MiniReview

Human cytomegalovirus infection of breast milk

Kei Numazaki *

Department of Pediatrics, Sapporo Medical University School of Medicine, S.1 W.16 Chuo-ku, Sapporo 060, Japan

Received 12 February 1997; revised 2 April 1997; accepted 15 April 1997

Abstract

Human cytomegalovirus is the most common cause of congenital and perinatal infections throughout the world. Primary infection with human cytomegalovirus usually follows a benign course, but the virus remains latent or persistent in the host cell thereafter. Understanding the epidemiology of human cytomegalovirus is a key element in the development of strategies for prevention of infection. Although the actual sites of latency or persistence of human cytomegalovirus infections are still controversial, peripheral blood mononuclear cells and endothelial cells appear to be major sites of infection. Persistent infections caused by human cytomegalovirus could be augmented by a decrease in major histocompatibility complex expression as well as by virus-mediated immune dysfunction. It is possible that specific cellular interactions as well as production of several cytokines are necessary for the reactivation of human cytomegalovirus. Breast-fed infants are susceptible to human cytomegalovirus infection from breast milk. Human cytomegalovirus was isolated more frequently from breast milk at more than 1 month after delivery than fromcolostrum or early breast milk. Human cytomegalovirus DNA was also not detected in colostrum, but was found in breast milk samples 1 month after delivery. To clarify the role of milk cells and whey in vertical infection by breast feeding, we separated breast milk into milk cells and whey and examined each fraction. Human cytomegalovirus was isolated more frequently from milk whey samples than from cell samples. Human cytomegalovirus particle shedding into whey may be more important in vertical infection by breast milk than cell-to-cell transmission. The supernatant of colostrum did not exert an inhibitory effect on human cytomegalovirus-infected cells. Serum levels of cell free soluble interleukin-2 receptor of mothers with DNA-positive milk at 1 month after delivery were significantly higher than those of mothers with DNA-negative milk. It is likely that levels of factors such as soluble interleukin-2 receptor in serum are related to the reactivation of human cytomegalovirus which occurs locally in the mammary gland of the lactating mother after delivery. This minireview focuses on recent advances in the study of human cytomegalovirus infection of breast milk.

Keywords: Human cytomegalovirus; Breast feeding; Reactivation; Breast milk; Antiviral agent

1. Introduction

The human cytomegalovirus (HCMV) genome is double stranded DNA, 230 kb in size, the largest human beta-herpes virus, and has unique short and long regions. HCMV has the largest genome encoding more than 200 potentially immunogenic proteins. The characteristic of latency and reactivation is shared with other members of the herpesvirus family. After infection, the viral genome is transcribed in a regulated sequence, resulting in the serial transcription of three different classes of mRNA, immediate early (IE), early (E), and late (L).
HCMV is the commonest cause of congenital and perinatal infections throughout the world (Table 1). However, the prevalence of congenital HCMV infection varies widely between different populations. Almost 90% of the Japanese population are seropositive by 20 years of age. As a result of transmission during the course of delivery, by ingestion of infected breast milk, and by blood transfusion, perinatal infections are much more prevalent than congenital infections. Perinatal HCMV infection often involves the hepatobiliary tract but rarely causes clinical manifestations in normal individuals. However, on the basis of the results of clinical and serological studies of HCMV excreters, it has also been postulated that some infected infants experience hepatitis, hepatosplenomegaly, pneumonitis or a mononucleosis syndrome. Generally, HCMV infections are effectively controlled by the immune system without the ultimate clearance of the virus. Seropositivity for antibodies against HCMV is indicative of latent infection, but unreliable as a predictor for the risk of recurrence.

2. Latent infection and reactivation of HCMV

There is a risk of reactivation of HCMV infection which occurred in the perinatal and neonatal periods, whereas the risk is low when the primary infection occurs during adulthood [1]. After primary infection, HCMV becomes latent, and peripheral blood mononuclear cells (PBMCs) appear to be one of the principal sites of persistent infection [2–4]. Some degree of differentiation may be necessary for permissive infection of freshly isolated human monocytes with HCMV [5,6]. Latent HCMV burden and risk of recurrence were reported to be related to the extent of virus multiplication during primary infection. HCMV latency differs from herpes simplex virus latency by its wide organ distribution. The presence of latent HCMV in multiple organs provides the molecular basis for recurrence from multiple organs.

