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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

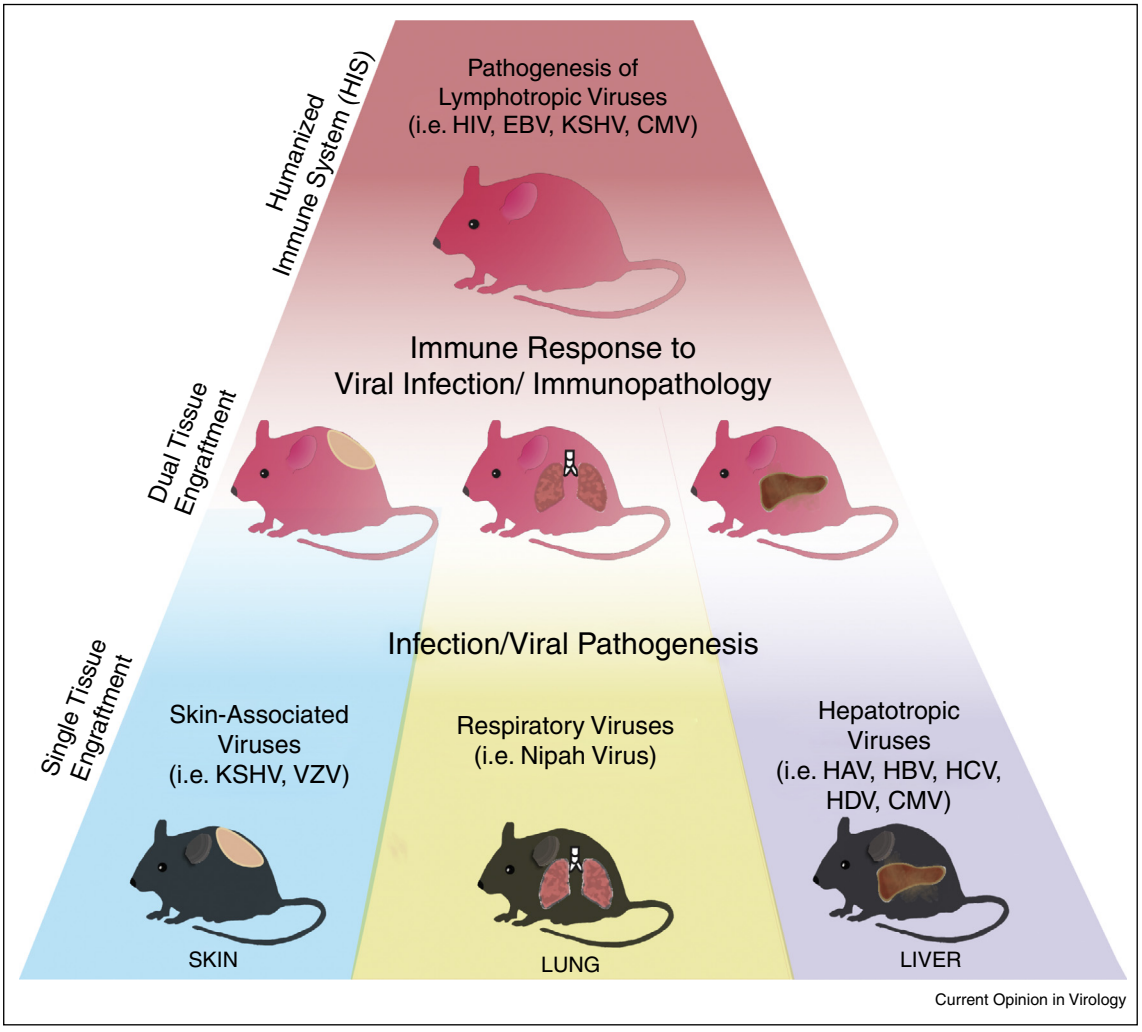
Pathogen	Disease/symptoms	Host factors needed at different steps of the viral life cycle in humans	Factors restricting infection in mice
HIV (as reviewed in [59])	Leads to decreased levels of CD4+ T cells, ultimately resulting in AIDS	Entry: CD4, CCR5, CXCR4 (some T-tropic HIV-1 viruses can use the murine 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

