

Pathogenesis, Diagnosis and Therapeutics in Human Cytomegalovirus Infection

Silva DFL^{1*} and Medeiros RC²

¹Department of Virology and Molecular Biology Laboratory, Evandro Chagas Institute, Brazil

²Department of Tropical Medicine Nucleus, Federal University of Pará, Brazil

***Corresponding author:** Dorotéa de Fátima Lobato da Silva, Virology and Molecular Biology Laboratory/IEC/SVS/MS, BR 316, 67030-000, Ananindeua, Brazil, Tel: 91 3214-2092; Email: dorotealobato@iec.pa.gov.br

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ABSTRACT

Cytomegalovirus is a beta-herpes virus that causes various severe conditions, particularly when associated with reduced immune response of the host. The infection may be symptomatic, with clinical features similar to infectious mononucleosis; however, in most cases, cytomegalovirus infection progresses in an asymptomatic or subclinical manner. Elucidation of the mechanisms of infection could contribute to the control of the disease and the development of novel therapeutics, particularly in patients with a deficient immune response because the virus usually does not cause serious injury and is not life threatening in immunocompetent patients. In this article, we will review some aspects of the pathogenesis, diagnosis and therapeutic resources for the treatment of cytomegalovirus infection.

Keywords: Cytomegalovirus; Pathogenesis; Therapeutics; Treatment; Diagnosis

INTRODUCTION

Human cytomegalovirus (**HCMV**) is one of the main causative agents of several pre- and postnatal infections. Infections by this virus may be either symptomatic or asymptomatic and in immunocompetent patients the latter is the most frequent [1,2]. The primary viral infection can occur during different periods of life and may be related to specific factors, including

socioeconomic factors, high endemicity, and the presence of the agent in various body fluids [3-5]. Moreover, CMV infection can occur in different stages of life. During intrauterine development (prenatal or vertical infection), which may cause congenital infection; at birth or in the first few feedings (perinatal infection); or at a later stage (postnatal or horizontal infection) [5].

Primary infections can occur by natural or iatrogenic routes [6]. Alternatively, the virus can remain in the body indefinitely, in this case, immunological and viral factors may trigger CMV activation from the latency state, thereby causing recurrent infections [7-11].

Viral reactivation is common in immune compromised individuals [12-14] and may affect many body systems, such as the central nervous system (**CNS**), the visual, gastrointestinal, and respiratory systems, among others [4,15-19]. Although uncommon, this type of infection can also [8,20] occur in immunocompetent patients.

Clinical evaluation is not sufficient to diagnose CMV, and specific laboratory tests are required. These laboratory tests generally involve detection of IgG and IgM antibodies or molecular methods that detect and quantify the viral genome, the latter of which can be helpful for the diagnosis of secondary and subclinical infections [21,22]. Treatment usually involves several options, such as combined antiviral therapy, introduction of immunoglobulins, and reducing immunosuppression [23]. The most commonly used drugs are ganciclovir, forcartet, and cidofovir [24-26] and ganciclovir was the first drug licensed for the treatment of CMV infection in the Brazilian population.

In this review, we aim to describe some of the key aspects of HCMV pathogenesis, diagnosis, and therapeutic approaches.

PATHOGENESIS

HCMV is the largest member of the *Herpesviridae* family, *Beta-herpesvirinae* subfamily, and *Cytomegalovirus* genus; this virus is also known as human herpesvirus-5 (**HHV-5**), salivary gland virus, HCMV or CMV. Each mature virion particle measures around 200 nm and is enveloped by an outer membrane corresponding to the phospholipid envelope, which delimits the tegument [27] containing important proteins for viral replication. Additionally, the virus has a capsid with icosahedral symmetry consisting of 162 capsomeres; this capsid contains the viral DNA genome, which is double-stranded and linear and consists of 230 kbp with over 190 coding regions [28-30].

The viral particles are thermolabile and exhibit an average viability of 45 minutes at 37°C as well as sensitivity to low pH, lipidic solvents, and heat [9,31]. The virus has a slow replication *in vitro* in cell culture, with a preference for human fibroblasts, but a fast replication in living organisms [32,33].

Viral particles are formed by four isomers produced by inversion of two genomic regions, i.e., the unique long (**UL**) and unique short (**US**) regions [34]. In these regions, there are sequences encoding the virion proteins, including proteins of the capsid, tegument, and envelope [35].