During active infection with HCMV, viral antigen is consistently present in peripheral blood leukocytes [7]. Transfusions containing leukocytes have been found to be a significant source of HCMV infection. Peripheral blood monocytes are difficult to infect with HCMV in vitro, and viral gene expression cannot be reproduced in peripheral blood cells of healthy people [8,9]. Both peripheral blood mononuclear and polynuclear cells have been found to express viral antigens [10]. Detection of HCMV in the polymorphonuclear leukocyte fraction of peripheral

<table>
<thead>
<tr>
<th>Country</th>
<th>No. investigated</th>
<th>Incidence (%)</th>
<th>Symptomatic cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>England (Stern, 1997)</td>
<td>4259</td>
<td>0.37</td>
<td>–</td>
</tr>
<tr>
<td>England (MacDonald, 1978)</td>
<td>9223</td>
<td>0.40</td>
<td>–</td>
</tr>
<tr>
<td>Canada (Larke, 1980)</td>
<td>15212</td>
<td>0.42</td>
<td>7.8</td>
</tr>
<tr>
<td>Sweden (Allers, 1982)</td>
<td>4421</td>
<td>0.42</td>
<td>–</td>
</tr>
<tr>
<td>England (Peckham, 1982)</td>
<td>14220</td>
<td>0.29</td>
<td>4.7</td>
</tr>
<tr>
<td>Japan (Chiba, 1990)</td>
<td>7995</td>
<td>0.39</td>
<td>10.0</td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>MHC class I or II antigen</th>
<th>Decreased or increased</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHC class I</td>
<td>Decreased</td>
<td>Enhanced degradation of MHC class I heavy chains in endoplasmic reticulum Inhibition of MHC class I antigen expression by IE gene product</td>
</tr>
<tr>
<td>MHC class I</td>
<td>Increased</td>
<td>Augmentation of IFN-β upregulation of MHC class I antigen</td>
</tr>
<tr>
<td>MHC class II</td>
<td>Decreased</td>
<td>Inhibition of IFN-γ upregulation of MHC class II transcription</td>
</tr>
<tr>
<td>MHC class II</td>
<td>Increased</td>
<td>Enhancement of IFN-γ upregulation of class II antigen expression</td>
</tr>
</tbody>
</table>

Modified from the review of Rinaldo [16].
blood has been reported in patients infected with human immunodeficiency virus (HIV) and in other immunocompromised patients [1]. However, it is hard to detect viral antigens in the lymphocytes of immunocompromised hosts, who usually have few PBMCs. Characterization of HCMV antigen-positive T lymphocytes was possible in non-immunocompromised infants with liver dysfunction associated with perinatal primary HCMV infection [11].

Activation of CD8+ and CD4+ lymphocytes requires recognition of viral antigens as short peptides bound to major histocompatibility complex (MHC) class I and class II antigens on the surface of antigen-presenting cells (APC), such as monocytes and macrophages. HCMV can both increase and decrease expression of MHC class I and II antigens. HCMV IE gene products can globally block MHC class I presentation and prevent recognition of infected cells by cytotoxic T lymphocytes (CTL). Interference with normal MHC class I assembly and expression may have implications for restriction of the diversity of the CD8+ CTL repertoire directed against HCMV antigens [12]. Only a few host CD8+ CTL specific for IE gene products are present in seropositive individuals. Selective abrogation of IE peptide presentation by a HCMV matrix protein with associated kinase activity was demonstrated [13]. It was also suggested that modification of a viral protein can result in limiting access to the processing machinery and evasion of CTL.