Figure 1



Humanized mice for study of viral pathogenesis. The direct cytopathic effects of a virus on a particular tissue can be studied in mice engrafted with a single human tissue. Skin engraftments have been utilized to study KSHV, where mice exhibit the development of skin lesions and latent infection in cells with altered morphology [43]. Similarly, VZV-infected humanized mice form lesions and have necrosis of multiple skin layers [44]. Respiratory viruses, such as Nipah Virus, can be studied in mice following engraftment of human fetal lung tissue, resulting in viral infection at high titers specifically in the lungs [62]. Following human liver engraftment, mice are susceptible to infection with both HCV [41] and HBV [42]. Lymphotropic viruses, such as HIV-1 and EBV, have been studied in humanized immune system (HIS) mice. However, these HIS mice engrafted with other human tissues have also proven their utility for studying human immune responses exhibited during viral infection.

of the human immune response in humanized mice following DENV infection by mosquito bite is an encouraging step [21].

Human herpesviruses (HHVs)

The nine HHVs are prevalent and can establish long-lasting latent infections, leading to skin lesions, epilepsy, cancer, and autoimmune disease (for review see [22]).

Epstein Barr Virus (EBV) is widespread and linked to ~2% of human tumors originating in lymphocytes and epithelial cells [23]. Only B cell EBV infection can be studied in humanized mice, and different stages of the latent and lytic viral life cycle have been observed in these cells [24,25]. EBV-associated malignancies have also been studied in HIS mice. A viral mutant lacking EBV latent nuclear antigen 3B (EBNA3B) led to formation of large B cell lymphomas in HIS mice [26•]. Further research on this strain has provided evidence that various EBNA3 antigens regulate expression of the chemokine CXCL10, leading to reduced T cell action [27]. EBV-associated hemophagocytic lymphohistiocytosis, with a pathology highly similar to that seen in humans [28], and erosive arthritis [29] have both been observed in

humanized mice. Finally, HIS mice have also been a platform for examining the role of innate immunity in EBV infection (reviewed in [30]).

Human cytomegalovirus (HCMV) is the most common causative agent of congenital viral infection, resulting in children with growth defects or, most detrimental, CNS injury. Additionally, immune-compromised patients, such as individuals living with AIDS or recent organ transplant recipients, are also at risk for HCMV-mediated disease [31]. CMV is found in numerous species, but the determinants of species tropism are not yet defined. While rodents and other animals have been utilized to study congenital CMV infection via their species-specific virus [32,33], it is still not possible to specifically model congenital HCMV infection. However, progress is being made — immunocompromized mice engrafted with human CD34+ hematopoietic stem cells were able for the first time to establish both systemic HCMV infection and also viral latency and reactivation [34]. Even more recently, HCMV infection was established in the human hepatocytes of a human liver chimeric mouse, building toward a resource for *in vivo* testing of candidate therapies [35].

Human T cell leukemia virus type 1 (HTLV-1) is strongly linked to the development of adult T cell leukemia/lymphoma (ATL) and the inflammatory disease HTLV-1-associated myelopathy/tropical spastic paraparesis. HIS mice are primarily used to study human T cells during early HTLV-1 infection and initial stages of HTLV-associated diseases. These mice are able to reproduce some aspects of infection in humans, such as CD4+ T cell lymphomas [36] and symptoms associated with changes in human thymopoiesis [37]. Efforts to improve the adaptive immune response in CD133+ mice by injecting human hematopoietic stem cells into the bone marrow of these mice has led to more consistent B-to-T-cell ratios over time and is thus a better approach for studying ATL development [38].

Finally, immunocompromised mice with human skin transplants have been utilized for studying the herpesviruses most highly associated with skin lesions, such as Kaposi's sarcoma-associated herpesviruses [39] and Varicella-zoster virus [40].

Hepatitis B and C Virus (HBV and HCV)

HBV and HCV together infect ~490 million individuals worldwide, causing liver cirrhosis, fibrosis, and hepatocellular carcinoma if left untreated. These two viruses robustly infect only chimpanzees and humans. In the absence of a permissive mouse model, numerous transgenic strains expressing individual or combinations of HCV gene products were developed to study HBV and HCV-induced liver disease (Table 2, see [41] for HBV and [42] for HCV for in-depth reviews on existing humanized mouse models for these viruses). HBV transgenic mice have contributed substantially to our understanding of many aspects of HBV biology, immunobiology and pathogenesis (reviewed in [43]). In contrast, reports on the histopathology in HCV protein transgenic mice differ vastly depending on the expressed HCV gene product, mouse background or differences in the promoters used for the expression of viral proteins. Their utility is further lessened as they are not bona fide infection models as any pathological processes develop in the absence of the inflammatory milieu established during chronic infection.

