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Review

# Studies of retroviral infection in humanized mice

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ABSTRACT

Many important aspects of human retroviral infections cannot be fully evaluated using only *in vitro* systems or unmodified animal models. An alternative approach involves the use of humanized mice, which consist of immunodeficient mice that have been transplanted with human cells and/or tissues. Certain humanized mouse models can support robust infection with human retroviruses including different strains of human immunodeficiency virus (HIV) and human T cell leukemia virus (HTLV). These models have provided wide-ranging insights into retroviral biology, including detailed information on primary infection, *in vivo* replication and pathogenesis, latent/persistent reservoir formation, and novel therapeutic interventions. Here we describe the humanized mouse models that are most commonly utilized to study retroviral infections, and outline some of the important discoveries that these models have produced during several decades of intensive research.



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## Introduction

Infectious agents exact a devastating daily toll of morbidity and mortality worldwide (WHO, 2014). This not only creates millions of personal tragedies each year, but also deeply affects wider communities as the economic and social impact of infectious disease is

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borne by society as a whole. Fortunately, the application of basic and translational scientific research coupled with careful clinical studies has produced a range of vaccines and therapeutic agents that can prevent, treat, or cure previously common human diseases. These successes are built on a foundation of studying the causative organism in relevant biological systems that are suitable for investigating its replication and pathogenesis, and which in turn may be used to evaluate new therapies.

Researchers in the biological sciences are continually striving to develop improved *in vitro* approaches that mimic natural hosts more completely. However, *in vitro* systems represent isolated situations that cannot fully emulate the complexity of an *in vivo* environment. In some cases unmodified animals have proven to be suitable models for studying human pathogens, but this method carries the limitations that the microorganism must be able to replicate efficiently and ideally should cause disease within the animal host species, and that interaction with animal rather than human cells and tissues are being studied.

A powerful complementary approach to *in vitro* or unmodified animal studies is the use of humanized mice. These are mice that have either been engineered to express human genes or (as will primarily be discussed in this review) immunodeficient mice that have been reconstituted with human cells and/or tissues. One particular area of research in which humanized mice have proven useful is in the field of retrovirology. Retroviruses are important human pathogens, and humanized mice have provided important insights into many aspects of retrovirus biology, including transmission, *in vivo* replication and pathogenesis, latent/persistent reservoir formation, and evaluation of novel therapeutic interventions. The purpose of this review is to provide a background on retroviral studies in humanized mice, explain the main types of humanized mouse models currently in use, and provide some examples of the many important areas of retrovirus research that have benefited from the use of humanized mice.

## *Exogenous retroviruses in humans*

Currently, four exogenous retroviruses have been identified that are endemic in certain human populations: human immunodeficiency virus type 1 and 2 (HIV-1 and HIV-2), and human T cell lymphotropic virus type 1 and 2 (HTLV-1 and HTLV-2). Additional retroviruses including HTLV-3, HTLV-4, and human foamy virus (Mahieux and Gessain, 2011; Meiering and Linial, 2001) are occasionally found in rare individuals, but this is generally the result of infrequent zoonotic transmission of related simian viruses. Consequently, the majority of humanized mouse studies of retroviruses have focused on HIV, and to a much lesser extent on HTLV.

HIV is a lentivirus which infects and depletes CD4+ T cells, leading to the development of acquired immune deficiency syndrome (AIDS), which typically manifests approximately 10 years after primary infection with the virus (Barre-Sinoussi et al., 1983; Gallo et al., 1983). HIV originated in nonhuman primates in West Africa. The most common strains of HIV type 1 (HIV-1) are derived from a related simian immunodeficiency virus (SIV) $SIV_{CPZ}$ present in chimpanzees (*Pan troglodytes troglodytes*), whereas HIV-2 is derived from $SIV_{SMM}$ in sooty mangabeys (*Cercocebus atys*) (Hirsch et al., 1989; Keele et al., 2006; Sharp and Hahn, 2011). Over the past century, more than 10 different strains of either HIV-1 or HIV-2 have jumped into humans through independent zoonotic transmission events. The most prevalent of these (HIV-1, group M) was transmitted into humans approximately 100 years ago and is responsible for well over 90% of global HIV infections (Worobey et al., 2008). HIV-2 also causes AIDS but is associated with lower viral loads and reduced person-to-person transmission rates, and has not spread substantially from West Africa (Reeves and Doms, 2002; Sharp and Hahn, 2011). Overall, approximately 35 million people are currently infected with HIV and a similar number have already died of AIDS (UNAIDS, 2013).

