

Chapter 2

Overview of Human Cytomegalovirus Pathogenesis

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Abstract

Human cytomegalovirus (HCMV) is a human pathogen that infects greater than 50 % of the human population. HCMV infection is usually asymptomatic in most individuals. That is, primary infection or reactivation of latent virus is generally clinically silent. HCMV infection, however, is associated with significant morbidity and mortality in the immunocompromised and chronic inflammatory diseases in the immunocompetent. In immunocompromised individuals (acquired immune deficiency syndrome and transplant patients, developing children (in utero), and cancer patients undergoing chemotherapy), HCMV infection increases morbidity and mortality. In those individuals with a normal immune system, HCMV infection is also associated with a risk of serious disease, as viral infection is now considered to be a strong risk factor for the development of various vascular diseases and to be associated with some types of tumor development. Intense research is currently being undertaken to better understand the mechanisms of viral pathogenesis that are briefly discussed in this chapter.

Key words Human cytomegalovirus, Viral pathogenesis, Immunocompetent, Immunocompromised, Vascular disease, Oncogenesis, Congenital infection, AIDS patients, Transplant patients

1 Introduction

Human cytomegalovirus (HCMV) is a prevalent infectious agent affecting the health of the human population. In a simple sense, HCMV pathogenesis can be broken down to that observed in immunocompetent hosts and that observed in immunocompromised hosts [1, 2]. HCMV pathogenesis in immunologically normal individuals is usually considered less severe when compared to the morbidity and mortality seen in immunocompromised individuals. Severe complications such as pneumonia, retinitis, hepatitis, encephalitis, and disseminated HCMV disease with multiorgan involvement are extremely rare in immunologically healthy people [1–4]. The majority of HCMV infections in the immunocompetent are asymptomatic [1]; however, primary infection can result in a mononucleosis-like syndrome [3]. In addition, data from both clinical and experimental studies now define a potentially strong role for HCMV infection in the development and/or severity of inflammatory

cardiovascular diseases, and HCMV infection has been linked to the development of certain types of cancers [2, 5–8].

In immunocompromised individuals, HCMV infection can result in severe disease [1, 2]. For example, in patients undergoing immunosuppressive therapies, such as in transplant and cancer patients, and in patients with acquired immunodeficiency syndrome (AIDS), HCMV infection is of significant clinical concern. In addition, HCMV is one of the leading infectious agents causing congenital infection [9]. Thus individuals with suppressed or underdeveloped immune systems are prone to severe disease following primary HCMV infection or reactivation of latent virus. This short chapter focuses briefly on some of the consequences of HCMV infection in the immunocompetent and the immunocompromised.

2 Pathogenesis in Immunocompetent Hosts

2.1 *Infectious Mononucleosis*

The most common clinical manifestation of HCMV infection in the immunocompetent host is a self-limiting febrile illness that resembles the infectious mononucleosis resulting from infection with Epstein-Barr virus (EBV) [3, 10]. The clinical picture of HCMV mononucleosis is typically indistinguishable from EBV mononucleosis, with the exception that pharyngitis, adenopathy, and splenomegaly occur less commonly with HCMV infection [1, 3, 10, 11]. In addition, HCMV mononucleosis is a heterophile-negative mononucleosis accounting for approximately 10 % of mononucleosis diagnoses [1, 3, 10]. Fever, malaise, myalgia, headache, and fatigue are the most commonly experienced signs and symptoms of the disease [1, 3, 10, 11]. A smaller number of patients can present with splenomegaly, hepatomegaly, adenopathy, and a rash [1, 3, 10, 11]. Laboratory tests commonly reveal lymphocytosis, activated or atypical lymphocytes, and abnormal liver function [1, 3, 12].

