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Review

Cytomegalovirus (CMV): Virology, pathogenesis and immunology

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ABSTRACT

Cytomegalovirus (CMV) belongs to the *Betaherpesvirinae* subfamily of *Herpesviridae* viruses. A primary CMV infection, which usually occurs in childhood, is asymptomatic in immuno-competent individuals. Usually, following the primary infection, the virus cannot be fully eliminated from the host, thus resulting in latent infection of the host's tissues, mainly in myeloid precursor cells. Although such latently infected CMV seldom causes severe infectious disease in an immunocompetent state, it does provoke various types of inflammatory diseases, such as pneumonitis, retinitis, gastritis, and colitis, in immunosuppressed patients. It has been recently reported that latent infection and the reactivation process of CMV are closely associated with the differentiation state of immature precursor cells and chromatin remodeling of the latently infected cells. Considering that symptomatic CMV diseases are observed in such immunosuppressed patients as transplant and AIDS patients, it is true that the host's immunity must have a crucial role in the pathogenesis of CMV. In addition, some gene products derived from human CMV act to induce immune evasion of the virus from the host's CD8 T cells. Therefore, immunity is closely related to the pathogenesis and regulation of CMV. In this review article, the relationship between CMV and immunity is discussed using the research results of our group and addressing other related published manuscripts.

KEYWORDS: cytomegalovirus (CMV), innate immunity, adaptive immunity, congenital infection, neonatal infection

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INTRODUCTION

Cytomegalovirus (CMV) is a ubiquitous beta human herpesvirus type 5. Compared to other human herpesviruses, CMV is the largest, with a genome of 235kb encoding 165 genes [34], and is morphologically indistinguishable from other human herpesviruses. The CMV virion consists of a double-stranded linear DNA core in an icosahedral nucleocapsid, enveloped by the tegument [26]. These components are enclosed in a lipid bilayer envelope that contains a number of viral glycoproteins. In 1970, the first human CMV (HCMV) strain was isolated by Thomas H. Weller [177].

CMV can be transmitted via urine, saliva, sexual contact, placental transfer, breast-feeding, blood transfusion, solid-organ transplantation (SOT), or hematopoietic stem cell transplantation (SCT) [146]. CMV is highly prevalent in most populations (the Japanese population is 70% seropositive, and more than 90% of the population is seropositive in developing countries). Usually, CMV asymptomatically infects the host during childhood, and establishes life-long latency. CMV shows its pathogenic properties in immunocompromised hosts such as organ transplant recipients, patients with AIDS,

and cancer patients. Presently, CMV infection is mostly controlled in immunocompromised patients by available antiviral drugs. In addition, congenital CMV infection occasionally causes microcephaly, sensorineural hearing loss and mental retardation. Therefore, CMV infection is still a major health problem, thus warranting strong preventive measures.

The CMV genome contains a number of accessory genes. Most of them are engaged in immune evasion or inhibition of cell death, possibly resulting in a symbiosis between the virus and host. CMV, like all herpesviruses, undergoes latency and reactivation in the host. CMV has been shown to infect a broad spectrum of cells *in vivo* [149]. However, the only cells that are permissive for CMV replication *in vitro* are human fibroblasts. In these cells, virus replication results in the formation of intranuclear and intracytoplasmic inclusion bodies, with the former full of nucleocapsids and the latter containing several dense bodies. $CD34^+$ myeloid progenitor cells are considered as the site of latency. However, the molecular mechanisms by which CMV establishes and maintains latency and is reactivated still remain poorly understood.

Virology: Replication, transmission, latency, and reactivation

CMV attaches to target cells through nonspecific interactions with glycosaminolycans followed by specific interactions with high-affinity cell surface receptors. The cell surface receptors include integrins, the epidermal growth factor receptor, the platelet-derived growth factor-alpha receptor, and heparan sulfate proteoglycans. Binding of CMV glycoproteins to the cell surface initiates both virion entry and various host cell responses. After fusion and penetration of CMV, viral nucleocapsids containing infectious DNA are translocated to the nucleus by a pathway that appears to involve microtubular transport.

Once the virion DNA has entered the nucleus, the transcriptional program is initiated by cellular factors that activate the expression of viral immediate-early (IE) genes. The expression of IE genes leads to the sequential expression of the viral early (E) genes, which are required for viral nucleic acid synthesis, including the viral DNA-dependent DNA polymerase, and the viral late (L) genes, which encode virion structural proteins.

The assembly of the CMV virion include steps involving capsid formation, viral DNA packing, and envelopment that are likely shared by all herpesviruses. The pathway of virus assembly involves both nuclear and cytoplasmic steps. Within the nucleus, the viral capsid is formed as a 130-nm icosahedral nucleocapsid by scaffold formation. Viral DNA is synthesized as long chains of genome-length DNA that are cleaved into unit length genomes during packaging and capsid maturation. Capsids are thought to exit the nucleus by sequential budding through the inner and outer leaflets of the nuclear membrane, tegument, and eventually become enveloped in the cytoplasm. Mature virions have a diameter of 180 nm (Fig. 1, [109]). The release of infectious viruses is thought to occur either by exocytosis or following the death of host cells late in infection.

