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Cytomegalovirus Tegument Proteins and the Development of Novel Antiviral Therapeutics

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1. Introduction

Cytomegalovirus (CMV) is a widespread pathogen that infects a majority of the world's population by early adulthood with approximately 50-85% of individuals over 40 being seropositive [1,2]. CMV can establish a life-long infection with its host by becoming latent during the lysogenic stage of the viral life cycle in which the virus becomes dormant and the shedding and production of infectious virions ceases [3]. However, the virus can later re-enter the lytic stage of the viral life cycle when presented with certain environmental cues, such as stress, thereby triggering the production of viral progeny and resulting in an acute infection of the host. Immunocompetent individuals generally display no symptoms of acute CMV infection, but CMV can cause morbidity and mortality in those with weakened or not fully developed immune systems [4]. The clinical manifestations of CMV infections in those with weakened immune systems, include spiking fever, malaise, leucopenia, encephalitis, pneumonitis, hepatitis, uveitis, retinitis, gastrointestinal disease and graft rejection [5,6]. If primary infection or reactivation of CMV occurs during pregnancy in women, serious complications will arise for the fetus or developing embryo. CMV is the leading cause of viral birth defects, including microcephaly, mental retardation, spastic paralysis, hepatosplenomegaly, anemia, thrombocytopenia, deafness, and optic nerve atrophy that subsequently leads to blindness [5, 7]. CMV is also responsible for 8% of infectious mononucleosis cases [8].

Although immunocompetent individuals generally display no symptoms of CMV infection, CMV has been implicated in playing a role in several proliferative and inflammatory diseases [9]. CMV has been linked with several forms of cancer, such as colon, breast, and prostate. Previously, CMV was regarded as having an oncomodulatory role in cancers by infecting tumor cells and modulating their malignant properties. It was hypothesized that tumor cells provided a genetic environment that allowed CMV to exert its oncomodulatory effects. CMV

was then identified as a potential therapeutic target in those tumors infected with CMV [10-12]. However, recent evidence supports oncogenic properties of CMV in certain cancers, such those as in salivary gland [13]. Furthermore, epidemiological and pathological studies suggest a strong link between CMV and atherosclerosis [9]. A potential mechanism for CMV in the pathogenesis of atherosclerosis involves the reactivation of virus followed by virus-induced enhancement of vascular inflammation and damage through smooth cell proliferation, uptake of low-density lipoproteins, and narrowing of the vessel lumen [1].

Antiviral agents that inhibit CMV viral replication exist, including ganciclovir, valganciclovir (the prodrug of ganciclovir), foscarnet, and cidofovir [1]. The primary mechanism of action of ganciclovir/valganciclovir against CMV is through the inhibition of the replication of viral DNA by ganciclovir-5'-triphosphate, which includes a selective and potent inhibition of the viral DNA polymerase [14]. Foscarnet, by comparison, interferes with the exchange of pyrophosphate from deoxynucleoside triphosphate during viral replication by binding to a site on the CMV DNA polymerase [15]. Similarly, cidofovir inhibits CMV DNA synthesis by DNA chain termination following incorporation of two consecutive cidofovir molecules at the 3'-end of the DNA chain [16]. Nonetheless, the antiviral agents commonly used to treat CMV infections suffer from high hematologic, renal, and neutropenia toxicity, low bioavailability and the development of drug-resistant virus strains [9,17]. Furthermore, there is no effective vaccine available.

The lack of an effective treatment for CMV infections has increased interest in the identification of targets for the development of novel CMV antiviral treatments. These include proteins found within the tegument of CMV virions. These proteins are abundant and play pivotal roles in the viral life cycle, including immune evasion, viral entry, gene expression, assembly of new virus particles and egress through an envelopment-deenvelopment-reenvelopment process [18]. The structure of the CMV tegument, the roles of some major tegument proteins in the CMV life cycle, and the therapeutic potential of CMV tegument proteins will be reviewed.

2. Cytomegalovirus structure and function of the tegument

CMV is a member of the *Betaherpesvirinae* sub-family of the *Herpesviridae* family, (Figure 1). The virion has an icosahedral protein nucleocapsid that contains the 235-kb double-stranded DNA. The capsid is surrounded by a proteinaceous tegument and an outer lipid envelope [1]. CMV virions gain entry into a host cell via a membrane fusion event involving the outer membrane of the cell and the glycoproteins located on the lipid envelope of the virion. When the cell membrane and lipid envelope fuse, the DNA-containing protein nucleocapsid and tegument proteins are released into the host cell. This initiates the lytic stage of the viral life cycle [19].

