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Review

Non-Human Primate Models of Enteric Viral Infections

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Abstract: There is an important role non-human primates (NHP) play in biomedical research. Phylogenetic proximity of any of the NHP species to *Homo sapiens* assures that much better translatability of research outcomes from model studies involving human diseases can be achieved than from those generated with other pre-clinical systems. Our group and others used during past two decades NHPs in research directed towards viral and autoimmune disorders of the gastrointestinal tract. This review summarizes progress made in the area of enteric viral infections and its applicability to human disease.

Keywords: Enteric Virus, Non-Human Primate, Virome, Macaque, Dysbiosis, Enteritis, Diarrhea, Animal Model, Rotavirus, Calicivirus, Gut Microbiome

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1. Introduction

The use of NHPs in biomedical research can be traced to early 20th century and discovery of ABO blood groups [1]. Since then, several of the NHP species, predominantly those kept in captivity, helped to facilitate progress in various biomedical areas including behavioral sciences, genetics/genomics, cancer, neuroscience, HIV/AIDS, cardiovascular and respiratory disorders, regenerative medicine, endocrinology, aging, immune-mediated disorders, and infectious diseases. From the infectious diseases point of view, simian model of AIDS was the one historically utilized most extensively and linked with seminal discoveries concerning retrovirus pathogenesis, viral transmission, reservoirs, vaccine and drug development [2], (Figure 1). In addition, there are emerging research areas exploiting those features of NHP models that cannot be easily duplicated *in vitro* or with alternative *in vivo* systems. In this short review the human health-relevant enteric viruses of NHP host origin are discussed as several of these represent current disease models of interest (https://nprc.org).

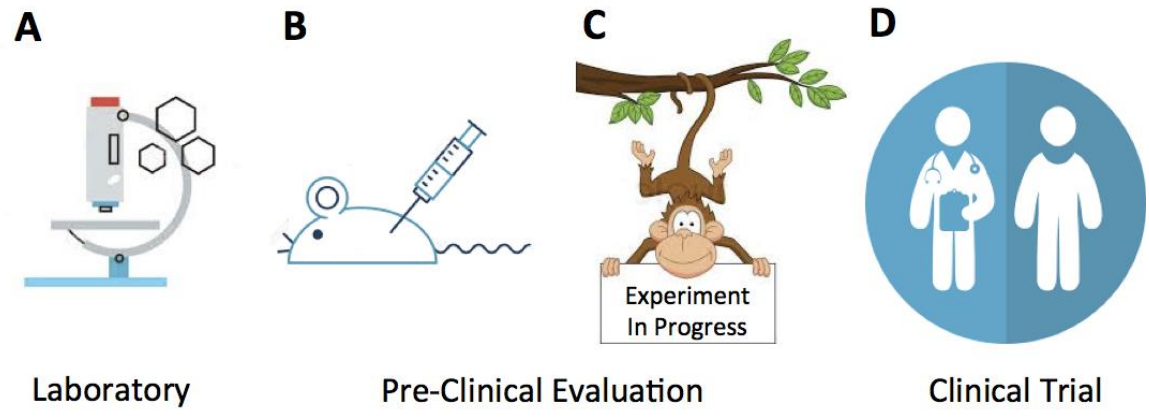

Figure 1. To maximize the potential of NHPs as pre-clinical models, it is essential to recognize function and information these models can generate in a cascade of events starting from the A) *in vitro* basic investigation, B) concept validation, C) pre-clinical trials, and ultimately, D) clinical studies.

The main two groups of enteric viruses discussed in this chapter (simian rotaviruses and rhesus enteric caliciviruses) were derived from the following NHP species: Vervet Monkey (*Chlorocebus pygerythrus*), Rhesus Macaque (*Macaca mulatta*), and Pigtailed Macaque (*Macaca nemestrina*) [3-11]. Notwithstanding, the serological and direct virus detection evidence indicates that numerous other NHP species including those living in captivity and wilderness can be infected and/or seropositive [7,12].

