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Congenital Cytomegalovirus Infection: Molecular Mechanisms Mediating Viral Pathogenesis

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Abstract

Human cytomegalovirus (CMV) is responsible for approximately 40,000 congenital infections in the United States each year. Congenital CMV disease frequently produces serious neurodevelopmental disability, as well as vision impairment and sensorineural hearing loss. Development of a CMV vaccine is therefore considered to be a major public health priority. The mechanisms by which CMV injures the fetus are complex and likely include a combination of direct fetal injury induced by pathologic virally-encoded gene products, an inability of the maternal immune response to control infection, and the direct impact of infection on placental function. CMV encodes gene products that function, both at the RNA and the protein level, to interfere with many cellular processes. These include gene products that modify the cell cycle; interfere with apoptosis; induce an inflammatory response; mediate vascular injury; induce site-specific breakage of chromosomes; promote oncogenesis; dysregulate cellular proliferation; and facilitate evasion of host immune responses. This minireview summarizes current concepts regarding these aspects of the molecular virology of CMV and the potential pathogenic impact of viral gene expression on the developing fetus. Areas for potential development of novel therapeutic intervention are suggested for improving the outcome of this disabling congenital infection.

Keywords

Cytomegalovirus; congenital CMV infection; immune evasion; pathogenesis; vaccine

INTRODUCTION

Cytomegalovirus (CMV) infections are ubiquitous in humans and usually asymptomatic in the immunocompetent host [1, 2]. However, some populations are at risk for serious CMV-associated disease. The medical importance of CMV is most significant in immunocompromised hematopoietic stem cell and solid organ transplant patients; individuals with human immunodeficiency virus (HIV) infection; and pregnant women [3-5]. Of particular concern is the problem of congenital CMV infection. CMV is the most common congenital viral infection in the developed world, occurring in to 0.4-2.3% of all newborns, depending upon the population studied [6]. The overall birth prevalence of

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congenital CMV infection is estimated to be 0.64% but varies considerably among different study populations. Factors associated with an increased risk of congenital CMV infection include non-white race, low socioeconomic status, premature birth, and the maternal CMV seroprevalence rate in the particular population under study, with higher seroprevalence rates being associated with increased incidence of congenital infection [7]. The public health impact of congenital CMV infection is striking; more children suffer from long-term sequelae as a result of congenital CMV infection than either trisomy 21 or fetal alcohol syndrome [8]. Sequelae of congenital CMV infection are particularly severe for the developing central nervous system (CNS) and include mental retardation, cerebral palsy, neurodevelopmental disorders, and sensorineural hearing loss (SNHL) [9]. Symptomatic and disabling congenital CMV can follow both primary and recurrent maternal infection [10-12] but it is generally believed that primary CMV infections are transmitted more frequently to the fetus, and are more likely to cause fetal injury, than are recurrent infections [13, 14]. This observation has helped to drive the effort to develop a CMV vaccine that might, if effective in conferring robust immunity to women of childbearing age, be able to interrupt maternal-fetal transmission and the attendant sequelae of resulting infection [15].

The pathogenesis of fetal injury in the setting of congenital CMV infection is poorly understood. Pathology associated with acute CMV infection has been attributed to lytic virus replication, with ultimate end-organ damage occurring either secondary to virus-mediated cell death, or from host inflammatory responses targeting virus-infected cells [16]. Factors that contribute to fetal injury include the timing of infection relative to the gestational age of the fetus [17]; the maternal immune status [13]; the extent of CMV-induced placental injury [18]; the magnitude of the viral load in the amniotic fluid [19]; the induction of host genes (fetal and/or placental) that occurs in response to infection and the pathogenic effects these gene responses may mediate [20]; and, possibly, the genotype of the particular strain(s) of CMV infecting the fetus [21]. In recent years, considerable effort has been expended to explore potential mechanisms by which specific CMV gene products may mediate pathogenesis. This improved understanding of the molecular and cellular biology of CMV infection may, in turn, yield novel approaches for future therapies. In this minireview, the molecular virology of CMV is reviewed, with an emphasis on the current level of understanding of potential virally-mediated molecular mechanisms of injury at the cellular level. CMV-encoded genes that perturb the cell cycle, modify apoptosis, modulate the immune response, and interact with cellular function are discussed. Future prospects for targeted interventions, particularly those that might protect the fetus, are suggested.

CMV: BASIC VIROLOGY

CMV is a member of the *Herpesviridae* family of viruses. It is one of the 8 human herpesviruses (HHV) [22]. All of these viruses are double-stranded, DNA viruses; all are capable of inducing chronic or latent disease in the infected host; and all share similarities in virion morphology and genome organization. CMV, designated as HHV-5, is a member of the betaherpesvirus subfamily of the *Herpesviridae*; this subfamily also includes the roseolaviruses, HHV-6 and HHV-7, which are responsible for the clinical syndrome of *exanthem subitum* (roseola infantum) in young children [22].

Virion Morphology and Composition

CMV, like other *Herpesviridae*, is an enveloped virus with a double-stranded DNA genome. The three distinct regions of the CMV virus particle include: an icosahedral *capsid*; the *tegument* layer; and an outer lipid *envelope*. The morphology of CMV is demonstrated in the electron microscopy (EM) studies shown in Fig. (**1**). The capsid, which comprises 162 capsomere subunits arranged in an icosahedral symmetry, houses the viral genome, and is classically highly electron-dense when imaged by EM [23]. In the virus particle, the capsid

is surrounded by the tegument, a protein-rich layer containing several of the dominant targets of the T-lymphocyte response to infection, including a 65-kilodalton (kDa) phosphoprotein (pp) referred to as pp65 [24, 25]. Surrounding the tegument is the envelope layer. The envelope contains several virally-encoded glycoproteins (g), including protein complexes designated as the gB complex, the gM/gN complex, and the gH/gL/gO complex. CMV-seropositive individuals mount an immune response characterized by neutralizing antibodies that target these glycoproteins; hence, recombinant forms of these proteins have emerged as potential candidates for subunit vaccine development [15, 26-28].

In addition to serving as targets of the humoral immune response, these glycoproteins also play a central role in the binding and entry of CMV into cells. CMV employs at least two distinct, cell-type specific mechanisms of cell entry. Entry of CMV into endothelial and epithelial cells is mediated by endocytosis in a pH-dependent fashion; in contrast, entry into fibroblasts is non-endocytic, and pH-independent [29]. CMV fibroblast entry is believed to be initiated by binding of virion-associated gB to an as-yet incompletely characterized cell surface receptor, followed by fusion with the cell membrane in a process that requires a complex of three other glycoproteins: gH, gL, and gO [29]. In contrast to the model of CMV entry for fibroblasts, endocytic entry of CMV into endothelial and epithelial cells requires a complex of gH, gL, and three other proteins encoded by a region of the CMV genome referred to as the UL128-131 gene locus [30], a region of the genome discussed in further detail in the next section of this review. Emerging insights into the nature of these protein/cell surface interactions has resulted in development of new therapeutic strategies for prevention of CMV infection, based on vaccines and immunotherapies that could, in principle, block cell entry, particularly at epithelial surfaces where primary infections are typically acquired.

