Journal of Infection and Chemotherapy 29 (2023) 1071–1074

Contents lists available at ScienceDirect

Journal of Infection and Chemotherapy

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

Case Report

Cell-mediated and humoral immune responses to human cytomegalovirus in pregnant women with vertically transmitted infection following primary infection: A case report

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ARTICLE INFO

Keywords:
Enzyme-linked immunosorbent spot assay
Congenital infection
Cytomegalovirus
Pregnancy
Cell-mediated immunity

ABSTRACT

Human cytomegalovirus (HCMV) is the major cause of neurological sequelae in infants. Immune control of primary HCMV infection appears to depend on the interaction between humoral and cell-mediated immune responses. We report the case of an HCMV-transmitter mother observed with dissociation between humoral and cell-mediated immune responses. The patient had immunoglobulin (Ig) G and M positivity at 11 weeks of gestation and showed fetal hyperechoic bowel and minimal ascites at 21 weeks of gestation. At 25 weeks of gestation, the polymerase chain reaction result for HCMV using amniotic fluid was positive. The numbers of spots in the enzyme-linked immunosorbent spot (ELISPOT) assay at 25, 36, and 39 weeks of gestation were three, five, and six spots/2×10^5 peripheral blood mononuclear cells, respectively. Furthermore, IgG avidity indexes (AIs) at 21, 25, 36, and 39 weeks of gestation were 37.6, 49.7, 72.5, and 74.3, respectively. At 40^{+1} weeks of gestation, the patient delivered a symptomatic infected newborn with a weight of 2,384 g (−2.6 SD) and a head circumference of 30 cm (−2.6 SD). The neonate had a petechial rash and bilateral hearing loss although did not show liver dysfunction or thrombocytopenia. Cranial magnetic resonance imaging revealed mild ventriculomegaly, left lateral/parietal polymicrogyria, and a punctate white matter lesion. This case showed that IgG AI increased with increasing gestational age, whereas the numbers of spots in the ELISPOT assay had no change. The dissociation between humoral and cell-mediated immune responses may be characteristic of the immune response of a transmitter mother.

1. Introduction

Human cytomegalovirus (CMV) infection is considered the most common fetomaternal viral infection, accounting for 0.3%–2.3% of all live births [1,2]. Congenital CMV infection is the leading cause of neurological sequelae in newborns. However, owing to the lack of effective intrauterine treatment and vaccine against the infection, there is no global consensus regarding routine maternal screening for HCMV infection [1,2]. Conversely, maternal serum screening is useful for identifying seronegative women and advising them on preventive measures [3].

Maternal screening is typically performed to identify CMV immunoglobulin (Ig) M-positive pregnant women who are suspected of primary infection during pregnancy. Subsequent IgG avidity testing is useful for differentiating patients with primary infection from those with CMV false IgM-positive and persistent IgM-positive. Pregnant women with a low IgG avidity index (AI) are recommended to undergo a confirmatory test using polymerase chain reaction (PCR) for CMV-DNA

Abbreviations: AI, avidity index; ELISPOT, enzyme-linked immunosorbent spot; HCMV, human cytomegalovirus; PBMC, peripheral blood mononuclear cells; PCR, polymerase chain reaction.

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https://doi.org/10.1016/j.jiac.2023.07.004
Received 2 March 2023; Received in revised form 5 June 2023; Accepted 10 July 2023
Available online 13 July 2023

in amniotic fluid samples [4]. However, several of these pregnant women may refuse to undergo this test owing to the risks involved in amniocentesis. To evaluate the cell-mediated immunity, CMV enzyme-linked immunosorbent spot (ELISPOT) assay is performed. This assay may have the potential to diagnose vertical transmission in utero.

Accordingly, we reported a case that performed the ELISPOT assay as a sequence of tests to diagnose the HCMV vertical transmission in utero.

2. Case report

A 21-year-old pregnant woman was referred to Miyazaki University Hospital because of suspected vertical transmission in utero. The pregnant woman has a healthy 10-month-old baby boy. The patient had a good nutritional condition and was not taking any medications, including steroids. She did not have any primitive or secondary immunodeficiency, including diabetes mellitus, cancer, or collagen disease. During the first trimester of her previous pregnancy, the patient had negative results for HCMV IgG and IgM antibody tests.

At 11^{+0} weeks of gestation during her present pregnancy, the patient showed positive test results for HCMV IgG (≥250 AU/mL) and HCMV IgM (1.62). Routine ultrasonography at 21 weeks of gestation revealed fetal hyperechoic bowel and minimal ascites. At 21 weeks of gestation, reexamination for HCMV serological tests was performed and revealed HCMV IgG, HCMV IgM, and IgG AI of 21.1 AU/mL, 0.73, and 37.6%, respectively. At 25 weeks of gestation, the HCMV ELISPOT assay revealed 3 spots/2×10^5 peripheral blood mononuclear cells (PBMCs) (Fig. 1). Moreover, at 25 weeks of gestation, the IgG AI was 49.7%. Thereafter, the ELISPOT assay at 36 and 39 weeks of gestation revealed 5 and 6 spots/2×10^5 PBMCs, respectively (Fig. 1). However, the IgG AI at 36 and 39 weeks of gestation were 72.5 and 74.3, respectively. The PCR result for HCMV using amniotic fluid samples at 25 weeks of gestation was positive.