The proteins in the tegument are excellent candidates for novel CMV antiviral treatments due to their abundance and significant roles in all stages of the viral life cycle. The tegument, located between the outer lipid membrane and the icosahedral protein nucleocapsid is largely unstructured and amorphous although some structuring is seen with the binding of tegument

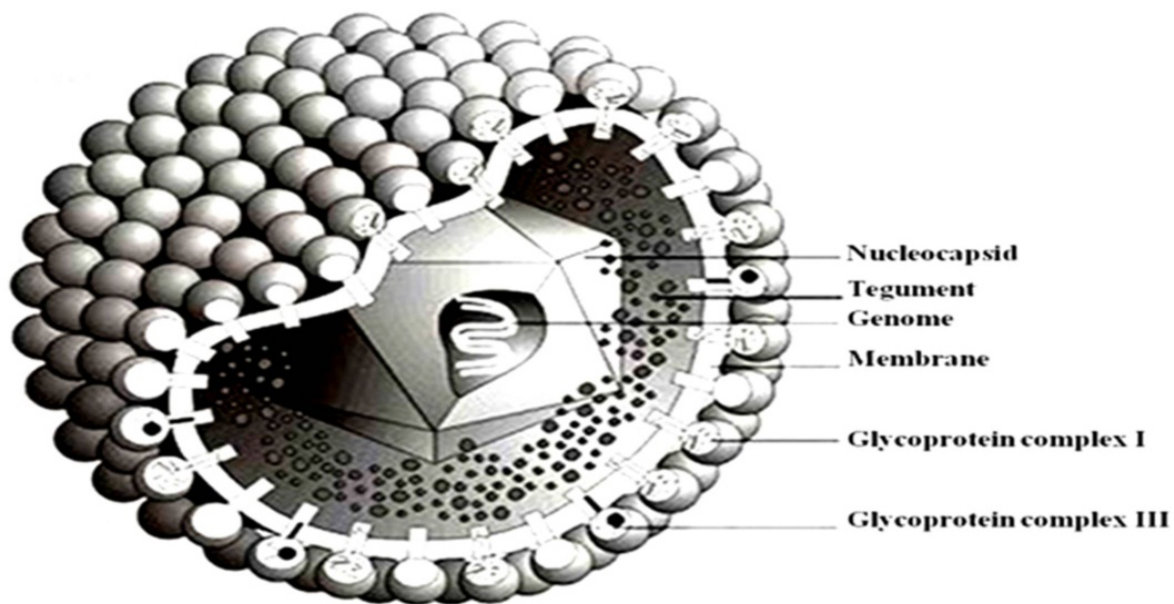


Figure 1. A cartoon depicting the structure of the HCMV virion. (Image obtained from (http://www.virology.net/big_virology/bvднаherpes.html website) courtesy of Dr. Marko Reschke in Marburg, Germany).

proteins to the protein capsid [20]. The tegument proteins comprise more than half of the total proteins found within infectious CMV virions [21]. Tegument proteins are phosphorylated and undergo other posttranslational modifications, but the significance of these modifications is unknown [19]. A common biochemical sequence to direct proteins into the tegument has not been identified, and the process of assembling the viral tegument upon viral egress and disassembly upon viral entry into cells is unclear [22]. Incorporation of proteins in the tegument is likely facilitated by the phosphorylation of the tegument proteins, their subcellular localization to the assembly site and their interaction with capsids or the cytoplasmic tails of envelope proteins [18].

As mentioned, the tegument proteins gain entry into the host cell along with the DNA-containing protein nucleocapsid upon fusion of the outer membrane of the host cell and the outer glycoprotein riddled lipid membrane of the CMV virion [19]. Once the tegument proteins are released into the cytoplasm, they become functionally active and participate in all stages of the viral life cycle [1].

3. Cytomegalovirus tegument proteins

The CMV tegument proteins play pivotal roles illustrated in Figure 2. The known or inferred functions of tegument proteins are presented in Table 1, compiled using a functional profiling of the CMV genome from a global mutational analysis [18,62,63]. Gene expression classifications are based on when expression occurs during the viral life cycle (Immediate-Early, Early, Early-Late, and Late). Finally, the tegument proteins are categorized based on their role in lytic

replication. Some proteins are essential for replication, while others are required for efficient replication (augmentative), or dispensable for lytic replication. The roles of major tegument proteins are presented in sections 3.1-3.4 in the context of the various stages of the CMV life cycle, which includes viral entry, viral replication and gene expression, immune evasion of the host, and assembly and egress of new infectious virions.

