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Current Opinion in Virology

Rhesus monkeys for a nonhuman primate model of cytomegalovirus infections

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Human cytomegalovirus (HCMV) is the leading opportunistic viral infection in solid organ transplant patients and is the most common congenitally transmitted pathogen worldwide. Despite the significant burden of disease HCMV causes in immunosuppressed patients and infected newborns, there are no licensed preventative vaccines or effective immunotherapeutic treatments for HCMV, largely due to our incomplete understanding of the immune correlates of protection against HCMV infection and disease. Though CMV species-specificity imposes an additional challenge in defining a suitable animal model for HCMV, nonhuman primate (NHP) CMVs are the most genetically related to HCMV. In this review, we discuss the advantages and applicability of rhesus monkey models for studying HCMV infections and pathogenesis and ultimately informing vaccine development.

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Current Opinion in Virology 2017, **25**:126–133

This review comes from a themed issue on **Animal models for viral diseases**

Edited by **James** and **Cris**

http://dx.doi.org/10.1016/j.coviro.2017.08.005

Introduction

Human cytomegalovirus (HCMV) is a ubiquitous herpesvirus that establishes persistent infection in its host. While infection of healthy individuals is generally asymptomatic, primary HCMV infection or viral reactivation in immunosuppressed patients, such as AIDS and transplant patients, can lead to dissemination and life-threatening end-organ diseases [1,2]. Congenital HCMV (cCMV) infection can also cause severe clinical outcomes to the developing fetus and impacts ~1 in every 150 (0.7%) newborns, making it the most common congenitally transmitted pathogen worldwide [3–5]. In the United States alone, an alarming 40,000 congenital infections occur annually and nearly 7,000 infants develop permanent neurological sequelae including microcephaly, sensorineural hearing loss (SNHL), and cognitive impairment [3–5].

Despite the significant global health impact of cCMV and opportunistic HCMV infections, there are no licensed vaccines or effective immunoprophylactic measures to prevent these infections, as clinically tested vaccine candidates have not been successful enough to move forward toward licensure [6,7]. One major challenge of studying HCMV infections and testing vaccines preclinically is that HCMV, like all β-herpesviruses, does not infect nonhuman species. Therefore, animal models rely on species-specific CMVs, which vary in their biological relevance to HCMV [8]. In this review, we discuss the suitability and application of the rhesus monkey (*Macaca mulatta*) nonhuman primate (NHP) model for studying CMV infections and disease and for testing immunotherapeutic and prevention strategies.

NHP CMV genomic and functional relevance to HCMV

Because CMV evolution has paralleled mammalian speciation, primate CMVs are more closely related to each other than to CMVs in small animals, such as rats, mice, and guinea pigs [9,10]. Chimpanzee CMV and rhesus CMV (RhCMV) genomes are most similar to HCMV, though chimpanzees are a less accessible animal model due to their endangered population status [10]. Genomic sequencing and annotating of RhCMV isolates 180.92 and 68.1 revealed that 60% of RhCMV open reading frames are homologous to HCMV proteins — notably those encoding structural, immune evasion, replicative, and regulatory proteins [10,11]. For instance, subunits of the pentameric complex (PC; glycoproteins gH/gL and UL128-131A), which is involved in epithelial and endothelial cell tropism and has been reported as the target for the most potently neutralizing antibodies against HCMV, are conserved in RhCMV [10–12,13••]. As with HCMV, the RhCMV subunits interact as a pentamer and are essential for epithelial cell viral entry [13••,14,15]. Vaccination of rhesus monkeys with a vector coexpressing the

RhCMV pentamer subunits induced neutralizing antibodies against RhCMV infection of epithelial cells and fibroblasts, which reduced plasma viral loads and supports a functional similarity between RhCMV and HCMV PCs [13••]. Due to these findings, a similar vector was designed to coexpress the HCMV pentamer subunits, and rhesus monkeys vaccinated with this vector stimulated antibodies that neutralized HCMV infection of human epithelial cells, placental macrophages, and fibroblasts [16•]. Interestingly, RhCMV 68.1, which lacks regions homologous to HCMV UL128-131A, has been used as a vector to express simian immunodeficiency virus (SIV) proteins and vaccination with this vector induced unconventional $CD8^+$ T cell responses, namely targeting of diverse, highly promiscuous epitopes presented by major histocompatibility complex II molecules [17,18]. Nearly 50% of vaccinated animals were protected after SIV challenge, and unconventional T cell responses only occurred when the RhCMV vector lacked HCMV UL128-131A homologs. Thus, the RhCMV PC bears functional relevance to its HCMV homolog and knockdown of certain regions within the pentamer may be advantageous for vaccine delivery.