At 40^{+1} weeks of gestation, the patient was induced labor owing to oligohydramnios and vaginally delivered a male newborn with a weight of 2,384 g (−2.6 SD) and a head circumference of 30 cm (−2.6 SD). Apgar scores at 1 and 5 min were 8 and 9, respectively. Umbilical arterial blood gas analysis revealed the following results: pH, 7.345; $PaCO_2$, 50.9; PaO_2, 9.4; Base excess, 0.9; and HCO_3, −27.7. The PCR test result using a neonatal urine sample on day 3 of life was positive.

The neonate had a petechial rash on the abdominal skin although no hepatomegaly. Neonatal laboratory findings did not show liver dysfunction, icterus, or low platelet counts. Ophthalmology assessment on day 4 of life revealed normal retinas bilaterally, and the auditory brainstem response testing on day 7 revealed bilateral sensorineural hearing loss (right, 40 dB; left, 60 dB). Cranial magnetic resonance imaging revealed mild ventriculomegaly, left lateral/parietal polymicrogyria, and a punctate white matter lesion (Fig. 2).

3. Discussion

We showed the chronological change of maternal IgG AI and the

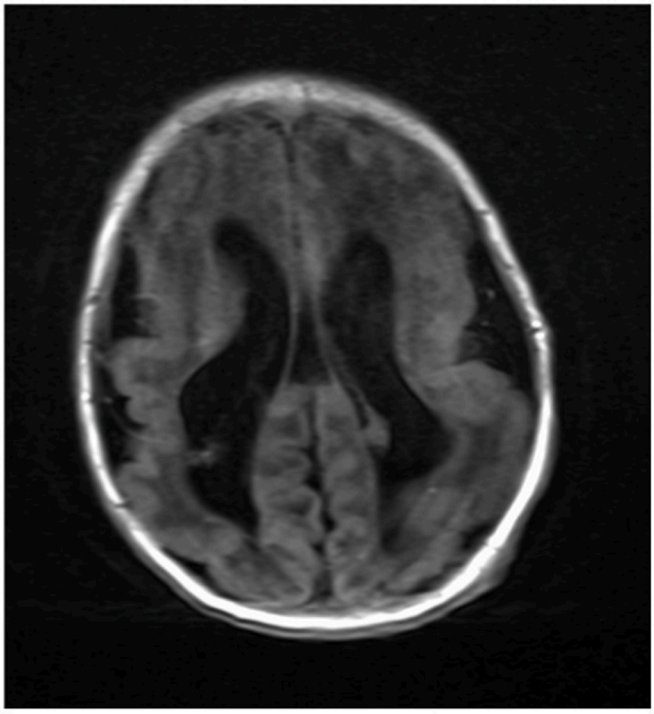

Fig. 2. Cranial magnetic resonance imaging findings.

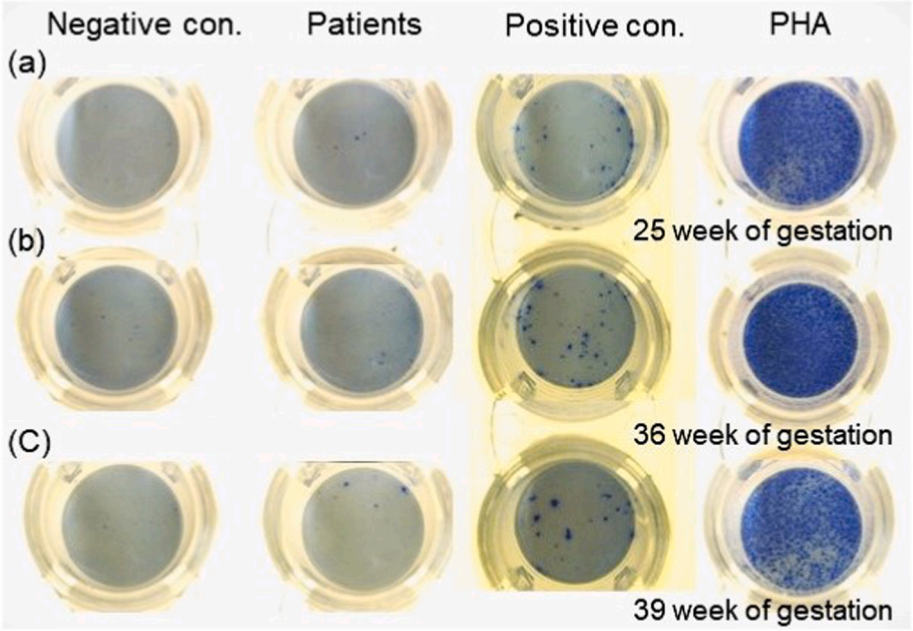

Fig. 1. Chronological changes of the human cytomegalovirus (HCMV) enzyme-linked immunosorbent spot assay result.
The numbers of spots for 2×10^5 peripheral blood mononuclear cells are three spots at 25 weeks of gestation (a), five spots at 36 weeks of gestation (b), and six spots at 39 weeks of gestation (c). Positive con: positive controls were obtained from three pregnant women with CMV IgG positivity in previous pregnancy. PHA: phytohemagglutinin.