In the viral envelope, there are 12 main glycoproteins, six of which are grouped in complexes of high molecular weight, designated gcl, gclI, and gclII [29,36] (Table 1). Among these glycoproteins, gB and gH play an important role in the virus-host relationship and are responsible for the penetration of the virus into the target cell, cell-to-cell propagation, and syncytia formation [37,38]. Additionally, these proteins are targets of neutralizing antibodies and potential candidates for the production of vaccines [39-42].

In the viral tegument, phosphoproteins are crucial for the regulation of HCMV genes and modification of the host cell metabolism. Among them, pp150 and pp65 have immunogenic importance, pp71 is important for virus replication and activates gene expression at the very early stage of the replicative cycle [9,43] and pp65, in addition to immunogenicity, is responsible for latency mechanisms and evasion of the virus from the immune system [44].

Table 1: Groups of glycoproteins in protein complexes of high molecular weight.

Molecular complex	Glycoproteins	Other denominations
gcl	gB	gA, gP55-116, PUL55 gP58, p130/55 gP ₅₂
gclI	gM	gPUL ₁₀₀
	gN	gPUL ₇₃
gclII	gH	P86, gPUL ₇₅
	gL	gPUL ₁₁₅
	gO	gPUL ₇₄

Source: Adapted from Rasmussen [29] and Pignatelli et al [36].

INFECTION AND VIRAL LATENCY

Klemola and Kaariainen [45] first described CMV infection as a condition similar to infectious mononucleosis caused by the Epstein Barr virus; thus, CMV infection was also known as a “mononucleosis-like” disease.

The incubation period for CMV ranges from 4 to 12 weeks, during which time it can be detected. In most cases, the infection progresses, it remains asymptomatic or subclinical in immunocompetent patients. However, when symptoms present, the infected individual may exhibit persistent fever, myalgia, and cervical lymphadenopathy [4]. The most severe symptoms are typically observed in infected fetuses and patients with exacerbated immunodeficiency [5,12-14,46] either in a disseminated manner or in specific organs [47].

In patients with immunodeficiencies, CMV is opportunistic, and infection is associated with diverse clinical signs and symptoms. For example, in human immunodeficiency virus

(HIV)-seropositive individuals, CMV infection compromises various organs, leading to the development of important diseases, such as retinitis in the visual system, interstitial pneumonia in the respiratory tract, hepatitis, arthritis, carditis, CNS diseases (e.g., encephalitis), and various infections of the gastrointestinal tract [17,48-50]. Retinitis is one of the main diseases caused by HCMV when CD4+ T lymphocytes reach less than 50 cells/mm³ blood [51-54].

In patients who have undergone transplant, the respiratory, visual, nervous, and gastrointestinal systems are compromised and may exhibit graft rejection [55-57]. In such cases, HCMV infection is further supported by some risk factors, such as latency of HCMV, serological profile of the donor/recipient, immune deficiency induced by the use of corticosteroid therapy after transplantation, and immune response to the graft [55,58,59].

In prenatal infection, only 10% of affected children may have alterations in different body systems, such as the central and peripheral nervous systems (e.g., cerebral and periventricular calcifications, microcephaly, hypotonia, difficulty with sucking, spasticity, hemiparesis, and seizures), hematopoietic system (e.g., jaundice, hepatomegaly, and/or splenomegaly), and locomotor system, in addition to chorioretinitis, sensorineural hearing loss, pulmonary complications, low birth weight, and prematurity [60,61]. These alterations may be related to infection of various cell types, such as epithelial cells, endothelial cells, fibroblasts, white blood cells, and specialized cells (e.g., neurons, retina, smooth muscle, gastrointestinal system, and hepatocytes), by CMV [7,9,48,62,63].

The mechanism of infection starts when the virus adsorbs the host cell through the binding of a complex of glycoproteins present in the viral envelope to receptors in the cell membrane. This glycoprotein complex [42,64-66] has specific membrane receptors, including soluble heparin, annexins, epidermal growth factor receptor (**EGFR**), and integrins [67,68] which hold the virus in the host cell (Figure 1).

After binding of the agent to the host cell, the viral and cell membranes are disrupted through activation of cell signaling pathways that promote changes in transcription, initiating a series of events that lead to lytic infection and latency. The infection begins with the release of the capsid and tegument proteins into the cytoplasm of the cell along with the ejection of viral DNA [44,69].