During the process of viral replication, a variety of types of CMV particles are generated. In cell culture, CMV infection leads to the assembly and release of, in addition to infectious virions, noninfectious defective particles termed “dense bodies” (DB), so designated because of their characteristic highly electron-dense appearance when imaged by EM. Another type of body, designated as a “noninfectious enveloped particle” (NIEP), is also generated during viral replication Fig. (**1**) [31, 32]. The structure and protein composition of NIEPs are comparable to those of virions, but they lack DNA and are therefore not infectious [31]. DBs are enveloped spherical structures that lack capsid proteins and DNA [32]. They consist mainly of viral tegument proteins and glycoproteins. In cell culture, the biology of DBs mimics that of infectious virus, since DBs enter cells efficiently and deliver their protein components intracellularly. Purified DBs are highly immunogenic when used to immunize animals, and sera from seropositive individuals are immunoreactive with these particles. For these reasons, DBs have been proposed as a novel CMV vaccine candidate [33]. In principle, DBs could induce a broad range of humoral and cellular immune responses to CMV tegument and glycoproteins, but, in contrast to live virus vaccines, would theoretically demonstrate a more favorable safety profile, given their lack of DNA and inability to replicate.

Recent work has demonstrated that CMV particles exhibit additional levels of complexity extending beyond the structural elements of the nucleocapsid, tegument, and envelope. Using CMV gene array technology, a novel class of viral RNA transcripts, termed *virion RNAs*, has recently been identified in infectious virions [34, 35]. These RNAs, which are packaged during virion assembly, are delivered to the host cell immediately on infection, potentially allowing viral gene products to be expressed in an infected cell before any viral transcription or host immune response occurs. The role of virion RNAs in pathogenesis is unknown at this time, but these transcripts may represent novel targets for therapeutics, such as anti-sense RNAs.

The CMV virion was initially characterized by analyses that were focused on identification of protein products that were, in turn, predicted by the nucleotide sequence of the virus. However, the advent of *proteomic* technologies has facilitated a more comprehensive analysis of the proteins present in CMV virions and DBs, and has yielded some surprising findings. A proteomic analysis of CMV virions using mass spectrometry was recently undertaken [36]. This study facilitated identification of viral and cellular proteins present in purified viral particles and dense bodies and allowed determination of the stoichiometry of the various protein species. A total of 71 CMV-encoded proteins and, intriguingly, over 70 *host* proteins were identified; interestingly, host proteins included cellular structural proteins, enzymes, and signal transduction molecules. DBs were found to contain only 29 viral proteins and reduced quantities of cellular proteins were present, compared to virions. These results underscore the complex interplay that exists between CMV and the cell it infects. More evidence for this complexity has been noted in recent spatial-temporal proteomic analyses that identified multiple cellular trafficking tracts involved in virion maturation, requiring host cell binding partners in the endosomal sorting complex and clathrin pathways [37, 38]. Considerable future work remains to characterize the virion proteome, to elucidate the process of virion assembly, and to define the relationship of the viral proteome to pathogenesis. Recently, an elegant analysis of protein-protein interactions contributing to CMV virion assembly was performed using yeast two-hybrid analysis. Over 1000 pairwise combinations for binary interaction were assessed in the two-hybrid assay, and multiple interactions involving tegument and capsid proteins were identified [39]. Continued examination of these protein-protein interactions should provide insights into the mechanisms of virion assembly and maturation, which in turn may yield novel intervention strategies.

CMV: VIRAL GENOME AND MOLECULAR GENETICS

Genome Structure, Organization, and Replication

CMV has the largest genome of any human viral pathogen. The CMV genome consists of approximately 235 kilobase pairs (kbp) of DNA, existing in a complex conformation [40]. The genome is organized as two regions of *unique* sequences, the unique long (UL) and unique short (US) areas, flanked by two sets of terminal and internal *inverted repeat* sequences (TRL/IRL) and (IRS/TRS) Fig. (**2**). The presence of these two unique regions bracketed by repeats allows generation of four potential isomeric conformations for a single unit length genome, depending upon the relative positioning of the unique segments during genome packaging into virions. This feature of CMV genome structure, in turn, likely contributes to the generation of genetic diversity among progeny virions following DNA replication.

By convention, CMV genes are designated numerically, left-to-right, based upon the sequence of open reading frames (ORFs) identified in the UL and US genome segments. Viral gene expression is complex and highly regulated through interactions between virally-encoded proteins and host cellular factors [40]. The replication cycle of CMV is divided temporally into three regulated phases: the immediate early (IE) phase, the early phase, and the late phase. IE messenger RNA (mRNA) is transcribed within the first few hours after infection of the cell and these transcripts encode IE proteins that modulate both host and viral gene expression, including transactivators of transcription of other viral gene promoters. Early gene products include DNA replication proteins and other structural proteins. Late gene products are typically transcribed at ~72 hours post-infection and include structural proteins and other proteins involved in virion assembly, structure, and egress.

Originally, the designation of CMV ORFs was based on the DNA sequence analysis of a highly laboratory-adapted strain of CMV, the AD169 strain [42]. One of the most important

advances in the study of CMV genetics came with the discovery that laboratory-adapted strains of CMV, such as AD169 and another lab-adapted strain, the Towne strain, undergo significant mutations, deletions, and rearrangements during the process of tissue culture adaptation [41]. In recent years, detailed DNA sequence comparisons of the Towne, AD169, and a less-attenuated strain of CMV, the Toledo strain, have been performed. The Toledo genome was discovered to contain a DNA segment of approximately 13 kbp that was not found in the Towne genome and a segment of approximately 15 kbp that was not found in the AD169 genome. These novel sequences were located at the unique long (UL)/internal repeat (IRL) boundary, within the L component of the viral genome, and contained multiple potential ORFs that were not present in AD169. This region of the genome was designated the *ULb' region*. A total of 22 previously-unrecognized ORFs (denoted *UL133* to *UL154*) were identified in this "wild-type" region of the CMV genome Fig. (**2b**). These observations suggested that the true number of genes carried by wild-type CMV was much higher than those originally identified by the sequencing of laboratory-adapted strains and that genes had been overlooked because of the large-scale deletion and rearrangement of the ULb' region that occurs during laboratory passage of virus isolates. The potential selective advantage driving the deletion of these genes during passage of virus in fibroblast cells is unknown. Subsequent studies have compared the patterns of genomic deletions and rearrangements present in the AD169 and Towne strains of CMV [44] and carefully analyzed the genomes of unpassaged clinical isolates. These studies have suggested that the true number of genes encoded by CMV is 165-173 [45-48]. The continued study of the molecular biology of low-passage "clinical isolates" of CMV is a high-priority area for future work. Increasingly, it is becoming clear that there are not only extensive sequence variation amongst CMV genomes, but also great functional variation in genome structure. These variations include genome size, inversion, orientation, and coding potential, and occur even within the most highly conserved genes. Some of the factors contributing to this divergence include nucleotide polymorphism, DNA strand composition asymmetry, variation in codon usage, and variability in the rate of evolution in conserved genes from strain to strain [49, 50]. The application of techniques that allow DNA sequencing to be performed directly from clinical specimens containing CMV [51], such as urine or saliva from a congenitally-infected child, should enable the collection of more authentic data regarding the CMV genome. This, in turn, is likely to yield new insights into the pathogenicity of heretofore uncharacterized virus genes.