HCMV ELISPOT assay results during pregnancy in the congenital CMV case. Consequently, the IgG AI increased with increasing gestational age, whereas the numbers of spots in the ELISPOT assay had no change.

We suspected that the mother had the primary infection during early gestation owing to negative HCMV IgG and IgM antibody titers at the previous pregnancy, positive HCMV IgG and IgM antibody titers at the present pregnancy, relatively low IgG AI at 21 weeks of gestation, and IgG maturation during pregnancy.

The IgG AI is considered a significant assay for interpreting HCMV IgM-positive results [1]. Specifically, low IgG AIs are considered indicative of a recent (less than 3–4 months) primary infection, whereas high IgG AIs indicate a previous infection [1]. Furthermore, it has been reported that IgG maturation in pregnant women with vertical transmission is faster than that in pregnant women with non-vertical transmission [5,6]. IgG maturation, in this case, showed similar changes to these previous studies.

We used the HCMV ELISPOT assay to evaluate cell-mediated immune response in pregnant woman. This assay detects interferon-gamma produced by antigen-stimulated PBMCs [7]. This assay detects both $CD4^+$ and $CD8^+$ T-cell responses [7].

Results regarding cell-mediated immune response in pregnant women with vertical transmission are conflicting. Saldan et al. [8] and Forner et al. [9] showed that an increase in HCMV ELISPOT levels was statistically associated with fetal transmission. Their results are completely different from ours. In their study, the pregnant women with vertical transmission showed a large variety of the numbers of spots in the ELISPOT assay. Moreover, they did not track the subsequent progress of the ELISPOT results in the same pregnant woman. This difference may indicate differences in cell-mediated immunity at the individual level.

On the other hand, some papers showed that cell-mediated immunity was not enhanced in pregnant women with vertical transmission. Revello et al. [10] and Lilleri et al. [11] reported a delay in the development of the HCMV lymphoproliferative response. Lilleri et al. also demonstrated a delay in the re-expression of $CD45RA^+$ T_{EMRA} cells in both HCMV-specific IFN-γ$CD4^+$ and $CD8^+$ T cells [12]. Fornara et al. revealed a delay in IL-2 production by HCMV-specific $CD4^+$ T cells [13]. Furthermore, Mele et al. showed a late development of long-term IL-$7R^{P\circ S}$ memory HCMV-specific $CD4^+$ T cells compared with IL-$7R^{neg}$ T cells [14]. Immune control of primary maternal HCMV infection appears to depend on the interaction of humoral and cell-mediated immune responses. In a multivariate logistic regression analysis conducted by Fornara et al., a higher cultured ELISPOT response, which is predominantly based on the lymphoproliferative activity of HCMV-specific $CD4^+$ T cells, was independently associated with a lower risk of vertical transmission, a higher avidity index, and a lower DNAemia level [15]. Further investigations are warranted to elucidate cell-mediated immune response after CMV infection.

As previously mentioned, several attempts have been made to predict fetal transmission using several immunological prognostic markers; however, nothing is definitive. The detection of CMV-DNA in amniotic fluid samples using PCR is the only prenatal test to confirm the diagnosis of congenital CMV infection. However, as amniocentesis, which is used to extract amniotic fluid, is an invasive procedure, an alternative testing method is warranted. Therefore, if congenital CMV infections can be prenatally diagnosed based on their cellular immune response to CMV using the ELISPOT assay in pregnant women with low IgG AI, it will be clinically relevant.

The immune control of HCMV in primary maternal infections appears to depend on humoral and cell-mediated immune response interaction. The control of HCMV infections is multifactorial and complicated. To understand the immune response in maternal HCMV infection with vertical transmission, further studies are required.

In conclusion, in pregnant women with vertical transmission, IgG AI showed a rapid increase with increasing gestational age, whereas no change was noted in the numbers of spots in the ELISPOT assay. This result may indicate the dissociation in response during pregnancy between humoral immunity and cell-mediated immunity in the pregnant woman primarily infected by HCMV with vertical transmission.

ICMJE statement

All authors meet the ICMJE authorship criteria.

Authorship contributions

MK and NY contributed to designing and conceptualizing the study and drafted the manuscript. SM and YK contributed to data collection and revising of the manuscript. LY, and TM contributed to data analysis, and revising of the manuscript. All the authors read and approved the final version of the manuscript as submitted and agree to be accountable for all aspects of the work.

Funding

This study was supported by JSPS KAKENHI [grant number JP21K09452].

Ethical approval

Written informed consent was obtained from the patient for publication of the paper.

Declaration of competing interest

None.

Acknowledgment

None.

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