To study HBV and HCV infection in mice, humanization of the mouse liver by xenoengraftment of permissive human primary hepatocytes has been explored. Most of the commonly used xenorecipients share common features: they are immunodeficient to prevent xenograft rejection and often suffer from an endogenous liver injury to promote hepatocyte proliferation and provide human donor cells a competitive growth advantage over mouse hepatocytes. In 2001, the development of an Alb-uPA/Rag-2 mouse, which could be engrafted with primary human hepatocytes, was successfully infected with

Table 2

Examples of transgenic mice and their associated phenotypes in studying HBV and HCV

Pathogen	Component of virus expressed	Resultant phenotype in mouse
HCV (as reviewed in [42])	Core	Apoptosis of hepatocytes, lipogenesis
	NS4B	No liver disease observed
	E1-E2-NS2	Liver injury
	Core-E2	No liver disease observed
HBV (as reviewed in [41])	HBV surface antigen (HBsAg) and pre-S and X antigens	No viral replication or signs of liver disease
	X gene	Tumor formation in the liver
	Hepatitis B core antigen (HBcAg)	T cell tolerance in response to HBcAg, but no liver disease observed
	1.3 HBV-DNA	High viral particle production

HBV [44] and also HCV [45]. To improve robustness and throughput, several other immunodeficient liver injury models, including fumaryl acetoacetate hydrolase deficient mice (FAH−/− [46]), MUP-uPA [47] and Alb-HSV1-tk [48], have been generated. These mice can be engrafted to very high levels with human hepatocytes and subsequently become susceptible to HBV and HCV infection. Human liver chimeric mice have been critical tools for studying HBV, HCV and HDV infections but also serve as important tools for preclinically assessing the efficacy of novel therapeutics. However, HBV and HCV pathogenesis, human adaptive immune responses and vaccine development can only be studied in a mouse model harboring both a human liver graft and a functional human immune system. Several groups have now reported that dual engraftment of components of a human immune system and a (matching) human liver can be achieved in a single recipient [49,50]. When infected with HBV [51] or HCV [52], dually engrafted mice indeed mount virus-specific immune responses and develop histopathological features reminiscent of liver disease in humans. However, the difficulties of donor matching for the two tissue compartments, variation in the level of engraftment, the low throughput and the limited functionality of the engrafted human immune system lessen the utility of this model.

An inbred mouse model with inheritable susceptibility to HBV or HCV would overcome the technical difficulties of the xenotransplantation model. The challenge is to systematically identify and overcome any restrictions to viral growth in murine cells. Both the HBV and HCV life-cycles are blocked at numerous steps. For HCV, the minimal set of human specific entry factors, that is human CD81 and occludin (OCLN) have been identified, facilitating HCV uptake into mouse cells *in vitro* [53] and *in vivo* [54]. The entire HCV life-cycle can be recapitulated in mice transgenically expressing human CD81 and OCLN with severely impaired antiviral innate immunity [55••]. The recent identification of human taurocholate co-transporting polypeptide (hNTCP) as an HBV receptor [56••] is a promising first step toward creation of a mouse model with inheritable susceptibility to HBV infection. However, it should be noted that there are still numerous blocks to overcome. While HBV assembly and release are supported in mouse hepatocytes, expression of hNTCP does not render mouse cells permissive for HBV uptake, pointing toward post-attachment and post-entry blocks. These include, but are not limited to, the inability of HBV to form covalently closed circular DNA, its main transcriptional template.

Conclusions

The use of humanized mice in infectious disease research provides a forum for studying viruses previously less accessible due to their species tropism. With so many types of humanized mice now available, researchers will continue to improve and expand upon these models. The research discussed here has provided invaluable lessons for handling emerging viral threats, as exemplified by the quick development of a humanized mouse for studying Middle Eastern Respiratory Syndrome (MERS [57•]) and a lung xenotransplantation model for the emerging Nipah Virus [58••]. As viruses continue to evolve and adapt to new hosts, humanized mice will be an indispensable tool for studying pathogenesis and will increase the likelihood of developing more efficacious therapeutics.

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