2.2 *Viral Role in Vascular Disease*

Mounting evidence suggests that HCMV infection is an etiologic/co-etiological agent in the development and/or severity of inflammatory cardiovascular diseases [7, 8, 13]. Beginning in the 1980s, several studies established a potential link between HCMV infection and the development of atherosclerosis [14–17]. A prospective study conducted from 1987 to 1992 examined medical records, including anti-HCMV antibody titers taken more than a decade earlier from patients with carotid intimal-medial thickening (IMT) and their age- and gender-matched controls [17]. Investigators observed higher anti-HCMV antibody titers in those patients with IMT than in the control group, consistent with HCMV infection being a possible factor/cofactor in the development of disease [17]. Additional serological studies identified a correlation between HCMV seropositivity and the severity of various cardiovascular

diseases, and HCMV nucleic acid and antigen have been detected in tissue sections taken from atherosclerotic lesions [16, 18]. A link has also been established between HCMV infection and coronary restenosis [19, 20]. For example, HCMV seropositivity and higher HCMV IgG titers were shown to correlate with the incidence of restenosis following surgical intervention to treat atherosclerosis [19]. Studies have also linked HCMV infection to transplant vascular sclerosis [8, 21, 22]; and it has been reported that patients with coronary artery disease have higher blood serum levels of C-reactive protein, a marker of the inflammatory response, that correlates with HCMV seropositivity, suggesting that inflammation resulting from HCMV infection may serve as a risk factor for vascular disease [23]. HCMV infects endothelial cells, smooth muscle cells, and monocytes *in vivo* and *in vitro*, all of which, when aberrantly activated, can directly contribute to the development of cardiovascular disease [24–30]. As an example, evidence shows that monocytes can migrate into arterial tissue, differentiate into macrophages, and engulf oxidized low-density lipoproteins, becoming the foamy macrophages that accumulate in arteries and atherosclerotic lesions [reviewed in ref. 25]. Because HCMV infection of monocytes has been shown to alter many of these processes [24, 31], it is plausible to hypothesize that viral infection of monocytes and the resulting biological changes in these cells contribute to atherosclerotic disease. The same idea holds true for HCMV infection of vascular endothelial cells and smooth muscle cells. Animal studies in various rodent models have demonstrated (essentially confirming Koch's postulates) that CMV infection is linked to endothelial damage, monocyte infiltration, foam cell accumulation, and vascular disease [30, 32–36]. Thus, both clinical and experimental studies have provided strong evidence that CMV infection promotes vascular disease at almost every stage of the disease process (enhancement of the proinflammatory response, vascular injury, increased migration and proliferation of smooth muscle cells, migration of monocytes into lesions, formation of foamy macrophages, plaque development, and other biological changes consistent with a role in vascular disease).

2.3 Possible Viral Role in Oncogenesis

The potential relationship between HCMV infection and cancer has been debated for decades, and during that time, HCMV has been argued to be associated with a variety of malignancies [6]. Seroepidemiological studies, as well as detection of viral nucleic acids and/or antigens in malignant tissues, are suggestive of an etiologic role for HCMV in the development of various types of cancers [6, 37–41]. However, it remains unclear whether HCMV is the causative agent of any of these cancers, as the virus has not been shown to transform normal cells and is not generally thought of as an oncogenic virus in the classical sense [6]. On the other hand, HCMV does possess many of the molecular hallmarks of the

