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Recent Advances in the Prevention and Treatment of Congenital Cytomegalovirus Infections

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Continued but slow progress has led to recent advances in our understanding that congenital cytomegalovirus (CMV) infection has occurred. We understand that the most severe congenital disease occurs following a primary maternal infection during pregnancy. We now have the ability to accurately diagnosis a primary maternal infection using serologic studies of single serum sample. For pregnant women with young children, we know that child-to-mother CMV transmission can probably be prevented by hygienic intervention, and that for pregnant women who have acquired a primary CMV infection, mother-to-fetal transmission is probably preventable using CMV hyperimmune globulin. Although additional studies are needed, treatment of congenitally infected fetuses or newborns should be possible using either CMV hyperimmune globulin or antiviral agents such as ganciclovir or its derivates. Finally, recent evidence indicates that CMV replicates in the placenta, impairs development, and causes inflammation and dysfunction. This plus the reversibility of many manifestations of congenital infection in the fetus and newborn indicate that congenital CMV disease is in part a syndrome of placental insufficiency.

KEYWORDS cytomegalovirus, pregnancy, placental infection, placental enlargement, hyperimmunoglobulin, antiviral therapy

Progress in preventing congenital cytomegalovirus (CMV) infection has been remarkably slow, even though a 1999 Institute of Medicine report, *Vaccines for the 21st Century*, stated that development of a CMV vaccine was the highest priority for new vaccines.[1] Although government and industry funding for this effort remains inadequate, two CMV vaccines are being evaluated, screening programs and interventions have been studied, and more is known about the mechanism of transplacental virus spread and the natural history of congenital infection. Nevertheless, CMV transmission continues imperceptibly, resulting in damage to about 8000 infants in the United States each year.[2] Without a vaccine, recent efforts have focused on developing interventions that would justify universal screening of pregnant women. Here, we review recent developments in our understanding of the pathogenesis, treatment, and prevention of congenital CMV infection.

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Natural History of Maternal Infections

Primary Versus Recurrent Maternal Infection

Nearly all symptomatic congenital infections occur when a woman sustains a primary infection with CMV either during or just before pregnancy.[2] Infection appears to be associated with progressively increasing viral transmission rates by gestational age, but infections early in gestation probably result in more severe congenital disease.[3,4] If infection occurs after conception, approximately 50% of fetuses will become infected, and approximately one-half of those will have symptoms at birth.[3] If infection occurs in the 6 months before conception, transmission to the fetus and symptoms at birth will occur at a lower rate. In a recent study of 12 women who acquired a CMV infection between 2 and 18 weeks before pregnancy, only 1 of the newborns was asymptomatically infected.[5] This rate is higher than expected for naturally im-

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mune women, but much less than if following a primary infection after conception.

Although congenital infections do affect infants born to mothers who are seropositive before pregnancy, they rarely result in symptomatic or severely affected infants. Such infections are called "recurrent" infections and are caused either by reinfection with new CMV strain or reactivation of a latent infection. The congenital infection rate in infants born to mothers with preconception immunity is between 0.2% and 2%. There is indirect evidence that reinfection of seropositive mothers with new strains of CMV can occur.[6]

Risk Factors for Maternal Infection

The most important risk factor for maternal CMV infection during pregnancy is frequent and prolonged exposure to young children.[7] Once infected, children less than 2 years of age excrete virus in both saliva and urine for an average of 24 months. Hence, seronegative women who have contact with young children are more likely to become infected than are women who do not. At least half of the women of middle and higher socioeconomic status in the United States are seronegative for CMV. These women are often exposed to infected young children in the home or in daycare, and 50% of these women will acquire a CMV infection within 1 year. Thus, they are at high risk for delivering an infant with symptomatic congenital CMV infection.

Both cellular and humoral immunity to CMV are important factors in viral transmission during pregnancy; women with impaired cellular immune responses (eg, those with AIDS or those receiving immunosuppressive therapy) are more likely to transmit the virus to the fetus. Neutralizing titers and IgG avidity to CMV antigens are both inversely correlated with transmission.[3,8] Given the reduction of disease severity in infants born to seropositive mothers, it is presumed that in recurrent infections, preexisting immunity reduces or eliminates maternal viremia and is therefore protective to the fetus. The frequency of CMV transmission to the fetus and disease are associated with viral load, as measured by polymerase chain reaction (PCR) either in fetal amniotic fluid or in the newborn's plasma.[9]

The best method for the serologic diagnosis of asymptomatic maternal primary infection is seroconversion; however, this is rarely, if ever, achieved because universal serial serologic screening of pregnant women is not a standard practice in the United States. The detection of IgM antibodies in maternal sera can be helpful but is not without problems. Although IgM antibodies to CMV occur in all primary infections, they may also occur after reactivation or reinfection and remain present for months. Hence, finding IgM to CMV in a single serum sample is not definitive for a primary CMV infection.