The presence of HCMV-infected endothelial cells in patients with an active HCMV infection indicates association of widespread occult vascular damage [14]. HCMV-related transplantation atherosclerosis has been recognized as a major cause of allograft failure in long-term cardiac transplant recipients. HCMV is thought to induce an inflammatory reaction of polymorphonuclear leukocytes against arterial endothelial cells. Endothelial cells are one of the major targets for HCMV infection and may also represent a site of persistence. Recently, Sedmak et al. [15] reported that HCMV can downregulate the expression of MHC class II glycoproteins induced by interferon-gamma (IFN-γ) on infected endothelial cells. HCMV-infected APC, such as monocytes-macrophages or endothelial cells, would be unable to present viral antigens acquired exogenously and endogenously. Similar alterations in MHC class I glycoprotein expression have been reported [16]. These findings suggest potential mechanisms for the persistence of HCMV infection by evasion of host immunosurveillance (Table 2). Persistent infections caused by HCMV could be augmented by a decrease in MHC expression as well as by virus-mediated immune dysfunction.

HCMV can contribute to the disease process during an abortive infection, which is characterized by viral gene expression limited to IE gene products without viral replication. IE gene products also affect the expression of many human cellular genes. Previous findings have raised the possibility that HCMV contributes to the development of restenosis of coronary arteries. It was reported that in approximately one third of patients with restenosis, the lesion contained HCMV DNA sequences. Smooth muscle cells grown such lesions express a IE gene product, IIE84, and IIE84 binds to and inhibits the p53 tumor suppressor gene product. HCMV infection has been shown to activate NF-xB, a transcription factor involved in stimulation of a broad range of genes. IIE72, another IE gene product, also increases the expression of the scavenger receptor gene.

Following primary infection, production of IgG and IgM antibodies, responses by CTL, activation of natural killer (NK) cells, and antibody-dependent cellular cytotoxicity (ADCC) occur. Alteration of
MHC class I expression could affect lysis of HCMV-infected endothelial cells by CD8+ CTL. A decrease in MHC expression may not only enhance persistence of HCMV infection but encourage reactivation. IE, glycoprotein B (gB), and non-envelope structural virion such as the matrix protein pp65 have been shown to serve as target antigens for the MHC class I-restricted CD8+ CTL [17]. Responses of CTL and NK cells to HCMV also represent the predominant mechanism necessary for resistance to and recovery from HCMV infection [18]. It was also reported that HCMV evolved a unique mechanism for selectively limiting the presentation of the potentially immunogenic IE protein, which might preclude IE-specific CTL from providing protective immunity to HCMV infection [19]. An alternative basis for the alloreactivity and autoimmunity induced by MHC class II antigen expression could be molecular mimicry of HLA-DR by the IE-2 antigen of HCMV. The IE-2 protein has immunological cross-reactivity with HLA-DR, in that antibodies generated against this IE-2 polypeptide react with the HLA-DR β chain. Activation of CD8+ cells was associated with recovery from both primary and secondary infections and with a low risk of relapse after antiviral therapy. Increased numbers of subsets of CD8+, CD57+ cells correlated with previous HCMV infection [20].

HCMV hepatitis is often recognized in both normal and immunocompromised hosts and in patients with both primary and reactivated HCMV infections. Although infantile HCMV hepatitis was speculated to be caused by primary infection in the perinatal period, immunological conditions of the hosts may modify the clinical manifestations. We investigated the role of PBMCs, especially CD8+ T lymphocytes, in infants with liver dysfunction associated with perinatal primary HCMV infection, by flow cytometry and the polymerase chain reaction (PCR) [11]. Expression of HCMV antigens in CD8+ cells was also found in patients with liver dysfunction associated with perinatal primary HCMV infection. HCMV infection of CD8+ cells may play an important role in the pathogenesis of CD8 activation. Extrahepatic HCMV infection producing an immunological reaction may also be necessary to give rise to neonatal and infantile hepatitis.