small DNA tumor viruses (altering p53 and Rb function, inducing cellular proliferation, enhancing cellular survival, etc.), suggesting at least from a molecular standpoint that it could be an etiologic agent in tumor development and/or progression [42–46]. It has also been suggested that HCMV infection might produce an environment of a “smoldering inflammation,” which in turn could mechanistically promote oncogenesis in a similar manner to that defined for the hepatitis viruses [47]. In keeping with this idea, it has been proposed that “oncomodulation” could describe HCMV’s effect on tumor cells; in that HCMV could infect tumor cells and enhance their malignancy, thereby promoting tumor progression without being an oncogenic virus per se [48–51]. The body of evidence supporting a possible role for the virus in tumor growth continues to expand, with investigators focusing on both clinical and experimental aspects of HCMV cancer research. HCMV genome and antigen indicative of a persistent low level of replication have been detected in tumor cells, but not in the surrounding tissue, of a variety of malignancies including colorectal cancers [38], malignant gliomas [37, 41, 52, 53], prostate cancers [39], and breast cancers [40]. HCMV has also been proposed to be a co-etiological agent in the development of certain types of cancers [54]. A recent study aimed at establishing the clinical relevance of HCMV infection in malignant glioblastomas grouped patients based on the level of HCMV-infected tumor cells and uncovered a relationship between the level of infection and the patient’s life expectancy: patients with low-level HCMV infection outlived those with higher levels of HCMV infection [5, 55]. There are multiple molecular mechanisms, which are proposed to contribute to HCMV-induced oncomodulation. A recent review by Michaelis et al. provides a comprehensive description of the mechanisms by which viral proteins and noncoding RNAs alter the molecular and functional properties of HCMV-infected tumor cells [6]. Experimental evidence suggests that HCMV alters the cell cycle and inhibits apoptosis in infected cancer cells, thereby promoting proliferation and survival of the cells [6]. HCMV infection also appears to influence invasion, migration, and endothelial adhesion of malignant cells, potentially contributing to metastatic complications in HCMV-infected patients [6]. In addition, HCMV infection has been shown to promote angiogenesis, a process that is central to the initiation and progression of malignancies [6, 26, 56]. Infection with HCMV also diminishes cancer cell immunogenicity and causes chromosomal abnormalities in infected cells [6, 57]. Nevertheless, the relationship between HCMV infection and cancer is unclear/unresolved and remains an important question in the area of HCMV pathogenesis. Additional research is needed to define if and how HCMV infection modulates and/or is an etiologic agent in tumor initiation and/or progression.

3 Pathogenesis in Immunocompromised Hosts

3.1 *Congenital Infection*

Congenital HCMV infection occurs when the virus crosses the placental barrier allowing transmission of the virus from mother to baby. This process can occur following primary infection of the mother or from reactivation of latent virus in the mother [1, 9]. It is estimated that in developed countries, up to 7 % of seronegative mothers will develop a primary infection, and from this group, approximately 30–50 % will transmit the virus to the fetus [9, 58–61]. However, only around 10 % of congenitally infected newborns show disease symptoms [9]. If the woman was seropositive before conception, the risk of a newborn being congenitally infected is low and oscillates at about 1 % in developed countries (higher frequencies of infection are reported in developing countries), with a small number of children being severely affected by the virus [9, 60, 62, 63]. Congenitally infected babies can have a multisymptomatic disease affecting many organ systems and ranging from pneumonia, through gastrointestinal and retinal diseases, to central nervous system (CNS) diseases [64, 65]. Congenital HCMV may also manifest as a hematologic disease with, for example, thrombocytopenia and hemolytic anemia being commonly observed abnormalities [1]. Additionally, congenital HCMV infection may result in jaundice, hepatitis, hepatosplenomegaly, petechiae, and thrombocytopenia in the infected neonate. Although symptoms generally subside a few weeks after birth, the disease can be severe for some newborns, even leading to neonatal death in a small percentage of cases [9, 60, 64, 66]. Greater than 50 % of cases of symptomatic congenital HCMV infection present with abnormalities in the CNS [60, 62, 63, 67]. These abnormalities often cause a range of neurological symptoms, such as mental retardation, diminished motor skills, and hearing and/or vision loss [1, 9, 66–68]. CNS sequelae also present (~10 %) in newborns that are asymptomatic at birth for congenital HCMV infection and mainly cause hearing loss [9, 62, 69, 70]. Additionally, it has been documented that symptomatic congenital HCMV infection with a higher severity of disease occurs more often if the mother was exposed to a primary infection during pregnancy [62, 71]. However, because congenital infection can also occur in seropositive mothers [9, 72], it is not entirely clear if seropositivity per se diminishes the incidence of congenital infection. Based on the prevalence and severity of disease, congenital HCMV infection is considered as a leading cause of CNS damage in children [1, 9, 62].

