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Study of viral pathogenesis in humanized mice

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Many of the viral pathogens that cause infectious diseases in humans have a highly restricted species tropism, making the study of their pathogenesis and the development of clinical therapies difficult. The improvement of humanized mouse models over the past 30 years has greatly facilitated researchers' abilities to study host responses to viral infections in a cost effective and ethical manner. From HIV to hepatotropic viruses to Middle East Respiratory Syndrome coronavirus, humanized mice have led to the identification of factors crucial to the viral life cycle, served as an outlet for testing candidate therapies, and improved our abilities to analyze human immune responses to infection. In tackling both new and old viruses as they emerge, humanized mice will continue to be an indispensable tool.

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Introduction

Viruses make a staggering contribution to morbidity and mortality in the human populations of both industrial and developing countries. At least 500 million people are chronically infected with hepatitis B (HBV) or C viruses (HCV), placing them at risk for developing severe liver disease. 33 million individuals are infected with HIV, leading to 1.7 million AIDS-related deaths every year. Of the approximately 400 million people who contract dengue virus (DENV) annually, almost 100 million present with clinical symptoms. 60–90% of the global population is infected with herpes simplex viruses (HSV), resulting in orolabial and genital lesions. Human cytomegalovirus (HCMV), which persistently infects 40% of the world, can be life-threatening for newborns and immunocompromised individuals.

Many of the viruses causing disease in humans have a narrow host range, often limited to humans and closely related non-human primates (NHPs). This has created challenges in studying the pathogenesis of human-tropic viruses as experiments in NHPs are hampered by logistical, financial, and ethical concerns. This creates a pressing need for more tractable small animal models to study existing and emerging viral diseases. In the last few decades, humanized mice have emerged as a solution to this problem. Humanized mice can be generated by expressing human genes whose products are needed for viral infection (Table 1), such as entry factors, or through xenotransplantation of hematopoietic stem cells (creating human immune system mice, known as HIS) and/or other human tissues (Figure 1).

This paper highlights the recent progress and challenges in studying viral pathogenesis in humanized mice. We will discuss four groups of human-tropic viruses — HIV, DENV, herpesviruses, and hepatitis viruses — as examples of diseases for which specific types of humanized mice were and still are enabling experimental platforms. Using these examples, we will provide a general outlook on how humanized mice can be adapted and refined through genetic host adaptations and/or co-engraftment of multiple tissues to facilitate analysis of other viral infections.

Human immunodeficiency virus (HIV)

In 2013 alone, 1.5 million people worldwide died from AIDS, and 33 million were cited as living with HIV. Besides humans, only chimpanzees are readily susceptible to HIV, but since they usually do not progress to AIDS, they have not gained traction as HIV animal models. In searching for alternatives, it was shown that smaller NHPs, specifically rhesus macaques, were susceptible to simian immunodeficiency virus (SIV), leading to AIDS-like symptoms. To improve the utility of this model, chimeric viruses closely resembling HIV-1, namely simian-human immunodeficiency virus (SHIV) and simian-tropic HIV (stHIV), were generated [1].

Despite intense efforts, it has not yet become possible to genetically overcome species barriers and recapitulate the HIV life-cycle in small animal models. Advances have been made, but they are primarily focused on establishing HIV uptake in mice [2]. Since HIV is a lymphotropic virus primarily infecting CD4 T cells, engraftment of human immune system components proved a viable approach to establish HIV infections in a small animal model. Early models pioneered by McCune and colleagues, based on engrafting xenorecipients with human fetal thymic or lymph node implants, demonstrated that an acute infection of human lymphoid organs with HIV-1 can be

Table 1

Prominent examples of factors allowing or restricting aspects of different viral life cycles