3. Role of breast milk in the vertical transmission of HCMV

A high prevalence of HCMV infection is associated mainly with universal breast feeding practices rather than with crowding or poverty. In Japan, over 90% of healthy adults acquire the anti-HCMV IgG antibody and over 60% of healthy infants are infected with HCMV during the first year of life [21]. As the incidence of HCMV infection is affected by ethnic and socioeconomic backgrounds, the lower rate may be due to a change in the life style of young Japanese people. Congenital HCMV infection causes severe disease and sequelae, such as microcephaly, intracranial calcification, chorioretinitis, hepatospleenomegaly, petechiae, and jaundice [22]. Since Diosi et al. [23] succeeded in isolating HCMV from breast milk, breast milk has been considered one of the most important sources of mother-to-infant infection. Hayes et al. [24] isolated HCMV from breast milk of 17 out of 64 (27%) seropositive women and most of the isolates were obtained after the first week. Based on urine isolation, Stagno et al. [25] reported that breast-fed infants are more frequently infected with HCMV than bottle-fed infants. Moreover, Dworsky et al. [26] reported that consumption of infected breast milk led to infection in 69% of infants. The presence of HCMV was more frequently observed in breast milk than in other sites such as vaginal secretions, urine and saliva. HCMV DNA was not found in colostrum, but was detected in breast milk samples 1 month after delivery [27]. Breast feeding seemed to be associated more closely with vertical infection than contact with an infected genital tract [28].

Infants who were fed on breast milk for over 1 month were infected more frequently, and the incidence of infection in infants was significantly higher when the infants were fed by mothers who shed HCMV into their milk [26]. The population of T lymphocytes in PBMCs of pregnant women was clearly decreased. A decrease in helper T cells and an increase in suppressor T cells were also observed. This suppresses the cellular immune response and may affect the response of NK cells which are associated with protection against viral infection. The number of cytotoxic T cells may be decreased during this period. In HCMV-seropositive women, cervical
HCMV shedding increases in late pregnancy [28]. This reactivation of HCMV may be responsible for the suppressive cellular immune response. In postpartum women, the state of cellular immunity is thought to be similar to the state in late pregnancy. The suppression of cellular immunity is thought to induce a localized reactivation in the mammary gland and to induce a large amount of HCMV shedding into the colostrum. For most viruses including HCMV, although transmission has been documented as evidenced by seroconversion, no serious illness in the neonate secondary to breast feeding has been reported [29].

Most of the viruses in the human herpesvirus family are transmitted by cell-to-cell contact. Cell-to-cell contact is also the main method of vertical transmission for human T-lymphotropic virus type I [30,31] and human immunodeficiency virus type 1 [32]. Human breast milk contains many different types of cells associated with immune reactions [22]. Although it is thought that PBMCs and polymorphonuclear cells are susceptible to HCMV [10,33], it has not been clarified whether milk cells are susceptible to this agent.

4. Relationship between antiviral agents or cytokines and HCMV infection of breast milk

Breast feeding is the major factor during the first year of life with HCMV excretion rates of more than 50% observed in countries where the majority of women were seropositive and breast feed their infants [34]. HCMV is transmitted from mothers to infants through human milk and the ensuing infection is generally without clinical symptoms [29,35]. The presence of specific antibodies to HCMV in human milk or the ability of the milk to neutralize the virus in vitro does not prevent transmission. To clarify the role of milk cells and whey in vertical infection by breast feeding, we separated breast milk into milk cells and whey and examined each fraction [36]. Although HCMV DNA was detected in milk cells, the rate of detection in whey was higher. We found HCMV IE DNA mainly in the fraction of liquid supernatant by PCR. HCMV was isolated more frequently from milk whey samples than from cell samples. HCMV particle shedding into whey may have a more important role in vertical infection by breast milk than cell-to-cell transmission.

Fresh human milk contains components that provide specific and non-specific defences against infectious agents. Some cytokines that have the function of interference with virus, carrier proteins such as lactoferrin, and some non-immunoglobulin antiviral factors are secreted in breast milk. Lactoferrin and other iron-binding proteins present in colostrum and milk have bacteriostatic activities. The presence of these substances may also be associated with the small amount of HCMV shedding into colostrum and early breast milk. Recently Harmsen et al. [37] suggested that native lactoferrin from human milk could completely block HCMV infection. Potential antiviral effects of a mixture of milk constituents have been suggested [38]. Lactoferrin is produced by lactating women and secreted in their milk. Lactoferrin levels drop during lactation. Lactoferrin acts as an iron-binding protein and may interfere with binding of HCMV to cell surface heparan receptors. However, a direct interaction of the protein with the virus cannot be excluded. Harmsen et al. [37] also speculated that HCMV was present in milk but could not be cultured on human fetal lung fibroblasts due to the much higher lactoferrin concentration in colostrum. They also reported that the transmission of HCMV through infected leukocytes could be an important source of infection.