The tegument compartment contains the majority of the virion proteins, with the most abundant tegument protein being the lower matrix phosphoprotein 65 (pp65), called unique long 83 (UL83). The tegument proteins are important for the assembly of virions and the disassembly of the particle during entry. Another function is immunomodulation of the infection. The host cell endoplasmic reticulum-Golgi intermediate compartment-derived lipid bilayer envelope surrounding the tegument contains at least 20

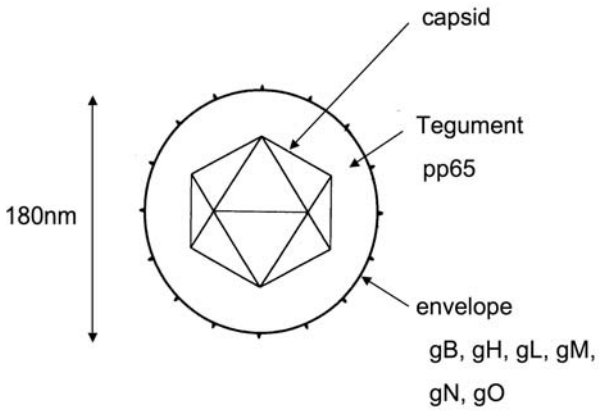

Fig. 1. The basic structure of human cytomegalovirus (HCMV).

virus-encoded glycoproteins that are involved in cell attachment and penetration [109]. These include glycoprotein B (gB, UL55 gene product), gH (UL75 gene product), gL (UL115 gene product), gM (UL100 gene product), gN (UL73 gene product), and gO (UL74 gene product) ([173], Fig. 1).

CMV transiently expresses a unique subset of viral genes in the absence of productive virus replication, and the virus does not initiate a productive infection within cells before the establishment of latency. However, the mechanism by which CMV establishes and maintains latency and is reactivated remains poorly understood. During latent infection, the CMV genome is estimated to be carried in 0.004-0.01% of mononuclear cells from granulocyte colony-stimulating factor-mobilized peripheral blood or bone marrow, with 2-13 genome copies per infected cell [153]. The exact site of latent infection has not been determined, but appears to be in cells of the myeloid lineage [148]. Genomic DNA can be detected in various cell types, including monocytes, macrophages [155], lymphocytes [143], $CD34^+$ bone marrow cells (105), immature dendritic cells (DC) [144], and endothelial cells [149]. CMV can enter the cell, but the transcriptional repression of the major IE (MIE) promoter suppresses the production of new virions [151]. The active viral replication is directly related to the state of cell differentiation. For example, differentiated cell types permit viral replication, but the undifferentiated cells are nonpermissive for viral replication ([151], Fig. 2). Nevertheless, nonpermissive cells do play an important role in the dissemination of the virus throughout the body.

Elucidation of the latency controlling factors will be required to restrict CMV disease. CMV-associated latency specific transcripts (CLTs), which are encoded within the MIE promoter region of the CMV genome, have been identified by experimental infection of granulocyte-macrophage progenitor cells [85, 86]. However, the functions of CLTs remain undefined. CLTs derived from the UL111.5A region, encoding a variant of the viral interleukin-10 (IL-10) homolog, have been detected within latently infected granulocyte-macrophage progenitor cells and in naturally infected bone marrow and granulocyte colony-stimulating factor mobilized blood samples [72]. The UL138 open reading frame (ORF) detected in latently infected $CD14^+$ monocytes and $CD34^+$ progenitor cells from CMV-seropositive donors was the first viral sequence proven to be functionally required for CMV latency [57]. Furthermore, a CLT that is antisense to UL81-82 of CMV has been identified [7]. The function of this CLT is the inhibition of the expression of the UL82 protein (pp71), which activates viral IE transcription and thus plays a role in initiating lytic infection [20].

It is well known that CMV reactivation can be detected in response to immunosuppression, inflammation, infection, or stress ([89, 113, 127], Fig. 2). The reactivation of CMV from latency is a key step in the pathogenesis of CMV infection. The mechanism of CMV reactivation has not been elucidated. However, tumor necrosis factor alpha (TNF-α) and cyclic AMP are considered to be key factors ([45], Fig. 2). TNF-α binds to the TNF receptor of latently infected cells, inducing the activation of protein kinase C and NF-κB and, subsequently, the transcription of the CMV IE genes, which triggers the onset of virus replication [126, 162]. The reactivation of CMV can also be achieved through stress catecholamines, epinephrine, and norepinephrine, which increase the concentrations of cyclic AMP, thus resulting in the stimulation of the IE enhancer/promoter [127]. In turn, proinflammatory prostaglandins stimulated in the course of various inflammatory processes also promote viral reactivation through the cyclic AMP pathway [84].

Pathogenesis

Infection of immunocompetent adults

Primary CMV infection in the immunocompetent adult is usually asymptomatic and rarely causes illness. Primary CMV infection is clinically indistinguishable from primary Epstein-Barr virus infection. The complications of primary CMV infections include arthralgia and arthritis, myalgia, lymphadenopathy, hepatomegaly, splenomegaly, ulcerative colitis, pneumonitis, hepatitis, aseptic meningitis, and myocarditis [51].

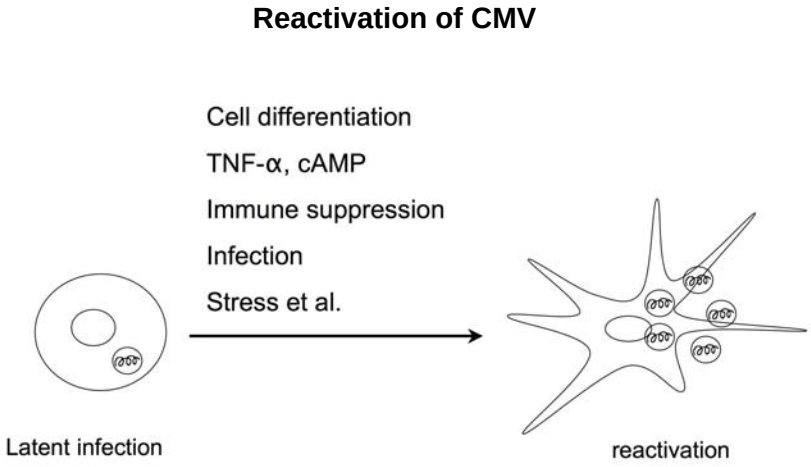

Fig. 2. Various factors affected upon the reactivation of CMV.