HTLV is likely to also have originated in non-human primates, which carry related simian T cell lymphotropic viruses (Proietti et al., 2005). However HTLVs have potentially been present in humans for thousands of generations (Weiss, 1996). HTLV-1 infects around 10–20 million people worldwide and is endemic in regions of Japan, Caribbean islands, South America, and equatorial Africa. The majority of HTLV-1-infected individuals remain asymptomatic, but approximately 5% of carriers go on to develop adult T cell leukemia (ATL) or HTLV-associated myelopathy/tropical spastic paraparesis (HAM/TSP) (Proietti et al., 2005). More rarely, other conditions including infective dermatitis and HTLV-associated uveitis are also caused by HTLV-1 (Proietti et al., 2005). HTLV-2 has not been causatively linked to any disease.

These retroviral infections of humans, particularly the devastating HIV epidemic, provided a strong impetus for the development of animal models that would allow their study in relevant *in vivo* systems.

## *Animal models of retroviral infection*

Many aspects of retroviral infection cannot be adequately investigated in infected humans. This is in part because detailed studies of *in vivo* retroviral replication and pathogenesis necessitate that infections be performed under controlled conditions with pre-defined viral variants. Invasive sampling of tissues at specific post-infection timepoints is also often required. Furthermore, the risks associated with experimental treatments are often too great to perform early efficacy testing in infected patients, and instead can only be reasonably performed in relevant animal models.

Early virus challenge experiments showed that HIV cannot infect unmodified small animals (Morrow et al., 1987). Consequently, most *in vivo* modeling of HIV has focused on alternative approaches. HIV-1 can infect chimpanzees but ethical concerns and their endangered status, coupled with other issues including infrequent and slow development of disease (Keele et al., 2009) now effectively preclude the use of chimpanzees in laboratory HIV research. Certain SIVs can infect and cause AIDS-like disease in non-human primates such as rhesus (*Macaca mulatta*), cynomolgous (*Macaca fascicularis*), or pigtailed (*Macaca nemestrina*) macaques. These models have therefore been used to investigate replication and pathogenesis of a virus that is closely related to HIV, with many of the findings subsequently translated to HIV (Gardner and Luciw, 2008; Hatziioannou and Evans, 2012; Letvin et al., 1985). A variety of modified chimeric SIV/HIV viruses termed simian-human immunodeficiency viruses (SHIVs) have also been created, which can replicate in nonhuman primates (Hatziioannou and Evans, 2012). This allows, for example, antiretroviral drugs or vaccine candidates that are directed towards HIV rather than SIV proteins to be evaluated in simian models. Non-human primate models have limitations in that they are expensive and individual studies are usually restricted to relatively small numbers of animals. The required use of an SIV or SHIV strain of virus also means that some aspects of the interaction between wild-type HIV and human cells or tissues cannot be adequately studied in these models.

Mice have been used to model human disease for many years. Studies with mouse models benefit from their small size, relatively short generation time, and the fact they share many features of physiology and immune function with humans. However mice cannot be infected by HIV, and no murine lentivirus that might serve as a natural model for HIV infection of mice has been found. Transgenic mice that have increased susceptibility to HIV infection through the engineered expression of human HIV entry receptors (CD4 and CCR5) have produced useful information, but are limited