Studying Rhesus Cytomegalovirus (RhCMV) to Develop Therapeutics for Human Cytomegalovirus (HCMV)

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Abstract:
Cytomegalovirus (CMV) is an evolutionarily divergent virus with unique species specificity limiting its ability to infect multiple hosts. The form of the virus specific to humans is known as human cytomegalovirus (HCMV). HCMV possesses pathogenic significance especially in immunocompromised individuals. As there remains a need to explore HCMV, limitations arise from the host restriction and inability to conduct vaccine development studies in human hosts. Rhesus Cytomegalovirus (RhCMV) is another opportunistic virus naturally isolated in Rhesus macaques and it contains most of HCMV gene families. In this review, primary research articles were sourced for information to better understand the relationship between HCMV and RhCMV. It explores how studying RhCMV in its natural host presents a preeminent model for prevention of HCMV infection and will aid in the development of vaccines and therapeutics.

1. Introduction

HCMV is a double stranded DNA virus and member of the herpesviridae family (16). It was first isolated in the year 1881 in the kidney cells of a luetic stillborn child and thought to be protozoan cells (34). Between the years 1956-1957, this double stranded DNA virus was individually isolated by different scientists; Thomas Weller, Margaret Smith, and Wallace Rowe in humans and in mice (18). One of the defining characteristics of these isolates was intranuclear inclusions found on the cells (18). Weller had isolated the virus from a child initially thought to have congenital toxoplasmosis (38). It was then that Weller named the virus "Cytomegalovirus", alluding to the big cells that result from its infection. HCMV infects various cell types such as epithelial cells, endothelial cells, smooth muscle cells, fibroblasts, monocytes, and macrophages (32) which play a role in HCMV dissemination from host to host. It is important to note that HCMV infection and disease are not to be used interchangeably; infection represents the presence of viral proteins or nucleic acid in any body fluid or tissue regardless of symptoms, while disease is characterized by infection with attributable signs and symptoms of tissue-invasive disease (19).

Just like any other herpesvirus, HCMV is able to establish latency. Latency is a state where the virus is not cleared but is non-infectious and non-replicating for the lifetime of the host

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(35). The virus is able to establish latency through mechanisms of transcriptional silencing (13). It also has a lytic phase which may precede latency during natural infection. During the lytic phase, the virus is capable of releasing new virions and is highly infectious. Latent infection may be triggered to lytic infection by inflammation, bacterial infection and other injuries common to many cell types (13).

For a virus to survive, it must elude the immune surveillance of its host (31). HCMV is highly adept in escaping immune detection due to its ability to establish latency amongst other mechanisms. In addition, CMV's species specificity has made it harder to develop vaccines for target human populations. This review provides an overview of HCMV's characteristics and infection and gives prominence to how RhCMV in the rhesus model is suitable for providing more insight on HCMV.

2. **Epidemiology**

HCMV can be transmitted through bodily fluids, sexual contact, transplacentally from mother to child, and through breast milk. HCMV infection is endemic amongst various populations. Data shows that IgG antibodies (which indicate a previous HCMV infection) are found in approximately 60% of adults in developed countries and 100% in developing countries (15). In the United States, over 50% of adults are infected with HCMV by the age of 40, and approximately one in three children are infected with HCMV by the age of five (9) yet, there is no FDA-approved vaccine against HCMV (7). So far, treatments for HCMV infection include ganciclovir and valganciclovir which have side effects such as headache, dizziness, tremors, nausea, diarrhea, fever, renal dysfunction, and bone marrow suppression (23).

Congenital HCMV infection occurs in approximately 35% of pregnancies in which maternal primary infection is present (36). HCMV infection is a leading cause of disability due to hearing loss, impaired vision, cognitive impairments and neuromotor deficits (28). HCMV can also spread from mother-to-child through breast milk. The epidemiological impact of breast milk mother-to-child-transmission is amplified by the fact that infants shed the virus in saliva and urine for years (28). Because of this, children could further transmit HCMV to other family members or neighbors that they come in contact with, hereby increasing its prevalence.

