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Human cytomegalovirus and neonatal infection

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ABSTRACT

Human cytomegalovirus is an ancient virus that has co-evolved with humans. It establishes a life-long infection in suspectable individuals for which there is no vaccination or cure. The virus can be transmitted to a developing fetus in seropositive pregnant women, and it is the leading cause of congenital infectious disease. While the majority of infected infants remain asymptomatic at birth, congenital cytomegalovirus infection can lead to substantial long-term neurodevelopmental impairments in survivors, resulting in considerable economic and social hardships. Recent discoveries regarding cytomegalovirus pathophysiology and viral replication cycles might enable the development of innovative diagnostics and therapeutics, including an effective vaccine. This Review will detail our understanding of human cytomegalovirus infection, with an in-depth discussion regarding the viral genome and transcriptome that contributes to its pathophysiology. The neonate's clinical course will also be highlighted, including maternal and neonatal testing, treatment recommendations, and long-term outcomes.

1. Introduction

Human cytomegalovirus (HCMV) transmission occurs from persistent and prolonged viral shedding by asymptomatic individuals through body fluids (i.e., saliva, urine, breast milk, cervical secretions, and tears), which can last months or even years (Forte et al., 2020; Fornara et al., 2022). HCMV can also be acquired by sexual intercourse (Forte et al., 2020), transfusion of infected whole blood (Shigemura et al., 2019), or organ transplantation from seropositive donors (Singh et al., 2020). Conversely, congenital CMV (cCMV) infection mainly occurs following hematogenous placental transmission of the virus to the fetus during gestation (Hughes et al., 2021). Infants can also acquire postnatal HCMV infection through direct infant contact with infected epithelial cells or body fluids during childbirth (Pass et al., 1982), as well as breast milk consumption from seropositive women (Volder et al., 2021; Bapistella et al., 2019; Furui et al., 2018). While HCMV infections are usually mild or asymptomatic, progression to severe or life-threatening disease may occur in persons with deficiencies of host adaptive immune responses or those exposed to immunosuppressive therapies (Hughes et al., 2021).

Up to 90 % of adults worldwide (range 60−90 %) are HCMV seropositive, with an increased prevalence in those with lower socioeconomic status and non-Caucasian backgrounds (Zuhair et al., 2019). HCMV is not highly contagious, with a basic reproductive number of ~1.7–2.4, indicating that, on average, an infected person transmits the virus to two susceptible individuals (Griffiths et al., 2001; Mayer et al., 2017). Viral shedding by seropositive individuals ranges between 0 % and 70 % depending on the person's socioeconomic standing, education level, age, race, and ethnicity (Fowler et al., 2022). HCMV infection can be defined by four subtypes including (Hui et al., 2022; Paris et al., 2023; Scaramuzzino et al., 2022): (1) *primary* infection that occurs when an individual acquires the virus for the first time and lacks previous immunity; (2) *latency* occurs after the primary infection, where viral replication is very low, and the person remains asymptomatic; (3) *reactivation* during which viral replication is triggered, usually due to stress or other medical conditions, from a latent infection; and (4) *reinfection* that occurs following infection with a new strain despite natural immunity (i.e., superinfection).

In the United States, between 0.5 % to 2 % of all live births (or about 30,000 infants every year) will acquire congenital CMV (cCMV) infection (Boppana et al., 2013; Manicklal et al., 2013), with an estimated economic cost between $2-$6.6 billion (Grosse et al., 2021) (Fig. 1). HCMV is the most common etiology of congenital infection, as it establishes life-long infection in affected individuals (Fowler et al., 2018; Pesch and Schleiss, 2022). Although most neonates (90 %) with cCMV demonstrate no clinical symptoms, about 8000 infants will experience long-term neurodevelopmental impairments or may die from cCMV-associated complications (Boppana et al., 2013; Dollard et al.,

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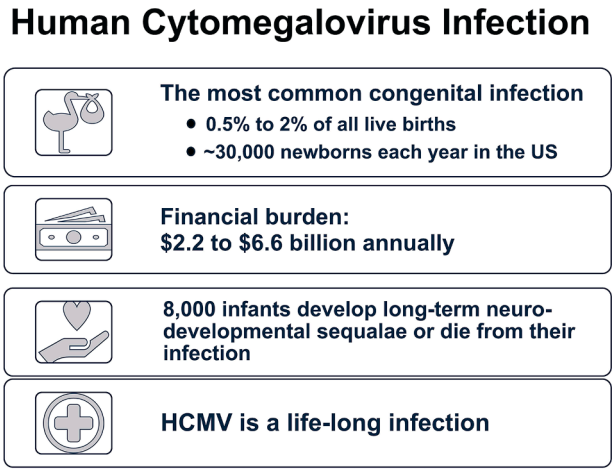


Fig. 1. Human cytomegalovirus infection.

2007). While a primary maternal infection is usually associated with more severe sequelae in the offspring, prior maternal immunity does not entirely shield the newborn from developing sensorineural hearing loss (SNHL) or other adverse neurologic conditions (Hughes et al., 2021; Boppana et al., 1999; Ross et al., 2006). cCMV with severe clinical features can occur irrespective of the trimester during which primary maternal infection occurs but is more common when the pregnant woman is infected during the first trimester (Committee on Infectious Diseases et al., 2021; Périllaud-Dubois et al., 2021).

This Review will detail our understanding of human cytomegalovirus infection, with an in-depth discussion regarding the viral genome and transcriptome that contributes to its pathophysiology. The neonate's clinical course will also be highlighted, including maternal and neonatal testing, treatment recommendations, and long-term outcomes.

2. Characteristics of cytomegalovirus

HCMV, or human herpesvirus 5 (HHV-5), is an ancient herpesvirus believed to have originated from a common ancestor more than 400 million years ago (Forte et al., 2020; Davison, 2011). Predating the emergence of mammalians by more than 300 million years, this timetable allowed the co-evolution of humans and HCMV over the subsequent 60–80 million years. A member of the *Herpesviridae* family, subfamily *Betaherpesvirinae,* HCMV derived the name "cytomegalovirus" from changes in the host cell following viral infection. Specifically, HCMV induces the development of one or more intra-cytoplasmic inclusion bodies, consisting of newly produced viruses and lysosomes, and a sizeable intranuclear inclusion body that together causes the infected cell to become voluminous or attain cellular cytomegaly (Gugliesi et al., 2020).

HCMV is one of eight double-stranded DNA human herpesviruses known to induce chronic or latent disease in an infected host (Gugliesi et al., 2020). Its DNA genome is the largest virus known to infect humans (at 230–250 kb pairs) and can encode nearly 165 genes, four non-coding RNAs, and 14 miRNAs (Forte et al., 2020; Sijmons et al., 2015; Suárez et al., 2019). Of the protein-coding genes, 43 to 44 are core replication genes common to all herpesviruses, while ~30 are unique to beta-herpesviruses (Forte et al., 2020). Despite its generous size, HCMV is solely dependent upon the host cell transcription machinery and viral tegument proteins for protein synthesis and is, therefore, incapable of *de novo* viral protein translation (Bresnahan and Shenk, 2000; Dicker et al., 2021).