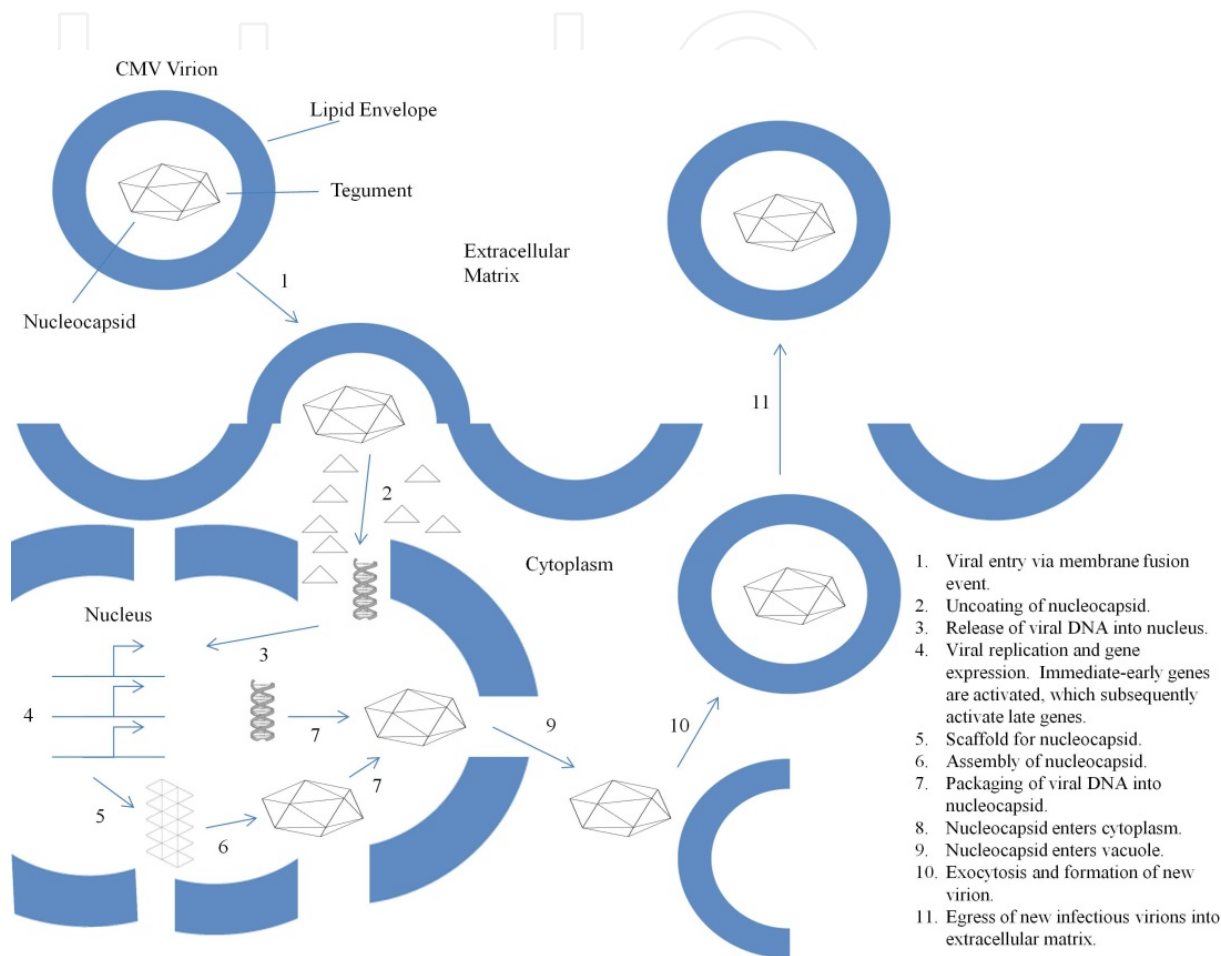


Figure 2. Illustration of the CMV life cycle from viral entry to egress of new infectious virions.