Congenital and neonatal infection

Congenital CMV infection causes severe morbidity and mortality in newborns and is the leading infectious cause of poor hearing and a large contributor to neuro-developmental abnormalities in infants [47, 134]. It is currently accepted that congenital CMV infection may be the consequence of either a primary or recurrent maternal infection [160]. The risk of primary infection in a seronegative mother is 1-4%, which carries a 30-40% risk of congenital infection [79, 158]. Recurrent infections may consist of either reactivation of the virus strain causing primary infection or reinfection by a new virus strain. The incidence of symptomatic congenital CMV infections in immune mothers has been shown to be similar in primary or recurrent maternal infections [14]. In addition, symptomatic congenital CMV infections appear to be mostly caused by reinfection in mothers with a new CMV strain during pregnancy [15]. On the other hand, congenital infections following reactivated, but not reinfected, maternal infections are almost always asymptomatic [159].

The rate of vertical transmission was found to be 0.2-2.2% in immune mothers undergoing recurrent infection during pregnancy and 20-40% in pregnant women with primary infections. Thus, the ratio of transmitting to nontransmitting mothers is about 1% between those with recurrent and those with primary infections. Transmission of CMV *in utero* may follow either primary or recurrent infections, due to the latency following primary infection and periodical reactivation of CMV replication causing recurrent infections [1, 142, 161]. It is commonly recognized that primary CMV infections are transmitted more frequently to the fetus and are more likely to cause fetal damage than recurrent infections [48]. In addition, it seems that primary infection occurring at an early stage of pregnancy is related to a worse outcome [39, 158].

About 10-15% of congenitally infected infants are symptomatic at birth, exhibiting intrauterine growth retardation, hepatitis with jaundice and hepatosplenomegaly, thrombocytopenia with petechiae, pneumonitis, and severe central nervous system damage with microcephaly, intracerebral calcifications, chorioretinitis, and sensorineural hearing loss [79, 99]. In symptomatic infants, a mortality rate of 30% has been reported, while many others display serious neurological, visual, and hearing impairment [99]. The majority of congenitally infected infants are asymptomatic at birth, however, 10-17% subsequently develop hearing defects or neurodevelopmental sequelae [51].

In the case of primary infection, the antiviral immune response begins with virus transmission to the fetus, whereas in the case of recurrent infection, virus transmission occurs in the presence of both humoral and cell-mediated immune responses. As a result, viremia occurs as a rule only in primary infections [132], whereas it

is either absent or undetectable in recurrent infections of an immunocompetent host [132], and is common in recurrent infections of immunocompromised patients [44, 73, 112]. Since, following primary CMV infection, intrauterine transmission occurs in approximately 30% of cases, an innate barrier seems to partly inhibit vertical transmission [39, 158]. Furthermore, a similar event seems to occur among infected newborns, as fewer than 15% of newborns show symptomatic infections in the great majority of cases resulting from primary maternal infection [1, 39]. Although existing immunity does not prevent transmission of the virus to the fetus, reactivated infections are less likely to cause damage to infants than primary infections [48]. Therefore, the risk of symptomatic congenital infections is even markedly lower in reactivated maternal infections [1, 158].

The mechanisms of CMV transmission to the fetus are still poorly understood. It has been reported that about 15% of female patients who experience a primary infection during the first months of pregnancy abort spontaneously, showing placental (but not fetal) infection [63]. Subsequently, placental infection has been shown to be consistently associated with fetal infection [111]. A better understanding of the mechanisms of CMV transmission to the fetus is thus required to elucidate the major steps during placental development. The development of the placenta requires differentiation of specialized epithelial stem cells, called cytotrophoblasts, in both floating villi and anchoring villi. Cytotrophoblasts fuse into multinucleate syncytiotrophoblasts covering the villous surface. The syncytiotrophoblasts are in direct contact with maternal blood, mediating transport of multiple substances to and from the fetus. Cytotrophoblasts also form cell columns and then invade maternal arterioles by replacing endothelial and smooth muscle cells, thus generating a hybrid cell population of fetal and maternal cells inside uterine vessels. Syncytiotrophoblasts upregulate the expression of the neonatal immunoglobulin G (IgG) Fc receptor, involved in the transport of maternal IgG to the fetus [91, 147]. The invading cytotrophoblasts initiate the expression of adhesion molecules, such as integrin $\alpha 1\beta 1$, and proteinases, which are required for invasion, in addition to molecules inducing maternal immune tolerance, such as human leukocyte antigen (HLA)-G [87] and interleukin-10, which suppress the activation of MMP-9 [135, 136]. In guinea pigs (gp), the placenta behaves as a reservoir in which gpCMV replicates prior to being transmitted to the fetus [92].

In the case of congenital CMV infection following recurrent maternal infection, it must be considered that the placenta is a hemiallograft inducing local immunosuppression in the uterus [46, 136]. This may cause reactivation of latent virus in the macrophages of the uterine wall, with CMV transmission to the invading cytotrophoblasts. Therefore, the virus can spread to anchoring villi and subsequently to the fetus [111]. CMV establishes a true latent infection in $CD14^+$ monocytes, which can be reactivated upon allogeneic stimulation of monocyte-derived macrophages from healthy blood donors [156]. Reactivation of latent CMV is dependent on the production of gamma interferon (IFN-γ) during the differentiation process [157].

In primary infection, leukocytes carry the infectious virus and transmit CMV infection to the uterine microvascular endothelial cells [53, 132]. These cells are in direct contact with cytotrophoblasts of anchoring villi invading maternal arterioles and hybrids of maternal-fetal cells. The infected cytotrophoblasts may transmit CMV infection to the underlying tissues of villous cores, including fibroblasts and fetal endothelial cells, thereby spreading the virus to the fetus [150]. In the case of primary maternal infection, infected maternal leukocytes transmit CMV infection to the villous stroma through breaches of the syncytiotrophoblast layer [66, 71]. Furthermore, another hypothesis suggests that maternal low avidity IgG-coated CMV virions are transported to the fetus by a process of transcytosis through intact syncytiotrophoblasts [46]. Finally, syncytiotrophoblasts may be directly infected, but the infection likely proceeds slowly and remains predominantly cell-associated until the infected cells can be eliminated during the normal physiological turnover [66, 154].