Antibody avidity is a better method for maternal diagnosis.[10] As an indirect measure of the tightness of antibody binding to its target antigen, avidity increases in the first weeks and months after a primary infection. Currently, apart from seroconversion, the combination of anti-CMV IgM and low-avidity anti-CMV IgG is the best way to diagnose a primary maternal infection.

Examination of amniotic fluid may be a helpful adjunct in prenatal diagnosis. Although viral culture of the amniotic fluid is 100% specific, it often yields false-negative results. PCR, especially after 21 weeks' gestation, is both sensitive and specific for fetal infection.[3] A diagnosis of fetal CMV infection alone is insufficient to predict whether the newborn will be symptomatic, but fetal abnormalities or placental enlargement detected by ultrasound are predictive of disease and long-term sequelae.[3,11]

Fetal Outcomes and Syndrome Manifestations

About 10% of infants with congenital CMV infection have signs and symptoms at birth; 90% are asymptomatic. Some of the initially asymptomatic children develop sequelae later in life, such as progressive sensorineural hearing loss. Some of these children are born of mothers with recurrent infections.[12]

In a recent study, fetal and placental ultrasound findings were predictive of symptomatic newborn disease.[11] Fetal findings included one or more of the following: ventriculomegaly, microcephaly, intrauterine growth restriction (IUGR), ascites, organomegaly by ultrasound, pyelectasis, megaloureter, and periventricular or hepatic and intestinal echodensities. Placental findings were related to an increase in maximal placental thickness (see below).

Symptomatic infants have a constellation of clinical features that reflect placental dysfunction and probable viral infection of the central nervous system of the fetus. Many of the signs and symptoms overlap with those of other congenital viral infections. The symptoms that occur in one-half or more of CMV-infected symptomatic infants include petechiae and thrombocytopenia, hepatosplenomegaly, liver disease as manifested by jaundice (elevated direct bilirubin) and hepatic transaminases, IUGR, microcephaly, and intracranial calcifications.[13] One or more symptoms of neurologic involvement also occur in over one-half of symptomatic newborns, including seizures, chorioretinitis (and other ocular abnormalities), hypotonia and a poor suck, elevated cerebrospinal fluid protein (>120 mg/dL), and hearing deficit.[13] Some of the neurologic manifestations may be due to intrauterine hypoxia. Others, such as sensorineural hearing loss (either bilateral or unilateral), are more likely to be due to viral infection and inflammatory effects on the fetus. The evidence for this is that hearing at birth may be normal, but hearing loss can be slowly progressive over the first 5 to 10 years of life.[14]

Prevention of Maternal Infection during Pregnancy

Possible approaches to preventing congenital CMV infections include changes in hygienic behavior for seronegative pregnant women, administration of CMV hyperimmune globulin

(HIG) to pregnant women with a primary infection, and vaccines administered to girls or women well before pregnancy.

Two studies were done to determine whether changing protective behaviors prevents child-to-mother transmission of CMV during pregnancy.[15,16] One studied 166 seronegative mothers with a child <36 months of age who attended 1 of 124 child care centers.[16] For each child care center, women who were either pregnant or attempting to conceive (ie, not using contraception) were randomly assigned to either a control group or an intervention group. Mothers in the intervention groups were given instructions for frequent hand washing, wearing gloves for specific childcare tasks, and avoiding various types of intimate contact with their child. All women and their children were monitored for CMV infection every 3 months until delivery or, in women attempting conception, for 12 months; 7.8% seroconverted. Logistic regression analysis revealed only 2 independent predictors of maternal infection: a child shedding virus at any time (50% of children became infected after the mother's enrollment in the study) and a mother attempting pregnancy at the time of enrollment. For 41 women with a child shedding CMV, 10 of 24 who were not pregnant at enrollment became infected, compared with only 1 of 17 women who were pregnant at enrollment ($P = 0.008$). In several studies, only 1 of 31 pregnant women acquired a CMV infection during pregnancy, compared with 60 of 147 nonpregnant women ($P < 0.0001$).[15-17] Therefore, intervention before pregnancy is ineffective, but pregnant women with a child in daycare should be given the option of serologic testing. Intervention for pregnant women should be effective as they are more motivated to adhere to recommendations than nonpregnant women.