3.1. Viral entry

Mechanistically, the CMV virions enter host cells through a membrane fusion event involving the host cell's outer membrane and the glycoproteins located on the lipid envelope of the CMV virions [19]. The receptors on the viral envelope connect to complementary receptors on the cell membrane of the host cell. This initial interaction makes the cell susceptible to further interactions that allow the membranes to fuse, and the subsequent disassembly and release of the viral genomic DNA and tegument into the host cell. It is believed that several tegument proteins mediate delivery of the DNA-containing nucleocapsid to the nuclear pore complex and the release of the viral DNA into the nucleus [18]. Comparisons with other herpesviruses suggest that the UL47 and UL48 CMV tegument proteins, along with microtubule motor

proteins, facilitate the delivery of the CMV nucleocapsids to the nuclear pore complex and the release of the CMV genome into the nucleus [18,64]. Pp150 may also play a role in viral entry due to its tight association with the CMV capsids [65].

3.2. Viral replication and gene expression

Once the viral genome enters the nucleus of the host cell, the viral immediate-early genes are expressed through their activation by the pp71 tegument protein, which initiates the lytic stage of the viral life cycle and the subsequent replication of the 235-kb double-stranded CMV DNA genome [46]. It is important to note though that the expression of the immediate-early genes can be repressed resulting in a latent infection that is characterized by the minimization of viral gene expression and the inhibition of the assembly and egress of new viral progeny [1,66]. Although pp71 is known to play a pivotal role in the expression of the immediate-early genes during the lytic stage of the CMV life cycle, the gene products of UL35 and UL69 have been implicated in gene expression [18]. However, more research is necessary to identify the other tegument proteins involved in viral gene expression.

3.3. Immune evasion

CMV evades the host cell immune system through the targeting of intrinsic, innate, and adaptive immune responses by several different tegument proteins, including pp65, pp71, and IRS1/TRS1. pp65 the major tegument protein involved in immune evasion of the host as well as IRS1/TRS1 modulate the innate and adaptive immune responses. pp71 modulates the intrinsic immune defense through its neutralization of the Daxx-mediated repression of immediate-early gene expression. Without immune evasion, the lytic stage of the viral life cycle is inhibited. The roles of pp65 and pp71 in modulating the host cell immune response is further discussed below in sections 4.1 and 4.3 respectively [18].

3.4. Assembly and egress

After viral DNA replication, the immediate-early gene products, which include several tegument proteins as seen in Table 1, turn on the expression of viral late genes [1]. The viral late proteins are mainly structural components that assist in the assembly and egress of newly formed infectious CMV virions [1]. The primary tegument proteins involved in assembly and egress are pp150, pp28, and UL97. pp150 directs the movement of the cytoplasmic capsids to the site for particle formation, while pp28 directs the enclosure of the tegument proteins and DNA-containing nucleocapsids within an enveloped particle [18,26,52]. UL97 phosphorylates the tegument proteins through its kinase activity, which may facilitate the incorporation of the tegument proteins into new infectious virions [18,30]. Once the virions are packaged, they are shed from the host cell through an exocytosis mechanism which uses the host cell's transport system to enclose vacuoles containing the newly synthesized infectious virions for release into the extracellular space.

Gene (Protein)	Expression	Essential for Lytic Replication	Known or Inferred Function	Reference
UL23	Early-Late	Dispensable	Involved in events immediately after virus penetration	[23]
UL24	Early-Late	Dispensable	Involved in events immediately after virus penetration	[23]
UL25	Late	Dispensable	Structural protein	[25]
UL26	Early-Late	Augmentative	Transcriptional activation	[24]
UL32 (pp150)	Late	Essential	Virion egress (directs capsid to the site of final envelopment)	[26]
UL35	Late	Augmentative	Viral replication and particle formation	[27]
UL36	Immediate-Early	Dispensable	Control of a caspase-independent cell death pathway	[28]
UL38	Immediate-Early	Augmentative	Control of apoptosis	[29]
UL43	Late	Dispensable	Involved in events immediately after virus penetration	[23]
UL44	Early	Essential	CMV DNA polymerase processivity/transcription factor	[31]
UL45	Late	Augmentative	Influences virus growth at low multiplicities of infection	[32]
UL47	Late	Augmentative	Release of viral DNA from nucleocapsid Disassembling of virus particles	[33]
UL48	Late	Essential	Deubiquitinating protease Release of viral DNA from capsid	[34]
UL50	Early	Essential	Egress of nucleocapsids	[35]
UL53	Late	Essential	Egress of nucleocapsids	[36]
UL54	Early	Essential	CMV DNA polymerase	[37]
UL56	Early-Late	Dispensable	DNA packaging	[38]
UL57	Early	Essential	Single-stranded DNA-binding protein	[39]
UL69	Early-Late	Augmentative	Nuclear export of unspliced mRNAs Arrests cell cycle in G1 phase	[40]
UL71	Early-Late	Augmentative/ Essential	Late envelopment	[41]
UL72	Late	Dispensable	Inactive	[42]
UL76	Early	Augmentative/ Essential	Modulation of gene expression	[43]