The diagnosis of primary CMV infection in a pregnant patient is usually made by detection of

seroconversion. The presence of CMV-specific IgM antibodies in pregnant patients is not related to primary CMV infection during pregnancy. The IgG avidity test may be of great help in both confirming and clarifying the clinical significance of the IgM antibody. When a primary CMV infection is diagnosed or suspected, a prenatal diagnosis should be offered to a pregnant woman to confirm whether CMV infection has been transmitted to the fetus. The detection of virus or virus products in the blood of the mother may further confirm or substantially support the diagnosis of a primary infection.

Infection in immunocompromised patients

CMV is a serious opportunistic infection in immunocompromised hosts such as human immunodeficiency virus (HIV)-infected patients and transplant patients on immunosuppressive medication due to their impaired adaptive immune system. A major risk factor for CMV disease in CMV-seropositive HIV-infected patients is a $CD4^+$ T cell count below 100 cells/μl. The incidence of CMV in HIV-infected patients has significantly declined with the use of highly active antiretroviral therapy (HAART) [117, 139]. Although the incidence has declined, CMV infection continues to be problematic for HIV patients, and it is suggested that CMV infection can directly or indirectly accelerate the progression to AIDS and death [60, 139, 177]. The most common symptom of CMV disease in HIV patients is retinitis, which accounts for 85% of all cases, and is characterized by hemorrhagic retinal necrosis [163, 182]. The availability of HAART has led to a new symptom, called immune recovery vitritis, associated with posterior segment inflammation. This occurs almost exclusively in patients with a previous history of CMV retinitis as the $CD4^+$ T cell count reconstitutes on antiretroviraltherapy [77]. Other symptoms of CMV-associated disease include enterocolitis, gastritis, esophagitis, hepatitis, and encephalitis.

In SOT and allogeneic SCT (allo-SCT) recipients, CMV is regarded as the most significant infectious pathogen. More than half of SOT recipients show evidence of CMV infection, with 10-50% of patients developing symptomatic disease, depending on the serostatus of the recipient and donor [137]. The matching of CMV-seronegative recipients to CMV-seronegative donors is ideal. The highest risk for CMV infection is the combination of a CMV-seronegative patient receiving an organ from a CMV-seropositive donor, with disease being more severe in this group of patients, due to their lack of a host-derived CMV-specific immune response [95]. Other risk factors for CMV infection include the type of organ transplantation [147], coinfection with human herpesvirus type 6 [41], and the type and intensity of the immuno-suppressive therapy, including the use of antibodies to T cell receptors (TCRs) [10, 123]. Clinically, acute CMV infection in the SOT patient causes fever, leukopenia, malaise, arthralgia, and a mascular rash, or as tissue-invasive disease, which presents as hepatitis, pneumonitis, enterocolitis, encephalitis, chorioretinitis, nephritis, cystitis, myocarditis, or pancreatitis. The diagnosis of CMV disease is made according to clinical signs and symptoms in conjunction with the detection of CMV in the blood and in the involved tissues [69]. In addition to causing end-organ disease, CMV has also been indirectly associated with a number of disease in SOT patients. These include graft rejection, renal artery stenosis, coronary artery stenosis, bronchiolitis obliterans, and vanishing bile duct syndrome.

CMV infections following SCT occur more frequently than in SOT patients due to a prolonged period of immunodeficiency and the potential for reactivation of latent viruses [17, 22]. Primary CMV infection develops in 30% of seronegative recipients, and reactivation of CMV occurs in 80% of patients who are seropositive before transplantation [97]. The risk of CMV infection following allo-SCT is affected by the patient's serostatus, age, source of donor stem cells, degree of HLA disparity, use of T cell-depleted grafts or anti-T cell antibodies, conditioning regimen, posttransplant immuno-suppression, time to engraftment, and prophylaxis for acute graft-versus-host disease [63]. The most common clinical symptom of CMV disease during the early SCT period are pneumonitis and enterocolitis. It was shown that late CMV disease developed in about 20% of patients after transplantation, with a mortality rate of 46% [12].

Immune responses against CMV

Innate immunity

The innate immune system plays an important role in the protection against CMV infection and in priming the adaptive immune response. CMV is a potent immunogen that triggers strong immune responses from all arms of the immune system. It is becoming increasingly apparent that CMV is subject to innate sensing by Toll-like receptors (TLRs). The stimulation of TLRs by CMV activates signal transduction pathways, which induce the production of inflammatory cytokines that recruit cells of the innate immune system, and the upregulation of costimulatory molecules such as CD80 and CD86, which are important for the activation of adaptive immunity [27]. In humans, CMV has been demonstrated to activate and signal through interactions with gB/gH and TLR2, which triggers inflammatory cytokine induction [13]. In mice, TLR9 and TLR3 have also been proven to be critical components of the innate immune system against MCMV. Once TLRs recoginaize viral compornents, their signaling pathways are activated, which leads to the production of IFN-α/β by DCs and macrophages. And subsequently, IFN-α/β is induced by natural killer (NK) cells (Fig. 3).

NK cells and IFN-α/β play a major role in the innate control of MCMV replication [141], and MCMV encodes several gene products which target these defenses [38, 93]. NK cells are an integral part of the innate immune response to CMV. Cmv1 contained in the NK gene complex on mouse chromosome 6 controls both the survival and viral titers in the murine spleen [140], and this resistance is mediated by the murine NK cell activation receptor, Ly-49H, contained in the NK gene complex [18]. In humans, NK cells are also critical for controlling CMV infection [11]. In renal transplant patients, NK activity was shown to increase during both primary and recurrent CMV infection, thus indicating that NK cells contribute to the recovery from CMV infection [174].