Prevention of fetal infection by HIG was recently evaluated.[3] By serologic screening, 181 asymptomatic pregnant women with a primary CMV infection were identified. For women with a primary infection at <21 weeks' gestation or for those who refused amniocentesis, HIG (100 U/kg) was offered monthly until delivery. Of 126 women (mean gestational age at infection, 14.3. ± 7 weeks) who did not receive HIG, 56% delivered infected infants, compared with 16% of 37 women (mean gestational age at infection, 13.2 ± 5.5 weeks) who received prophylactic HIG ($P < 0.001$).

Work is in progress to develop vaccines against CMV. These experimental vaccines include a live attenuated strain Towne and a recombinant protein vaccine that uses the major glycoprotein B of CMV and an adjuvant (MF/59).[18,19] Work in this area has been slow but steadily ongoing, and clinical trials are in phase II.

Pre- and Postnatal Treatment of Congenital CMV Infections

Prenatal Therapy

Despite advances in the diagnosis of maternal–fetal CMV infection, an effective therapy is unavailable. Pregnancy termination is often offered as an option when affected or infected fetuses are identified by ultrasonography or amniocentesis, respectively. Recent case reports have focused on the safe administration of oral ganciclovir to mothers of CMV-infected fetuses. An HIV-positive woman was treated with intravenous ganciclovir from 30 to 34 weeks' gestation, followed by neonatal plasma ganciclovir concentrations of 0.8 μg/mL measured at 2 hours after birth.[20] This was within the median effective inhibitory dose, which ranges from 0.2 to 1.6 μg/mL.[21] In another report, ganciclovir given orally to a pregnant woman with CMV DNA in the amniotic fluid reached amniotic concentrations higher than the minimal inhibitory dose, and the neonate was CMV-free.[22] Two case reports showed no teratogenicity of ganciclovir given in the early stages of pregnancy.[23,24] The actual efficacy of ganciclovir remains to be defined in controlled trials.

HIG was used to treat a mother with one of her twin fetuses with a CMV infection and IUGR.[25] Response to HIG was suggested by the twin's growth and decreased placental thickening and cord edema. At birth, the male twin was uninfected, and the female twin was infected but healthy, with normal psychomotor development. Subsequently, a multicenter prospective cohort study of 157 pregnant women with confirmed primary CMV infection evaluated the use of HIG.[3] Of these women, 148 were asymptomatic and were identified by routine serologic screening; 8 had symptoms and laboratory abnormalities consistent with CMV infection; and 1 was identified because of abnormal ultrasound fetal images. Forty-five women in the therapy group had a primary infection more than 6 weeks before enrollment, underwent amniocentesis, and had CMV DNA or culture-positive amniotic fluid. Thirty-one of these women received CMV HIG (200 U per kg of the mother's body weight). Nine women received 1 or 2 additional intraamniotic infusions because of persistent fetal abnormalities on ultrasonography. Fourteen women who declined HIG were the controls, and half of them had fetuses/infants with symptomatic CMV infection. In contrast, only 1 of the 31 women who received HIG had a diseased infant at birth (adjusted odds ratio, 0.02; $P < 0.001$), although 15 were carrying fetuses with ultrasonographic evidence of infection. In particular, 9 neonates were healthy despite prenatal ultrasound signs of involvement with the following systems: cerebral (5 with ventriculomegaly and 2 with periventricular echodensities), hepatic (2 with hepatic echodensities and 1 with hepatosplenomegaly and ascites), intestinal (4 with echodensities), and renal (2 with pyelectasia, one of whom also had megaloureter involvement). Administration of HIG to the mother and fetal ultrasound abnormalities before treatment were independent predictors of fetal outcome ($P < 0.001$). No adverse effects of HIG were observed.