Investigations of natural HCMV infection are complicated because of its restrictive tropism, which limits experiments outside cultured cells (Momtaz et al., 2021). Despite this drawback, much has been learned regarding the HCMV transcriptome over the last few years, particularly concerning the different phases of human infection (i.e., lytic, latent, and reactivation stages). The viral genome consists of two distinct regions, the unique short (US) and unique long (UL) segments, that are flanked by terminal repeats [i.e., terminal repeat long (TRL) and terminal repeat short (TRS)] and internal repeats [internal repeat long (IRL) and internal repeat short (IRS)] (Fig. 2) (Dooley and O'Connor, 2020). Of the two regions, the UL has been shown to encode many genes involved in immune evasion and cell tropism (Griffiths and Reeves, 2021). One unique portion, known as the *ULb′* region, is believed to be essential for viral dissemination and latency and is contained in all clinically relevant HCMV strains (Le-Trilling et al., 2023; Bughio et al., 2013; Dutta et al., 2015). Studies in infected cultured fibroblasts, however, have demonstrated the loss of the *ULb′* region after several viral passages, suggesting these genes are nonessential for viral replication (Le-Trilling et al., 2023; Cha et al., 1996).

All herpesviruses share a highly regulated tripartite temporal gene regulation pattern, with the expression of immediate early (IE) genes followed by early (E) and then late (L) genes (Isomura et al., 2011). Viral lytic replication is driven by the expression of IE genes following the induction of the major immediate early (MIE) promotor (Mason et al., 2023). The MIE locus contains two essential genes, *UL122* and *UL123*, which are transcribed by host RNA polymerase II and encode the proteins IE1 (IE72) and IE2 (IE86), respectively (Li et al., 2020; Rozman et al., 2022; Schwartz et al., 2023). The IE2 protein appears to be the most important of the two and is a master regulator of lytic viral expression (Chaturvedi et al., 2020), with direct control of E gene expression (Mason et al., 2020). IE1 and IE2 proteins contribute to the pathogenesis of HCMV infection, as each can activate intracellular promotors, including c-myc, hsp-70, c-fos (Hagemeier et al., 1992), nuclear factor kappa B (NFκB), SP-1, and mitogen-activated protein kinase (MAPKs) (Boldogh et al., 1991; Kowalik et al., 1993), to attenuate innate and adaptive host responses and promote of viral replication (Fig. 2) (Schwartz et al., 2023). IE1 mainly regulates type-I interferon (IFN) production, a critical cytokine that inhibits HCMV replication through induction of antiviral IFN-stimulated genes (ISGs), including signal transducer and activator of transcription (STAT) (Pham et al., 2021). At the same time, IE2 inhibits cytokine production through the stimulator of interferon genes (STING) and NFκB (Kim et al., 2017). By exploiting host cell proteins, HCMV can ultimately facilitate viral gene transcription, nuclear export, mRNA translation by the ribosomes, and post-translational modifications of cellular proteins to favor viral over host gene expression (Fu et al., 2019; Griffante et al., 2021).

3. HCMV pathogenesis

HCMV enters mucosal epithelial cells during the initial stages of primary infection, replicating and establishing its lytic cycle. The released viral progeny is highly infectious and can spread to other susceptible cell types, including endothelial cells, fibroblasts, and smooth muscle cells, mainly through cell-to-cell transmission, but can also transpire by cell-free mechanisms (Day et al., 2020; Schultz et al., 2020). While fibroblasts and smooth muscle cells enable robust viral replication, mucosal epithelial and endothelial cells experience only low-level virus shedding (Collins-McMillen et al., 2018a; 2018b). As a result, a weaker host immune response is elicited that supports the establishment of chronic infection, prolonged viral shedding, and HCMV transmission to other susceptible hosts (Collins-McMillen et al., 2018a; Bentz et al., 2006). This phenomenon is demonstrated clinically by infectious viruria of infants with cCMV and viral shedding in the breast milk of seropositive lactating women (Azenkot et al., 2019).

Viral entry into host cells is mediated by the HCMV major envelope glycoprotein B (gB) through membrane fusion (Fig. 3) (Liu et al., 2021). In fibroblasts, this step is followed by a more stable and specific interaction between the cell membrane receptor platelet-derived growth factor receptor-alpha (PDGFRα) and integrins and the HCMV trimeric complex gH/gL/gO (Buehler et al., 2019; Wu et al., 2017). Conversely,

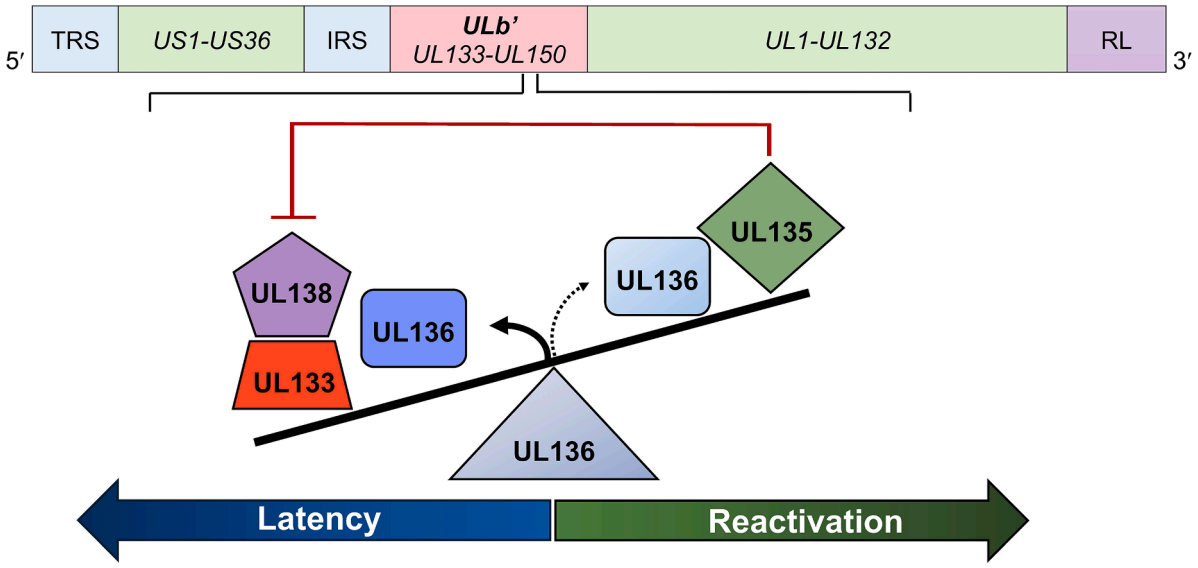


Fig. 2. A schematic representation of the human cytomegalovirus genome.
The UL133-UL138 gene locus is highlighted within the ULb′ region. Three major transcripts, UL135, UL136, and UL138, are important contributors to the different stages of infection. While UL135 and UL138 have an antagonistic relationship to promote reactivation and viral latency, respectively, UL136 contributes to both. LUNA (latency unique natural antigen) works synergistically with UL133 and UL138 to encourage viral latency. The dark arrow represents strong activation, while the dotted arrow denotes a more dampened activation.

the viral pentamer complex (gH/gL/UL128, UL130, UL131A) is essential for HCMV infection of myeloid, epithelial, and endothelial cells (He et al., 2022) through binding to its cognate ligand, the epidermal growth factor receptor (EGFR) (Wang et al., 2003). Fusion of the virion envelope to the host cell and subsequent endocytosis expedite the transport of the viral capsid and tegument proteins into the cytoplasm (Buehler et al., 2019; Chan et al., 2009). The capsid is then transported to the nucleus, where the naked viral DNA is released, and the once linear viral genome circularizes to exist as a genetic element called an episome, which quickly associates with histone proteins (Albright et al., 2022) and becomes tethered to the host chromosome (Lyon et al., 2020; Mauch--Mücke et al., 2020).