A schematic representation of the CMV genome is illustrated in Fig. (**2b**) [46]. The *UL133-151* genes in this figure correspond to the ULb' region, the region of the genome that is subject to deletion and rearrangement during passage of CMV in cell culture. Interestingly, the laboratory-passaged strain, Towne, was used as a vaccine against CMV in a number of clinical trials prior to the complete characterization of its genome structure [15]. Its relative lack of therapeutic efficacy as a vaccine may relate, at least in part, to some of the mutations and genomic rearrangements that occur during tissue culture attenuation, possibly due to deletion of genes that encode proteins that are important in protective immunity. The putative role that many of the specific CMV genes and gene families play in pathogenesis is summarized in the following sections of this review. However, since the function of many CMV ORFs remains unknown, this understanding is, at best, a very incomplete one.

Molecular Mechanisms of Pathogenesis: Effects of CMV on Cell Biology

Injury to the fetus is mediated by a complex balance of the pathogenic effects of viral infection and the maternal immune response to infection. What is known about the potential role of *virally-encoded gene products* in mediating this injury? In the absence of an animal model to study congenital human CMV infection (discussed below), most studies have, by

necessity, been performed in *in vitro* systems, by definition in the absence of a compensatory immune response. The following section provides a brief overview of work from many laboratories that has advanced our knowledge of viral genes and pathogenesis, summarizing some of the *in vitro* models employed. In some circumstances the putative pathologic effect of a CMV-encoded gene is believed to be mediated directly by that gene product; in other situations, CMV infection leads to downstream effects on cellular gene expression that then may potentially lead to pathologic outcomes, as illustrated Fig. (**3**). In many circumstances, CMV gene products mediate more than one pathogenic effect and there is considerable overlap across these categories.

Modification of Cellular Apoptosis Mechanisms by CMV—Programmed cell death, or apoptosis, is a mechanism whereby damaged or infected cells are eliminated from tissue by an "auto-destructive pathway." The apoptotic process is essential for the elimination of damaged or poorly-developed cells during organogenesis and is also considered a critical defense mechanism to eliminate virus-infected cells from the host. Apoptosis plays a central role in embryogenesis. Spatial and temporal regulation of cellular apoptotic machinery is essential for normal development and homeostasis of the developing fetus in all multicellular organisms [52]. Thus, any impact of CMV infection on cellular apoptotic pathways could play a potential role in teratogenic events occurring during *in utero* infection.

Two predominant pathways mediate apoptosis in the mammalian cell [52, 54]: the *intrinsic pathway*, which triggers cellular sensor proteins such as p53 and initiates a cascade of biochemical signals leading to the mitochondrial release of cytochrome C, and the *extrinsic pathway*, which is activated by external signals primarily involving the immune system. Activation of the extrinsic pathway leads to phosphorylation of receptor death domains, as found in the tumor necrosis factor (TNF) receptor family and Fas, by their respective ligands. Apoptotic signals, both intrinsic and extrinsic, converge to induce the activation of the *caspase* proteases, leading to proteolysis of the cell architecture, metabolic derangement, genomic fragmentation, and, eventually, cell death.

CMV has evolved several mechanisms to delay the intrinsic apoptotic signaling pathway. The viral IE proteins, IE1 (IE72) and IE2 (IE86), are known for their ability to inhibit apoptosis [55]. CMV-mediated inhibition of late apoptotic events is associated with sequestering of the tumor suppressor protein, p53, by IE2 [56]. CMV encodes two other genes that suppress apoptosis in the late replication phase. *UL36* encodes an inhibitor of caspase 8 activation (vICA), and *UL37* (exon 1) encodes a viral mitochondria-localized inhibitor of apoptosis (vMIA) [57-59]. The vICA protein inhibits apoptosis by binding to procaspase 8 and preventing its activation to an active form, while vMIA inhibits apoptosis by interacting with Bax (a pro-apoptotic molecule) and sequestering it within the mitochondrial membrane in an inactive form, a process that requires association of vMIA with internal lipid rafts in the endoplasmic reticulum and mitochondria-associated membranes [60]. CMV replication requires expression of vMIA and deletion of the *UL37* gene in a recombinant virus can only be overcome by inhibiting cellular apoptotic responses using caspase inhibitors or by supplying other anti-apoptotic proteins to the cell [61]. Interestingly, an anti-apoptotic function has recently been attributed to another CMV gene product, but it is an RNA molecule, and not a protein, that mediates this effect. This RNA, the 2.7β (TRL4/IRL4) RNA, binds mitochondrial respiratory complex I, helping to maintain mitochondrial ATP production late in viral infection and preventing formation of death-induced reactive oxygen species [62].

Why does CMV encode such elaborate mechanisms to inhibit apoptosis? The presumed benefit of the anti-apoptosis mechanisms encoded by CMV may relate in part to the slow

replication cycle of the virus. Inhibition of apoptotic mechanisms presumably helps sustain this slow replication cycle and may promote the propensity of CMV to establish chronic or latent infection. The impact of inhibition of apoptosis for the fetus could be quite profound and this could help explain elements of CMV-induced teratogenesis, since apoptosis is, as noted, a critical element of normal fetal development and organogenesis. A better understanding of these mechanisms could conceivably lead to targeted cellular therapies to correct dysregulated fetal development. Establishment of improved animal model system to study CMV pathogenesis will also shed light on pathogenesis. CMVs are highly species-specific and human CMV will not infect laboratory animals [63]. Remarkably, it has recently been shown that viral modulation of apoptosis contributes substantially to the species-specificity of CMVs. If apoptosis is blocked, murine and rat cytomegaloviruses are able to replicate in human cells [64]. Mutations in another murine CMV coding region, the M112-113 locus, have also been shown to help override the interspecies barrier, apparently through mechanisms other than modulation of apoptosis [65]. Potentially, the continued elucidation of the mechanisms that dictate the species-specificity of CMVs could be of great value in developing future models in which human CMV could be used to infect animals - a breakthrough that, in turn, could allow preclinical efficacy evaluation of human CMV-specific vaccines and antivirals.

CMV Infection and the Cell Cycle—Like apoptosis, cell-cycle regulation plays a critical role in fetal development. This is particularly important for the developing CNS. Recent findings in rodents and primates indicate that regulation of the cell cycle, specifically of the G1 phase, has a crucial role in controlling area-specific rates of neuron production and the generation of normal neuron architecture [66]. Intriguingly, CMV infection has been shown in a number of studies to have a striking impact on cell cycle progression. In addition to their role in inhibiting apoptosis, CMV IE gene products have been shown to modify cell cycle progression. IE proteins, IE72 and IE86, as well as proteins encoded by the *UL82* and *UL69* genes, alter cell cycle progression in human fibroblasts by interacting with cell cycle regulatory proteins. In quiescent fibroblasts, CMV infection activates cell cycle regulatory proteins at early time points (6-12 hours) post-infection and accelerates cell cycle progression from G_0/G_1 to early S phase. At later stages of infection, when viral DNA replication is initiated, progression of cellular DNA synthesis is inhibited and the cell cycle is arrested at the G_2/M stage. After CMV-mediated arrest, chromosome segregation and cytokinesis are then blocked [67-71]. Recently, a role for the major tegument protein, pp65 (UL83 gene product) has also been suggested in the arrest of cell proliferation in G_1/S, since the accumulation of this protein in the nucleolus is very prominent in G_1 and G_1/S, but very minimal in S or G_2/M [72].