Gene (Protein)	Expression	Essential for Lytic Replication	Known or Inferred Function	Reference
UL77	Early	Essential	DNA packaging/cleavage	[44]
UL79	Early-Late	Essential	Promotes the accumulation of late viral transcripts	[45]
UL82 (pp71)	Immediate-Early	Augmentative	Degrades Daxx; facilitates Immediate-Early gene expression	[46]
UL83 (pp65)	Early-Late	Dispensable	Endogenous kinase activity	[47,58,59]
			Associated kinase activity	
			Evasion of adaptive immunity	
			Evasion of innate immunity	
UL84	Early	Essential	CMV DNA replication	[48]
UL88	Late	Dispensable	Unknown	N/A
UL93	Late	Essential	Virion packaging	[49]
UL94	Late	Augmentative/ Essential	Putative DNA-binding protein	[50]
UL96	Early	Augmentative/ Essential	Preserves the integrity of the nucleocapsid during translocation from the nucleus to the cytoplasm	[51]
UL97	Early-Late	Augmentative	Kinase that phosphorylates UL44	[30,60,61]
			Stimulates DNA replication, assembly, and egress	
			Cyclin-dependent kinase-like functions	
UL99 (pp28)	Late	Essential	Directs the enclosure of enveloped virus particles	[52]
UL103	Late	Augmentative	Regulates virus particle and dense body egress	[53]
UL112	Early	Augmentative	CMV DNA replication	[54]
IRS1/TRS1	Early-Late	Augmentative/ Essential	Inhibits PKR antiviral response	[55]
			Virion assembly	
US22	Early	Dispensable	Involved in events immediately after virus penetration	[23]
US23	Early	Augmentative	Colocalization with pp65	[57]
US24	Early	Augmentative	Activation of viral gene expression	[56]

Table 1. CMV tegument proteins and their known or inferred function.

4. Cytomegalovirus tegument proteins as potential antiviral targets

Since the CMV tegument proteins play pivotal roles in all stages of the lytic stage of the viral life cycle, they are candidates for novel antiviral treatments. An antiviral agent able to bind to

a major tegument protein and inhibit its function would prevent CMV from replicating its viral DNA genome and producing new infectious virions. This would have great therapeutic value. Examples of how tegument proteins could be targeted therapeutically are presented below (See Figure 3).

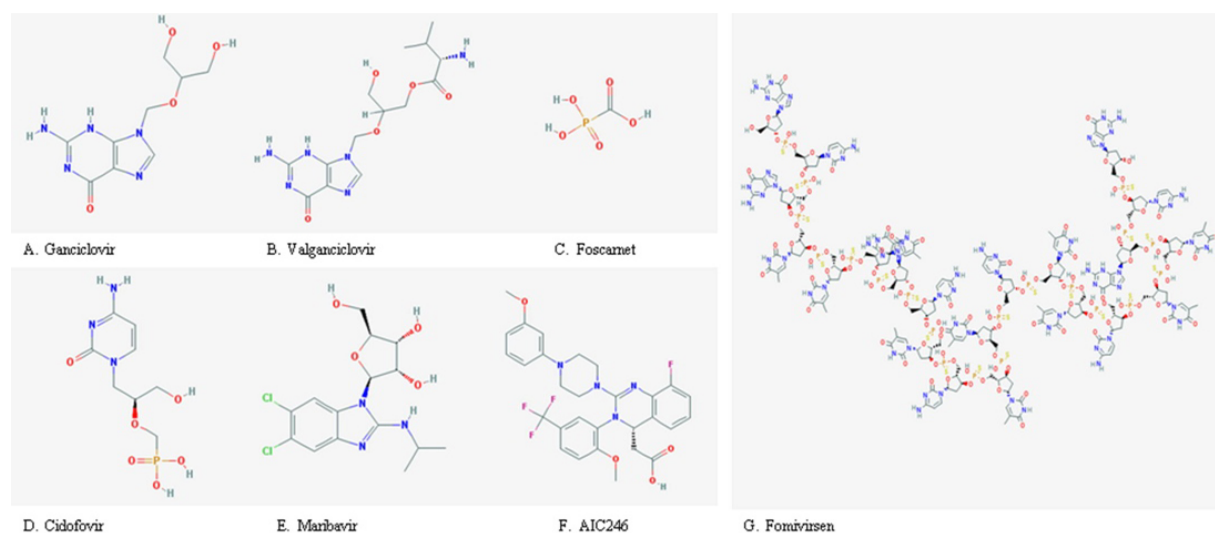


Figure 3. Current and developmental antiviral agents that target acute CMV infection.