Following lytic infection, latency ensues. Latency is established in mature, circulating myeloid cells through silencing of the MIE locus and inhibition of IE gene expression, which occurs either by: (1) transcriptional repression of the promoter region or (2) chromatin remodeling via methylation or acetylation of the associated histone proteins (Gugliesi et al., 2020; Smith et al., 2021; Zalckvar et al., 2013). Suppression of IE genes results from upregulation of the synergistic HCMV genes *UL138* (Lee et al., 2015) and latency unique natural antigen (*LUNA*) (Dutta et al., 2015; Keyes et al., 2012). This collaborative relationship is demonstrated in cell cultures, where the loss of *LUNA* resulted in a 100- to 1000-fold decrease in *UL138* expression in fibroblasts and CD14+ cells, respectively (Keyes et al., 2012). *UL138* expression may also be heightened by activating the EGFR/MEK/ERK signaling pathway (Buehler et al., 2019) through MIE locus methylation and transcription repression (Reeves and Compton, 2011). Alternatively, latency may be encouraged through inhibition of *UL135* expression, which is expressed during early productive infection and leads to EGFR turnover (Buehler et al., 2016; Rak et al., 2018; Moy et al., 2023). Therefore, *UL138* and *UL135* have an antagonist relationship, with striking defects in the initiation of viral replication in fibroblast following the disruption of *UL135* (Buehler et al., 2016; Moy et al., 2023).

HCMV is a slow replicating virus, with a low copy number of one to eight viral genomes per infected cell, and only 0.004–0.01 % of mononuclear cells from seropositive granulocyte colony-stimulating factor (G-CSF)-stimulated donors carry latent viral genomes (Slobedman and Mocarski, 1999). Therefore, infected cells must be kept alive for a prolonged period of time (days to weeks) to ensure the replication and proliferation of their progeny. This adaptation is accomplished by activating the PI(3)K/Akt/mTOR signaling cascade (Cheng et al., 2022), which promotes cell survival through the targeted transcription of anti-apoptotic factor Mcl-1 (Kim et al., 2017; Mahmud et al., 2020) and heat shock protein 27 (HSP27) (Peppenelli et al., 2016). These proteins suppress the MIE promoter to drive latency and prolong the lifespan of HCMV-infected monocytes (Reeves et al., 2012; Collins-McMillen et al., 2015), uninfected monocytes (Collins-McMillen et al., 2018b), and $CD34^+$ hematopoietic stem and progenitor cells (HSPCs) (Buehler et al., 2019). After the first 48 h post-infection, HCMV induces a shift from Mcl-1 to Bcl-2 as the primary anti-apoptotic promoter of monocyte survival (Reeves et al., 2012; Collins-McMillen et al., 2015). Bcl-2 then binds to and inactivates Bax, preventing its insertion into the mitochondrial membrane (Zhang et al., 2013) and blocking the subsequent release of cytochrome C. These actions abrogate the caspase cascade, providing an ancillary mechanism that delays cellular apoptosis and triggers macrophage differentiation (Mahmud et al., 2020; Peppenelli et al., 2016; Zhang et al., 2013). Activation of the PI3K/NFkB signaling pathway in differentiating macrophages enhances the extravasation of these cells from the bloodstream into organ tissues to encourage viral replication and the establishment of chronic infection (Leone et al., 2019; Chan et al., 2008).

Infected monocytes also travel back to the bone marrow, transmitting the virus to $CD34^+$ HSPCs via the EGFR/PI3K/Akt signaling pathway. This action creates a secondary latency reservoir that further supports chronic infection (Kim et al., 2017; Cheng et al., 2017). This unique form of latency has been termed "*quiescent infection*" (Cheung et al., 2023; Shen et al., 2020), as it describes transcriptional silencing and the absence of *de novo* virion production. Following reactivation signals, infected $CD34^+$ HSPCs preferentially differentiate into myeloid progenitors and latently infected monocytes (Kim et al., 2017; Galinato et al., 2019) through upregulation of *UL133* (Umashankar et al., 2014). Progeny cells travel to host organs and tissues, becoming replication-permissive macrophages that begin the expression of immediate early (IE) genes (Smith et al., 2004; Taylor-Wiedeman et al., 1994). While HCMV latency within HSPCs can last for an extended period (decades), viral reactivation is usually triggered by an allogenic stimulation (Söderberg-Nauclér et al., 1997), immune insult (Paixão et al., 2020), or other differentiation signals (Söderberg-Nauclér et al.,