The positive clinical results were supported by the immunological studies performed in a subgroup of HIG-treated patients before and after the infusions or in untreated patients at enrollment and after about 2 months.[3] HIG-treated women showed a significant increase ($P < 0.001$) in CMV-specific titers and IgG avidity after infusion and at the end of pregnancy, compared with untreated women. Treated women had a significant decrease ($P < 0.01$) in number, percentage, and cytotoxic activity of natural killer lymphocytes (CD56+ CD16+) and activated cells (HLA− DR+) at the end of preg-

nancy, compared with untreated mothers. These changes may be due to HIG inhibition of CMV replication, because natural killer and DR+ cells are increased at the onset of a primary CMV infection. However, the increased number and activity of these immune cells is associated with a high production of cytokines, such as tumor necrosis factor alpha, which can contribute to the immune-mediated fetal damage.[26,27] Thus, HIG decreases the pathogenic effects of CMV by neutralizing antibodies and immunomodulatory effects suggested by the increased IgG concentration and avidity, decreased number of natural killer and DR+ cells, and decreased cytotoxic activity.

The efficacy of HIG in humans is supported by its in vitro activity against CMV and by studies in guinea pigs going back 25 years.[28-30] Pregnant guinea pigs have been challenged with guinea pig (gp)CMV before or after passive administration of neutralizing antisera to either whole virus or gB, a glycoprotein that induces neutralizing antibodies.[28,29] Passive administration of immune serum to whole virus significantly increased fetal survival from 51% to 77% when administered before gpCMV challenge and to 81% when given after viral challenge.[29] In these experiments, immune serum did not affect the rate of fetal infection, indicating that the immune serum was therapeutic. In other guinea pig experiments with immune sera to purified gB, there was reduced fetal infection, placental inflammation, fetal death, and IUGR.[28] Ten of 12 fetuses of control (treated with nonimmune globulin) pregnant dames died, compared with 3 of 23 pregnant dames treated with immune globulin to gB. This effect was independent of whether immune globulin was administered before or after the challenge virus.[28] Additional high-titer immune globulin given before or after maternal challenge significantly reduced the rate of fetal infection from 39% (9 of 23 fetuses infected) to 0% (0 of 18 fetuses infected). Immune globulin to gB administered before or after maternal challenge also significantly reduced placental inflammation and IUGR, as measured by fetal weight. Thus, there are several plausible mechanisms for the therapeutic efficacy of HIG: immunomodulatory effects, reduction of viral load, and/or decreased placental inflammation resulting in increased blood flow with enhanced fetal nutrition and oxygenation.

Postnatal Therapy

HIG has not been directly evaluated for the treatment of neonates with symptomatic congenital CMV disease, but observations of neonates with transfusion-acquired CMV infections suggest that it may be effective.[31,32] Premature neonates born of seronegative mothers develop symptomatic CMV infection acquired from transfused blood products, but the premature newborns from seropositive mothers remain asymptomatic after receiving the same blood products. After birth, the infant's maternal antibody to CMV declines rapidly, and by 8 weeks of age only 10% to 20% remains. Even at this age, although not protected against transfusion-acquired infection, newborns are protected against disease.

Ganciclovir may be used to treat neonates or infants with congenital CMV disease. This drug is active only after phosphorylation to ganciclovir triphosphate, which is recognized as guanosine triphosphate by the viral DNA polymerase, with consequent inhibition of CMV replication. Since the first phosphorylation requires the presence of viral-encoded (UL 97 gene) phosphotransferase, ganciclovir is active only in infected cells. Potential adverse effects of ganciclovir in neonates include transient neutropenia, which may necessitate dose adjustment or interruption of therapy.[33-37]

Regarding the efficacy of ganciclovir, numerous studies have been published. Apart from a few infants with severe pneumopathy or liver disease, all treated infants were symptomatic and had at least one neurologic manifestation: microcephaly, seizures, abnormal cerebrospinal fluid, imaging abnormalities (calcifications, periventricular echodensities, cortical atrophy, ventriculomegaly, cystic leukomalacia, cerebellar hypoplasia, cerebral dysplasia by abnormal neuronal migration, large cisterna magna, intraventricular adhesions, hypoplastic corpus callosum, echogenic enhancement in the caudothalamic grooves, lenticulostriate vasculopathy, and periventricular pseudocysts), hearing loss, or chorioretinitis.[36,38,39]

A pilot study in 1994 compared 2 regimens of ganciclovir treatment in 12 infants with severe neurologic manifestations.[35] Group 1 (6 infants) received ganciclovir 5 mg/kg twice daily for 2 weeks only, whereas group 2 (6 infants) received ganciclovir 7.5 mg/kg twice daily for 2 weeks, followed by 10 mg/kg three times a week for 3 months. In group 1, viral shedding disappeared in 3 infants, whereas in group 2, all 6 infants stopped shedding virus. In all infants in the study, viral shedding reappeared after ganciclovir treatment was interrupted. Two infants of group 1 and 4 of group 2 had normal neurologic outcomes at 18 months of age. In 1 infant in group 2, who was born to a mother with recurrent infection, microcephaly resolved. Two infants with initial chorioretinitis had normal eye examinations at 18 months of age. Three infants (2 in group 1 and 1 in group 2) developed bilateral hearing loss that was detected before treatment.

