

1. Background

Cytomegalovirus (CMV) primary infection during pregnancy can lead to congenital CMV infection to fetuses [1], result in severe clinical complications [2] or defects like hearing loss [3–7]. Congenital CMV infection is also caused by multiple CMV infections during pregnancy [8]. Multiple strains of CMV are known to infect humans [9–17]. One reason is that CMV evades from CD8+ T cells [18]. Another reason is that the neutralizing antibodies against the primary infection are not sufficient to protect against infection with another CMV strain [19,20]. CMV can be classified into at least two serotypes, AD169 type (AD) and Towne type (To), based on polymorphisms in the glycoprotein H (gH) neutralizing epitope, useful for the identification of the history of CMV infection in

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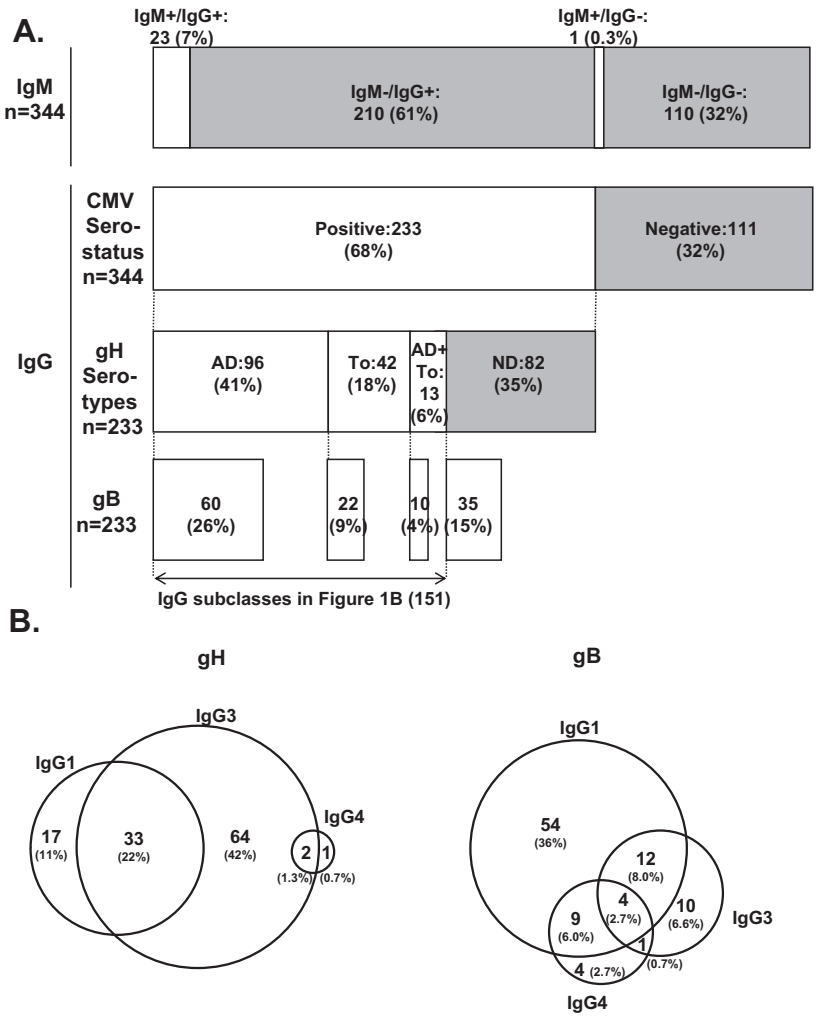

Fig. 1. Seroprevalence of CMV in the middle stage of pregnancy in healthy women. (A) CMV antibodies were screened using serum samples from women in the 2nd trimester of pregnancy. CMV-specific IgG or IgM antibodies were detected using commercial kits. The CMV serotype was determined by serotype-specific ELISA analysis targeting the CMV gH region. AD, AD169 strain; To, Towne strain; ND, not determined; Open, positive or determined; close, negative or undetermined. (B) Distribution of CMV IgG subclasses was determined by ELISA analysis targeting the CMV gH or gB regions.

transplantation patients [21] as well as in congenital CMV cases [2,22]. In this study, we analyzed the serological status of healthy pregnant women, and neonates with congenital CMV infection and their mothers to better understand the mode of transmission in cases of fetal CMV infection.

2. Objective

To investigate the prevalence and risk of maternal CMV primary infection and reinfection during pregnancy, based on serological analyses of pregnant healthy women, and pairs of neonates with congenital CMV infection and their mothers.

3. Study design

3.1. Specimens

Sera from 344 healthy women in the 2nd trimester of pregnancy were collected at the National Center for Child Health and Development. Sera from 18 cases of congenital CMV infection, identified in the screening program [23], were also collected within a month after identification. Case #20026 presented with hearing loss and was treated with oral valganciclovir for 6 weeks from 5 months of age [24]. Case #19389 had normal hearing capability at 6 months, but hearing loss was observed at one year. DNA was isolated from urine specimens using a QIAamp DNA mini kit (Qiagen, Valencia, CA) according to the manufacturer's protocol.

3.2. CMV-specific serological status

CMV serostatus was determined using commercial ELISA kits (Enzygnost Anti-CMV IgM and IgG, Siemens, Munich, Germany) and ELISA using the gB epitope [21]. For serotype-specific anti-CMV IgG, gH epitopes from the AD and To strains were used for our previously established ELISA method [21]. We modified the serotype-specific ELISA by using HRP-conjugated anti-human IgG1, IgG2, IgG3 and IgG4 antibodies (Beckman Coulter Inc., Fullerton, CA) to determine the subclasses of anti-CMV IgG for gH and gB.

3.3. Determination of the CMV gH genotype by DNA analysis

The nucleotide sequences of the CMV gH gene from neonates were determined by fluorescent dye-terminator sequencing and CMV genotype-specific real-time quantitative PCR (qPCR) as described previously [22,25].