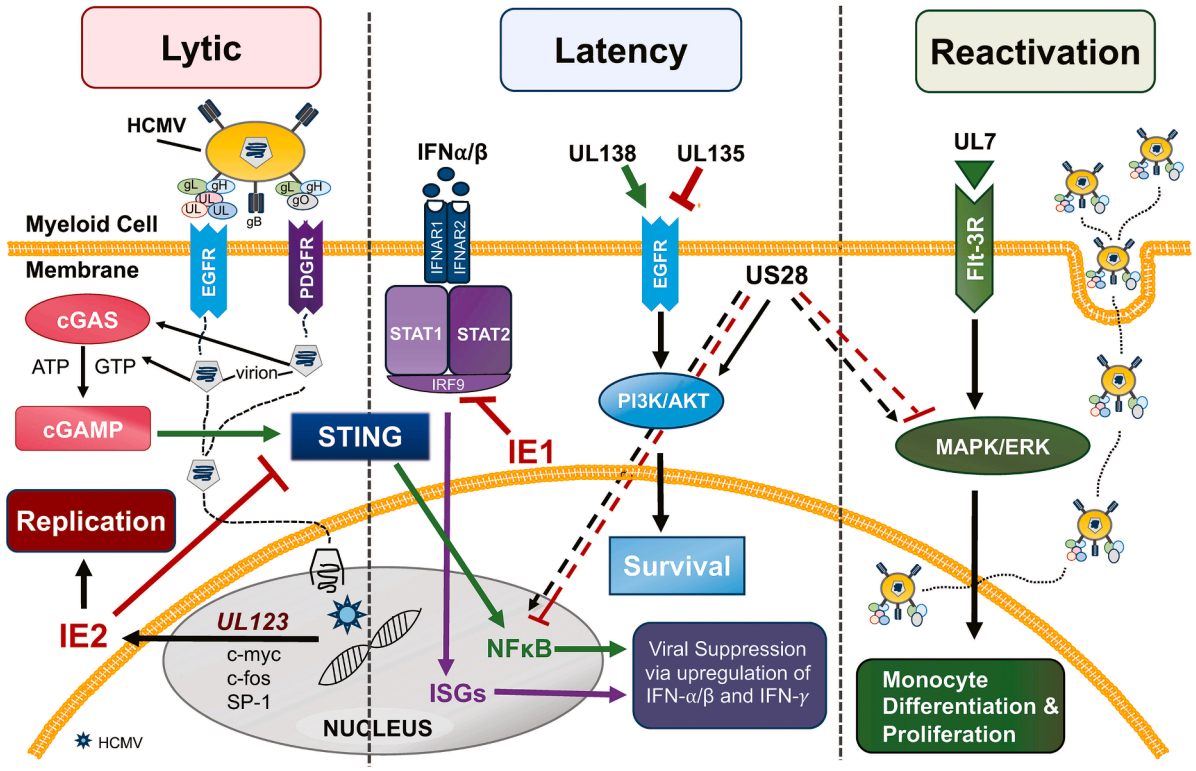


Fig. 3. Viral regulation of host cellular signaling during the stages of human cytomegalovirus infection.
In early lytic stages, human cytomegalovirus (HCMV) gains entry into fibroblasts via binding of the cell membrane receptor platelet-derived growth factor receptor-alpha (PDGFRα) HCMV trimeric complex gH/gL/gO. Conversely, the viral pentamer complex (gH/gL/UL128, UL130, UL131A) binds to its cognate ligand, the epidermal growth factor receptor (EGFR), to infect myeloid, epithelial, and endothelial cells. The HCMV virion can trigger the production of interferons (IFNs) through cGAS/cCAMP activation of signal transducer and activator of transcription (STING), while transcription of UL123 promotes IE2, which enhances viral replication. IFN-α/β can induce the expression of interferon stimulating genes (ISGs) after binding to and triggering the dimerization of IFN-α/β receptor subunit 1 and 2 (IFNAR1and IFNAR2), which subsequently forms the ISGF3 (IFN-stimulated gene factor 3) complex with (IFN regulatory factor 9) IRF9. UL135 and UL138 exhibit an antagonistic relationship during latency, where UL138 encourages latency, but UL135 drives reactivation. The function of US28 depends on whether the monocyte is differentiated. In undifferentiated cells, US28 attenuates cellular signaling of MAP kinase and NF-kB to support epigenetic suppression of the major immediate early promoter (MIEP) to prevent lytic expression (Krishna et al., 2017). In contrast, US28 activates these signaling pathways in differentiated myeloid cells to drive IE expression and viral reactivation (Krishna et al., 2017). Reactivation is driven by UL7 through the Flt-3R/MAPK/ERK signaling pathway to trigger monocyte differentiation and proliferation in host tissues. This schematic demonstrates how each viral component either promotes (solid green and black arrows) or inhibits (solid red crosses) cellular signaling events during the different stages of infection. Dashed black arrows and red crosses indicate upregulation or down-regulation (respectively), depending on myeloid differentiation.

2001). Spontaneous viral reactivation also occurs about 2–3 weeks after a primary infection (Smith et al., 2004), establishing a chronic infection in tissue-resident macrophages and adjacent epithelial layers.

Following reactivation signals, infected CD34$^+$ HSPCs preferentially differentiate into myeloid progenitors and latently infected monocytes (Kim et al., 2017; Galinato et al., 2019) through upregulation of *UL133* (Umashankar et al., 2014). Progeny cells become replication-permissive macrophages by activating immediate early (IE) viral gene expression in various organs and tissue sites (Smith et al., 2004; Taylor-Wiedeman et al., 1994). HCMV latency within HSPCs can last for an extended period (decades), with viral reactivation usually triggered by an allogenic stimulation (Prockop et al., 2023), an immune insult (Paixão et al., 2020), or other differentiation signals (Crawford et al., 2021).

HCMV also encodes four virally-encoded G-coupled protein receptors (vGPCRs), including US28, US27, UL33, and UL78, which are synthesized de novo during lytic infection and incorporated into the mature viral particle. Three of these vGPCRs (US28, UL33, and UL78) are expressed during latency (Davis-Poynter and Farrell, 2022; Krishna et al., 2021). In particular, US28 is essential for the establishment and maintenance of latency stages of HCMV infection in monocytes (Krishna et al., 2017) and CD34$^+$HSPCs (Humby and O'Connor, 2015) by targeting STAT3 (Zhu et al., 2018), c-fos/c-jun/AP-1 (Krishna et al., 2019), NFkB, and MAPK (Krishna et al., 2017; Elder et al., 2019) to silence the MIE promotor and suppress lytic infection (Fig. 3). By driving the expression of IE genes, US28 also encourages cellular differentiation and viral replication (Medica et al., 2023). These paradoxical actions are primarily driven by the phenotype of the myeloid cell. That is, US28 attenuates cellular signaling of MAP kinase and NF-κB to support epigenetic suppression of the MIEP to prevent lytic expression in undifferentiated cells (Krishna et al., 2017). Conversely, in differentiated cells, US28 activates these signaling pathways to drive IE expression and viral reactivation (Krishna et al., 2017). US28 can also be abrogated by the expression of *UL33* and *UL78*, which block the US28-mediated activation of NFκB (Krishna et al., 2021). In immunocompetent patients, overwhelming sepsis or a surge in pro-inflammatory cytokines can trigger NFκB expression, causing de-repression of the MIE locus and activation of lytic gene expression (DeMeritt et al., 2004). Viral reactivation can also be driven by the HCMV gene *UL7*, which acts as a ligand for the cellular receptor Flt3 to induce the PI3K/AKT and MAPK/ERK signaling pathways to encourage the differentiation of monocytes and CD34$^+$ HSPCs (Crawford et al., 2018).

Interferon (IFN) production is a critical human innate immune response to viral infection. While type-I IFNs (i.e., IFN-α/β) are produced by most somatic cells, type-II IFN (IFN-γ) is primarily generated by immune cells and links innate and adaptive immune responses to viral infections (Lum et al., 2024). IFN is essential for the repression of

viral transcription, with reactivation of HCMV demonstrated following inhibition of IFN signaling (Schwartz et al., 2023; Shnayder et al., 2020; Holzki et al., 2015). During pregnancy, robust IFN-γ responses have been reported in cCMV-infected fetuses (Ouellette et al., 2020). IFN production is stimulated after induction of the immune sensor cyclic guanine monophosphate-AMP synthase (cGAS) following the detection of the HCMV double-stranded DNA (dsDNA) genomes (Albright et al., 2021). cGAS then synthesizes cyclic guanine monophosphate-AMP (cGMP), which, in turn, binds to and activates the stimulator of interferon genes (STING; Fig. 3) (Albright et al., 2021). Activation of STING leads to a cascade, culminating in the upregulation of NFkB expression in the nucleus that drives the transcription of IFN genes. This signaling pathway plays a vital role in response to HCMV in fibroblasts, endothelial cells, macrophages, and dendritic cells (Albright et al., 2021). Alternatively, IFN-α/β can induce the expression of interferon stimulating genes (ISGs) after binding to and triggering the dimerization of IFN-α/β receptor subunit 1 and 2 (IFNAR1and IFNAR2), which subsequently forms the ISGF3 (IFN-stimulated gene factor 3) complex with (IFN regulatory factor 9) IRF9 (Ashley et al., 2019). The UL138 protein can inhibit this signaling pathway during infection to regulate lytic replication in fully differentiated cells and during latency within incompletely differentiated myeloid cells to control the accumulation of IFN-β during both lytic and latent infection (Albright et al., 2021).

Cell-free HCMV fragments can also trigger polymorphonuclear cells to produce pro-inflammatory mediators that enable infected peripheral blood monocytes to extravasate into peripheral organ sites and drive the further recruitment of naïve monocytes and neutrophils to these sites (Day et al., 2020; Schultz et al., 2020). Despite the common presence of highly fragmented HCMV genomes in the serum and plasma of seropositive humans, this DNA is not infectious. This concept is demonstrated by the absence of HCMV transmission to seronegative patients following the transfusion of leukocyte-depleted blood products from seropositive donors (Eisenfeld et al., 1992; Gilbert et al., 1989).

