

Review Article

SCID Mouse-Human Lung Xenograft Models for Studying Viral Infection and Pathogenesis

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ABBREVIATIONS

HCMV: human cytomegalovirus; HZ: Herpes Zoster; FDA: Food and Drug Administration; NiV: Nipah Virus; VZV: Varicella-Zoster Virus; MERS-CoV: Middle East Respiratory Syndrome Coronavirus; ZIKV: Zika Virus; RSV: Respiratory Syncytial Virus; SCID: Severe Combined Immunodeficiency; rOka: recombinant pOka VZV strain

INTRODUCTION

Many viruses exclusively infect humans, which hampers *in vivo* studies of their replication, tissue tropisms, and pathogenesis. To address this limitation, humanized severe combined immunodeficiency (SCID-hu) mouse models, carrying biologically relevant human tissues or cells, have been extensively used to reproduce human disease phenotypes caused by viral infection [1]. The purpose of the SCID-hu mouse models varies depending on the type of tissue or cell transplants it receives, which most commonly include human liver, thymus, hematopoietic stem cells, skin, dorsal root ganglion, and lung [1]. Here we review recent progress in the application of SCID mouse-human lung xenograft models, or SCID-hu lung mouse models for virus research, as well as insights into the pathogenesis of virus-induced lung diseases gained from these useful tools.

Creation of the SCID-hu lung mouse model

An ethical statement is critical when performing research on human fetal tissues. The National Institute of Health (NIH) has a regulatory process for obtaining and using human fetal tissues for research. For the establishment of SCID-hu lung mouse models, human fetal lung tissues can be obtained from companies like Advanced Bioscience Resource (ABR), Inc., which is a non-profit tissue procurement organization that provides human fetal tissues to requesting scientific investigators? C.B-17 SCID mice, which are homozygous for the Prkdc^{scid} mutation

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and lack both T and B cells [2], are typically used for the tissue engraftment. Human fetal lung tissues are dissected into 2-8 mm size fragments under sterile conditions before being implanted under the flank skin or the kidney capsule. After 1-2 months, the lung xenografts will expand, develop mature structures closely resembling normal human lung, and can be surgically exposed for virus inoculation. The SCID-hu lung mouse model has been used to study lung infection of various viruses, including varicella-zoster virus (VZV), human cytomegalovirus (HCMV) and Nipah virus (NiV) (see below). By using this model, both viral factors and host factors that determine viral lung infection can be analyzed in a microenvironment similar to normal human lung tissue *in vivo*.

The SCID-hu lung mouse model for VZV infection

VZV, also known as human herpesvirus-3 (HHV-3), is a human herpesvirus of the α -herpesvirus family. The primary infection of VZV causes chickenpox and the virus will establish latency within the neurons of the cranial nerve and dorsal root ganglia. Because of this latency, immune deterioration through factors such as age and medical conditions can cause VZV to become active again as herpes zoster (HZ), or shingles [3]. Another serious complication from VZV is varicella pneumonia, which can cause severe respiratory failure in adults and immunocompromised individuals. Over the years, VZV has mainly been studied in human cell cultures since the virus cannot infect standard animal models. The usage of the SCID-hu mouse models with implants of human skin, T cells, lung and dorsal root ganglion allow for studying VZV infection, tissue tropisms and pathogenesis within various human tissue microenvironments *in vivo* [4-7].

In a study of VZV in SCID-hu mice, the mice were engrafted with human fetal lung tissues. The implanted lung xenografts were then injected with the cell-free virus of rOka, a recombinant Oka expressing firefly luciferase, or mock-infected as a control. To approximately measure viral replication, bioluminescent

signals were measured once every two days for 24 days, and results showed that the rOka-infected lung xenografts displayed viral spread and replication of VZV, unlike the mock-infected xenografts. The virus actively replicated and infected both the alveolar epithelial and mesenchymal cells of the lung xenografts. This viral growth additionally resulted in the upregulation of 14 pro-inflammatory cytokines because of the lack of human immune cells in the SCID-hu model. Clusters and individual nucleocapsids, some of which were wrapped by membrane vesicles and underwent second envelopment, were also observed in the nucleoplasm of the lung cells of the rOka-infected xenografts, unlike in the mock-infected controls. However, some common traits of VZV infection in humans were not shown in the rOka-infected lung xenografts: tissue structures remained intact in the uninfected areas outside the viral lesions, no staining was observed, and no cell fusion or syncytia formation was shown. Despite this, because of its ability to replicate VZV pathogenesis in a microenvironment similar to human lung tissue, the SCID mouse-human lung xenograft model could aid in future antiviral developments and treatments for VZV-related lung diseases, as well as provide answers as to why immunocompromised individuals are more prone to severe pulmonary complications as a result of VZV infection [[6].