The virally-mediated inhibition of cellular DNA synthesis appears to be critically important for ensuring viral replication, since introduction of a mutation in the IE86 protein results in a recombinant CMV that is unable to block cell cycle progression. As a result, this recombinant virus replicates very poorly in cell culture [73]. What is the presumed benefit to CMV conferred by the presence of viral genes that modify the cell cycle? Modulation of cell cycle progression in infected cells may enable CMV to maximize the availability of the cellular DNA replication machinery, without competition from the cellular genome for the same resources [74]. This interplay between CMV and the cell cycle may impact the cell types infected or injured in the developing fetus. In the developing brain, CMV productively infects astrocytes and neural precursor cells, cell types known for their ability to undergo cell division *in vivo*. However, neurons, a terminally differentiated cell, are not permissive to productive viral infection [75].

Although CMV mediates cell-cycle arrest, the virus must also allow a mechanism that allows newly replicated viral genomes to egress from the nuclear lamina during viral DNA

replication, in order to complete the viral life cycle. To ensure that this occurs, CMV encodes a protein, the UL97 gene product, which mimics the cellular protein, Cdc2/cyclin-dependent kinase 1, a kinase that normally triggers the entry of cells into mitosis. This cellular kinase is normally responsible for breaking down the nuclear lamina during mitosis [76, 77]. Thus, the UL97 gene product provides a mechanism allowing the completion of viral replication, in spite of the overall effect of cell cycle arrest mediated by infection. The identification of the ability of the UL97 protein to break down the nuclear lamina identifies *UL97* as a potential novel antiviral drug discovery target, since inhibition of this process would greatly impair the ability of CMV to complete its replication cycle.

Impact of CMV on Chromosomes—One of the more intriguing observations in recent years regarding the impact of CMV infection on the biology of the cell has been the discovery that CMV infection may leads to *chromosomal breakage*. These studies provide a provocative hypothesis relating to the putative role of CMV as a teratogen [42, 78, 79]. In a study using human fibroblasts, infection with CMV during the S-phase of the cell cycle resulted in two specific chromosome 1 breaks, at positions 1q42 and 1q21. Purified virions, and not infected cell supernatants alone, were responsible for the effect, which could be blocked by co-incubation of virus with neutralizing antibody. UV-inactivated virus was as efficient as untreated virus in inducing specific damage to chromosome 1, suggesting a requirement for viral adsorption/penetration, but not viral gene expression [80]. The implications of chromosomal breakage or injury to the developing fetus are self-evident. Provocatively, in initial studies identifying injury to chromosome 1, two loci were identified near the break point that were of particular interest with respect to potential molecular mechanisms of CMV-induced birth defects: DFNA7 and USH2A. The DFNA7 gene has been linked to the inheritance of an autosomal dominant, nonsyndromic, progressive form of hearing loss [81]. Perturbation of the DFNA7 gene caused by CMV-induced breakage could conceivably be linked to the development of progressive SNHL. The USH2A gene, physically closest to the most prevalent CMV-induced break described in cell culture, encodes a protein important in the pathogenesis of Usher's Syndrome Type II, an autosomal recessive disorder responsible for both SNHL and blindness [82, 83]. Subsequent fine-mapping techniques pinpointed CMV-induced genetic damage to 1q23.3, within a maximal region of 37 kb. A breakpoint was noted between DFAN7 and another hearing impairment locus, DFNA49. This breakpoint was noted to be in close proximity to the MPZ gene previously shown to be involved in autosomal dominant Charcot-Marie-Tooth syndrome with auditory neuropathy [84]. These observations suggest a possible relationship between these chromosomal breaks and the CMV-induced sequelae of SNHL and visual impairment. To date, the precise CMV gene(s) responsible for these chromosomal breaks have not been identified. However, a recent study in stably-transfected cells suggested that some CMV-induced chromosomal aberrations could be attributed to a specific CMV gene, *UL76*, although the chromosome(s) targeted in these cells were not specified [85].

Impact of CMV on Cellular Metabolism—In addition to study of the viral proteome, mass spectrometry has been employed to examine the impact of CMV infection on the *cellular* proteome. In a study of the impact of CMV infection on cellular metabolism [86], mass spectrometry was employed to measure the levels of intracellular metabolites at multiple time points after infection of fibroblasts. These studies demonstrated that, as the infection progressed, the levels of metabolites involved in the glycolysis pathway, the citric acid cycle, and pyrimidine nucleotide biosynthesis markedly increased. Peak levels of multiple metabolites during infection far exceeded those observed during normal cellular growth and homeostasis, indicating that CMV induces a distinct metabolic program. In a followup study [87], a flux measurement approach based on mass spectrometry was used more precisely to quantify changes in metabolic activity following viral infection.

Significant increases occurred in flux through the tricarboxylic acid cycle and fatty acid biosynthesis pathway. Intriguingly, pharmacological inhibition of fatty acid biosynthesis in turn suppressed the replication of CMV. The concept of targeting the CMV-induced perturbations on cellular metabolism as an antiviral strategy was further validated in additional recent studies of the impact of infection on the glycolytic pathway. CMV infection was found to target calmodulin-dependent kinase kinase 1 (CaMKK1) expression, increasing the levels of CaMKK1 mRNA and protein. Inhibition of CaMKK severely attenuated production of viral progeny and blocked viral DNA replication [88]. Pharmacologic inhibition of CMV-induced fatty acid synthesis and/or activation of glycolysis may represent novel antiviral intervention strategies for control of CMV infection, and future metabolic profiling may identify additional metabolic targets that could be exploited for development of antiviral therapies.

CMV Genes Impact the Host Immune Response: Evasion and Inflammation—

Throughout the course of its co-evolution with the human host, CMV has developed a number of mechanisms that modify the interaction between the virus and the immune system. Many of these immune modulation genes are homologs of normal cellular immune effectors, and are believed to have been 'hijacked' from the host genome. Some of these gene products interfere with host innate and/or adaptive immune responses, toward a presumed goal of promoting immune *evasion*; others appear to play a pro-inflammatory role, seemingly to promote *inflammation* that, in turn, could facilitate widespread dissemination CMV in the infected host. These functions are summarized in Table **1**, and are briefly reviewed below.

Modulation of Cytokine and Chemokine Responses: The CMV genome encodes several proteins that have the functional of *cytokines*, as well as *chemokines* (CKs; low-molecular weight "chemotactic cytokines" with characteristic highly conserved cysteine motifs [89]). The best-characterized cytokine homolog is a CMV homolog of inter-leukin (IL)-10, encoded by the *UL111A* gene. Although the CMV IL-10 has only limited homology (27%) to human IL-10 [90] it can, like human IL-10, induce a potent anti-inflammatory response [91-93]. CMV IL-10 may, by down-regulating host inflammation, diminish the immune response to infection. The host immune response, in turn, may target CMV IL-10 as a countermeasure; it was recently demonstrated that 28% of sera from CMV-seropositive blood donors contain antibody to viral IL-10, and that these antibodies not only potently inhibited the binding of CMV IL-10 to cellular receptors, but also specifically inhibited CMV IL-10-induced signaling through the JAK-STAT kinase pathway [94]. CMV IL-10 may contribute to fetal pathogenesis by virtue of an effect on placental development, where it impairs trophoblast invasiveness; impaired placentation, in turn, may compromise normal fetal growth and development [95, 96].