In summary, neutrophils, monocytes, and macrophages possess all the necessary components to promote viral dissemination to establish chronic or latent infection in host cells via repression of the MIE locus and chronic EGFR and PI3K signaling. Therefore, the MIE locus is a dynamic focal point where host and viral signals are integrated to enable an HCMV infection to transition between active (lytic), latent, and derepressed (reactivation) stages of infection. **Clinical reactivation** refers to the detection of viremia in seropositive individuals, while **cellular reactivation** is the reappearance of viral replication in differentiated permissive cells (Griffiths and Reeves, 2021). Viral-induced immunopathology and viral lysis are critical contributors to the establishment of HCMV infection. Detecting many viral transcripts in healthy seropositive donors may reflect the reactivation of the latent virus and transcription of viral genes in newly infected cells (Forte et al., 2020).

3. Congenital versus acquired HCMV infection

Nearly 60 % of reproductive-aged females (15–44 years of age) in developed countries are HCMV seropositive, with rising seroprevalence associated with advancing age and varied by differences in race and ethnicity (Zuhair et al., 2019; Fowler et al., 2022). In the United States, the highest cCMV prevalence is reported in black (9.5/1000 live births), followed by Hispanic white (3.0/1000 live births) and non-Hispanic white (2.7/1000 live births) newborns (Committee on Infectious Diseases et al., 2021). These disparities persist even after controlling for country of birth and parental income, education level, marital status, family size, and availability of medical insurance (Sapuan et al., 2022). In general, the highest-risk groups for HCMV infection are those providing care to young children (i.e., daycare workers, teachers, pediatric nurses, and mothers) and adolescents (Gugliesi et al., 2020).

During pregnancy, up to 3 % of seronegative women experience a primary HCMV infection (Gugliesi et al., 2020; Ben Shoham et al., 2023), but the mechanisms by which HCMV can be transmitted to the fetus remain unclear (Lindholm and O'Keefe, 2019; Uenaka et al., 2019). While women with primary HCMV infections are most likely to pass the infection to their fetus, most (two in three) newborns diagnosed with cCMV are born to seropositive mothers (Scaramuzzino et al., 2022). In these situations, fetal infection is caused by the reactivation of latent virus or reinfection with a new viral strain (Scaramuzzino et al., 2022). Notwithstanding the high number of seropositive women, transmission of HCMV to the developing fetus is very low at 1 % (James and Kimberlin, 2016), and the prevalence of cCMV in their offspring is between 0.4−6.1 % (Coppola et al., 2019; Lanzieri et al., 2014).

Fetal HCMV infection is routinely diagnosed after the 21st week of gestation and at least six weeks after a maternal lytic infection via amniocentesis and the detection of viral DNA in the amniotic fluid (Leruez-Ville et al., 2020) (Fig. 4). While detecting HCMV in the amniotic fluid is the current "gold standard" to diagnose fetal infection, HCMV serologies (IgM and IgG) are also routinely used in clinical practice. Serologies are recommended if the pregnant woman experiences flu-like symptoms (i.e., fever, malaise, headache) that cannot be attributable to another infection or have fetal ultrasound findings concerning for HCMV infection (Kettler et al., 2023; Rawlinson et al., 2017). The addition of IgG avidity testing, which measures the binding strength between the virus and host IgG antibodies, is also routinely employed to better inform on past versus primary infections (Torii et al., 2019). That is, high avidity is routinely observed in established or later stages of infections, while low avidity is common with primary infections.

After birth, cCMV is primarily diagnosed by the detection of HCMV DNA in the saliva, urine, blood, or cerebrospinal fluid (CSF) via nucleic acid amplification tests [NAAT; i.e., polymerase chain reaction (PCR) assays] within the first three weeks of life. After this period, distinguishing between postnatally acquired versus congenital infection becomes difficult. However, the clinical finding of brain calcifications in the periventricular, ependymal, or subependymal region appearing as points or that occasionally outline the ventricles should raise clinical suspicion for cCMV, as these observations are usually pathognomonic for cCMV (Committee on Infectious Diseases et al., 2021; Ijezie et al., 2023). Whereas serologies are commonly used as a diagnostic tool during pregnancy, these assays are not recommended for newborn testing because detecting anti-HCMV IgG cannot discriminate between maternal and neonatal infection. Furthermore, IgM is poorly sensitive and is not specific for cCMV (Torii et al., 2019; Zelini et al., 2022).

Although most cCMV infections are asymptomatic following birth (90 %), between 60 %−90 % of symptomatic and up to 15 % of asymptomatic cases will develop long-term neurologic sequelae, including neurodevelopmental impairment, ophthalmic abnormalities, and SNHL (Cannon and Davis, 2005; Andriesse et al., 2006; Boppana et al., 2005). Notably, more children are adversely affected by cCMV infection than more well-known conditions, including Down syndrome, fetal alcohol syndrome, and spina bifida (Cannon and Davis, 2005). Factors that contribute to fetal injury include the: (1) gestational age of the fetus at the time of infection (Pass et al., 2006), (2) status of maternal immunity (Hughes et al., 2021); (3) extent of HCMV-induced placental injury (Fisher et al., 2000); (4) burden of the viral load in the amniotic fluid (Lazzarotto et al., 2000); (5) genotype of the particular HCMV strain(s) infecting the fetus (Arav-Boger et al., 2006); and (6) magnitude of the fetal/maternal immune response to infection (Schleiss, 2011). Infants with symptomatic cCMV also exhibit heightened emotional sensitivity, problems with school maturity at six years of age, and balance disturbances compared with those who remain asymptomatic or exhibit only mild clinical findings (Yamaguchi et al., 2022). cCMV is also the leading nongenetic cause of SNHL, occurring in almost half of symptomatic and 7 % of asymptomatic cases (Ouellette et al., 2020). However, a quarter of neonates with cCMV who have normal hearing screens after birth will develop SNHL later in childhood (Ouellette et al., 2020). Other signs and symptoms of cCMV are outlined in Table 1 (Scaramuzzino et al., 2022).

Infants who acquire HCMV postnatally via transmission from

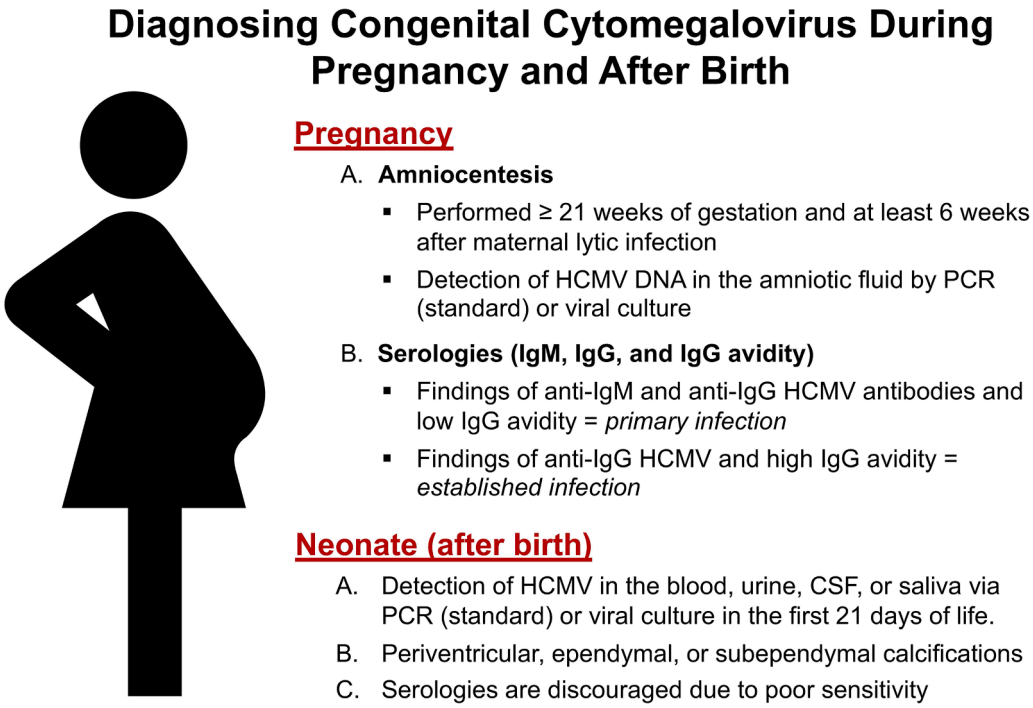
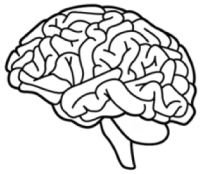
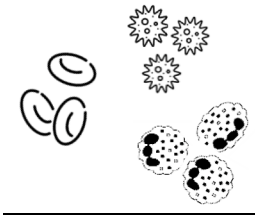