The SCID-hu lung mouse model for NiV infection

NiV is an emerging zoonotic virus that causes severe respiratory illness, acute lung injury, and encephalitis, which has a 92% mortality rate in humans [8]. Human-to-human transmission of NiV, as well as animal-to-human, has previously been reported [9-11]. Typically, NiV infects endothelial cells in the lung, but there is limited research data following viral infection, due to the lack of human tissue samples. The histopathological data of NiV infected lungs obtained after necropsy shows the hemorrhage, necrosis, and inflammation of lung epithelium. NiV has been found to be able to infect and replicate to higher titers in the lungs of animal models such as the hamster, ferret, and African green monkey [10,12-15], but a human lung xenograft mouse model of NiV had not yet been established when these studies were being conducted. The exact mechanism of NiV pathogenesis in humans is still unknown, and there is an urgent need to study the molecular mechanism of NiV pathogenesis in order to develop anti-viral drugs and vaccines. It has been reported that the respiratory epithelium plays a major role in early viral infection; therefore, a lung xenograft mouse model for NiV is critical to study viral pathogenesis in the lung and to test the efficacy of antiviral drugs. A human lung xenograft mouse model has recently been developed for the study of NiV infection as a result [16]. The SCID mice (Jackson Laboratory) were transplanted with human fetal lung tissues (obtained from ABR Inc) on the dorsal muscle fascia in the dorsal subcutaneous space. This xenograft mouse model of the human lung demonstrated that following transplantation, lung tissues rapidly develop mature structures resembling those of adult lungs', including vascular vessels, cartilage, ciliated pseudostratified columnar epitheliums, and primitive air space filled with mucus and surrounded by flat epithelium [16]. In this model, NiV was found to replicate in the respiratory epithelium of small airways and bronchi as well as mesenchymal cells. Moreover, NiV was also detected in the endothelium cells, cells that are commonly targeted by the virus. Furthermore,

intraperitoneal inoculation of NiV led to the infection of human lung xenografts, suggesting hematogenous viral spread. In this mouse model, NiV was able to replicate to higher titers in human lung tissue and cause pulmonary syncytia formation after three days of post-infection. Histopathological analysis showed that NiV infection caused extensive necrosis, syncytia formation and loss of alveolar architecture in the lung xenografts. The infection stimulates a strong inflammatory response caused by an acute lung injury. This mouse model will be very helpful in unraveling the host-virus interactions and can be used for many other pathogens that cause respiratory diseases.

The SCID-hu lung mouse model for HCMV infection

HCMV, or human betaherpesvirus 5, is a large DNA virus that infects people of all ages. HCMV infects 60-70% of adults in developed countries and more than 90% of adults in developing countries. HCMV infection is usually asymptomatic in healthy people, but immunocompromised patients and patients with organ transplant are at greater risk of infection. Moreover, HCMV is a leading cause of birth defects in newborns, affecting 1% of all births worldwide [17]. Mature children and immunocompromised patients are typically associated with life-threatening respiratory lung-related disease from HCMV infection. Studies have also shown that HCMV mainly targets the lung for infection and replication in fetuses, as well as in newborns [18]. HCMV is uniquely a human pathogen and does not infect animals [19], which brings up the need for an *in vivo* animal model that can be infected with HCMV. A SCID-hu mouse model with human fetal lung xenografts was generated by implanting the fetal lung tissue under the kidney capsular membranes of C.B-17-SCID mice [20]. The clinical VR1814 strain of HCMV was then allowed to infect the lung xenograft, and results demonstrated that the virus efficiently replicated for a period of two weeks and formed large viral lesions. HCMV was found to have replicated in alveolar epithelial and mesenchymal cells, similar to congenital HCMV infection of the fetal lung. Additionally, HCMV glycoprotein B was readily detected by immunofluorescence and western blot, confirming viral infection in the lung xenografts. The anti-HCMV drug, ganciclovir and valganciclovir, treatments reduced the viral load in the lung of SCID mice, suggesting applications for antiviral drug screening. Staining in the lung xenografts confirmed viral replication in the alveolar epithelial cells, similar to natural HCMV infection. Moreover, the immunostaining infected xenografts showed that HCMV impaired the secretion of surfactant proteins from alveolar epithelial cells. This mouse model recapitulates the fetal as well as neonate lung development and represents an important tool to study HCMV, as well as other human pathogens that target the lung.