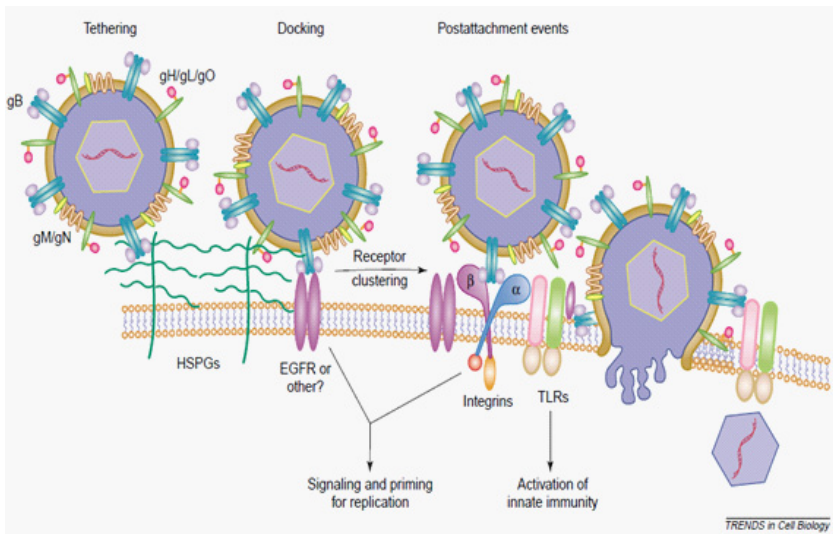


Figure 1: Mechanism of CMV infection in the host cell.

Source: Compton [27].

The phosphoproteins pp65 and pp71, located in the viral tegument, migrate to the nucleus of the permissive cell and initiate the infection [33,44]. The pp71 protein activates the expression of an immediate early (**IE**) cluster of genes, which initiates both lytic infection [43] and latent infection in the host [44,70]. The pp65 protein participates in the escape of the virus from the immune system, promoting viral latency by inhibiting natural killer (**NK**) cells on the infected cells [44]. This protein also plays an important role in the degradation of the alpha chain of HLA-DR and the phosphorylation of viral proteins of the immediate early stage, i.e., blocking the presentation of viral antigens to the MHC class I system [44,71,72]. Consequently, it is possible for the virus to remain in the cell for a long period of time without recognition by the host immune system.

Lytic infection occurs through three major events: 1) production of regulatory proteins, 2) production of DNA polymerase, and 3) production of structural proteins for the assembly of new viruses [46]. In non permissive cells, the infection is interrupted by blocking the expression of viral genes of the immediate early (**IE**) phase, preventing replication and simultaneously leading to latency [27,70]. However, these cells may permit the continuation of viral replication during cell differentiation; for example, monocytes are not permissive, but become permissive during the differentiation of the cells into macrophages [62], maintaining the replication and lytic phases of the virus [43].

DIAGNOSIS

In the absence or presence of clinical manifestations, the clinical assessment of infection requires a laboratory-based diagnosis. Specific laboratory tests can be performed considering

various aspects of pathogenesis, such as cellular changes, antigenic proteins, antibodies, and the viral genome.

Several laboratory methods are used to support the clinical diagnosis, including serological methods for the identification of IgG and IgM antibodies and direct or indirect immune fluorescence, which can detect anti-HCMV antibodies or viral proteins such as phosphor protein pp65 using the antigenemia technique [73,74]. Detection can also be performed using a method to isolate the virus; this method, while very laborious, is an efficient diagnostic approach for congenital infection, enabling the detection of the viral cytopathic effect [75-77].

With the introduction of molecular biology, it is possible to detect the viral genome in different stages of infection. DNA detection based on scoring of the viral load by quantitative reverse transcription polymerase chain reaction (**qPCR**) has also been widely used as a high-sensitivity method [78-80]. Analytical techniques based on these methods, such as restriction fragment length polymorphism (**RFLP**) and sequencing, have also been used and are important for determining the possible emergence of resistance to drugs used to treat CMV infection and to assist in therapeutic decisions [22,23,81].