In addition to the IL-10 homolog, CMV encodes two ORFs with homology to the "CXC" family of CK [89]: these are *UL146* and *UL147* [97]. Notably, these genes are encoded within the ULb' region of the CMV genome, a region (as already noted) that is associated with enhanced pathogenesis of the virus but is also subject to deletion and rearrangements during cell culture passage Fig. (**2b**) [41]. The functionality of these CKs and their role in the *in vivo* contribution to pathogenesis contributed by these ORFs is unclear, although the UL146 protein has been shown to be able to induce chemotaxis and intracellular calcium release in neutrophils [97]. It has been suggested that these proteins may function to promote neutrophil-mediated dissemination of CMV in the infected host. Other CMV genes have been noted to have homology to the CCCK family, in particular the *UL128* and *UL130* genes; however, the true function of these proteins appears to be related to their ability to form complexes with CMV glycoproteins that mediate CMV entry into endothelial and epithelial cells, and not through any intrinsic CK activity [30, 98].

Finally, as well as encoding cytokine and CK homologs, the CMV genome also encodes homologs of their cellular *receptors*. The best-characterized putative cytokine receptor is a member of the *TNF receptor* superfamily encoded by the *UL144* gene [98-100], another gene found in the ULb' region of the genome. The natural ligand for this receptor remains undefined, although the protein does up-regulate NF-kappaB expression and enhances expression of the CK receptor, CCL22 [101]. Since CCL22 attracts T-helper (Th)2 cells, UL144 could function as an anti-inflammatory effector, compatible with a role as an immunoevasive protein. CK receptors are members of the *G-protein coupled receptor* (GPCR) family [89] and there are at least 4 potential GPCRs encoded by the CMV genome: *UL33*, *UL78*, *US27*, and *US2*8 [102, 103]. Of these, the *US28* gene is the best studied [104]. US28 can bind ligands from multiple different CK classes and this, in turn, can trigger calcium mobilization, compatible with a putative functional role of US28 as a signaling molecule. However, the role of US28 in the virus life cycle or in pathogenesis remains unclear. US28 may exist to sequester host CKs (functioning as a "CK sink") in order to promote evasion of the immune response; alternatively, US28 may function to activate signaling pathways that contribute to viral replication or spread, compatible with a pro-inflammatory function.

Modulation of Innate Immunity: One of the most important effectors of innate immune responses to CMV infection is the *natural killer (NK) cell*. NK cells play a critical role in cytotoxic killing of virus-infected cells at very early time points post-infection. NK responses depend upon the relative contributions of inhibitory receptors and activating receptors expressed on the cell surface. CMV has evolved several genes that interact with NK-mediated pathways, exploiting both inhibitory and activating pathways. One of the first such identified proteins in HCMV, gpUL18, is encoded by the gene *UL18* and appears to exert its NK evasion effect by binding the NK cell inhibitory receptor, LIR-1, with a higher affinity than the host major histocompatability complex (MHC) class I molecule [105-107]. Recently, it has been shown that cells expressing another HCMV MHC class I homolog, the product of the *UL142* gene, are protected from NK cell lysis [108]. The NK evasion effect is related to the ability of gpUL142 to down-regulate an NK activating ligand, MICA, that is a ligand for the activating NK cell receptor, NKG2D [109]. A number of other CMV genes, include *UL40*, *UL83*, and *UL141*, also appear to function as NK cell evasins [110].

CMV also encodes a number of functions that *interfere with type 1 interferons* (IFNα and IFNβ), a critical aspect of the host innate immune defense against viral infection [111]. The IE1 (IE72) and IE2 (IE86) proteins interfere, through distinct mechanisms, with induction of transcription of IFN-responsive genes, and with activation of the IFNβ promoter, respectively [112-114]. CMV also expresses proteins that interact with IFN-induced gene products. Among the most effective of these immune evasion functions are associated with the proteins encoded by TRS1 and IRS1. These proteins interfere with the *protein kinase R* (PKR) IFN-induced antiviral defense pathway. PKR is activated by double-stranded RNA (dsRNA), which is produced during CMV genome replication. In the presence of dsRNA, PKR undergoes a conformational change, dimerizes, autophosphorylates, and then phosphorylates the α subunit of eukaryotic initiation factor 2α, leading to inhibition of translation initiation and of viral replication [115, 116]. The CMV-encoded proteins TRS1 and IRS1 are capable of binding dsRNA, hence promoting evasion of the PKR pathway of host innate defense [117, 118].

Evasion of Adaptive Immunity: Although innate immune responses represent the "first line of defense" against CMV infection, long-term disease control depends upon T cell-mediated immune responses, particularly cluster designation (CD)8^+ T cell responses. To counteract these responses, CMV encodes genes that prevent T cell control of infection through interference of both the MHC class I and class II antigen presentation pathways

[119]. Notably, most of these genes map to the US region of the CMV genome and have characteristics of glycoproteins. They are resident to the endoplasmic reticulum (ER) of the CMV-infected cell. The *US3* gene encodes a protein that binds MHC class I molecules and retains them in the ER, in the process preventing antigen presentation to $CD8^+$ T cells [120, 121]. The *US2* and *US11* gene products bind to MHC I molecules, leading to their translocation from the ER to the cytoplasm, where they undergo proteasome-mediated degradation [122, 123]. The *US6* gene encodes a protein that downregulates MHC class I expression [124, 125]. Down-regulation of MHC class I expression could make infected cells susceptible to NK cell mediated-lysis, which (as noted above) is a likely explanation for why CMV encode class I homologs as putative NK evasins [105-107].

Intriguingly, it has been suggested that MHC class I downregulation may play a role in fetal pathogenesis at the level of the fetal cytotrophoblast, the cell layer separating the maternal and fetal circulation in the placenta [126]. Fetal cytotrophoblasts do not express the "classical" HLA antigens, HLA-A and HLA-B, but express the "non-classical" class I HLA-G and HLA-C antigens, which inhibit NK-mediated cell lysis, in the process protecting the placental-fetal unit from being rejected as a foreign allograft. Notably, CMV US3 and US6 can down-regulate the cell-surface expression of both HLA-G and HLA-C in trophoblast cells; if such down-regulation resulted in increased NK lysis of placental trophoblast, this observation could provide a mechanistic explanation underlying CMV-related fetal injury [126]. However, when recombinant CMVs with deletions in these genes were tested in an *ex vivo* model of trophoblast differentiation and invasion, HLA-G expression continued to be down-regulated even in the absence of US3 and US6, implying that another viral function is responsible [127].

CMV-Encoded microRNAs: Like many viruses, CMV encodes a number of microRNAs (miRNAs). The miRNAs are approximately 22 nucleotide non-coding transcripts that mediate gene expression at the post-transcriptional level [128-130]. To date, approximately 15 miRNAs have been identified in CMV-infected cells. Many cellular processes, including apoptosis, cell cycle events, and immune response, are regulated by miRNAs. Recently, a novel mechanism of NK cell evasion was attributed to a CMV miRNA. As previously noted, one way in which CMV inhibits immune cell activation is by inhibiting NK cell receptor-activating ligands. One such example is the protein product of the *UL142* gene, which downregulates the activating ligand, MICA [109]. It was recently demonstrated that another NK cell activating ligand, MICB, is downregulated by CMV, and this effect is mediated by a miRNA encoded by the *UL112* locus [131-133]. Other CMV miRNAs appear to regulate viral gene expression and replication in cell culture [134, 135]. In rodent models of CMV infection, miRNAs play a role in tissue persistence and latency in the salivary gland, possibly mediated by an immunoevasive effect [136, 137]. Another study recently identified multiple cellular targets for a specific CMV miRNA, miR-US25-1. This miRNA was observed to bind target sites in the untranslated leader regions of many cellular mRNAs, in the process mediating significant reduction in gene expression. Interestingly, many of the genes targeted by this miRNA are associated with cell cycle control, suggesting a previously unrecognized mechanism by which CMV modulates the cell cycle [138]. Adding complexity to this rapidly emerging field of study is the observation that CMV not only encodes its own miRNAs, but also alters expression of cellular miRNAs, in the process facilitating viral replication in the CMV-infected cell [139]. Continued study of viral and host miRNAs is likely to lead to development of new oligonucleotide-based therapeutic approaches designed to modify the impact these sequences play on CMV replication and cellular physiology [140].