4.1. pp65

A good tegument protein to target would be pp65, since it is the most abundant tegument protein [67]. pp65 is implicated in counteracting both innate and adaptive immune responses during CMV infections. It invokes humoral and cellular immunity and is the dominant target antigen of cytotoxic T lymphocytes [67]. In addition to pp65 being antigenic, it can also be considered a good antiviral target due to its immunomodulatory role. pp65 prevents immediate-early proteins from being recognized by components of the immune system in addition to inhibiting the synthesis of the various components involved in the host cell's immune response through its associated enzymatic kinase activity [58,59]. Thus, if you can inhibit the function of pp65, the host cell immune response would be able to inhibit the CMV viral life cycle.

Recent evidence in support of pp65 as a target for a novel antiviral treatment strategy concerns its subcellular localization during the lytic stage of the viral life cycle. pp65 migrates to the nucleoli at the early stages of infection, which suggests a functional relationship between its localization and the lytic stage of the viral life cycle [68]. However, pp65 begins to migrate to the cytoplasm 48 hours into the lytic cycle through cyclin-dependent kinase activity and a Crm 1 exporter mediated migration [69,70]. It is likely that pp65 does not migrate independently to the nucleoli as it remains in the cytoplasm in the absence of other components of CMV [71, 72]. This is significant as the localization patterns of the tegument proteins are correlated with their function [73]. Furthermore, pp65 has a bipartite nuclear localization signal, which implies that the nuclear localization signal is in a region that is initially inaccessible to importin proteins

[74]. Another tegument protein may bind to pp65, inducing a conformational change that allows the inaccessible nuclear localization signal to be recognized by nuclear transport molecules, triggering the characteristic nuclear localization of pp65 and the initiation of the lytic cycle [71,72]. Importantly pp65 co-localizes with the tegument protein associated with the CMV US23 gene product [57]. Thus, the US23 gene product could be the other tegument protein necessary for pp65 to enter the nucleoli (also see section 4.2 UL97). Thus, two monoclonal antibodies could be developed to target antigens present on the surfaces of pp65 and the US23 gene product, which would prevent them from interacting and inhibit the development of the lytic stage of the CMV life cycle.

Additionally, pp65 can also be used to develop a vaccine to prevent or inhibit CMV infection. An excellent example of pp65 being utilized to develop a novel vaccine to prevent CMV infection concerns the two plasmid DNA vaccine, ASP0113. Currently, ASP0113 is in Phase I/II clinical trials to prevent CMV infection in solid organ transplant recipients at high risk for CMV infection. The vaccine is comprised of the highly immunogenic pp65 tegument protein and the gB CMV surface protein, which utilizes the ability of pp65 to induce cell-mediated response and gB to induce humoral immune system responses in those infected with CMV. Results show a reduction in the number of CMV episodes and the time to initial viremia [75].

4.2. UL97

The UL97 gene product is also a tegument protein that could be targeted for the development of a novel antiviral therapeutic. UL97 stimulates DNA replication, assembly, and egress in addition to being a known kinase homologue [30,76]. UL97's kinase activity makes CMV susceptible to the current CMV antiviral ganciclovir, a synthetic 2'-deoxy-guanosine analogue. UL97 phosphorylates this synthetic analog to a deoxyguanosine triphosphate analogue, which competitively inhibits the incorporation of it by viral DNA polymerase. This competitive inhibition results in the termination of CMV DNA elongation [77].

Currently, a specific inhibitor of UL97 protein kinase activity, maribavir, inhibits viral replication and is in clinical trials [78]. Maribavir is a benzimidazole riboside and inhibits CMV DNA assembly as well as the egress of CMV nucleocapsids from infected host cells [78]. Maribavir was given fast track status by the Food and Drug Administration and is being investigated in Phase 2 clinical trials using a high dose treatment option for clinically significant CMV viremia.

The UL97 gene product also shows due to its association with pp65. UL97 and pp65 act directly to form a complex during viral replication. When UL97 is genetically ablated or pharmacologically inhibited, pp65 localizes in unusual refractile bodies, which suggests that UL97 is essential for the successful localization of pp65 to the nucleus of the host cell at the beginning of the lytic stage of the viral life cycle [79]. Similarly to US23, UL97 may direct pp65 into the nucleus to initiate the lytic stage of CMV replication [71,72]. Furthermore, a pp65 deletion mutant exhibits modest resistance to maribavir [80]. pp65 may negatively regulate UL97 by sequestering kinase that would be available to promote viral replication [81]. The interaction between pp65 and UL97 could influence pp65-mediated immune evasion, because the

presentation of viral immediate-early proteins to T cells is blocked when the proteins are phosphorylated by UL97 kinase [81,82].