3.2 *Infection of Infants*

An infant's immune system does not generally fully develop until approximately 6 months after birth. Thus, they have a lower ability to mount effective immune responses to pathogens. Nevertheless,

maternal antibodies transferred through the placenta and antibodies transferred through breast milk generally serve to protect newborns from infections during their early life.

In regard to HCMV infection, newborns can be infected in utero (as discussed above), via intrapartum transmission, or via consumption of breast milk containing the virus [67]. It is estimated that HCMV is shed in around 10 % of women from the vagina or cervix [1, 73, 74] and that the rate of intrapartum transmission from virus shedding mothers is around 50 % [75]. Human breast milk is also considered to be a common means for mother-to-infant transmission of viruses. The rate of newborn HCMV infection via this route strongly correlates with the length of time that the baby is nursed. It has been thought that approximately 40–60 % of breast-fed infants will be infected by HCMV at the end of their first year of life if fed by seropositive mothers [67, 76]. This high rate of transmission is consistent with studies that have documented that up to ~95 % of tested milk samples are positive for HCMV DNA [67, 77, 78].

Although full-term newborns usually do not present with significant disease if infected early in life, there have been reported cases of hepatomegaly, elevated hepatic enzymes, and inflammation of lung tissue [79–81]. The risk of complications, however, rises in preterm babies. For example, there is an association between HCMV disease manifestation and premature infants of seronegative mothers that received blood from seropositive donors. In these reported cases, symptoms suggested multiorgan dysfunction [82, 83]. In addition, it has been found that preterm neonates are infected at a higher rate than full-term infants if nursed by seropositive mothers [76, 78]. In preterm infants, the risks of complications associated with infection include thrombocytopenia, neutropenia, apnea, liver dysfunction, sepsis syndrome, and a mononucleosis-like disorder [78, 84]. Limiting preterm newborns to blood products from seronegative donors or by eliminating leukocytes from the transfused blood reduces the transfusion-acquired HCMV disease in these infants [83, 85, 86]. Although, the health-related issues resulting from HCMV infection via intrapartum or postnatal transmission are not usually associated with adverse outcomes in full-term babies, the role of infected infants in the epidemiology of HCMV spread is significant, as they can shed the virus for years, thereby enhancing viral transmission [1, 9, 61, 74]. This point may be especially relevant to pregnant seronegative women who have older children in day care, as it increases their risk of exposure to the virus.

3.3 Infection of Immunocompromised Hosts

HCMV is considered to be one of the most common opportunistic pathogens seen in immunocompromised patients. These patients are at risk for viral-mediated disease as a result of a primary infection, reinfection (of an already seropositive host), and reactivation of latent virus. It has been documented that the stronger the

suppression of the immune system, the greater the risk for HCMV-mediated disease [1]. Allogeneic stem cell transplant patients and AIDS patients are characterized as having the most severe disease manifestations. HCMV infection and disease are also seen in solid organ transplant and cancer patients undergoing immunosuppressive therapy [1]. Clinical manifestations in these patients can range from a short febrile illness to multiple organ system involvement.

Although the investigation of the impact HCMV infection has on immunocompromised patients is complex, studies performed on those patients have allowed a better understanding of HCMV infection, immune control of the virus, and viral-mediated diseases. For example, these studies have provided evidence about the reinfection of patients with new strains of virus [87–89], the importance of humoral and cell-mediated immunity in limiting HCMV infection [9, 90, 91], and evidence that a CMV vaccine may be efficacious [9, 92]. Additionally, the investigation and generation of new antiviral drugs has been influenced by the need for better management of HCMV infection in immunocompromised patients [93].

