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Rhesus CMV: an emerging animal model for human CMV

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Abstract

Human CMV is the predominant infectious cause of congenital birth defects and an opportunistic pathogen in immunosuppressed individuals, including AIDS patients. Most individuals are infected early during their life followed by life-long latent infection. During this latent phase, frequent reactivation and antigen production continue to stimulate the immune system. While the immune response is able to control the virus, it is unable to eradicate it. Moreover, super-infection by different CMV strains has been observed despite a strong immune response. Long-term immune stimulation by CMV has also been implicated in immune senescence and chronic conditions such as atherosclerosis. CMVs are highly species-specific and the relatedness of CMV genomes exactly mirrors the relatedness of their hosts. Thus, each CMV species is highly adapted to its respective host species, but is unable to infect other, even closely related hosts. While fascinating from an evolutionary perspective, this host restriction prevents studying HCMV in experimental animals. Exceptions are severely immunocompromised mice, e.g. SCID mice, or SCID/NOD mice, which might allow partial reconstitution of CMV infection in rodents. More practical however, is to study CMVs in their natural host, e.g. murine, rat or guinea pig CMVs. However, while these small animal models have many advantages, such as the availability of inbred animals as well as lower cost, the limited homology of the viral genomes with HCMV limits the functional analysis of homologous gene products. The closest relative to HCMV is chimpanzee CMV (CCMV), but this is not a practical animal model since chimps are a protected species, extremely expensive and of very limited availability. In contrast, rhesus macaques are a more widely used experimental animal species and, while more distant than CCMV, rhesus CMV (RhCMV) contains most of the HCMV gene families thus allowing the study of their role in acute and latent CMV infection. In this review we will discuss the current state of developing RhCMV as a model for HCMV.

Keywords

Cytomegalovirus; CMV; Rhesus cytomegalovirus; Rhesus CMV; Cytomegalovirus animal models

Introduction

Human cytomegalovirus (HCMV), a β-herpesvirus, is a successful and widespread pathogen [1]. Immunological and PCR-based assays revealed that 60–100% of the adult population is infected with the virus, with frequency of infection highest in urban areas. Infection is generally asymptomatic in healthy individuals, but the virus is a major cause of morbidity and mortality in immunocompromised hosts, who are less able to control primary infection or reactivation of latent virus. Indeed, HCMV is an opportunistic infection during AIDS and represents a

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common, life-threatening post-transplant complication in allograft recipients [2]. Congenital infection is also a serious problem with HCMV being the largest infectious cause of birth defects in the United States [3]. Total health care costs related to HCMV disease in the US alone exceed $4.4 billion annually with costs for CMV-induced sensorineural hearing loss and mental retardation alone exceeding $1 billion [4]. After initial infection, CMV persists in the host indefinitely and viral shedding in multiple body fluids (saliva, tears, urine, genital secretions, breast milk) can occur years after initial exposure. Like other herpesviruses, CMV can manifest latent infection of its target cells [5]. However, the frequency of shedding [6], kinetics of reactivation after transplantation [7], and the high CMV-specific T cell response [8] suggest that active CMV replication, or at least in some cases gene expression [9], occurs frequently within the infected host. This persistent, life-long infection together with the continuous stimulation of the immune system has also been implicated in chronic diseases, such as atherosclerosis, restenosis and transplant vascular sclerosis (reviewed in [10]).

One of the limitations of studying CMV is the fact that HCMV does not infect animals. This species-specificity is a characteristic trait of β-herpesviruses in general, and therefore, viral evolution has accompanied mammalian speciation such that each species has their own uniquely adapted CMV, and CMV relatedness generally parallels species relatedness [11]. Thus, the genomes of primate CMVs (human [12], chimp [13], rhesus [14]) are much more closely related to each other than rodent CMVs (mouse [15], rat [16], guinea pig [17]). Consequently, animal models for CMV depend on studying the CMVs of the respective mammal species. The most widely used model is murine CMV (MCMV), which can address many fundamental questions for biology, pathogenesis and immunology of cytomegalovirus [18,19]. However, the genomes of MCMV and HCMV are quite different and many “non-essential genes”, i.e. genes that are not required for growth in vitro, are also non-conserved in MCMV or other rodent viruses.