Similar to the PC, gB, which is essential for viral entry into all host cell types and is an immunodominant target for neutralizing antibodies, shares 60% identity (75% similarity) between RhCMV and HCMV at the amino acid level [19]. Researchers also found that HCMV monoclonal anti-gB antibodies cross-react and cross-neutralize with RhCMV gB (RhgB) [20]. Anti-RhgB antibodies have been shown to develop following primary RhCMV infection in rhesus monkeys and to possess neutralizing capabilities, which recapitulates humoral responses to HCMV [21]. In fact, a study recently isolated an anti-RhgB monoclonal antibody from a rhesus dam following viral challenge [22•]. In light of gB containing numerous neutralizing epitopes and two phase II clinical trials finding that a gB subunit vaccine was partially protective against HCMV acquisition, the genomic and functional similarities between RhCMV and HCMV gB, in addition to the PC, are particularly important for an animal model for CMV infection and vaccine immunity [23,24]. Because of the accessibility of rhesus monkeys and the parallels between RhCMV and HCMV genomes and proteins' functions, we will focus this review on the rhesus monkey/RhCMV model as an NHP model for CMV infections.

RhCMV acquisition and persistence

Like HCMV in humans, RhCMV is largely prevalent among rhesus monkey populations and causes life-long infection [25,26]. Nearly half of rhesus monkey infants in breeding colonies are seropositive for RhCMV by 7 months of age, which is just after maternal antibody wanes, and almost all seroconvert by 1 year of age [27,28]. High frequencies of CMV-specific $CD4^+$ and $CD8^+$ T lymphocytes are detected in naturally infected CMV-seropositive macaques, with prolonged virus shedding and impaired $CD4^+$ T lymphocytes commonly being observed in the first 2–3 years of infection [28–31]. Immune evasion mechanisms that contribute to infection persistence have been found to be similar between rhesus monkeys and humans. Specifically, induction of the cellular interleukin-10 signaling pathway and avoidance of natural killer cell activation, via preventing NKG2D ligand surface expression, contributes to RhCMV and HCMV viral persistence [32–35].

Despite the ubiquitous and persistent nature of RhCMV, efforts using early weaning of young infants to develop specific pathogen-free rhesus monkey colonies were successful in generating RhCMV-seronegative rhesus monkey colonies, which can be used to characterize primary RhCMV acquisition following direct inoculation and to evaluate prophylactic strategies [36]. For instance, prime/boost immunizations containing either RhgB or a combination of RhgB, phosphoprotein 65 (pp65), and immediate-early 1 (IE1) proteins have been tested in RhCMV-naïve, healthy adult rhesus monkeys for their effect on RhCMV acquisition and virus shedding using subcutaneous inoculation with epithelial cell-tropic RhCMV UCD52 [37]. In the mock-immunized controls, challenge virus was detectable in plasma, saliva, and urine within 4 weeks of challenge and high levels of virus persisted in the saliva and urine throughout 21 weeks of observation. This study's immunizations were partially effective, with the combined RhgB/pp65/IE1 vaccine leading to reduced oral shedding in half of the vaccinees. Similar studies have also employed subcutaneous and/or intravenous (IV) inoculation of RhCMV-seronegative monkeys to characterize primary RhCMV infection and to assess prevention strategies, including prime/boost immunizations with the RhCMV PC or with RhgB/pp65/IE1, which both partially reduced RhCMV plasma viral loads [13••,26,38]. Thus, direct inoculation of RhCMV in naïve rhesus monkeys via subcutaneous or IV routes causes quantifiable, persistent infection, which allows assessment of the efficacy of prophylactic strategies. Oral RhCMV challenge and natural vertical transmission models via co-housing with RhCMV-seronegative monkeys are also being trialed as more physiologically relevant challenge models that can better recapitulate human CMV transmission [39].

CMV infection in immune compromised populations

RhCMV-SIV coinfection

Like humans, adult macaques usually manifest with overt CMV disease only under immunosuppressive conditions that result in reactivation of latent RhCMV infection [40–44]. The SIV-rhesus macaque model is the leading animal model of AIDS and recapitulates many features