Adaptive immunity

Humoral immune responses

The humoral immune response plays an important role in the protection against CMV infection. The neutralizing antibodies to CMV restrict viral dissemination and limited the organ injury due to the disease (Fig. 4). The major target for neutralizing antibodies to CMV is gB and gH [16, 103, 129]. The gB protein is involved in cell

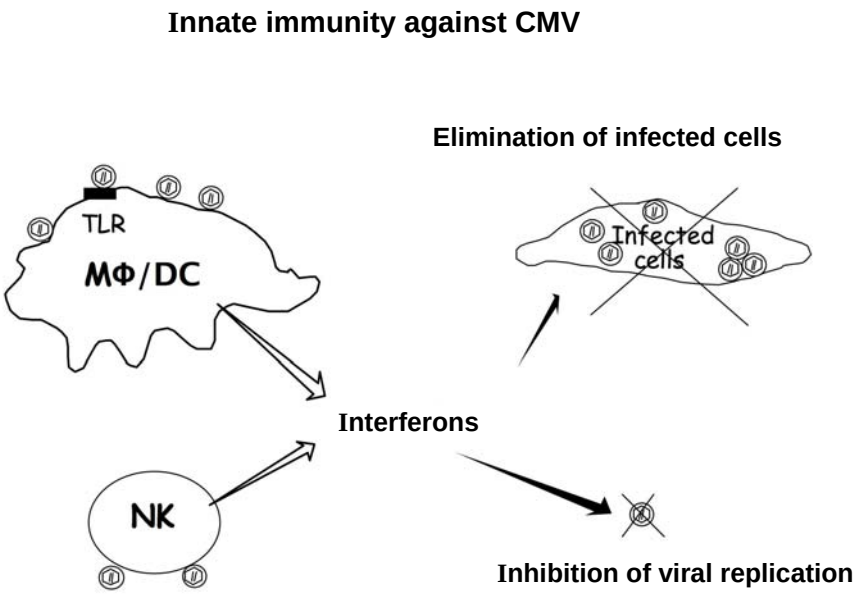

Fig. 3. Innate immunity against CMV.

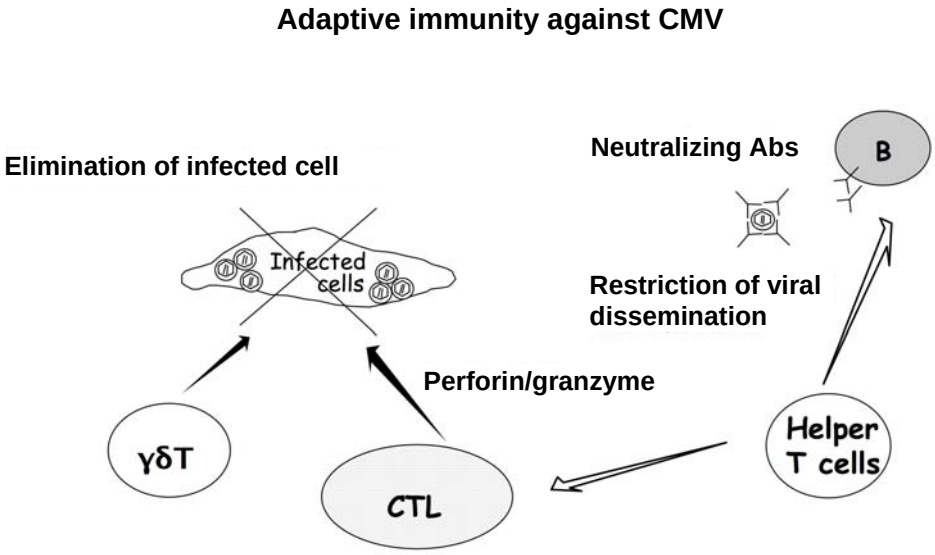

Fig. 4. Adaptive immunity against CMV.

attachment and penetration, and the gH protein is involved in the fusion of the viral envelope with the host cell membrane. The transfer of antibodies from a CMV-seropositive mother to a newborn infant was shown to be protective against CMV infection from seropositive blood transfusions [181]. It is well known that the ratio of vertical tranmission in a CMV-seropositive mother is lower than that in a CMV-seronegative mother.

T cell-mediated immune responses

The cell-mediated immune response is the predominant mechanism by which CMV replication is controlled. It is clear that γδT cells, $CD4^+$ T cells, and $CD8^+$ T cells are all important for controlling and restricting viral replication in hosts with persistent CMV infection.

The γδT cell subset comprises less than 6% of T cells in the blood of healthy humans, but represents a more substantial fraction of lymphoid cells in areas of the body exposed to the external milieu, such as the intestinal mucosa [37]. It has been demonstrated that γδT cells play an important role in host immunity to viral infections, including herpes simplex virus type 1 and MCMV [23]. An accumulation of γδT cells has been shown to occur in the salivary glands of MCMV-infected mice ([23], Fig. 4), while the depletion of γδT cells resulted in significantly increased MCMV titers [115]. In renal transplant patients, a significant increase in the levels of circulating γδT cells from 5 to 40% of total T cells was coincident with active CMV infection [36], and delayed γδT cell expansion was associated with prolonged and elevated antigenemias and increased severity of CMV disease [90]. It has been indicated that γδT cells specific for CMV have a potential crossreactivity, because CMV-specific γδT cells are cross-reactive against intestinal tumor epithelial cells [62]. Therefore, these data indicate that γδT cells are associated with the anti-CMV immune response.

The role of the major histocompatibility complex (MHC) class I-restricted $CD8^+$ T cell immune response against CMV is also clearly apparent (Fig. 4). In MCMV-infected mice, the selective depletion of $CD4^+$ T cells resulted in an increased incidence of recurrent MCMV infection [122]. $CD4^+$ T cells have been shown to contribute to the control of primary MCMV infection in mice that were long-term depleted of $CD8^+$ T cells before infection [76].