The closest relative of HCMV that infects primates is chimpanzee CMV (CCMV) [13]. However, chimpanzees are a protected species, costly and only available in very small numbers, which limits their use as a model for HCMV. In contrast, rhesus macaques (RM) are a widely used and available species for non-human primate (NHP) studies. Like all mammals, RM harbor their own species of CMV, RhCMV [20]. In this review we will summarize the current knowledge of RhCMV pathogenesis, gene functions and its usefulness as animal model for HCMV.

Pathogenesis of RhCMV

Similar to human CMV, the majority of monkeys within a rhesus population are positive for CMV, usually measured by serology. For instance, 95% of RM at “monkey temples” in India tested positive for RhCMV [21]. Similarly, close to 100% of RM in primate centers are CMV positive [22]. Most monkeys seroconvert during the first year of their life [23]. Once infected, NHP continue shedding virus for the rest of their lives in urine and saliva [6,20]. Thus, to study primary CMV infection in RM, CMV-free animals need to be kept separate from the rest of the colony, which adds considerable cost and logistical challenges to this animal model. Experimental infection of naïve animals (oral or i.v.) was shown to result in initial viremia in the blood and virus could be detected in various organs, particularly the spleen [24]. All infections were asymptomatic, similar to primary CMV infection of adult humans. To facilitate detection of CMV both in vivo and in vitro, Peter Barry and colleagues recently developed a recombinant CMV expressing GFP [25] as well as RhCMV strain 68.1 in a bacterial artificial chromosome, which greatly facilitates generating recombinant viruses [26]. Importantly, both recombinant viruses retained their ability to infect RM suggesting that molecular manipulations do not reduce RhCMV pathogenicity. Taken together, these data suggest that the pathogenesis, prevalence, and transmission of RhCMV are highly similar to that of HCMV.

RhCMV as a model for CMV-mediated congenital defects

Primary infection of seronegative pregnant women is a well-known cause of birth defects, particularly sensorineural defects [3]. In addition, recent observations suggest that hearing loss in children born to latently infected mothers occurred at the same frequency, albeit with slower progression, as in infants from primary infected mothers [27]. The close developmental, immunological, anatomical, and biochemical similarities between RM and humans render RhCMV particularly suited for the development of models for congenital CMV infection (recently reviewed in [28]). While natural congenital infection is difficult to detect in a monkey colony, intrauterine inoculation is being used to study the effect of CMV infection on the developing fetus. In one study it was shown that intra-amniotic inoculation with RhCMV leads to CNS lesions in the newborn [29]. However, more severe disease was observed upon intra-cranial inoculation of the fetus [30]. Similar to humans, severity of neurological defects was much higher when infections occurred during the first trimester of infection [28]. Thus, intrauterine infection with RhCMV clearly resembles some of the features of congenital HCMV infection. However, there is also a need to develop models that permit studying the impact of vertical transfer of RhCMV from pregnant mothers to infants, particularly during primary infection with RhCMV. The generation of CMV-free animal colonies at various primate centers should facilitate the development of such models.

RhCMV as model to test CMV anti-viral drugs

Rhesus macaques are routinely used at late stages of preclinical testing of vaccines as well as drugs in many areas of research. The main reason to use primates for these studies is that bio-availability and toxicity profiles obtained in primates are likely more predictive for humans than those obtained in small animal models. Primary and long-term RhCMV infected RM are thus ideally suited to examine the impact of novel drugs as well as anti-CMV vaccines. For instance, the pharmacokinetics of Ganciclovir, a well-established anti-CMV drug, was tested in blood and CNS in RM and was found to be similar to humans [31]. Moreover, RhCMV was found to be equally susceptible to Ganciclovir as HCMV and the activity of the Ganciclovir-kinase, UL97, was found to be similar [32]. RhCMV also showed comparable sensitivity to HCMV for benzimidazole nucleosides, a promising new class of anti-CMV drugs [33]. Thus, RhCMV will be an excellent model to test new anti-CMV drugs in vivo.