3.3.1 Infection of Transplant Patients

Complications resulting from HCMV infection of transplant patients significantly increase the overall cost and length of hospitalization for the patient [94, 95]. Viral reactivation is a common cause of HCMV infection in patients receiving solid organ or stem cell transplants [96, 97]. Usually, the virus is detected in the blood or excretions at around 4–8 weeks after transplantation [1, 98]. Nevertheless, primary HCMV infection of transplant patients is usually considered to show more complications than those arising in patients in which active virus is the result of reactivation of latent virus or reinfection (of a seropositive individual) [1, 96, 98, 99]. The most common symptoms of HCMV disease in solid organ transplant patients are fever, leukopenia, malaise, joint pain, and macular rash. However, more severe complications, such as pneumonitis, gastrointestinal ulceration, abnormal liver function, accelerated coronary artery disease, fungal and bacterial infection, and impairment of and/or rejection of the graft, have also been reported [1, 98]. The most common manifestation of HCMV infection in stem cell patients is an interstitial pneumonia that unfortunately has a high mortality (>50 %); mortality is still high in the presence of active antiviral treatment [96, 99–102]. Cases of gastrointestinal disease, hepatitis, encephalitis, and retinitis have also been described [96, 99, 102, 103].

The severity of complications caused by HCMV infection in transplant patients has set the stage for the improved management of HCMV disease. In general, pre-transplant donor and recipient screening as well as posttransplant screening for the presence of HCMV are performed, along with the preemptive and prophylactic administration of antivirals [96, 97, 99, 104, 105]. By having those protocols in place, the rates of HCMV disease and

infection-related deaths have been significantly reduced [103]. However, late-onset HCMV disease remains a significant problem in these patients and is responsible for the high mortality in these patients [97, 99, 101, 106–108].

3.3.2 Infection in AIDS Patients

HCMV infection is considered a leading opportunistic pathogen in AIDS patients and has been associated with progression of HIV infection [1, 9, 109–112]. The serological prevalence of HCMV is evident in nearly all adults and about half of the children seropositive for HIV infection [1]. It was estimated that approximately 40 % of adults and about 10 % of children with AIDS showed manifestations of HCMV disease before the introduction of highly active antiretroviral therapy (HAART) [1, 113, 114]. Common manifestations of HCMV disease in AIDS patients are retinitis, esophagitis, and colitis; case reports have also documented encephalitis, neuropathy, polyradiculoneuritis, pneumonitis, gastritis, and liver dysfunction [114]. Because of the use of HAART, incidence of each of these pathologies has significantly decreased in treated patients [9, 115–117]. Nevertheless, there is evidence that HCMV infection remains an independent predictor of morbidity and mortality in AIDS patients [111, 118, 119]. HCMV infection has long been attributed to the progression of HIV infection and morbidity in these patients, although mechanistically it remains unclear how HCMV may affect the outcome of HIV-infected patients (outside of HCMV's role as an opportunistic pathogen). Some examples for how HCMV may alter the course of infection include transactivation of the HIV promoter [109], changes in Fc receptor expression [110], altered immune activation [112], and increased T cell senescence [120]. Regardless, of the mechanism for how HCMV may affect the outcome of HIV-infected patients, it is very clear that these two pathogens possess a partnership and that even in the day of HAART, HCMV remains an important pathogen in AIDS patients.

4 Conclusions

HCMV remains an important pathogen of humans because of its high rate of dissemination in the human population and the multitude of disease pathologies caused by or associated with infection. HCMV-mediated disease can loosely be grouped into the diseases observed in immunocompromised individuals and the diseases observed in immunocompetent individuals. In immunocompromised people, HCMV infection can cause severe disease and affect a variety of organ systems. In healthy people, HCMV infection is generally thought of as benign; however, with the association of viral infection with cardiovascular diseases and now with several cancers, this idea of the virus generally being benign needs to be revisited and the virus thought of as a significant pathogen to all

hosts. Overall, HCMV is a complex pathogen with an interesting and diverse pathobiology. Additional studies into the basic aspects of the biology of the virus to the specific mechanisms that directly cause disease are needed. In addition, new treatment options and the generation of an effective vaccine are needed to counter the morbidity and mortality associated with infection.

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Human Cytomegaloviruses

Methods and Protocols

Yurochko, A.D.; Miller, W.E. (Eds.)

2014, XI, 368 p. 40 illus., 22 illus. in color., Hardcover

ISBN: 978-1-62703-787-7

A product of Humana Press