CMV-Encoded Molecular Mechanisms of Vasculopathy and Vascular Injury—

Another mechanism by which CMV infection may mediate injury to the host is through

infection of the vasculature. A number of vascular diseases have been hypothesized to be caused by CMV infection [141, 142]. Vascular diseases that have been associated with CMV include atherosclerosis, restenosis (typically occurring following coronary artery angioplasty), and transplant vascular sclerosis. The pathogenesis of these disease associations is uncertain. It has been hypothesized that these diseases are the result of mechanical and/or immune-mediated injury, followed by inflammation. Accordingly, CMV-encoded immune modulation genes have been implicated as potential mediators for endovascular injury and inflammation. This hypothesis holds that CMV infection plays a role in the migration of smooth muscle cells (SMC) from the vessel media to the vascular intima, which is followed, in turn, by cellular proliferation and inflammation. This sequence of events is theorized ultimately to lead to narrowing of the blood vessel, with the resultant compromise in tissue perfusion promoting pathologic consequences.

One CMV gene theorized to play a role in CMV-induced endovascular infection and injury is the product of the US28 gene. As already noted [104], US28 is a GPCR that interacts with a number of CK ligands. In functional studies, US28 mediates vascular SMC migration, compatible with its proposed role in CMV-associated vascular injury [143]. CMV-induced cellular proliferation may be driven in part by interactions between IE proteins and the p53 protein. Apoptosis of SMC can be induced by p53 but the CMV IE2 protein can protect these cells from p53-mediated apoptosis [144]. Moreover, the IE2 protein can also induce SMC proliferation and migration, suggesting another mechanism by which CMV infection and gene expression could promote a proproliferative effect [145]. CMV infection also stimulates the synthesis of IL-6, which upregulates the anti-apoptotic protein, survivin, in endothelial cells; this sequence may in turn play a role in neo-angiogenesis and in the acceleration of vascular diseases such as atherosclerosis and transplant vasculopathy [146].

Although these studies suggest mechanisms by which CMV infection could contribute to vascular injury and disease, what is the relevance to congenital CMV pathogenesis? Although most vascular diseases, particularly atherosclerosis, evolve over decades, vascular compromise could be of great potential importance for the health of the placenta and fetus. Vascular insufficiency due to CMV-mediated injury of endothelial cells could, in turn, compromise perfusion to the developing fetus or to the placenta, resulting in hypoxia and growth restriction. In an animal model of CMV pathogenesis, end-organ pathology in the setting of experimental infection was associated with significant disseminated vascular pathology, which evolved rapidly over a period of only a few weeks following experimental infection [147]. Vascular injury compromising perfusion of the fetal brain at critical windows of development is associated with the fetal brain disruption sequence, a syndrome characterized by multiple CNS anomalies. Conceivably, CMV-induced vascular injury could play a major role in this sequence; indeed, congenital CMV infection has been implicated as a causative factor in previous reports of this syndrome in the literature [148].

CMV-Encoded Molecular Mechanisms of Latency, Persistence and Oncogenesis—In addition to exploiting the immune response in such a way as to facilitate establishment of a *persistent* infection, cleared slowly (if at all) by the host immune response, CMV also appears to encode specific gene products that result in the establishment of a *latent* infection, resulting in a quiescent virus reservoir ready to be reactivated following appropriate provocative stimuli. The bone marrow appears to be the site where CMV latency is established [149], although the cell type where the latent viral genome is maintained remains incompletely characterized. Latent CMV genome has been described residing in both CD14+ monocytes and CD33+ myeloid precursor cells [150, 151]. Allogeneic stimulation has been successful in inducing CMV reactivation in CD14+ monocytes in the presence of T lymphocyte cytokines, providing strong experimental evidence for this cell as a site for latency [152, 153]. On the other hand, there is evidence for

latency-specific transcription from CD33+ progenitor cells, both in the context of *in vivo* and *in vitro* infection. Interestingly, these RNAs are transcribed in an “antisense” orientation relative to the IE transcription unit [154, 155].

In addition to these cell types, CMV also infects CD34+ hematopoietic progenitor cells and CMV DNA can be detected in CD34+ cells from healthy seropositive individuals [156]. Recently, an *in vitro* model to study CMV latency in these primitive hematopoietic progenitor cells was described [157]. In the context of this model, CMV gene expression in CD34+ cells during long-term bone marrow culture has been noted to be markedly different from that observed following infection in fibroblasts. Using this model, it was shown that low-passage strains of CMV, but not laboratory-adapted strains, could establish latent infection, suggesting that the ULb’ region of the CMV genome encoded a latency-associated gene. Further work demonstrated that this gene was the *UL138* gene. *UL138* encodes a ~21 kDa protein that localizes to the Golgi apparatus as an integral type I membrane protein, where it may contribute to latency by mediating some as-yet undefined aspect of protein trafficking [158, 159]. The implications of understanding the role this gene product plays in latency extend to the issue of congenital CMV, which is known to occur in women with longstanding immunity to the virus, due either to re-infection with a new strain, or reactivation of a latent strain [10-12]. Novel antivirals targeting this gene could be very valuable in preventing such reactivation events.

Many of the viral gene products that impact latency, cell cycle regulation, and cellular proliferation have been invoked in hypotheses linking CMV infection to *oncogenesis*. CMV has been linked to several malignancies, including colon cancer, malignant glioma, Hodgkin’s lymphoma, and cervical and prostate cancer [160]. As previously noted, CMV IE2 protein interacts with the tumor suppressor protein, p53 [144]. Given the central role of p53 in the pathogenesis of many human cancers [161], these interactions suggest molecular mechanisms by which CMV could trigger malignant transformation of cells. Notably, an interaction of the CMV UL97 gene product with another cellular tumor suppressor protein, the retinoblastoma gene product (pRB), has recently been identified [162]. This interaction is of potential interest with respect to oncogenesis, given the wellknown interaction of the human papillomavirus type 16 (HPV-16) E7 gene with pRB, and the role that this interaction plays in the pathogenesis of cervical cancer [163]. Interestingly, HPV-16 E7 can complement a laboratory-generated mutant of CMV from which the *UL97* gene has been deleted [164], suggesting that these disparate viruses have evolved similar genetic mechanisms to exploit and modify this regulator of cellular proliferation.

Other CMV genes have been implicated in the molecular pathogenesis of malignancy. These include the UL82 gene product, which binds to pRB and degrades it, stimulating cellular DNA synthesis [165, 166]. One of the first events following CMV infection in cell culture is the induction of proto-oncogenes, such as *c-myc*, and it has been postulated that this event could play a role in the pathogenesis of CMV-induced cancers [167]. Transforming activity has been mapped to other regions of the CMV genome. Within one such region, referred to as the mtrII locus, a 79 amino acid ORF was identified that was essential for maintenance of the cellular transformation phenotype [168, 169]. Subsequent studies demonstrated that this ORF was, in fact, the first exon of the CMV IL10 homolog [68]. Although the issue of whether CMV is an important co-factor in the pathogenesis of malignancies remains unsettled, these putative oncogenic/transforming gene products could be relevant to the problem of congenital CMV infection. It is possible that some of the putative mechanisms of *oncogenesis* could play a role in *teratogenesis* of the CMV-infected fetus, although to date there is no direct evidence that this is the case.