2. Conventional and specific pathogen-free (SPF) research colonies of captive NHPs

In order to maintain the colonies of captive NHPs free of microbial pathogens, the following six “conventional” simian pathogens are being tested at the U.S. National Primate Research Centers to assure seronegative status: 1) Simian immunodeficiency virus (SIV), 2) Simian T lymphotropic virus type 1 (STLV), 3) Simian retrovirus type D (SRV), 4) Herpes B virus, 5) Measles virus, and 6) *Burkholderia psedomallei*. With continuous improvement of primate-specific diagnostic assays and increased demand for disease-free primates in biomedical research, the additional six virus-specific diagnostic assays are currently being used: 7) Simian foamy virus (SFV), 8) Primate cytomegalovirus (CMV), 9) Rhesus rhadinovirus (RRV), 10) Simian varicella virus (SVV), 11)

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Simian vacuolating virus 40 (SV40), and 12) Lymphocytic choriomeningitis (LCV) (http://www2.tulane.edu/tnprc/microbiology/ resources/).

3. Enteric virus infections in captive NHPs

According to epidemiological surveys conducted during recent years by our group with participation of three National Primate Research Centers and three ZOOs in the U.S., a seasonal incidence of viral diarrhea-associated disease exists at captive NHP colonies [5,8]. Despite that viral diarrhea-linked morbidity is significant, current preventive measures do not target enteric viral pathogens as these persist within the colonies as well as in the environment in endemic form [5,13]. Understandably, the focus of the SPF eradication programs has been on blood-borne pathogens such as SIV, STLV, Herpes B, and others. Considering however that annual diarrhea-associated losses of production were conservatively estimated by our group at Tulane at hundreds of thousands USD per a single colony, it is evident that strategies that would help to reduce such losses, and to better define associated causes including viral diarrheas would be of great scientific (translational) and economic (animal health & breeding) benefits.

4. Simian rotaviruses

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The first rotavirus strain isolated from NHP species was SA11 [3]. SA11 was derived from vervet monkey in South Africa and used for decades in laboratories around the world as model to study rotavirus replication and life cycle. Another rotavirus strain RRV was isolated from rhesus monkey in 1980 [4]. The cell culture-adapted RRV was used for preparation of human-rhesus hybrid (reassortant) viruses that were employed by Wyeth Laboratories in 1998 for the formulation of first commercial rotavirus vaccine Rotashield. Despite its close to 100% efficacy, Rotashield had to be removed from the market in 1999 after it was determined that vaccination was associated with a 1:12,000 risk of intussusception in children. Subsequently, Rotashield was replaced by two attenuated, highly efficacious vaccines Rotarix by GlaxoSmithKline, and RotaTeq by Merck [14,15], which are still being used today. Another two simian (NHP) rotavirus isolates, TUCH and PTRV, were derived from captive rhesus and pig-tailed macaques, respectively [5,6]. It was shown that all of the known simian rotaviruses evolved by interspecies transmission and reassortment, i.e., by acquiring components of their RNA genome from the other, predominantly animal but also human rotaviruses [11].

When intragastrically inoculated into serum antibody-negative juvenile (<6 months-old) macaques, with no evidence of previous rotavirus infection, TUCH rotavirus was shown to consistently produce intestinal infection associated with peripheral, cell-mediated and antibody responses, as well as high levels of virus shedding [5,16]. The shedding in stools followed bell-shaped curve and decreased to baseline within two weeks after experimental inoculation [5].

Symptoms of clinical diarrhea were inconsistent or absent. Hence, ReCV macaque infection model is referred to as “shedding” rather than “diarrhea” model [5]. Nevertheless, another older study conducted in 1980 with a single (141 days-old) juvenile chimpanzee reported that when a SA11 simian rotavirus was orally administered to this animal, clinical symptoms of diarrhea and shedding took place over period of 9-days [17].