Colostrum and early milk are reported to contain abundant IgA and IgM that may be capable of neutralizing HCMV during the first few days of lactation [38]. IgA and IgM antibodies against HCMV are not associated with diminished HCMV shedding in colostrum and early milk, as HCMV DNA has not been detected in colostrum and early milk. Sequential quantitation of class-specific immunoglobulins in human colostrum and milk has demonstrated that the highest levels of s-IgA are present during the first few days of lactation. The antibodies may protect against viral disease by coating and blocking viral attachment and by neutralization of viral particles. IgA in colostrum and milk steadily declines during the first few months of lactation. Although lactoferrin also has anti-HCMV activity in vitro [36], in vivo roles for these antiviral agents in neonatal and maternal infections have yet to be clarified. The synergistic interaction between s-IgA and iron-
binding proteins such as lactoferrin has been speculated to have an important role in such defence [38]. The supernatant of colostrum from 10 mothers did not exert an inhibitory effect on HCMV-infected MRC-5 cells [39]. As viral DNA was not detected from colostrum and no anti-HCMV effects of liquid supernatant of colostrum were shown, an inhibitory effect of antibodies in colostrum was not proved (Table 3).

Although Van de Perre et al. [40] found HIV-1-positive breast milk samples more frequently in mothers with severe immune deficiency than in those who were immunocompetent, a systemic reactivation of HCMV lactating mothers is also unlikely. This fact is also supported by the results of serum levels of IgG and IgM antibodies in our previous study [36]. Seroconversion in mothers was not observed in the first month after delivery. This fact also suggests that mothers did not have HCMV viremia due to primary infection during the postpartum period. The excretion of HCMV into breast milk results more often from reactivation of latent maternal virus than primary infection.

Mononuclear cells of human breast milk have the potential to produce many different cytokines including tumor necrosis factor-alpha (TNF-α) and IFN-γ [41]. It is likely that specific cellular interactions as well as other cytokines are necessary for HCMV reactivation [42]. We also studied the relationship between active perinatal HCMV infection and serum levels of cytokines [43]. In the active phase of HCMV infection, serum titers of cell free soluble interleukin-2 receptor (sIL-2R) were correlated with clinical findings. Serum levels of sIL-2R of mothers with DNA-positive milk at 1 month after delivery were significantly higher than those of mothers with DNA-negative milk [44]. It was suggested that the presence of cytokines such as sIL-2R in serum was also related to the reactivation of HCMV which occurs locally in the mammary gland of the lactating mother after delivery.

5. Conclusions

HCMV causes serious disease in infants who acquire the virus in utero, and in patients who are immunosuppressed due to HIV-1 infection, organ transplantation, and immunosuppressive chemotherapy. Understanding the epidemiology of HCMV infection is essential for the development of strategies for prevention and therapy. The actual sites of latency or persistence of HCMV infections and the factors controlling latency and reactivation are still controversial. It is likely that specific cellular interactions as well as other cytokines are necessary for HCMV reactivation.

Perinatal HCMV infection refers to infection acquired during delivery through exposure to infected maternal genital secretions or acquired postnatally from ingestion of infected breast milk. Breast feeding is the major factor during the first year of life with HCMV excretion rates of more than 50% observed in countries where the majority of women are seropositive and breast feed their infants.

We conclude that HCMV excreted into milk whey may be more important in vertical infection than milk cells infected with HCMV for breast-fed infants. The presence of cytokines may also be related to the reactivation of HCMV which occurs locally in the mammary gland of the lactating mother after delivery.

Acknowledgments

This work was supported by a research grant from the Ministry of Education, Science and Culture of Japan (07670883).

References