Rhesus macaques were also used to test the effect of anti-CD40 and anti-CD28 antibodies to prolong renal allograft survival. Interestingly, there was a significant reactivation of RhCMV in anti-CD40/CD28-treated animals with severe systemic manifestations of disseminated CMV disease [34]. This observation suggests that monitoring RhCMV will be an important aspect during the pre-clinical testing of immune suppressive treatments.

Opportunistic infection and AIDS

Human CMV is a well-known opportunistic infection in AIDS patients. A common manifestation is CMV retinitis, which has become rare thanks to the availability of highly active antiretroviral therapy (HAART). Ocular CMV-infection was also noticed in SIV-infected monkeys [35]. However, a more common occurrence is disseminated CMV disease in animals developing spontaneous SAIDS [36] or upon experimental infection with SIV [37]. Affected organs include the gastro-intestinal tract [38,39]. The occurrence of disseminated CMV in SIV-infected animals is thus a unique and highly relevant model to study the interaction of these two viruses. The reactivation of CMV from latency and the development of disseminated CMV correlated with the deterioration of CMV-specific cellular immune responses [40]. In a progressive study, a clear correlation was observed in the magnitude and rate of decline of CMV-specific antibodies and $CD4^+$ and $CD8^+$ T lymphocytes and the degree by which macaques progressed to CMV disease [41]. These data indicate that both humoral and cellular

immune responses seem to protect from developing CMV disease and it is the combined decay that results in AIDS-opportunistic infection. The rhesus model also allows the study of the development of RhCMV and SIV infections upon co-infection with both viruses, thus mimicking situations where primary infection with each virus occurs almost simultaneously [42]. These experiments suggested that con-current primary infection with CMV could augment the development of AIDS.

RhCMV immunology

The immune response to CMVs and the components necessary for disease suppression have been well characterized using the MCMV model in mice as well as data from HCMV in humans. Thus far, RhCMV has largely been used as a tool by immunologists to help provide insight into the macaque immune response that provides critical information for the development of the RM model in general. This includes the characterization of the $CD4^+$ $CD8^+$ T cell population [43], the characterization of the cytolytic properties of $CD8^+$ T cells [44], defining of the memory T cell population [45], and the description of useful techniques such as polychromatic flow cytometry analysis of immune cells during infection [46].

Another focus of immunological study using RhCMV has been characterizing the response to RhCMV during SIV infection. This helps to establish the model for CMV pathogenesis during AIDS (see previous section). CTL responses to RhCMV were similar in both SIV-negative animals and those infected with pathogenic and attenuated SIV strains [47]. However, the frequency of CMV-specific $CD4^+$ T cells was found to be reduced in SIV-positive macaques [40]. Furthermore, the regulatory T cell response to RhCMV is delayed compared to SIV [48].

Similar to HCMV in humans, RhCMV gB appears to be a strong target of the humoral immune response. gB antibodies are detected in the serum of infected animals and there is a strong correlation between neutralizing and gB-specific antibody levels [49].

Genomics

The RhCMV genome (strain 68.1) is 221,459 bp in length, slightly smaller than HCMVs 229,354 bp [14]. The genomes are colinear and share a similar structure, although unlike HCMV, RhCMV does not appear to isomerize [14]. Initially 230 open reading frames (ORFs) were predicted within strain 68.1, although this number was later proposed to be modified to 260 [50]. Of these, 135 are homologous to known HCMV proteins (Fig. 1). These homologs include members of the RL11, UL25, UL82, US1, US6, US12, US22, and seven-transmembrane protein families. Large loci of RhCMV-specific genes are located at the ends of the RhCMV genome and in the rh165–180 ORFs, a region at the same position as the U_L/b′ region of HCMV, which is deleted or truncated in laboratory strains [51]. There are currently two full-length RhCMV strains sequenced [14,50]. The two genomes are 97% identical at the nucleotide level, although strain 180.92 is 5,781 nucleotides shorter than strain 68.1, is missing 10 ORFs found in strain 68.1, and contains 8 ORFs not identified in strain 68.1 [50]. This indicates variability between RhCMV strains not unlike differences seen between strains of HCMV. Along with the identification of orthologs in CCMV, the overall similarity between RhCMV and HCMV has helped redefine the coding potential for HCMV [13,52]. And while there is a large amount of coding potential seemingly unique to RhCMV, many RhCMV proteins have maintained functional homology to HCMV despite limited sequence homology [53].