In immunocompetent individuals, HCMV infection is often asymptomatic or mildly symptomatic; however, for those who are immunocompromised, it may lead to serious diseases. One of the most common diagnoses of HCMV disease occurs in organ transplant recipients due to transmission from transplanted organs or reactivation of a latent infection due to immune suppression. The virus induces a cytopathic effect on the recipient's organs and systems causing broad and febrile complications such as pneumonia, gastrointestinal tract disease, hepatitis, encephalitis, and retinitis that are sometimes fatal (5). Since HCMV disease can be a major cause of morbidity and mortality in these populations, prophylactic measures must be taken before and even after organ transplantation.

HCMV is also a common opportunistic infection comorbid with AIDS. HCMV in the blood (viremia) is reported to be a predictor of many CMV associated end-organ diseases (30). In 85% of AIDS related cases, the end-organ disease caused is retinitis (15). Aside from retinitis, other incidences of HCMV associated end-organ diseases include pneumonitis, hepatitis, and adrenalitis (14). When HCMV disseminates in a host with HIV infection, it can stimulate the host's immune response which leads to the production of antigens that consequently stimulate HIV replication (14). Taking this into consideration, it is important to explore how HCMV influences the course of HIV disease and AIDS in order to manage the co-existence of HCMV and HIV/AIDS in target populations.

HCMV threatens the quality of life for many people regardless of age or gender, but people from lower socio-economic backgrounds are even more at risk of its attack. With HCMV's significant prevalence in the general population, there is an urgent need to develop novel therapies that will control the spread and manage infections of this virus.

The Rhesus Model

Rhesus Cytomegalovirus, RhCMV is another opportunistic virus that naturally infects Rhesus macaques (Macaca mulatta). It has co-evolved with its host and is also highly species-specific. Rhesus macaques normally live in large mixed cohorts (i.e some are infected with RhCMV and some are not) where it has been shown that RhCMV spreads throughout the cohort as a function of the persistent shedding of the virus in bodily fluids of infected animals and the repeated mucosal exposure of uninfected animals to the virus (11). Much like HCMV, RhCMV can be detected in various cell types in the body and sheds in bodily fluids (2).

Scientists have isolated different strains of RhCMV for investigative purposes and the most common are strains 68-1 and 180.92 (3). RhCMV strain 68-1 is a prototypical strain that was isolated from urine of healthy rhesus macaques in 1968 and propagated in human fibroblasts (10). A complete genomic sequence analysis of RhCMV strain 68-1 revealed that it contained 135 out of 260 open reading frames (ORFs) which are homologous to HCMV, and these include include members of the RL11, UL25, UL82, US1, US6, US12, US22, and seven-transmembrane protein families. (33). Out of other animal specific variants CMV which include murine CMV (MCMV), chimpanzee CMV (ChCMV), Guinea pig CMV (GPCMV), Cynomolgus CMV (CyCMV) and many more RhCMV is the closest evolutionary animal model to HCMV (24, 20) so it is able to serve as an alternative animal model to study HCMV infections.

Studies have been done to characterize RhCMV gene products. Its immediate early 1 and 2 (IE1/IE2) gene and promoter region indicated a conservation of gene structure, protein sequence and transcript with HCMV's IE1/IE2 (1, 6). RhCMV also encodes homologs to HCMV phosphoprotein 65 (pp65) which is a target for the immune response to HCMV (39). The conservation of the immune system targets IE1/IE2 and pp65 makes RhCMV a practical model for studying potential HCMV vaccines (33).

Another characteristic RhCMV shares with HCMV is a viral interleukin-10 (vIL-10) homolog known as RhCMVIL-10. The role of RhCMVIL-10 in regulating a host's immune response is among the popular studies for vaccine targeting against RhCMV infection. One study which utilized vaccines to disrupt RhCMVIL-10 mediated signaling showed that the destruction of RhcmvIL-10 restricts long-term features of primary RhCMV infection such as the amount of RhCMV that can be detected in the saliva and urine of vaccinated/challenged rhesus macaques (12). The evidence suggests that vIL-10 in HCMV could also suppress the functions of cell types that are critical in containing virus dissemination and help shape long-term immunity during the earliest virus-host interactions (12).