Fig. 4. Diagnosis of congenital CMV during pregnancy and after birth.

Table 1
Common symptoms associated with congenital cytomegalovirus (Scaramuzzino et al., 2022; Akiva et al., 2023).

Organ	Clinical findings
	1. Small for gestational age 2. Intrauterine growth retardation 3. Prematurity 4. Petechial rash 5. Blueberry muffin rash
	1. Periventricular calcifications 2. Chorioretinitis 3. Failed hearing screen with increased risk for sensorineural hearing loss 4. Microcephaly/macrocephaly 5. Congenital hydrocephalus
	1. Hepatomegaly 2. Conjugated hyperbilirubinemia 3. Hypertransaminemia 4. Jaundice
	1. Anemia 2. Thrombocytopenia 3. Neutropenia

maternal cervical secretions or ingestion of breast milk do not typically develop clinical illness or sequelae because of the presence of passively transferred maternal antibodies (Committee on Infectious Diseases et al., 2021; Nijman et al., 2014). However, infants born prior to 32 weeks of gestation and/or have a birthweight <1500 g are at the greatest risk for developing symptomatic infection compared to full-term, healthy infants. A study from Bimboese et al. (2022) from Australia demonstrated that nearly 88 % of seropositive mothers (49/56) who delivered < 32 weeks of gestation or < 1250 gs birthweight shed HCMV in their breast milk in the first six weeks postpartum. Of the 58 infants exposed to HCMV-positive breast milk, 47 % (27/58) developed qPCR-confirmed viruria by 14 weeks of age. Even though there were no significant differences in gestational age, birth weight, incidence of bronchopulmonary dysplasia, or necrotizing enterocolitis between the HCMV-positive and negative groups, the CMV-confirmed groups had a longer length of stay (104 vs. 79 days, $P = 0.04$) and more episodes of prolonged neutropenia. Neutropenia was also the most common clinical sign of HCMV at the time the first urine sample converted to HCMV-positive (44 %). Of the CMV-positive infants, 30 % (8/27) remained asymptomatic, 48 % (13/27) had mild symptoms, and 22 % (6/27) experienced severe symptoms (i.e., respiratory deterioration, apnea, new CPAP requirement, new intubation, and increasing oxygen requirement).

Postnatal cases of HCMV infection have a variable incubation period and onset of viruria, with a mean time of HCMV DNA detection in the urine of approximately seven weeks (range 3–24 weeks) (Committee on Infectious Diseases et al., 2021). Infants with postnatal HCMV infection also have significantly reduced urine viral load compared with cCMV (Nijman et al., 2014; Nijman et al., 2012). Clinical signs and symptoms of acquired infection vary with the age and immune status of the neonate. Some infants will present with an infectious mononucleosis-like syndrome with prolonged fever and mild hepatitis. Others may exhibit more severe infection, with end-organ disease (i.e., pneumonia, retinitis, meningoencephalitis, colitis, or transverse myelitis) or HCMV syndrome (i.e., fever, thrombocytopenia, leukopenia, and mild hepatitis) if immunocompromised (Committee on Infectious Diseases et al., 2021). Historically, scientists believed postnatal asymptomatic HCMV infection and latency posed minimal health risks

to the host. However, mounting evidence suggests adverse health outcomes with increasing age in the pathogenesis of cardiovascular disease (Jia et al., 2017; Tan et al., 2022; Chen et al., 2021), with HCMV DNA identified in atherosclerotic plaques (Yaiw et al., 2013). HCMV has also been associated with immune dysfunction (Hastie et al., 2023; Ivanov et al., 2023; Karandikar et al., 2023) and frailty (Carvalho and C., 2018; van Sleen et al., 2023) in aging populations.

To summarize, SNHL is the most common complication of cCMV infection, accounting for 21–24 % of cases of hearing loss in young children (Manicklal et al., 2013). cCMV infection is associated with substantial financial and social burdens, with an estimated 2.6-fold increase in direct medical cost compared with healthy controls during the first year of life (Weil et al., 2022). While in-utero transmission can occur both during primary infection and in mothers with prior immunity (Forte et al., 2020), the mechanisms associated with HCMV transmission during pregnancy are only recently being elucidated as investigative methods and technologies continue to evolve and enable these critical discoveries.

4. Maternal and neonatal cytomegalovirus testing

HCMV replication is strictly regulated during primary infection or following reactivation and occurs when infected monocytes differentiate into macrophages (Smith et al., 2004; Chan et al., 2012). In general, only a small proportion of circulating monocytes (0.01 %) contain cell-associated viral genome in seropositive individuals (Min et al., 2020). However, HCMV viremia consists of intracellular infection of myeloid cells and the small, fragmented, non-infectious viral DNA found in the plasma. Differences between the two should be considered when ordering routine HCMV assays, as their results may have significant clinical implications (Tong et al., 2017). HCMV DNA loads in plasma are usually less than that reported in whole blood (Rzepka et al., 2022), as the size of the PCR amplicon used in the assay kit can vary considerably [up to 100-fold in copy number between assays, or from 50 to 350 base pairs (bp)] (Peddu et al., 2020). The use of cell-free DNA (cfDNA), routinely sent for non-invasive prenatal aneuploidy screening, has been shown by Peddu et al. (2020) to increase the sensitivity of HCMV testing in plasma when employing an amplicon size $\leq$ 86 bp compared with standard qPCR assays that have amplicon sizes $>$ 105 bp. Moreover, cfDNA fragment size associated with HCMV was shorter than human chromosomes [103 vs. 172 base pairs(bp), $p < 0.0001$] and more accurately predicted discrepancies in plasma viral loads measurements by different PCR assays.