In healthy CMV-infected infants, prolonged viral urinary and salivary shedding is linked to a persistent and selective deficiency of CMV-specific $CD4^+$ T cell immunity. Low levels of CMV-specific $CD4^+$ T cells also correlate significantly with susceptibility to infectious

complications with CMV in lung transplant recipients. After renal transplantation, the clinical symptoms of CMV have been shown to be preceded by a decrease in the levels of CMV-specific $CD4^+$ T cells and an increase in viral load. In addition, the effector-memory $CD4^+$ T cells are necessary to control viral replication and for recovery after infection [50]. In bone-marrow transplantation (BMT) recipients, the presence of a detectable $CD4^+$ T-helper (Th) response has been associated with protection from CMV infection [64, 88]. Furthmore, recovery of $CD4^+$ CMV-specific Th cells is required for the endogenous reconstitution of $CD8^+$ cytotoxic T lymphocytes (CTL) and the persistence of adoptively transferred T cells [131]. The adoptive transfer of predominantly $CD4^+$ CMV-specific T cells lead to a significant reduction of viral load in allo-SCT patients [58].

An extremely high frequency of $CD4^+$ T cells in healthy seropositive individuals is committed to anti-CMV immunity. CMV-exposed individuals devote approximately 10% of their circulating $CD4^+$ memory T cell population to this virus. Analysis of the specificity of the CMV-specific $CD4^+$ T cell response has indicated apparent broad antigen recognition. Although gB-specific $CD4^+$ T cell responses are most frequently detected in healthy subjects, a large number of precursors specific for TRL14 and UL16 can be detected in a small number of individuals [166].

The role of $CD4^+$ T cells in latent infections has been considered to be indirect through the maintenance of virus-specific antibody responses and expansion of the $CD8^+$ T cell populations [33, 175]. However, the direct role of CMV-specific $CD4^+$ T cells was reported, and gB-specific $CD4^+$ T cells with cytotoxic activity from healthy seropositive individuals and pregnant patients have been expanded *in vitro* [42, 68]. Furthermore, the acquisition of direct cytolytic activity by pp65-specific $CD4^+$ T cells has been shown to occur as a function of the differentiation state [21]. Evidence for a direct cytolytic role for gB-specific $CD4^+$ CTLs *in vivo* was provided by a study where $CD4^+$ T cells directly purified from the blood secreted granzyme B, in response to glial cells expressing endogenous gB [65]. Characterizations of these gB-specific $CD4^+$ CTLs have identified the highly immunodominant peptide epitope, DYSNTHSTRYV, from gB, which is restricted through HLA-DRB [42]. In another study, TCR-Vβ13.1 $CD4^+$ T cells recognized the HLA DR7-restricted CMV-specific $CD4^+$ T cell epitope, DYSNTHSTRYV [31].

$CD8^+$ T cells are the most important component in the immune control of MCMV infection [122]. In human fetuses, mature and functional $CD8^+$ T lymphocytes have been shown to expand *in utero* in response to a primary CMV infection [101]. IFN-γ-producing CMV-specific $CD8^+$ T cells also appear to be protective against CMV-associated retinitis in HIV-infected patients [70]. A crucial role of $CD8^+$ T cells in the control of CMV infection was also confirmed in BMT patients. The development of CMV-specific CTL responses following BMT has been shown to correlate with protection and recovery from CMV disease [94]. It has been shown that more than 50% of patients lacking a detectable anti-CMV T cell response developed CMV disease [131]. In addition, the infusion of donor-derived CMV-specific $CD8^+$ T cells effectively restored antigen-specific cellular immunity in allogeneic BMT recipients, with the immune reconstitution coincident with protection from CMV-associated clinical complications in the recipients [133]. A similar observation of $CD8^+$ T cell immunity has been shown in the SOT setting.

The analyses of virus-specific T cell responses in renal transplant recipients demonstrated the presence of dominant $CD8^+$ T cell responses that may limit viremia and protect against CMV disease [128, 130]. In lung transplant recipients, the acquisition of CMV-specific $CD8^+$ T cell immunity, in addition to $CD4^+$ T cell immunity, was associated with both freedom from CMV disease and the preservation of allograft function compared with those who failed to develop CMV immunity [145]. Furthermore, in a study involving heart and lung transplant recipients, high frequencies of IE-1-specific $CD8^+$ T cells were shown to correlate with protection from CMV disease [19].

The specificity of the $CD8^+$ CMV-specific T cell response and the viral proteins to which they are directed has been comprehensively examined in

healthy CMV-seropositive donors. It has been shown that the $CD8^+$ or $CD4^+$ T cells are directed toward more than 70% of the ORFs, using overlapping from 213 ORFs and *ex vivo* T cell assays [100]. The $CD8^+$ CMV-specific T cell response is considerably diverse, with recognition of a variety of structural, early, and late antigens, in addition to CMV-encoded immunomodulators, including pp28, pp50, pp150 gH, gB, unique short 2 (US2), US3, US6, US11, UL16, and UL18 [43]. These studies, in combination with the data reported by other groups, revealed that these responses were directed toward CMV-encoded proteins expressed at different stages of viral replication (IE, E, and L) and also proteins associated with the capsid, tegument, glycoprotein, and immune evasion. It can be seen that the most of the immunodominant antigens to which CMV-specific $CD8^+$ T cells respond are directed include UL123 (IE-1), UL122 (IE-2), and UL83 (pp65). The majority of T cell studies have focused on IE-1 and pp65.