RRV and TUCH rhesus-derived rotaviruses are currently being used in translational pathogenesis studies with murine model of biliary atresia, i.e., a neonatal obstructive cholangiopathy [18-20]. In pediatric patients, obstructive cholangiopathy often requires transplantation. Remarkably, the SRL peptide motif on VP4 protein of RRV but not TUCH rotavirus was found to be responsible for binding to target cells in this model, i.e., murine cholangiocytes [20]. Further preclinical studies might need to be performed directly with NHP hosts to elucidate the exact nature of cellular and molecular interactions during rotavirus-induced biliary atresia.

5. Rhesus enteric caliciviruses (ReCV)

No robust human-like models exist to study the pathogenesis and immunity of human noroviruses although studies are being conducted with human volunteers, mice models and intestinal organoids [21-29]. A taxonomically newer group of enteric caliciviruses of rhesus monkey host origin with the name Recovirus (ReCV) was isolated and characterized in 2008 [7,10]. ReCVs are

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closest relatives of human noroviruses and in contrast to noroviruses, can be grown *in vitro*. Epidemiological studies strongly indicate that ReCVs also infect humans [8,30]. Because of their biological properties, i.e., capability to grow *in vitro* and to cause diarrhea, ReCV macaque model is well positioned for studies where objective is to further elucidate features of human enteric calicivirus pathogenesis and immunity.

According to conventional pathogenesis paradigm concerning enteric viruses, small intestinal epithelium is the primary target of infection. Nonetheless, most of the attempts to demonstrate replication of enteric caliciviruses inside the enteric epithelium failed. The confocal microscopy imaging of infected intestinal tissues revealed that mouse noroviruses are crossing the intestinal epithelium while being carried by certain members of gut microflora that express virus-binding histo-blood group antigens (HBGA) [31]. It was proposed that HBGA-expressing bacteria carry noroviruses into deeper layers of intestinal wall where they enter the B and other cells [32]. Interestingly and coincidentally, few years prior to revelation of these important norovirus pathogenesis events, it was demonstrated that in human norovirus-challenged chimpanzees as well as in ReCV-challenged rhesus macaques, viral antigens appear not within but beneath the small intestinal epithelium – inside lamina propria [33,34]. Despite progress made in recent years, it is still not clear what exact genera/species of intestinal bacteria are facilitating enteric calicivirus transcytosis and systemic spread. It is also not clear if B cells serve as primary targets of infection or only as virus carriers. Giving the low rate of virus replication in these cells, it is more likely that

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B cells are not the targets but only vehicles to carry virus into other tissues. As ReCV macaque model would enable time-scaled experiments during which collections of intestinal biopsies, stools, peripheral blood and urine samples can be carried out, this model has strong potential to facilitate further discoveries.

6. Other enteric viruses

From the other groups of enteric viruses with capability to cause gastroenteritis in humans and animals, the following groups were confirmed in biological specimens from captive or semi-wild NHPs: enteric adenoviruses, enteroviruses, picobirnaviruses, coronaviruses, noroviruses, sapoviruses, astroviruses, anelloviruses, smacoviruses and parvoviruses [13, 35-39]. It is important to emphasize that many of these viruses can be found not only in symptomatic (diarrheic) but mostly in asymptomatic animals. Thus, to suggest pathogenic role in any species, experimental inoculations of virus-free subjects and reproduction of clinical or asymptomatic infection is required, consistent with previous studies (5,33,34).

7. Enteric virome and gut dysbiosis

Several studies suggested that enteric virome might be in primates affected by immunodeficiency and/or autoimmunity in a putative gut dysbiosis context [40-43]. For example, in SIV-infected wild gorillas (*Gorilla gorilla*) and

chimpanzees (*Pan troglodytes*), an abundance of viral families previously associated with gastrointestinal tract infections such as *Herpesviridae* and *Reoviridae* (gorillas), or *Circoviridae* and *Adenoviridae* (chimpanzees) were found increased [42,43]. This implies that selected enteric virome metrics might be utilized as markers of the disease progression/remission, and also be exploited for evaluation of novel therapies and vaccine approaches. Much remains to be discovered considering the complex nature of relationships concerning factors influencing the gut microbiome composition, and the impact of gut dysbiosis on overall health.

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