Virus Strain Variation—Given the plethora of putative pathogenesis determinants encoded by CMV, it is remarkable in many ways that the majority of congenitally-infected infants are *not* damaged by infection. As noted, the factors that impact the likelihood that an *infected* infant will be *affected* by CMV include: the timing of infection *in utero* [17]; the maternal immune status [13]; the status of the placenta [18]; and, possibly, the viral load in the maternal and fetal compartments [19]. Another variable that may impact the prognosis of congenital infection may be *viral strain variation*. It has been hypothesized that some clinical strains of CMV are intrinsically more harmful to the fetus, based on variability observed in genes implicated in viral pathogenesis. Experimental evidence to support this hypothesis is conflicting. Some subtypes of CMV classified on the basis of their *UL144* (TNF receptor homolog) sequence were described as being more likely to be associated with symptomatic disease in newborns [21], irrespective of the viral load. On the other hand, three subsequent studies were unable to confirm any association with *UL144* genotype and the outcome of congenital infection [170-172]. No differences in clinical outcome could be attributed to variants of the CK homologs, *UL146* and *UL147*, when genotypes based on variation in these genes were compared in congenitally-infected infants [172, 173]. When genotypes based on the sequence heterogeneity observed in the envelope glycoprotein gene, gN (*UL73*), were compared, congenital infection with one genotype, gN-1, was statistically associated with an improved prognosis with respect to long-term neurodevelopmental sequelae [174]. In another study of infants with congenital infection, the distribution of genotypes for the gB glycoprotein gene (*UL55*) showed significant differences, depending upon the disease classification observed, but no information was reported on long-term neurodevelopmental sequelae [175]. A report of the use of artificial neural network technology for predicting the outcome of congenital CMV infection on the basis of sequence data from four genes identified specific nucleotide sequences that were highly predictive of outcome [176]. If it can be confirmed that virus strain variation has a substantive impact on clinical outcomes in congenitally-infected infants, important implications for vaccine design could emerge. The application of high-throughput techniques for sequencing CMV genomes directly from clinical specimens (and hence obviating the need for passage of viral strains in tissue culture prior to sequence analysis) should aid in resolving this unanswered question [51].

Animal Models of Congenital Infection and Fetal Pathogenesis—Although *in vitro* systems have documented many putative mechanisms by which CMV infection interferes with cellular gene expression and cell biology, the authentication of these effects as critical events in the *in vivo* pathogenesis of congenital CMV has been difficult to establish. The primary challenge, as noted, is the species-specificity of CMVs, which makes it unfeasible to evaluate the pathogenesis of human CMV in an animal model [63]. This limitation has been overcome to some extent in studies of transgenic mice expressing the human CMV major IE promoter sequences as a transgene driving expression of a reporter gene, *lac Z*. In these studies, the tissue distribution of IE-mediated gene expression was defined in the developing CNS; target sites of gene expression included the cerebral cortex, cerebellum, and eighth cranial nerve [177]. Human CMV replicates in fetal thymic explants maintained in SCID/hu mice and this has been exploited as an *in vivo* model for testing antiviral therapies [178].

Although these experimental approaches have been useful, they do not authentically model the pathogenesis of CMV infection. Studies of *bona fide* pathogenesis, therefore, have typically required the study of species-specific CMVs in their respective animal hosts. The pathology of fetal infection with rhesus macaque CMV mimics that observed in infants [179], although the cost of primate studies limits the practical usefulness of this model. Among rodent models, murine cytomegalovirus (MCMV) has provided a useful model system in which to study fetal neuropathogenesis [180, 181] and teratogenesis [182, 183]. A

limitation of MCMV, however, is its inability to cross the mouse placenta and establish infection of the fetus. In contrast to MCMV, the guinea pig CMV is capable of establishing fetal infection, and this model has been valuable in the study of CMV vaccines and antiviral therapies, as well as the pathogenesis of fetal infection [184]. A key advance in the study of pathogenesis of CMV in all such models has been the ability to clone and maintain CMV genomes as infectious bacterial artificial chromosomes in *Escherichia coli* [185-187], allowing the powerful mutagenesis techniques available in prokaryotic systems to be applied to the generation of recombinant viruses with key mutations in pathogenesis genes. This approach has allowed the validation of the *in vivo* importance of many putative pathogenesis genes in animal models [188, 189] and this information in turn should help direct future, novel interventional studies designed to prevent CMV-associated injury in the newborn infant.

SUMMARY AND PERSPECTIVE

In summary, CMV has evolved highly complex sets of functions that have a profound effect on the biology of the cell and, presumably, on the human host. These effects, remarkably, are mediated not only by transcription of genes that modulate metabolism, senescence, cell cycle control, persistence, oncogenesis, and immune functioning, but also are mediated by regulatory and structural RNAs. From these experimental observations, hypotheses have been generated regarding the molecular pathogenesis of CMV-related injury and teratogenesis in the developing fetus. These include disruption of normal developmental pathways due to modifications of apoptotic and cell cycle pathways, altered perfusion of developing organ systems secondary to CMV-mediated endovascular injury, teratogenic CMV-mediated chromosomal injury, placental insufficiency with its attendant fetal compromise, and others. In spite of these remarkable advances in our understanding of how CMV interacts with the host at the cellular and molecular level, the precise mechanism(s) of CMV-related pathogenesis for the fetus remain largely unknown. Even less clear is what interventions can be anticipated to be of benefit to improve the outcome for the fetus, as evidenced by the fact that no randomized controlled trials exist, to date, supporting the use of any therapeutic interventions aimed at preventing CMV transmission or improving CMV-related outcomes for the fetus [190]. Even as much-needed vaccines and antivirals move forward in clinical trials, there is an important need to develop better model systems to study CMV-mediated injury of the developing fetus. Insights from such studies will in turn lead to novel interventions to help address this major public health problem.

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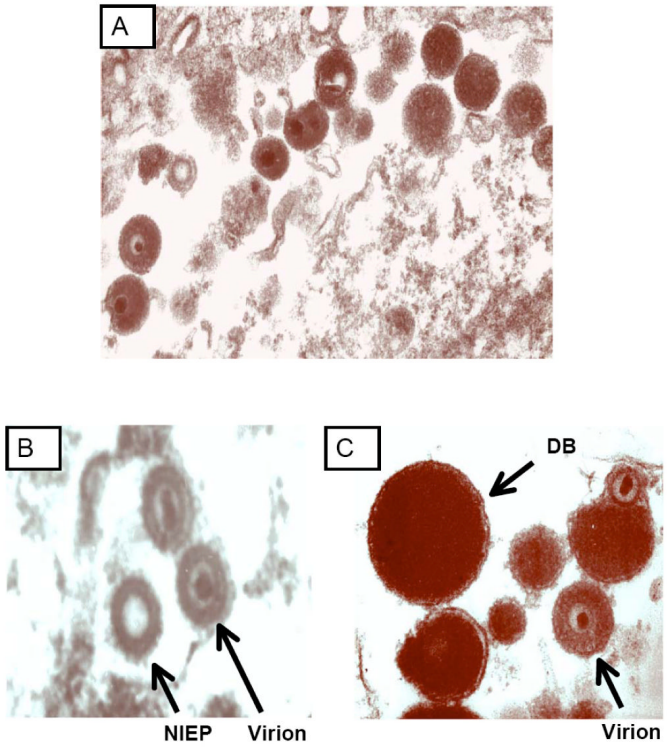

Fig. (1).