Identification of viral antigens that cause cell changes and characterize the disease is also widely used for diagnostic confirmation. Microscopy results obtained with histological and immunohistochemical methods are essential for confirming the presence of infection in a specific organ or to further verify the cause of death [17,77,82-85].

Serologic methods are effective in the diagnosis of primary infection; however, when the patient has recurrent or subclinical infections, sensitive techniques that provide an accurate diagnosis, such as molecular or antigenemia methods, are essential for initiating quick, effective treatment protocols.

TREATMENT

Therapeutic strategies usually involve several options, including combinations of antiviral drugs, introduction of immune globulins, and reduction of immune suppression [23]. Selection of the drug to be used in the treatment depends on the severity and location of the disease, drug resistance, and degree of immunosuppression and on whether the disease is a primary infection or viral reactivation. The most commonly used antivirals in the intensive treatment of CMV infections are ganciclovir, forcarnet, and cidofovir [26].

Ganciclovir (**GCV-TP**) was the first drug licensed for the treatment of CMV infection and has been the preferred therapeutic approach in patients with immunosuppression. The drug is in an inactive form and needs to be phosphorylated into the triphosphate form by both viral and cellular kinases to be active against infection [25,86]. In this form, the drug is similar to guanine and competes for the binding site on the DNA polymerase with dNTPs that should be incorporated into the new viral DNA strand during the replication process; this process is prevented when

the drug is in the active form [87]. When the drug binds to the site of the polymerase enzyme, incorporation of nucleotides in the DNA strand will not occur, and growth of that strand is interrupted [25,86,88] (Figure 2). GCV-TP has been used in both the prophylactic treatment and preventive treatment of kidney transplant patients because 30-75% of patients without preventive or prophylactic treatment develop HCMV infections [89].

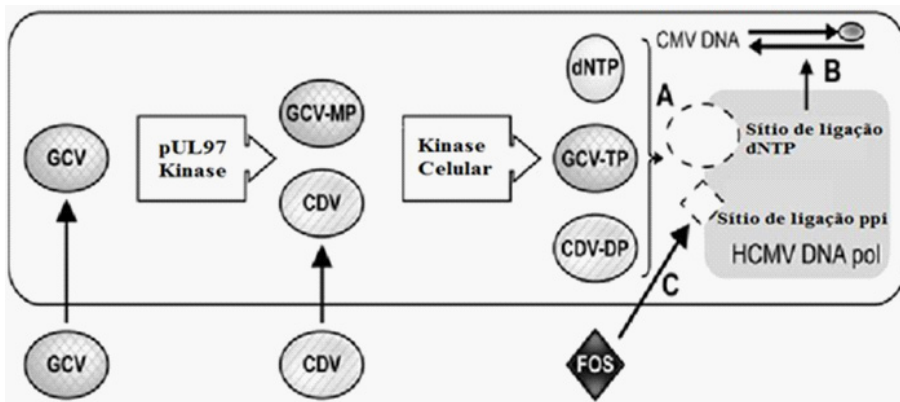


Figure 2: Schematic illustration of the phosphorylation of ganciclovir monophosphate into the triphosphate form, catalyzed by kinase pUL97, and binding of the drug to the viral DNA polymerase.

Source: Gilbert and Boivin, [86].

An alternative treatment is cidofovir, a drug that is similar to ganciclovir and requires phosphorylation into the diphosphate form by the viral kinase. Cidofovir also acts through a mechanism similar to that of ganciclovir, but is less tolerated by patients due to its nephrotoxicity [86,90]. Valganciclovir, a prodrug of ganciclovir that needs to be activated in the gut and liver, is another alternative treatment that functions through the same mechanism of action as ganciclovir [23]. This drug has been useful in the treatment of viral infections in renal transplant patients with clinical manifestations related to the pancreas and in clinical cases of retinitis in individuals with HIV/acquired immunodeficiency syndrome (**AIDS**) [89]. Table 2 shows the medication administration process for most drugs used in the treatment of CMV.

Table 2: Main drugs used in the treatment of viral disease.