The pulmonary-infiltrating $CD8^+$ T cells can exert an antiviral effect after MCMV clearance [121]. We identified that activated T cells accumulated in the lungs of MCMV-infected mice even after the virus could not be detected, and IE1-specific memory T cells were detected at 6-12 months after MCMV infection [167, 168, 169]. In addition, in the germfree (GF) mouse, which lacked any microbiota, the ratio of IE1-specific memory T cells reached its peak at 1 month after MCMV infection, and thereafter decreased. In contrast, in the specific pathogen free (SPF) mouse, the ratio of IE1-specific memory T cells at 6-12 months after MCMV infection was higher than that at 1 month [169]. These results suggest that microbiota profoundly affected the expansion of memory T cells. In fact, when GF mice were reconstituted with microbiota, the ratio of IE1-specific memory T cells in the MCMV-infected GF mice returned to the level observed in MCMV-infected SPF mice [169].

Memory T cells to MCMV-IE1 can cross-reactively recognize several epitopes derived from other microbiota. This is referred to as heterologous immunity [178]. Kersh and Allen [80] reported that αβT cells could recognize not only a ligand of a peptide bound to a self-MHC, but also a slightly altered ligand, which thus leads to a partial activation of the T cells. It has been reported that T cells were widely cross-reactive, in fact, $CD8^+$ T cells cross-reactively recognize two proteins within the same virus, similar proteins of closely related viruses, and different proteins from unrelated viruses [104]. The cross-reactive epitopes did not require significant amino acid homology and have been noted even between the proteins of viruses and bacteria [67].

Immunological memory can be divided into two phases: a short-term phase, lasting weeks to months, followed by a long-term phase, lasting years when clonally expanded effector and memory T cells are redistributed to the host's nonlymphoid tissue [98]. The persistent restimulation might promote short-term memory, whereas long-term memory was thought to be maintained independently of antigen [98]. In our work, the IE1-specific memory T cells detected at 6 months after infection might be considered to work in a long-term memory phase, which was maintained by the microbiota.

A number of studies have examined the impact of chronic CMV infection on memory T cell homeostasis and the differentiation phenotype of antigen-experienced $CD8^+$ T cells. Various phenotypic markers, including CD45RA, CD45RO, CCR7, CD27, CD28, CD62L, and CD57, in addition to functional markers such as the expression of IFN-γ, granzyme, and perforin, have been commonly used to study the differentiation and effector functions of naïve and memory antigen-specific T cells. During acute CMV infection, the main $CD8^+$ effector T cell population shows a $CD45RA^-$ $CD45RO^+$ $CD27^+$ $CD28^{+/-}$ $CCR7^-$ phenotype, while in chronic CMV infection, two types of CMV-specific T cells appear to exist: $CD45RA^-$ $CD45RO^+$ $CD27^-$ $CD28^-$ $CCR7^-$ effector-memory and $CD45RA^+$ $CD45RO^-$ $CD27^-$ $CD28^-$ $CCR7^-$ terminally differentiated effector T cells [3, 50]. It has been shown that the adoptive transfer of CMV-specific $CD8^+$ T cells derived from central memory T cells, which express CD62L and CCR7, but not those derived from effector memory T cells, persisted long term in the blood, and migrated to lymph nodes and bone marrow [9].

Immune senescence

CMV infection might be considered as an important factor driving immune senscence in elderly. A high proportion of $CD8^+$ T cells is committed to the anti-CMV response. A median of 10% of the $CD8^+$ T cells in the peripheral blood of healthy CMV carriers and up to 40% of $CD8^+$ T cells in the peripheral blood of elderly persons can be specific for CMV antigens [32, 55, 81, 116]. This proportion is increased with increasing age. In addition, the $CD8^+$ T cell response to CMV is due to the accumulation of oligoclonal T cells and a reduction in the naïve T cell pool [35]. This increase in virus-specific CTLs, called memory inflation, is a phenomenon seen with the $CD8^+$ T cell immune response to MCMV [78] and was shown to extend to the CMV-specific $CD4^+$ T cell response [124]. The CMV-specific $CD8^+$ T cell expansions are consistently oligoclonal. And CMV-specific $CD8^+$ T cells have a highly differentiated effector memory cell phenotype [82]. These data suggest that CMV may significantly contribute to immune senescence, which is characterized by a reduction in the levels of naïve cells, the accumulation of clonally expanded CD28 memory T cells, and a decline in immune responsiveness [119].

TCR selection is a highly complex process influenced by the functional avidity of the antigen-specific $CD8^+$ T cells. The high avidity and efficiency of endogenous viral epitope presentation, in combination with the biophysical characteristics of the HLA-peptide complex, are the major determinants which offer a competitive advantage for selection of the antigen-specific $CD8^+$ T cells into the memory repertoire. Dominant CMV-specific clonotypes selected into the long-term memory pool have been shown to have high functional avidity, while subdominant clonotypes which were contracted following primary infection were characterized by substantially lower avidity [125]. In addition, clonotypes with restricted TCR usage demonstrated more efficient recognition of virus-infected cells and put up a terminally differentiated phenotype compared to T cells expressing diverse TCRs [180].

CMV is associated with a cluster of immune parameters referred to as the immune risk profile, which develops in person over 80 years old, and is predictive of decreased immune function and poor patient survival [119]. The degree of the cellular immune response to various pathogens has been investigated in donors of different ages, showing that immunity to the influenza virus and varicella-zoster virus decreases with increasing age [5, 40]. In addition to CMV seropositivity, the parameters of the immune risk profile include an inverted $CD4^+/CD8^+$ T cell ratio, an increased proportion of highly differentiated $CD8^+$ $CD28^-$ T cells, the presence of $CD8^+$ T cell clonal expansions, and reduced mitogen-stimulated proliferative responses [119]. Additionally, the apparent immunodominance by CMV may inhibit responses to other pathogens, because CMV seropositivity is a cofactor of the progression of HIV to AIDS and is associated with lower success rates for influenza virus vaccination [60, 138, 176].

Immune evasion

Multiple mechanisms of immune evasion for CMV could be related to the pathogenic role of the virus. Recently, the expression of immune evasion genes US3, US6, and US11 of CMV has been investigated in the blood of solid organ transplant recipients, showing that, after clinical recovery, transcripts of these genes remain detectable, indicating that persistent low viral activity may have implications for long-term control of CMV infection [59].