Electron microscopic visualization of morphology of cytomegalovirus particles. Human foreskin fibroblasts were inoculated with the Toledo strain of cytomegalovirus and, following 7 days of incubation, cells were fixed with paraformaldehyde, stained with phosphotungstic acid, and visualized by EM (University of Minnesota). **A**) Image demonstrating cell-associated virus particles [virions and dense bodies (DB)]. **B**) Comparison of mature virion and non-infectious enveloped particle (NIEP, arrow), which contains capsid proteins but no DNA and is hence replication-defective. **C**) Comparison of virions and DB (arrow). In contrast to virions, DB are larger, contain no viral DNA and no capside proteins, and are non-infectious.

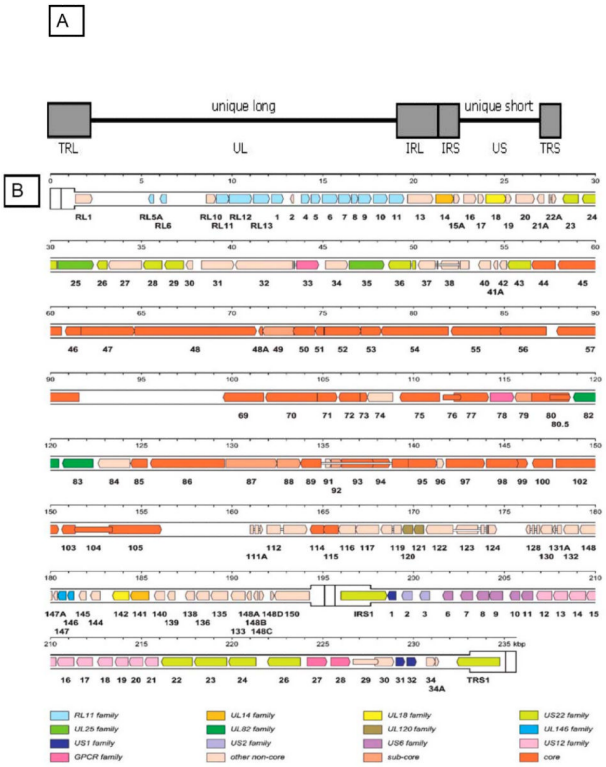

Fig. (2).

Schematic of CMV Genome. **A**) Structure of the CMV genome. The CMV genome is organized as two regions of unique sequences, unique long (UL) and unique short (US), flanked by two sets of inverted repeats (TRL/IRL) and (IRS/TRS) (light shaded boxes). UL - Unique long; US - Unique short; TRL - Terminal repeat long; IRL - Internal repeat long; inverted repeat of TRL; TRS -Terminal repeat short; IRS - Internal repeat short; inverted repeat of TRS. This genome structure allows one of four potential isomeric configurations to be represented in any given infectious virus particle. Figure originally published in [41] and used with permission. **B**) Genetic map of wild-type CMV. Related groupings of protein-encoding regions are indicated by colored arrows, with gene family nomenclature indicated below. Many CMV genes are spliced and these introns are indicated as narrow white bars. Colors also delineate whether genes are conserved in all *Herpesviridae* (core genes) or between the *Betaherpesviridae (CMV, HHV6, HHV7)* and *Gammaherpesviridae* (Epstein-Barr virus, HHV-8; sub-core genes). A group of 22 ORFs (ORFs ~133-150 in the UL region of the genome) are prone to rapid deletion/mutation/rearrangement when CMV is subjected to laboratory passage in fibroblast cell culture: this represents the so-called ULb' region of the genome (see text for details). Figure reproduced from [42] and used with permission, Copyright 2000, National Academy of Sciences, U.S.A.

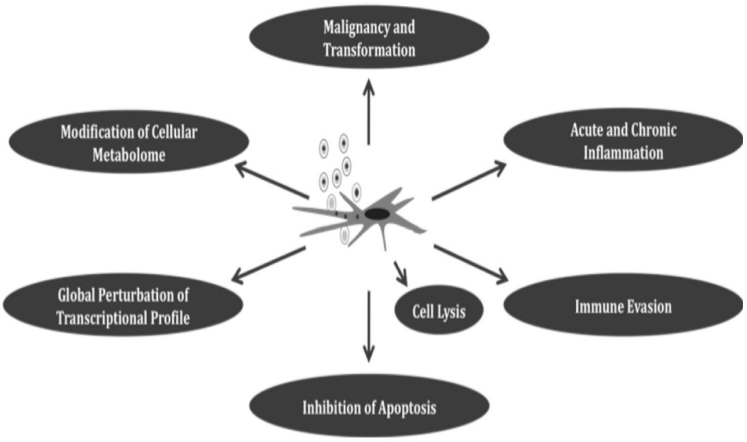

Fig. (3).

Impact of CMV infection on cellular physiology, gene expression, and function. Following CMV infection, a variety of fates potentially await the infected cell, depending on the target organ and embryological state of the cell. These include transformation/dysregulation of cellular replication; immune activation and/or immune evasion; modification of apoptosis mechanisms and perturbation of cell cycle regulation; changes in cellular metabolism; and global perturbations in transcriptional and proteomic profiles. Cell death and lysis may also occur following infection. The synergy of these effects on the infected cell likely mediates much of the pathology and pathogenesis associated with congenital infection.

Table 1

Cytomegalovirus Immune Modulation Genes

Cytomegalovirus Immune Modulation Genes	
CMV Gene(s)	**Putative Immunomodulatory Function**
UL16 *UL18* *UL142*	Homologs of MHC Class I molecules; promote natural killer (NK) cell evasion through binding of NK-inhibitory ligands, down-regulation of NK-activating ligands
UL40 *UL83* *UL141*	NK cell evasion genes; promote expression of non-classical HLA receptors; binding and inhibition of NK activating proteins; down-regulation of CD155 (activating receptor)
TRS1 *IRS1*	dsRNA binding proteins; prevent activation of PKR following CMV infection; evasion of interferon response
UL111A	IL-10 cytokine homolog; possible down-regulation of host inflammation to evade immune clearance
UL146 *UL147*	Homologs of C-X-C (alpha) chemokines; possibly function to promote widespread dissemination of infection in host
UL128 *UL130*	Homologs of C-C (beta) chemokines; immune function unclear; ancillary proteins involved in viral entry into epithelial cells
UL33 *UL78* *US27* *US28*	Homologs of G-protein coupled receptors; ligands may include multiple chemokines and cytokines; signaling molecules; may function as “chemokine sinks” to sequester immune activation proteins, compatible with an immune evasion role
US2 *US3* *US6* *US11*	Genes interfering with MHC class I antigen processing; prevent development of cytotoxic T-cell response to CMV through retention of MHC in ER; facilitation of degradation of MHC; downregulation of MHC expression; immune evasion function
UL144	Tumor necrosis factor receptor homolog; possible role modifying cytokine response to TH2 polarization; immune evasion
UL112	MicroRNA; functions at RNA not protein level; NK cell evasion