4.3. pp71

pp71 is also a major target for CMV antiviral research as it influences the activation of immediate-early gene expression at the start of the lytic cycle [83]. Mechanistically, pp71 activates viral gene expression through the neutralization of the effects of the cellular Daxx protein, which is recruited to promoters by DNA-binding transcription factors, resulting in the repression of viral transcription [84]. pp71 binds to two inherent domains on Daxx and induces its proteasomal degradation [85]. pp71 slows the intracellular transport of MHC class I molecules, which limits the display of CMV antigens on the surface of infected cells to cytotoxic T lymphocytes [86].

A UL82 gene deletion mutant may serve as a potential novel CMV vaccine candidate as it can enter cells efficiently and activate the innate immune response through interactions with cell surface receptors. However viral gene expression is disrupted during infection when cells are infected at a low multiplicity of the UL82 gene deletion mutant. The expression of the immediate-early genes that are involved in the host anti-viral response is blocked. One of the genes whose expression would be blocked is the immediate-early 2 (IE2) gene that can antagonize the host innate immune response by attenuating the interferon β response and blocking chemokine expression. If the expression of IE2 is blocked, the number of cytotoxic T lymphocytes and natural killer cells recruited to the infected cell would increase. Furthermore, viral replication is limited with low multiplicity of infection with the UL82 gene mutant as this would promote a robust anti-viral immune response. Thus, a novel antiviral treatment could target pp71 and its ability to control the activation of immediate-early gene expression [87].

4.4. Tegument structural proteins

The structural proteins of CMV with therapeutic potential include pp150, pp65 (above), pp28, pp38, and the gene products of UL55 and UL75, as they play pivotal roles in the CMV life cycle and are immunogenic. pp150, the second most abundant tegument protein behind pp65, plays a role in the assembly and egress of new infectious virus particles. It is necessary to incorporate nucleocapsids into these new infectious virus particles [21]. It is essential for maintaining the stability of the cytoplasmic capsids and directing their movement [1,88]. It also plays a role in the reorganization of the cytoplasmic assembly compartment during virion assembly [88]. pp28 is largely responsible for the cytoplasmic envelopment of tegument proteins and capsids during assembly and egress [89].

pp38 is a mitogen-activated protein kinase and has a critical function in CMV viral DNA replication. pp38 kinase activity is significantly increased after CMV infection, and inhibition of this kinase activity inhibits CMV-induced hyperphosphorylation of pRb and the phosphorylation of heat shock protein 27. This suggests that pp38 activation is involved in virus-mediated changes in host cell metabolism throughout the CMV infection [90].

The gene products of UL55 and UL75 by comparison have been shown to be involved Sp1 and NF-kappaB activation during the earliest stages of CMV infection via a cellular receptor-viral ligand interaction. This is based on the observation that the cellular transcription factors Sp1 and NF-kappaB are upregulated shortly after the binding of purified live or UV-inactivated CMV to the cell surface, which has also been seen in other systems where cellular factors are induced following a receptor-ligand interaction [91].

All tegument proteins elicit a strong humoral immune response [92]. This is significant, since the host immunological functions are clearly limit CMV-associated disease [93]. Novel therapeutics could take advantage of the highly immunogenic nature of the CMV structural proteins. Potentially, an antibody could be designed that recognizes the CMV antigens expressed on these proteins and could be exploited to deliver an active drug compound that can inhibit the lytic replication of the virus. This strategy is used to target certain cancers. Additionally, a monoclonal antibody could simply be used to help the immune system locate the CMV immunogenic structural proteins and end the CMV infection.

Additionally, structural phosphoproteins, such as pp65 and pp150, are good candidates for subunit vaccine development, since they elicit cytotoxic T lymphocyte responses. A CMV subunit vaccine would contain viral antigens without the CMV DNA genome. It would be less likely to cause adverse reactions and would be clinically valuable in view of the high hematologic and renal toxicity and low bioavailability of current antiviral treatments targeting acute CMV infection [94].