Antiviral drug	Doses	Indications	Toxicity
Ganciclovir	10-12 mg/kg/day	Retinitis; active infection; congenital infection; specific organ diseases in immunosuppression and compromised CNS	Bone marrow suppression; leukopenia
Foscarnet	90 mg/kg/12/12hours for 14-21 days, then 90mg/kg/day	HCMV retinitis in AIDS; specific organ diseases in immunosuppression; resistance or intolerance to blood toxicity to ganciclovir	Renal and bone marrow toxicity; not recommended during pregnancy
Cidofovir	5mg/kg1x/week, followed by 2x week/15 days	HCMV retinitis in AIDS; specific organ diseases in immunosuppression; resistance or intolerance to blood toxicity to ganciclovir	Renal toxicity (drug metabolites)
Valganciclovir	900 mg oral route 2x/day	Treatment and prophylaxis in kidney recipients and specific organ diseases in immunocompromised patients; retinitis in AIDS	Bone marrow toxicity (suppression and leukopenia)

Source: Adapted from Schleiss [87] and Ramanan; Razonable [88].

Due to the toxicity and emergence of strains resistant to antiviral drugs commonly used for the treatment of CMV, other drugs that are less toxic and have other targets are often used as alternative treatment options. These drugs, including maribavir, artesunate, leflunomide, sirolimus, and letermovir, can be used alone or with ganciclovir when low activity or drug resistance occurs [90,91].

Maribavir is a benzimidazole L-riboside with low cellular toxicity in oral presentation and is beneficial for treating patients infected with strains showing crossresistance to more than one drug. This antiviral treatment prevents the release of the viral nucleocapsid core and HCMV DNA replication by inhibiting the binding of the viral kinase at the adenosine triphosphate (ATP) binding site [86,90,92].

Alternative treatments for patients who are resistant to ganciclovir include artesunate and leflunomide, both of which exhibit low toxicity, however, leflunomide must be combined with other antivirals because it has only 50% anti-HCMV activity [93]. This drug is an immunosuppressant agent, it may also act in the synthesis of pyrimidines and affect the activity of the kinase, thereby interfering with the assembly of the viral capsid [87].

Sirolimus is an immunosuppressive agent that has indirect antiviral activity and has been implicated in reduction of the viral load in HCMV infection in solid organ and bone marrow recipients with resistance to antiviral drugs. Although the mechanism of action is not known, it is believed that sirolimus inhibits the proliferation of infected cells due to activation of specific signaling pathways [90].

Letermovir is a newer drug that is a non-nucleoside exhibiting low cell toxicity and good tolerance; this drug is used to control viral infections during the later stages of replication and is a potent inhibitor of infections caused by CMV. The mechanism of action of letermovir involves inhibition of replication in the terminase subunit of the UL 56 gene; thus, this drug is quite useful

in clinical cases of strains simultaneously resistant to several antiviral drugs commonly used against CMV [91,94].

Immunoglobulins (**Igs**) are also used in prophylactic treatment and are considered highly effective; however, the use of Igs should be combined with antivirals in order to neutralize the infectivity of the virus through a mechanism of action that targets the glycoproteins of the viral envelope. Igs should be administered as follows: 100-150 mg/kg after transplantation, repeated every 2-4 weeks for 4 months [87].

Despite the availability of other drugs with different mechanisms of action, great care is needed for the therapeutic management of these patients because CMV strains may become resistant to various antiviral drugs used either alone or in combination, such as ganciclovir, foscarnet, and cidofovir [95,96]. According to Lurain and Chou [90], prolonged antiviral use, therapeutic subdoses, and recurrence of HCMV infection are factors that must be considered during treatment in order to prevent the occurrence of resistance to available drugs.

Vaccines may be the best alternative to control infection; however, those prepared with different viral proteins of the envelope and tegument did not show high efficacy for stimulation of the immune response in the human body for prevention of disease and viral transmission [41]; therefore, it is necessary to implement strategic priorities to prevent serious health problems, such as CMV infection in immunodeficient patients.

PERSPECTIVES

Because HCMV is an opportunistic agent and causes severe human diseases, it is necessary to evaluate the introduction of preventive and educational measures, with the creation of protocols that aim to control and reduce the impact of CMV infection, specifically in cases of congenital disease and postnatal patients at high risk of infection, including immune deficient patients. In such cases, CMV infection should be subjected to compulsory notification as more effective measures, such as vaccines, have not yet been developed.

While there are no prophylactic measures to control this disease, there is a need to provide alternative drugs with other mechanisms of action in cases in which resistance to ganciclovir occurs, particularly if ganciclovir is the only licensed drug for the treatment of CMV infection in the population.

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