Functional characterizations of RhCMV proteins

Multiple RhCMV genes and gene products have already been characterized. Early studies on the RhCMV immediate early 1 and 2 (IE1/IE2) gene and promoter region showed a conservation of gene structure, transcription, and protein sequence with HCMV IE1/IE2 [54, 55]. gB, a major target of anti-HCMV antibodies, is also well conserved in RhCMV. The cloned RhCMV gB is proteolytically processed similarly to HCMV gB, portions of RhCMV gB cross-react with anti-HCMV gB antibodies, and RhCMV gB antibodies can be detected during RhCMV infection [56,57]. Phosphoprotein 65 (pp65) is another dominant target for the immune response to HCMV. RhCMV encodes two pp65 homologs. Much like HCMV pp65, Rh-pp65–2 localizes to the nucleus, is contained within the virion, and is the target of both humoral and cellular immunity [58]. The conservation of the immune system targets IE1/IE2, gB and pp65 makes RhCMV a viable model for studying potential CMV vaccines.

The ability to study HCMV-homologous immunomodulatory proteins in vivo also makes RhCMV attractive. HCMV inhibitors of apoptosis are conserved in RhCMV. Both the UL36 (viral inhibitor of caspase-8-induced apoptosis, vICA) and UL37 (mitochondria inhibitor of apoptosis, vMIA) homologs in RhCMV were able to prevent Fas-mediated apoptosis in HeLa cells, whereas the MCMV UL37 homolog M37 was not [59]. The IL-10 homolog of CMVs was first identified in RhCMV and was shown to be expressed in vivo, targeted by the humoral immune response, and to have immunosuppressive properties [60,61]. RhCMV also contains homologs of HCMV US2, US3, US6, and US11 [53]. This gene family inhibits expression of major histocompatibility complex class I (MHC I) molecules to avoid $CD8^+$ T-cell detection, and while MHC I modulators are found in rodent CMVs, the US2-US11 gene family is not. RhCMV contains five homologs to the HCMV chemokine receptor US28, a unique seven-transmembrane domain gene family not found in rodent CMVs. One of these, RhUS28.5, has been shown to have a similar ligand binding profile as HCMV US28 [62]. The US12 gene family, another seven-transmembrane domain gene family found only in primate CMVs, is also well conserved in RhCMV [63].

Among the RhCMV-specific genes is a cyclooxygenase-2 (COX-2) homolog that was shown to be a critical factor in viral growth in endothelial cells [64]. HCMV, while not having a COX-2 homolog, does upregulate cellular COX-2 upon infection, and COX-2 inhibitors prevent normal viral replication [65]. Lastly, RhCMV is able to block expression of interferon-stimulated genes (ISGs) by preventing activation of interferon regulatory factor-3 (IRF3) [66]. The failure to induce ISGs by an enveloped virus is thus far unique to RhCMV.

Concluding remarks

The development of RhCMV as an animal model for Human CMV has clearly come of age. Many useful tools have been developed in recent years that facilitate handling and manipulating the RhCMV genome such as bacterial artificial chromosomes [26] and telomerized rhesus fibroblasts [67]. In addition, immunological markers of rhesus immune cells have been developed [45] and several models of RhCMV pathogenesis, primary infection and re-infection are now available [24]. Due to cost and the unavailability of inbred and knockout animals, RM will not replace mice for many questions in CMV biology and immunology. However, for many important scientific questions, including vaccine development, drug-development, latent CMV infection and immune senescence, RhCMV is rapidly becoming the model of choice.