Pathogen	Disease/symptoms	Host factors needed at different steps of the viral life cycle in humans	Factors restricting infection in mice
HIV (as reviewed in [59])	Leads to decreased levels of CD4+ T cells, ultimately resulting in AIDS	Entry: CD4, CCR5, CXCR4 (some T-tropic HIV-1 viruses can use the murine ortholog of CXCR4) Post-entry: Cyclin T1	Transcription: low Tat activity (needs human cyclinT1 as cofactor for successful binding to trans-activation response element) Post-translation: excessive splicing of HIV-1 RNA Poor particle assembly
Polio virus [60]	Poliomyelitis, with paralysis in some individuals due to nerve cell damage	Entry: poliovirus receptor	
Measles virus [61]	Measles (also known as rubeola), which leads to respiratory infection	Entry: CD46	
HCV (as reviewed in [42])	Hepatitis C, which can lead to liver cirrhosis, fibrosis, and hepatocellular carcinoma	Entry: OCLN and CD81 (minimal necessary entry factors)	Replication: Innate immune responses
HBV [56••]	Hepatitis B, which has similar effects on liver health as hepatitis C	Entry: NTCP	Post-entry: no cccDNA formation; other post-entry restrictions unknown
Ebola virus [62]	Fever, diarrhea, and disrupted liver and kidney function; can lead to internal and external bleeding	Entry: Niemann-Pick C1	Unknown

followed in humanized mice [3]. With the improvement of xenorecipient strains and humanization protocols (extensively reviewed in [4]), HIS mice have deepened our knowledge about HIV viral transmission, immune responses to HIV and the efficacy of novel therapeutic interventions. The ability of HIV-1-infected cells to form latent reservoirs has been especially challenging in completely curing individuals of the virus [5]. Recently, several groups have shown that HIV-1 latency can be observed in humanized mouse models [6–8]. These mice have made it possible to model *in vivo*, for example, how treatment using broadly neutralizing antibodies in combination with inducers can prevent viral rebound following removal from antiretrovirals [9]. In hindering transmission, vectored immunoprophylaxis has shown promise as a way to obstruct intravenous [10] and mucosal transmission of HIV in humanized mice [11]. As the latter is the primary route by which individuals become infected, the *in vivo* model for mucosal HIV transmission is physiologically relevant and provides a venue for testing anti-viral therapies. Immune responses in HIS mice are suboptimal because of a variety of incompatibilities between the mouse and human immune system. Nonetheless, it was shown that in a particular version of HIS mice, so-called bone-marrow liver thymus (BLT) mice, the dynamic interplay of HIV-specific cellular immunity and viral escape from immune pressure can be accurately modeled [12••].

Dengue virus (DENV)

Dengue is a mosquito-borne disease, caused by DENV, a positive-sense, single-stranded RNA virus belonging to the family *Flaviviridae*. Four genetically and antigenically distinct serotypes, DENV-1 to DENV-4, have been described, annually causing ~390 million infections which range in severity from completely asymptomatic to lethal hemorrhagic fever or shock syndrome (DHF and DSS, respectively) [13]. Since a vaccine still does not exist, studying the immune response to DENV is of especial importance, as individuals with previous immunity are more susceptible to developing DHF and DSS [14,15]. Murine xenorecipient strains expressing HLA-A2 were injected with human blood-forming stem cells and demonstrated improved immune responses to tissue-culture derived DENV, especially in assessing human T-cell response to DENV during and after acute infection [16]. Additionally, it was shown that viremia can be suppressed by administration of direct-acting antivirals (DAAs) to humanized mice that displayed symptoms similar to those in humans following infection with a clinical DENV isolate [17], paving the way for creating and testing DAAs that could be utilized in treating DENV. However, while priming of DENV-specific B and T cell responses occurs at some level, it is not sufficiently robust in existing models. This poses challenges for untangling the mechanisms of why DHF/DSS is so much higher in individuals with secondary heterologous DENV infections. Further light has also been shed on identifying the cells targeted by DENV. Past research in humanized mice concluded that T cells were not infected by DENV [18,19], but two groups have recently observed evidence to the contrary [17,20]. Finally, since DENV is mosquito-borne, understanding transmission from host to vector and vice versa is important for examining viral spread in populations and preventing large-scale outbreaks. Thus, the examination for the first time