A larger phase II study compared two 6-week regimens of ganciclovir (8 mg/kg/d versus 12 mg/kg/d, in 14 and 28 infants, respectively) for toxicity, virologic response, and clinical and neurologic outcome.[37] The 12 mg/kg/d group showed a more pronounced antiviral effect in urine that was associated with a normal neurologic examination at 18 months of age. Data on audiologic performance, which were available for 30 of the 42 infants, did not differ by treatment group. Eleven of 13 infants with normal baseline hearing developed deafness. Of 14 infants with initial chorioretinitis (12 in the 12 mg/kg group), 8 had normal eye examinations at 6 months. Of the infants with baseline normal eye examinations, 3 developed retinal scarring. Eight of 33 children (24%) evaluated at $\geq$2 years of age had normal neurologic development, which did not differ by ganciclovir dosage. During therapy, the most significantly abnormal laboratory finding was absolute neutropenia, which was more frequent at a low dose (63% of children) than at a high dose (19% of children). A slight hypercreatinemia (always $>$2 mg/dL) was observed in 32% of children. Increased levels of liver enzymes (aspartate aminotransferase $>$250 IU/L; alanine ami-

notransferase >150 IU/L) were noted in 36% of both groups. Of four infants who died, 1 had concomitant HIV, syphilis, and CMV infections.

A subsequent randomized controlled trial of ganciclovir showed a beneficial effect on hearing deterioration in children with at least 1 neurologic manifestation.[33] Ganciclovir was given within the first month at 12 mg/kg/d intravenously for 6 weeks. The primary endpoint was improvement of brainstem-evoked potential between baseline and follow-up, or, for patients with normal baseline hearing, normal brainstem-evoked potential at both time points. Functional evaluation (results obtained with the better ear) was distinguished from biological evaluation (results with individual ears), and the latter represented the biological effects of ganciclovir. Among 42 infants followed, functional evaluation at 6 months and 1 year showed significantly less hearing deterioration in treated infants (0% and 21%, respectively) than in control infants (41% and 68%, respectively) ($P < 0.01$ at both ages). A significantly higher number of treated infants had normal or improved hearing compared with control infants. Neutropenia occurred more frequently (63%) in the treated group than in the control group (21%). The large proportion of unevaluable infants—58 of 100 subjects enrolled—raises concerns about follow-up bias.

Two case reports provide pharmacokinetic data on oral treatment with valganciclovir, the valine ester of ganciclovir.[40,41] One infant with encephalitis due to perinatal HIV-CMV coinfection was treated for 1 year. Valganciclovir inhibited CMV replication without side effects.[40] In another case, a continuous adaptation dose of 280 to 850 mg/m^2 was needed during 5.5 months of treatment of a symptomatic infant to achieve plasma levels that made CMV DNA undetectable in the urine.[41] Since valganciclovir has variable bioavailability, dosage adaptation related to the viral load in the urine could be a better marker of the drug's efficacy than pharmacokinetic monitoring.

Ganciclovir has potential toxicity: short-term, high doses of ganciclovir can inhibit spermatogenesis and induce possible carcinogenic effects in animals.[42,43] For this reason, foscarnet that blocks viral DNA polymerase was tested.[44] Foscarnet was administered for 4 months to an infant with multi-system CMV disease. At 1 year and subsequent follow up until 10 years, clinical outcome and psychomotor development were normal.

In conclusion, for infants with symptomatic congenital CMV infection, a longer duration of antiviral therapy appears to be associated with better outcomes.

Congenital CMV Infection and the Placenta

Development of the Hemochorial Human Placenta

IUGR associated with congenital CMV disease suggests placental deficiencies. Knowledge of the cellular and molecular processes involved in development of the human placenta is a prerequisite to understanding how infection impairs functions.[45] The embryo's acquisition of a supply of maternal blood is a critical hurdle in pregnancy maintenance. The mechanics of this process are accomplished by cytotrophoblasts, which are specialized epithelial cells of the placenta. Placentation, a stepwise process whereby cytotrophoblasts initiate blood flow to the placenta, entails differentiation along two pathways, depending on location. In floating villi, cytotrophoblasts fuse to form a multinucleate syncytial covering, attached at one end to the tree-like fetal portion of the placenta. The rest of the villus, covered by syncytiotrophoblasts, floats in a stream of maternal blood, delivering nutrients and, later in gestation, maternal IgG across syncytiotrophoblasts to the fetal bloodstream.