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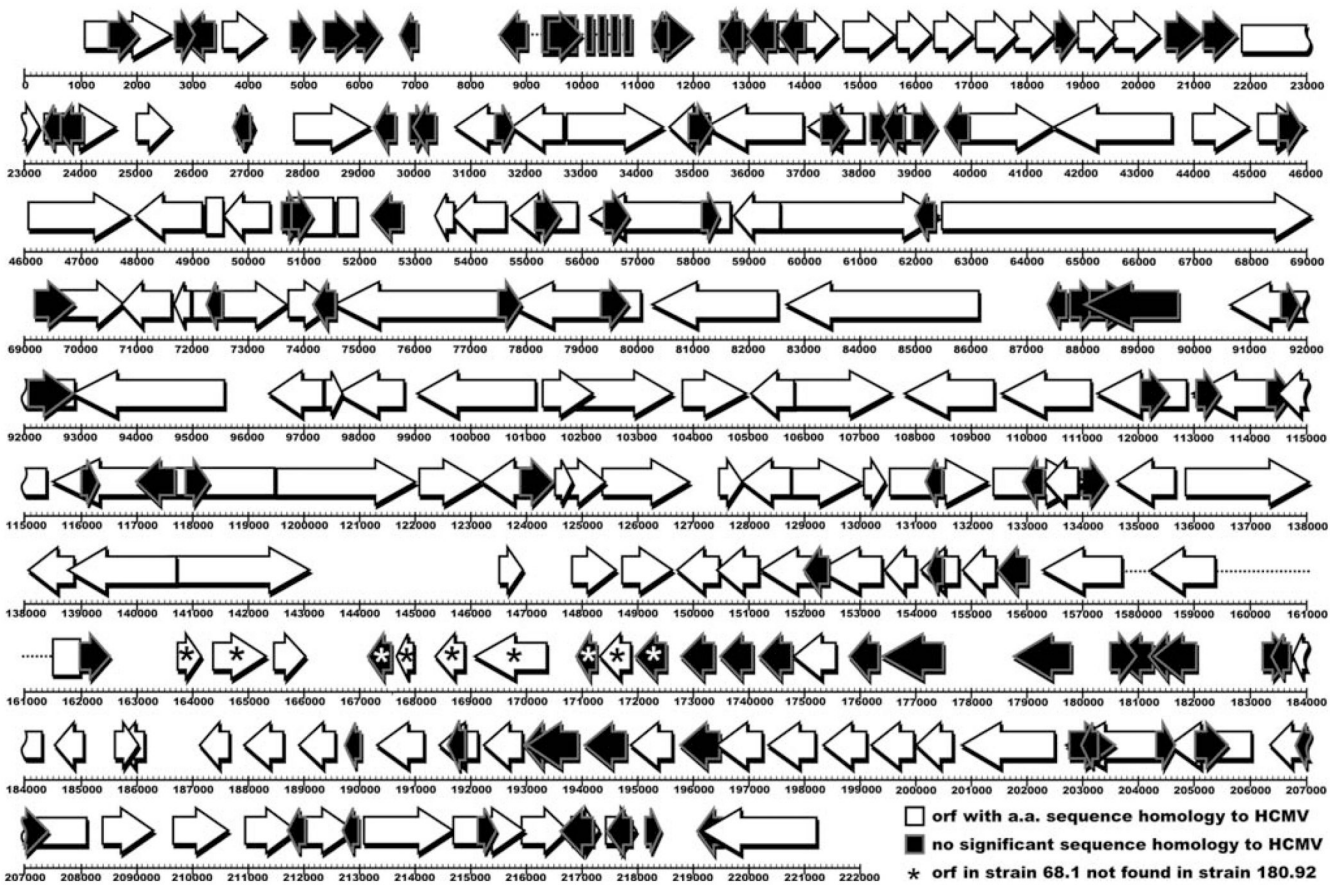

Fig. 1.

RhCMV open reading frame (*ORF*) homology to HCMV. This diagram details the predicted ORFs in the RhCMV genome (strain 68.1). ORFs in *white* have significant amino acid (a.a.) sequence homology to HCMV proteins, while ORFs in *black* have no significant homology to HCMV. *Asterisks* indicate ORFs in strain 68.1 not found in strain 180.92. *Rectangles* indicate known exons. *Dashed lines* indicate known introns. For a more detailed analysis see Refs. [14] and [50]