4.5. UL94

Although it is not critical for viral replication unlike the majority of potential CMV targets, UL94 could also be targeted by a novel antiviral agent. Studies of UL94 stop mutants show that UL94 plays a role in the secondary envelopment of viral particles. When the UL94 gene is absent or not functioning, the UL99 (pp28) tegument protein responsible for the cytoplasmic envelopment of tegument proteins during assembly and egress exhibits aberrant localization and there is a complete block of secondary envelopment of virions. Thus, UL94 functions late in the CMV lytic life cycle to direct pp28 to the assembly complex, facilitating the secondary envelopment of CMV virions [95].

If a molecule is able to target UL94 and inhibit its function, the assembly and egress of virion progeny will be blocked, since the secondary envelopment of CMV virions will not occur. A therapeutic targeting UL94 could utilize the interaction between UL94 and the pp28 structural protein. For example, a new antiviral could focus on the ability of pp28 to elicit a strong humoral immune response through the release of antibodies targeting the CMV specific immunoglobulin as mentioned above. Two monoclonal antibodies could also be developed to target pp28 and UL94 that could inhibit UL94 from directing pp28 to the assembly complex where new CMV virion progeny undergo secondary envelopment.

4.6. UL56

Currently, all of the licensed drugs used for the systemic treatment of acute CMV infection act through similar mechanisms as they target the viral DNA polymerase (UL54) [96]. With the emergence of ganciclovir-resistant strains of CMV, as well as cross-resistance to second-line agents (foscarnet and cidofovir), there is a need for new drugs [97].

A promising small molecule antiviral candidate, AIC246, is representative of the 3,4 dihydro-quinazoline nonnucleoside CMV inhibitors [98]. AIC246 acts through a unique mechanism distinct from that of the CMV DNA polymerase inhibitors. AIC246 blocks viral replication without inhibiting the synthesis of progeny CMV genomic DNA or viral proteins. Three pieces of evidence show AIC246 interferes with CMV DNA cleavage/packaging via a distinct molecular mechanism from other compounds that target the CMV viral terminase [99]. First, AIC246 does not affect CMV protein expression or CMV DNA replication, excluding the possibility of AIC246 acting through interfering with viral genome replication [99]. Second, genetic mapping of AIC246 resistance to the CMV open reading frame of UL56 shows that the viral terminase complex is involved in the action of AIC246 [99]. Third, a terminase cleavage assay showed potent inhibition of the formation of properly processed unit-length genomes [99]. Thus, AIC246 is a promising therapeutic candidate for the treatment of acute CMV infection through its unique mechanism of action involving the UL56 tegument protein involved in DNA packaging.

4.7. Antisense oligonucleotides

The expression of viral genes encoding proteins essential for the production of infectious virions can also be targeted. Fomivirsen, a 21-base phosphorothioate oligodeoxynucleotide complementary to the messenger RNA (mRNA) of the major immediate-early region proteins of CMV, can inhibit CMV gene expression through an antisense mechanism. Fomivirsen binds to the target mRNA transcripts of the immediate-early region 2 (IE2) that encodes several proteins responsible for the regulation of viral gene expression. This binding inhibits IE2 protein synthesis and the activation of immediate-early gene expression by pp71, which subsequently inhibits viral replication [100]. Fomivirsen is licensed to treat acute CMV infection and illustrates that CMV tegument proteins can be targeted indirectly.

5. Conclusion

CMV infects most individuals by early adulthood and is associated with morbidity and mortality, especially in those with poor immune systems. Furthermore, CMV has been implicated in inflammatory and proliferative diseases, such as cardiovascular disease and cancer. Antiviral agents able to inhibit the CMV replication cycle and the production of new infectious virions exist, but suffer from high levels of toxicity and low levels of bioavailability. CMV resistant and cross-resistant strains develop because all drugs target the viral DNA polymerase UL54. There is also no vaccine available to prevent acute CMV infection. However, several components of the CMV virion are promising targets for novel antiviral therapeutics.

that would inhibit the CMV lytic cycle and eradicate the virus from host cells. The most likely targets within the CMV virion are those proteins found within the tegument, which is a unique structure found in all members of the *Herpesviridae* family. Proteins that localize to the tegument play pivotal roles in all stages of the CMV life cycle. Novel antiviral therapeutics that target these proteins inhibit the CMV lytic cycle. Furthermore, the highly immunogenic nature of several tegument proteins makes them excellent candidates for subunit vaccines. In fact, several novel antiviral therapeutics and CMV vaccines based around the CMV tegument proteins are under development. Nonetheless, more research needs to be done to fully identify the function as well as the role in the lytic stage of the CMV life cycle.

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