To form anchoring villi, cytotrophoblasts undergo a novel differentiation program, switching from an epithelial to an endothelial phenotype that resembles vasculogenesis, controlled through the coordinated actions of numerous factors.[46] Differentiating cytotrophoblasts initiate expression of invasion-promoting endothelial integrins, vasculogenic factors, and receptors that allow them to mimic the surface of vascular cells.[47,48] Cytotrophoblasts also upregulate matrix metalloproteinases that degrade the uterine stroma[49] and express immune molecules—nonclassical MHC class Ib molecule HLA-G[50] and interleukin-10 (IL-10)[51]—that enable maternal tolerance. The cells express chemokine–receptor pairs that contribute to placental development and attract a specialized population of decidual leukocytes—natural killer cells (CD56+ CD16−), macrophages, and dendritic cell progenitors—to the pregnant uterus.[52] Cytotrophoblasts also express substances that influence vasculogenesis, including vascular endothelial growth factor family ligands and receptors that regulate cell survival at low oxygen levels.[53,54]

Early Gestation at the Maternal–Fetal Interface: A Hypoxic Environment

Dramatic changes in oxygen content of the placental environment occur during gestation. In the first trimester, differentiation occurs in a relatively hypoxic environment.[55] Cytotrophoblast invasion is confined to superficial portions of uterine blood vessels near the lumen, and blood does not flow to the intervillous space.[56] In the second trimester, cytotrophoblasts completely remodel the uterine vasculature and replace the endothelium up to the first third of the myometrium. By mid-gestation, cytotrophoblast differentiation becomes sensitive to hypoxia that inhibits invasion.[57] The hypoxia-inducible factor is a central mediator of cellular response to low oxygen and regulates expression of genes for cell survival, including vascular endothelial growth factor, which is modulated as cytotrophoblasts acquire an invasive phenotype. Cytotrophoblasts, which are relatively resistant to hypoxia, survive when they fail to access sufficient maternal blood, and cells proliferate and begin to differentiate but fail to complete integrin switching.[57,58] Invasion is impaired in pregnancy complications such as preeclampsia and is characterized by shallow cytotrophoblast invasion in the uterine vasculature and reduced maternal blood perfusion of the placenta.[59]

Patterns of CMV Infection in the Placenta Depend on Maternal Neutralizing Antibodies and Coinfections

Immunofluorescence analysis of paired decidual and placental biopsy specimens from early gestation showed that patterns of CMV replication vary depending on maternal immunity.[60-62] In highly infected placentas, cell islands in both decidual and placental compartments expressed viral replication proteins, and neutralizing titers were low. In the decidua, the uterine vasculature and interstitial cytotrophoblasts contained viral replication proteins, as did cytotrophoblasts and fetal capillaries in the adjacent placenta, suggesting transmission. In moderately infected placentas, the number of cells with viral replication proteins was reduced in the decidua and placenta, with occasional focal infection. This pattern predominated in women with low to intermediate neutralizing titers. PCR analysis confirmed the presence of CMV DNA, sometimes alone and sometimes in combination with herpes simplex viruses, chlamydia, and pathogenic bacteria.[63] In placentas without viral DNA and infection, few cells contained viral replication proteins in the adjacent decidua. This pattern predominated in women with intermediate to high neutralizing titers. Sometimes syncytiotrophoblasts and villus core macrophages contained vesicles with the CMV virion envelope glycoprotein gB without replication. Placental infection that leads to fetal transmission likely involves viral replication in the decidua, in cytotrophoblasts of placentas with the lowest neutralizing titers, and in cytotrophoblasts of some placentas with intermediate titers, including recurrent CMV infection.

A recent study of placentas from uncomplicated deliveries reported that more than 50% of biopsy specimens contained CMV DNA without other pathogens.[62] Analysis of virion proteins and antibody levels suggested that there was suppressed infection in placentas with moderate to high neutralizing antibody titers: 5% of the biopsy specimens from these placentas contained CMV DNA. Even when neutralizing titers were low, focal infection was seldom found in the placenta. Instead, leukocytes in fetal blood vessels in the villus core contained viral replication proteins. According to current diagnostic criteria, CMV proteins and DNA in leukocytes are markers for recent infection.[64] The results suggest that there is transplacental spread, virus replication, and dissemination in the fetus. Thus, congenital infection could be higher than estimated, as most infants appear asymptomatic at birth.