Infants with cCMV persistently shed very high levels of HCMV in their saliva and urine (median >12 months) (Forner et al., 2015; Gantt et al., 2017). Saliva is collected by oral (not throat) swab and is usually more convenient to obtain than urine, as urine production is limited during the first 24 h of life in normal, healthy infants. False-positive CMV PCR results are possible in breastfeeding infants tested by oral swab if the mother is seropositive and shedding virus, so confirmatory testing by urine PCR is required. PCR testing of neonatal saliva is shown to have high sensitivity (97–100 %) and specificity (99.9 %) as a screening method and has been validated in a large population-based cohort study (Dollard et al., 2021; Nagel et al., 2020; Ross et al., 2018). Moreover, PCR assays in saliva and urine performed similarly to HCMV viral cultures by the CMV and Hearing Multicenter Screening (CHIMES) study, providing a scalable diagnostic tool for high-volume screening (James and Kimberlin, 2016; Ross et al., 2014). However, HCMV levels in the blood are much lower than saliva or urine, which may contribute to decreased sensitivity of PCR testing of dried blood spots collected at birth (Gantt et al., 2017). Studies of HCMV loads in blood or urine have shown conflicting results regarding their predictive reliability for developing SNHL. Many studies show poor predictability of HCMV levels in blood and neurodevelopmental impairment (Ouellette et al., 2020; Yamaguchi et al., 2022; Marsico et al., 2019), while others support a possible correlation (Yamaguchi et al., 2022; Forner et al., 2015; Lanari et al., 2006).

Viruria is generally prolonged, with a mean duration of 4.55 years in asymptomatic neonates compared with 2.97 years with symptomatic infections (Noyola et al., 2000). Despite this difference, there were no associations between long-term growth or cognitive impairments and viral excretion duration. However, a higher number of children who shed HCMV for $<$ 4 years had SNHL and progressive SNHL compared to those with more extended excretion periods $>$ 4 years (Noyola et al., 2000). An additional study from Japan (Yamaguchi et al., 2022) reported higher urinary HCMV DNA copy numbers in infants with cCMV and SNHL than those with cCMV without SNHL ($p = 0.036$). Moreover, these investigators showed that the urinary HCMV viral burden was also significantly associated with brain abnormalities on magnetic resonance imaging (MRI; $p = 0.013$) at 18 months of age but not at six months. Differences in PCR assay kits and primers and timing of sample procurement and analysis after birth may account for some differences.

Neonatal screening in those infants with an abnormal hearing screen has been shown to facilitate the rapid diagnosis of cCMV, enabling timely initiation of antiviral treatment and focused observation with early intervention programs. Children who are diagnosed early benefit from timely fitting of hearing aids or cochlear implants to attenuate speech delay and enable the development of normal speech and cognitive development (Fourgeaud et al., 2022). A United States investigation conducted by Fowler and colleagues on behalf of the CHIMES Study (Fowler et al., 2017) found that 7 % of all HCMV-positive neonates did not pass their newborn hearing screen compared to 0.9 % of HCMV-negative controls ($P < 0.0001$). In those that did not pass their hearing screening test, 65 % were confirmed to have SNHL on diagnostic testing. Conversely, 3.6 % of infants with cCMV who passed there screening hearing test will later develop SNHL during early infancy. Therefore, in this cohort, newborn hearing screening tests failed to detect 43 % of infants with cCMV who later developed SNHL, supporting the establishment of universal cCMV testing after birth. Retrospective quantitative PCR (qPCR) testing of stored dried blood spot (DBS) cards, obtained shortly after birth for state newborn genetic and metabolic screens, may be used to facilitate a diagnosis of cCMV when the infection is considered beyond the newborn period (Gantt et al., 2017). As demonstrated by a study by Limaye and colleagues (Limaye et al., 2013), the CMV DNA load obtained from DBS cards correlated with that of plasma, with a 95 % limit of detection of the DBS assay estimated at 2700 plasma copies/ml [675 plasma International Units (IU)/ml with the technology used]. However, a negative test does not rule out the diagnosis of cCMV.

Therefore, when utilizing HCMV real-time PCR assays, clinicians should consider the following when making clinical management decisions: (1) the type of specimen (i.e., urine, saliva, whole blood, or plasma), (2) the limits of detection and quantification chosen by the clinical laboratory, (3) the linear range of the assay, (4) the assay's reproducibility within the institution, and (5) the considerable variability of viral load values among different assays (Kraft et al., 2012). Whole blood has been recognized as the optimal specimen for HCMV DNA load surveillance in peripheral blood (Gerna et al., 2011; Sanchez and Storch, 2002). In a recent survey, whole blood was the preferred blood compartment used in laboratories worldwide, where HCMV monitoring is performed by quantitative NAAT (Le Page et al., 2013). This preference is because whole blood enables the determination of both cell-free and cell-associated HCMV DNA (Pillet et al., 2014).

The American Academy and Joint Committee on Infant Hearing recommends at least one diagnostic audiology evaluation in all infants by 24 to 30 months of age, with "earlier and more frequent assessments in those diagnosed with cCMV" (Year, 2007). However, more detailed newborn testing criteria were released by the International Congenital Cytomegalovirus Recommendations Group released by the International Congenital Cytomegalovirus Recommendations Group (2017) (Rawlinson et al., 2017) and the Academy of Audiology (2023) (Kettler et al., 2023). While universal newborn cCMV screening is not currently

recommended, infants born to HCMV-infected pregnant women should be tested after birth. Infants who do not pass their newborn hearing screen on two or more attempts (one or both ears) should also be tested for cCMV. Additionally, infants born small for gestational age or display intrauterine growth restriction, hepatomegaly, a petechial or "blueberry" rash, microcephaly, and/or macrocephaly should also complete cCMV testing. Lastly, the finding of transaminemia, thrombocytopenia, or abnormal findings on cranial ultrasound or MRI should also prompt clinicians to test the newborn for cCMV infection (Table 1).

5. Treatments

Prevention of CMV infection through education and vaccination is of considerable public health importance (Staras et al., 2006). However, an effective HCMV vaccine has not been realized. Investigators have studied the efficacy of HCMV hyperimmune globulin (HIG) during pregnancy to inhibit HCMV fetal transmission, but the trial was stopped early because of no observed differences in the composite of cCMV infection or fetal or neonatal death. Participants who received HIG also had a higher incidence of headaches and shaking chills than controls, and one woman suffered a severe allergic reaction after the first infusion (Hughes et al., 2021).

Currently, there are five antiviral therapies approved for the treatment of HCMV-associated disease by the FDA, including ganciclovir (GCV), valganciclovir (VGCV), foscarnet, cidofovir, maribavir, and letermovir. Two of these, intravenous ganciclovir and oral valganciclovir are used to treat a subset of symptomatic neonates following viral detection (Dooley and O'Connor, 2020). Valganciclovir, the l-valyl ester prodrug of ganciclovir, is a standard oral therapy for neonates with cCMV infection, as plasma concentrations of ganciclovir are comparable to those achieved by the administration of the intravenous ganciclovir (Kimberlin et al., 2008). However, in infants with compromised gastrointestinal tracts due to necrotizing enterocolitis or other bowel maladies that limit the absorption of oral medications, GVC should be administered (Committee on Infectious Diseases et al., 2021). Dose-limiting toxicity, side effects (i.e., thrombocytopenia and neutropenia), the prolonged course (six months), and the development of drug-resistant infections limit the routine use of GCV and VGCV in neonatal patients (Committee on Infectious Diseases et al., 2021).