The inhibition of MHC class I-restricted antigen presentation plays a pivotal role on a major evasion mechanism [6]. An effective immune response to CMV is critically dependent on the generation of antigenic peptides, which can be presented in complex with MHC class I molecules to CTLs [30, 61]. During the IE phase of a CMV infection, a CTL response is directed against antigenic peptides derived from a 72-kDa IE-1 transcription factor. The matrix protein, pp65, which has kinase activity, can phosphorylate the IE-1 protein [54]. In turn, this response selectively blocks the processing and presentation of IE-derived antigenic peptides via the MHC class I pathway, and thus prevents an IE-1-specific CTL response. In addition, the CMV genome encodes five proteins, US2, US3, US6, US10, and US11, that block the generation and export of MHC class

I-peptide complexes and induce a rapid downregulation in MHC class I expression [2, 49, 74, 75]. Antigen presentation through the MHC class II pathway is also prevented by CMV through US2 targeting of the MHC class II DR-α and DM-α molecules for degradation by proteasomes and via the expression of proteins at the IE and E phases of a CMV infection (IE/E product), which interfere with the IFN-γ-induced expression of MHC class II molecules [106, 107].

NK cells selectively recognize and kill targets that lack cell surface-expressed self-MHC class I products [96]. This recognition is mediated by a complex balance of regulatory activating and inhibitory receptors on the surface of NK cells [118]. CMV-infected cells with downregulated MHC molecules would be expected to be vulnerable to NK-mediated lysis. However, CMV has responded by implementing various tactics to impede NK cell recognition, thus including the expression of virus-encoded MHC class I homologs to act as decoy proteins [108]. For example, the expression of the nonclassical class I molecule, HLA-E, is dependent on the binding of a signal peptide derived from other host MHC class I molecules, and suppresses NK cell recognition by binding the inhibitory CD94/ NKG2A receptor [170]. The UL40 gene product of CMV contains a sequence homologous to such signal peptides, which can substitute and upregulate cell surface HLA-E expression to protect virus-infected cells. CMV UL16 binds UL16 binding proteins (ULBPs) and also binds MHC class I chain-related gene B (MICB gene) [165]. The ULBPs are another family of ligands that activate the human NK cell receptor, NKG2D, and they have been reported to be upregulated in CMV-infected cells. UL16 can block the binding of NKG2D to ULBP1 and ULBP2, and to the MICB gene, consequently preventing the activation of NK cells [29]. Other mechanisms designed by CMV to evade NK cell killing include pp65 inhibition of the NK cell-activating receptor, NKp30 [4], a CMV UL122-encoded microRNA that downregulates MICB gene expression and subsequently reduces NK cell killing [164], the inhibition of NK cell-mediated lysis by CMV UL142 [179], and CMV UL141-mediated blocking of the surface expression of CD155 [171].

CMV also encodes a variety of other homologs with distinct subversive functions and which mimic the behavior of host proteins to divert the immune response. One such homolog is the human MHC class I homolog UL18, which, like MHC class I, binds β_2-microglobulin and peptides, but shows specific binding only with leukocyte immunoglobulin-like receptor 1, a receptor prominently displayed on monocytes and B cells [28]. The binding of leukocyte immunoglobulin-like receptor 1 to UL18 resembles the binding to MHC class I molecules [24]. However, the UL18 activity during viral infection remains unclear. In addition, CMV encoded homologs of seven transmembrane G-protein-coupled receptors, including UL33, UL78, US27 and US28 [25, 102]. US28 encodes a chemokine receptor that binds most human CC chemokines and CX3C chemokine fractalkin [52, 83, 114]. CMV also encodes a homolog of the immunosuppressive cytokine IL-10 (UL111a), a viral TNF receptor (UL144) [8], and a potent IL-8-like chemokine, which induces the chemotaxis of human peripheral blood neutrophils (UL146) [120].

Inhibition of apoptosis and necrosis

Programmed cell suicide (i.e. apoptosis) has the potential to serve as an effective strategy to restrict viral replication and spread at very early points in the first populations of infected cells. As a result, almost all viruses, including CMV, have developed strategies to block apoptosis at multiple levels. CMV encodes several gene products restricting the activation of both the intrinsic (i.e. mitochondria/Bcl-2-dependent) and extrinsic (i.e. death-receptor mediated) pathways, and these include UL36, UL37x1, UL38, and UL45 products in HCMV. M36, m38.5, M38, and M45, were also identified in MCMV. The UL36 and M36 proteins suppress caspase 8-induced apoptosis [152]. The product of the UL37x1 and m38.5 gene inhibits Fas-mediated apoptosis at caspase 8 activation and Bid cleavage, respectively [56]. The UL38 and M38 proteins have the potential to restrict ER stress-induced apoptosis [110]. UL45 encodes homologs of a ribonucleotide reductase, but the function of the UL45 protein is not clear yet. In the mouse, the M45 protein of MCMV contains both a receptor interacting protein homotypic interaction motif

(RHIM) domain as well as a ribonucleotide reductase homology domain. A functional M45 RHIM domain is required to block MCMV-induced endothelial cell death, indicating that MCMV infection triggers the initiation of necroptosis, but that M45 subsequently blocks it [172].

CONCLUDING REMARKS

We have discussed a number of the findings regarding the relationship between CMV and immunity, because immunity against CMV is closely related to the pathogenesis and regulation of CMV. As CMV infects more than half of the world population, a high priority for the scientific community is the development of an anti-CMV vaccine for use in combating congenital infection. The development of an effective vaccine for CMV-associated diseases remains a significant challenge. Significant advances both in the understanding of the immunobiology of CMV and in the diagnosis and treatment of CMV disease have been made. However, there are still many things that remain to be clarified with regard to the immune response to CMV. We hope that this review will allow for conceptualization of what needs to be accomplished for this purpose.

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