Virion Transcytosis and Infection of Cytotrophoblasts Expressing CMV Receptors

A puzzling pattern of CMV replication in floating villi in utero suggested a circuitous route for virus spread to the placenta that spares syncytiotrophoblasts but allows focal infection in underlying cytotrophoblasts.[60,61] Virion transport to susceptible cells that involves transcytosis in immune complexes was confirmed using villus explants in vitro.[65] Syncytiotrophoblasts express the neonatal Fc receptor that transports IgG from the maternal to the fetal circulation.[66] Immune complexes of CMV virions and IgG in secretions and blood that bathe the placenta are endocytosed by syncytiotrophoblasts, and some are transcytosed to cytotrophoblasts. In the presence of IgG with low neutralizing titer, focal infection can occur. Although replication is not usually found in CMV DNA-positive placentas from immune mothers with high neutralizing titer, syncytiotrophoblasts accumulate vesicles with IgG and virion gB.

A recent study of CMV virion receptors provided another explanation for why focal infection occurs in some villus cytotrophoblast progenitor cells but not others. Cell surface adhesion molecules for virions are developmentally regulated as trophoblasts progress from the fetal to the maternal compartment. Indeed, location of productive infection suggests virion engagement with receptors that are expressed during differentiation (Maidji E, Genbacev O, Chang HT, et al, manuscript submitted for publication, December 13, 2006). In floating villi, syncytiotrophoblasts express the epidermal growth factor receptor that binds CMV virions,[67] but they lack the integrin coreceptors $\alpha 1\beta 1$ and $\alpha V\beta 3$ used in fibroblasts and endothelial cells.[68,69] Strikingly, focal infection begins in villus cytotrophoblasts that upregulate αV integrin expression (Maidji E, Genbacev O, Chang HT, et al, manuscript submitted for publication December 13, 2006). Invasive cytotrophoblasts in decidua express integrins $\alpha 1\beta 1$ and $\alpha V\beta 3$ and upregulate the epidermal growth factor receptor, dramatically increasing susceptibility to infection. On the whole, the barrier function of the early gestation placenta is compromised by transcytosis of virion-containing immune complexes from the proximal infected decidua that reach villus cytotrophoblasts expressing virion receptors.

Infected Cytotrophoblasts Impair Differentiation and Functions

The early gestation placenta is not merely a passive conduit for virion transport to the fetal compartment. Infected cytotrophoblasts dysregulate key differentiation molecules that are required for interstitial and endovascular invasiveness at the level of transcription and protein expression.[60,70,71] These molecules include integrins for cell–cell and cell–matrix adhesion and the class I MHC molecule HLA-G for maternal immune tolerance for the allogeneic fetus. One viral gene product that functions as a pathogenesis factor at the uterine–placental interface, cmvIL-10, is an immunosuppressive cytokine[72] that impairs cytotrophoblast functions at multiple levels. For example, cmvIL-10 is strongly upregulated in infected cells and reduces matrix metalloproteinase-9 protein and activity.[71] Dysregulation of downstream effector molecules undermines key functions. Cytotrophoblast differentiation and invasiveness are markedly impaired by dysregulated integrin expression, reduced cell adhesion, and degradation of the extracellular matrix.[60,70,71] It is noteworthy that proteins dysregulated in CMV-infected cytotrophoblasts are among those altered in preeclampsia—a pregnancy complication characterized by poor placentation leading to hypoperfusion, oxidative stress, and IUGR.[73-75]

Placental Damage and Congenital Infection

In congenital CMV infection, IUGR can occur in the absence of fetal infection as a result of placental pathology.[76] Histological studies of infected placentas show distinctive changes, including villous fibrosis, calcification, and leukocytic infiltration.[77] Fibrin deposits that encase villi and damage syncytiotrophoblasts block contact with maternal blood. Thrombosis in chorionic (fetal) blood vessels and resulting avascular villi reduce transport of nutrients and oxygen to the fetal bloodstream. Paradoxically, active viral replication is seldom detected in the placenta at delivery, except in some cases of primary infection and severe symptomatic disease. Possibly, increased neutralizing titers and targeting of infection sites by innate immune cells limits CMV replication as gestation progresses.[61] Ongoing studies of placentas from infants with congenital infection, with and without HIG treatment,[3] revealed significant differences. Among these are morphological changes that suggest compensatory development of chorionic villi with intact syncytiotrophoblasts and blood vessels (E. Maidji and L. Pereira, unpublished observations). The renewed capacity of the placenta to transport oxygen, nutrients, and HIG in treated mothers could promote fetal growth, suppress infection, and prevent symptomatic disease.