A 2003 study by Kimberlin et al. (2003) reported neurodevelopmental outcomes of infants treated with GCV for six weeks. In this study, infants with confirmed cCMV via HCMV viruria and with evidence of neurologic involvement (i.e., microcephaly, intracranial calcifications, abnormal cerebrospinal fluid results, chorioretinitis, or hearing deficits) who received treatment had improved or maintained normal hearing compared with controls at 6 months (84 % versus 59 %, $P = 0.06$). Moreover, none of the infants who received GCV had worse hearing at 6 months compared with 41 % of control patients. A follow-up study by the same group in 2015 (Kimberlin et al., 2015) randomized neonates with symptomatic cCMV infection to receive VGCV treatment to compare six months versus six weeks of therapy. At six months, hearing in the best ear was similar between groups, and hearing in both ears was likely to improve or remain normal at 12 months in the 6-month treatment group compared with the 6-week group (73% vs. 57 %, $P = 0.01$) and this benefit remained at 24 months of age (77% vs. 64 %, $P = 0.04$). Infants also exhibited improved neurodevelopmental scores on the Bayley Scales of Infant and Toddler Development, 3rd edition, at 24 months, including the language-composite component ($P = 0.004$) and the receptive-communication scale ($P = 0.003$). Improved outcomes with a prolonged treatment course led these investigators to conclude that protracted HCMV viremia could be associated with cCMV-mediated neurologic sequelae and prolonged VGCV exposure might dampen viral loads to abrogate long-term neurologic effects during an important period of brain growth and development.

In 2015, Kawada et al. (2015) evaluated the efficacy of VGCV versus placebo for a six-week treatment period. Newborns were recruited into the study if they had abnormal hearing screening results or other clinical findings concerning for cCMV and had qPCR-confirmed HCMV DNA in saliva or urine specimens. A total of 158 infants were included (127 with abnormal hearing screen results and 31 infants with clinical concerns), and cCMV was identified in six and three infants, respectively. Of those found to have cCMV, six completed 6 weeks of VGCV therapy and only one case had improved hearing, but the other five cases had little or no improvement at one year of age. Among the nine identified patients, there was no difference in blood or urine HCMV load at diagnosis, but those with SNHL had significantly higher viral loads during VGCV treatment.

Given the above findings, antiviral therapy with either GCV or VGCV should be provided to patients with moderate or severe symptomatic cCMV disease with or without central nervous system involvement during the first 13 weeks following birth to improve audiologic and neurodevelopmental outcomes at 2 years of age. Antiviral drugs should be continued for 6 months and adjusted for weight gain (Committee on Infectious Diseases, 2024). Neonates with isolated SNHL should be treated for 6 weeks to improve audiologic outcomes (Committee on Infectious Diseases, 2024). Infants with asymptomatic cCMV infection should not routinely receive antiviral therapy outside ongoing research studies due to the lack of data suggesting benefit (Rawlinson et al., 2017; Committee on Infectious Diseases, 2024; Ross and Kimberlin, 2021). Similarly, neonates with mildly symptomatic infections have not been studied to define pharmaceutical efficacy (Committee on Infectious Diseases, 2024). Preterm infants with symptomatic cCMV infection (i.e., end-organ involvement) may receive parental GCV for two weeks with a reassessment of responsiveness to therapy. If the infant was found to benefit from antiviral therapy, then an additional one to two weeks of GVC can be administered intravenously if symptoms have not been resolved. In this patient population, VGCV is not considered an alternative to GVC because of the possibility of impaired gastrointestinal absorption in this clinical setting (Committee on Infectious Diseases, 2024). Importantly, these agents do not cure the host of the infection and are primarily effective against lytically replicating virus, as they fail to target the latent viral reservoir (Dooley and O'Connor, 2020). Antiviral resistance may be an underestimated emergent problem. While rare in neonates (~4 % of cases), antiviral drug resistance has been reported in several cCMV cases (Ross and Kimberlin, 2021; Torii et al., 2022; Garofoli et al., 2020; De Cuyper et al., 2023). During long-term antiviral therapy (>6 months), weight-based monitoring and viral load surveillance should be considered to prevent and rapidly identify the emergence of drug resistance (Ross and Kimberlin, 2021; De Cuyper et al., 2023).

6. Conclusions

Evidence supports both viral lysis and immunopathology as contributors to the establishment of human HCMV infection. Fibroblasts, epithelial cells, endothelial cells, smooth muscle cells, and macrophages are permissive to HCMV replication (Sinzger et al., 1995). HCMV attenuates the immune-responsive state of peripheral blood monocytes, regardless of the stage of maturation at which the cell is infected (Peppenelli et al., 2016; Jackson et al., 2021; Pocock et al., 2017). This anergic-like state is critical for establishing latency and eventual reactivation of the virus (Shnayder et al., 2020). While neutrophils and monocytes do not support complete lytic replication of HCMV, these cells are essential contributors to hematogenous viral dissemination of HCMV to organ systems that enable life-long persistence and latency to be established (Braun et al., 2022; Braun and Sinzger, 2021). Whereas monocytes and neutrophils are relatively short-lived cells (1–3 days), the slow replication cycle of HCMV (days to weeks) makes long-lived macrophages (months to maybe years) desirable cells to establish a chronic infection. HCMV employs a bi-phasic expression of anti-apoptotic cellular proteins, including Mcl-1 and HSP27 in the first

48 h and Bcl-2 after 72 h, to prolong monocyte survival by regulating caspase-3 activation in a time-dependent manner that coincides with the induction of monocyte-to-macrophage differentiation. Macrophages enable persistent viral release in organ tissues but are not a source of hematogenous viral spread because they are not bloodborne cells.

In immunocompetent individuals, a delicate balance exists between ongoing low-level HCMV lytic gene expression in the absence of viremia. However, unchecked stress on the host immune system may disrupt this balance to encourage reactivation and viremia, culminating in a potentially life-threatening disease (Griffiths and Reeves, 2021). This phenomenon may occur not uncommonly in the Neonatal Intensive Care Unit (NICU) but remains undiagnosed due to the lack of clinical suspicion, despite clinical symptoms and findings suggestive of viral infection. Currently, antiviral treatments remain restricted to defined patient populations due to evidence-based clinical benefits, documented toxicities, and the requirement for prolonged treatment courses. Whereas scientists once believed asymptomatic HCMV infection and latency posed minimal health risks to the host outside of the development of SNHL, mounting evidence suggests adverse health outcomes with advanced age in the pathogenesis of cardiovascular disease (Jia et al., 2017; Tan et al., 2022; Chen et al., 2021), immune dysfunction (Hastie et al., 2023; Ivanov et al., 2023; Karandikar et al., 2023) and frailty (Carvalho and C., 2018; van Sleen et al., 2023).

A critical need remains for developing a sensitive, inexpensive, and rapid diagnostics to identify neonates with cCMV. Early detection could facilitate close follow-up and early intervention programs to rapidly identify and provide treatments for associated neurodevelopmental impairments, including SNHL. Moreover, outcome data for the 90 % of neonates with cCMV that remain asymptomatic are restricted to limited observations over the first few years of life. As many of these infants remain undiagnosed, larger population-based studies are required to define the effects of cCMV and determine if any pharmaceutical intervention would prevent the development of SNHL or attenuate its effects once detected.

In closing, significant advances have occurred over the last five years to define the pathophysiology of HCMV in the human host. Viral and host genetic factors that fine-tune the delicate balance between lytic, latent, and reactivation stages of infection have been described. Incorporating this knowledge into emerging diagnostics may allow more accurate quantification of viral load in different tissue matrices, provide pertinent clinical information to distinguish between the various stages of infection, enable the detection of viral reactivation, and rapidly identify emerging antiviral resistance. It will take the proverbial village to expedite future investigations and trials that may attenuate neurodevelopmental impairments, impede the progression of SNHL as children age, and prevent disease progression in critically ill patients. However, financial, institutional, and educational support are necessary to accelerate the development of innovative and novel diagnostic and therapeutic approaches to this prevalent and devastating viral infection.

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Data availability

No data was used for the research described in the article.

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