Cellular receptors for the Fc domain of immunoglobulin G (IgG) (FcγRs) comprise a family of surface receptors on immune cells connecting humoral and cellular immune responses (4). They happen to be key determinants in antibody-mediated immune responses (20). RhCMV gene Rh05 (which is comparable to UL153 in HCMV) encodes an IgG-Fc binding glycoprotein (17, 20). Using a set of reporter cell lines expressing human and rhesus FcγRs, it was demonstrated that Rh05 protects infected cells from opsonization and IgG-dependent activation of host FcγRs (20). This feature could be employed as a mechanism that allows the virus to disseminate despite humoral and cellular responses (20) and as more studies are done with the rhesus model, the results will be able to discern the impacts of HCMV viral FcγRs on immunomodulation.

An important highlight is that RhCMV encodes five genes that share homology (i.e., similar structure or function) with HCMV chemokine receptor US28 namely, Rh214, Rh215, Rh216, Rh218 and Rh220 (17). These five genes encode proteins with seven transmembrane domains that are homologous to G protein-coupled receptors (GPCRs) (17). Clustal analysis showed that these genes vary from each other and HCMV US28 (29). It further revealed that Rh220 is able to bind to ligands like fractalkine (also known as CX3CL1) just like HCMV US28 (29). This raises the question: What other functional similarity does Rh220, and the other replicate genes share with US28? Further research would be useful in determining the evolutionary significance of these replicate genes in RhCMV and it could also help elucidate the significance of US28 in HCMV.

Viral GPCRs

GPCRs, also known as seven-transmembrane domain receptors, are found on the surface of the cell and produce a signal or response after the binding of a ligand. Once activated, the G proteins transduce signals from the cell surface to an effector molecule in order to modulate intracellular functions (17). GPCRs are popular drug targets because of their ability to regulate various physiological processes in the body. HCMV encodes four GPCR homologs namely: UL33, UL78, US27 and US28 (37). Among the four listed, three (UL78, US27 and US28) are known to play a role in HCMV dissemination and latency (37). It is currently hypothesized that HCMV's US27 and US28 genes arose via gene duplication (8). pUS27 has no known ligands to date but is found within the cell membrane of infected cells

(25). pUS28 on the other hand is a functional chemokine receptor that binds to various ligands (22).

A lot of studies have been conducted focused on characterizing the role of US28 during HCMV infection. Recent data revealed that US28-mediated signaling is important for maintaining latency (21). It was found that US28 is involved in the suppression of the major immediate early promoter (MIEP) which is a strong lytic promoter, and such activity is a major determinant for a successful latent infection (21).

US28 functions as a promiscuous chemokine 'sink' with an ability to bind to and internalize chemokines thereby limiting their dissemination and interfering with its host's immune activity (26, 25). US28 binds CXC and CC groups of chemokines which play a role in homeostasis and inflammation. In particular, it binds to CX3CL1(fractalkine) through a two-site interaction mechanism (27, 29). At the first site, the receptor N-terminal region binds a groove on the globular body of the chemokine. At the second site, the chemokine N-terminal peptide binds within a deep pocket formed by the receptor transmembrane helices (TMs) that functions as the receptor activation switch (27). Since CX3CL has the ability to mediate migration, proliferation and adhesion of cells, one implication of US28 binding to CX3CL1 is that it can activate new signaling pathways (26) which will in turn promote HCMV's survival in the host. Given that RhCMV's Rh220 shares a similar binding profile with HCMV's US28, Rh220 could be useful in investigating other implications of US28 binding to various other chemokines.

3. **Conclusion**

HCMV has undergone co-evolution with its hosts and as such, has increased its specificity to its host's species. The virus's ability to establish latency means it never clears from the host. Scientists are curious to understand the various ways it avoids and modulates its host immune system. RhCMV in the Rhesus model presents an adequate means for such studies. Particularly, characterizing RhCMV's US28 homologous genes would provide better insight on HCMV US28 as a tool for establishing latency and immune evasion. By further understanding viral latency in the Rhesus model system, we will be better informed on vaccine targets and drug developments against HCMV.

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