Effect of Primary Maternal Infection on Placental Size

Serologic testing for primary maternal CMV infection during pregnancy is not routine, but ultrasound studies, which are, can show abnormalities of the placental–fetal unit. Given this fact and the knowledge that CMV causes extensive acute and chronic placental inflammation, placental thickening was evaluated in women with primary CMV infections during pregnancy.[11] Ninety-two women with primary infection and 73 CMV-seropositive pregnant women without primary infection were studied. Thirty-two women were treated with HIG to either prevent or treat intrauterine CMV infection. Maximal placental thickness was measured by longitudinal (nonoblique) scanning, with the ultrasound beam perpendicular to the chorial dish. Programmed placental ultrasound evaluations were performed from 16 to 36 weeks' gestation.

At each placental measurement, women with primary CMV infection and a fetus or newborn with CMV disease had significantly ($P < 0.0001$) thicker placentas than women without infected fetuses, and these women in turn had significantly ($P < 0.0001$) thicker placentas than seropositive controls. After primary infection, for women with or without infected fetuses or newborns, treatment with HIG was associated with significant ($P < 0.001$) reductions in placental thickness. Placental vertical thickness values, predictive of primary maternal infection, were observed at each measurement from 16 to 36 weeks' gestation, and cut-off values ranged from 22 to 35 mm, with the best sensitivity and specificity at 28 and 32 weeks.[11]

It was concluded that primary maternal CMV infections and fetal or neonatal disease are associated with sonographically thickened placentas and respond to HIG administration.

Is the Congenital CMV Syndrome Due in Part to Placental Dysfunction?

Many symptoms of congenital CMV infection that are present at birth may not be due to a direct effect of the virus on the fetus. Rather, they may be due to an indirect effect of intrauterine infection on the placenta, which may be impaired in its capacity to provide oxygen and nutrients to the developing fetus. Several lines of evidence suggest this possibility.

(i) Many manifestations of congenital infection—IUGR, microcephaly, liver disease, hematopoietic abnormalities, and splenomegaly—resolve over the first weeks to months of life, concurrent with adequate oxygenation and nutrition of the newborn. These manifestations include some neonatal neurologic symptoms associated with intrauterine hypoxemia. For example, neonatal thrombocytopenia, present at birth in 75% of symptomatic CMV-infected newborns, is caused by impaired megakaryocytopoiesis and platelet production secondary to a pregnancy complicated by placental insufficiency and/or fetal hypoxia.[78] Fetal hypoxia leading to cerebral hypoxia and ischemia is a well-established cause of perinatal brain injury and may be associated with periventricular calcifications that occur in half of affected newborns. During intrauterine hypoxia in premature infants, the cerebral white matter is the site of injury and leads to periventricular leukomalacia.[79] This condition consists of focal cystic infarcts adjacent to the lateral ventricles and a diffuse gliosis that extends throughout the cerebral white matter.

(ii) Many infants born of mothers with primary or recurrent infection are asymptomatic and develop normally, despite viremia in utero and postnatally, and shedding of virus in urine and saliva for years after birth. Infants acquiring CMV postnatally exhibit similar patterns of viremia and viral excretion without symptoms. Even when CMV is acquired through transfusion by very low birth weight infants (<1250 g) of seronegative mothers, the infants may become ill but do not develop the symptoms acquired in utero after primary maternal infection.[31]

(iii) CMV infection is occasionally associated with a "blueberry muffin" syndrome, in which purpura is caused by extramedullary hematopoiesis indicative of intrauterine hypoxia.[80]

(iv) Hepatomegaly due to biliary obstruction, secondary to extramedullary hematopoiesis and erythrocytic congestion, is responsible for marked splenic enlargement in most symptomatic infants.[81]

(v) Administration of HIG to pregnant women with primary CMV infection is associated with the resolution of in utero signs of fetal infection detected by ultrasound and with the delivery of normal infants who develop normally.[3,11] Reversal of fetal symptoms suggests that HIG could improve placental function possibly by reducing inflammation that accompanies infection.

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