A human lung xenograft mouse model with expanded tropisms for human pathogens

Recently, a study has evaluated both lung-only humanized mice and bone marrow/liver/thymus-lung humanized mice to study lung infection of several emerging and clinically relevant human pathogens such as Middle East respiratory syndrome coronavirus (MERS-CoV), Zika virus (ZIKV), respiratory syncytial virus (RSV), and HCMV [21]. The results obtained in this study clearly demonstrate that the subcutaneous implantation of

human lung tissue in the backs of SCID mice resulted in a highly vascularized lung xenograft located just underneath the skin, allowing for easy accessibility and imaging [21]. This created humanized lung-only mice (LoM). The human lung tissues vascularized, persisted, and expanded as a human lung xenograft that supports the infection and replication of human pathogens, including viruses and bacteria. The lung xenografts were ectopic and not ventilated, but the xenografts still had well-defined structures that are characteristic of the human lung, including ciliated epithelium, airways, associated blood vessels, cartilage, and alveolar structures. The *ex vivo* culture of lung xenografts has the ability to produce human chemokines and cytokines, suggesting an ability to function similarly to normal human lungs. The authors of this study used immunostaining and histopathological analysis to show that LoM can be infected by a diverse set of pathogens including HCMV, MERS-CoV, ZIKV, RSV, and mycobacteria. Specifically, HCMV TB40/E, a clinical HCMV strain, was administered, and HCMV DNA and proteins were detected in the infected lung xenografts, demonstrating active viral replication. Ganciclovir treatment of LoM infected with HCMV reduced the viral load in the human lung xenografts.

The LoM mouse model can be used for virus replication and pathogenesis studies. To understand the role of host immunity in virus pathogenesis, a humanized BLT-L mouse model was also generated by implanting human lung tissue in BLT mice and was named the BLT-lung (BLT-L) model. The BLT-L mouse was created by implanting autologous human liver and thymus tissues under the kidney capsule and human lung tissue under the skin of the same preconditioned irradiated mouse, followed by bone marrow transplantation with autologous hematopoietic stem cells [21]. Therefore, a BLT-L mouse model consists of human liver and thymus tissues under the kidney capsule, along with human bone marrow tissue and human lung tissue. Therefore, the BLT-L model will have a systemic autologous human innate and adaptive immune response. The BLT-L mice also consist of human mesenchymal, epithelial, and endothelial cells. When infected with HCMV, the model shows viral gene expression and generates specific B and T cells response against HCMV infection. BLT-L mouse can be infected with a diverse set of respiratory pathogens including RSV, ZIKV, MERS-CoV, mycobacterium, HCMV, and other providing an opportunity to study their replication, pathogenesis, and host immune response activation in parallel. BLT-L mice recapitulate viral infection in humans with similar kinetics and replication, providing a valuable tool to study virus pathogenesis as well as host immune response [21].

DISCUSSION

It is often difficult to perform research on human-specific viruses because of their inability to infect standard animal models. Human-specific viruses, such as VZV, HCMV and HIV, only infect humans and lack animal models. This makes studies of viral replication and pathogenesis *in vivo* and preclinical trials of antiviral compounds extremely difficult. Therefore, a humanized mouse model is urgently needed.

The genetic mutation that SCID mice carry enables them to accept xenografts of human tissues and organs, creating humanized mice. One type of humanized mice is the SCID-hu lung mouse, which is created by grafting human fetal lung tissues

underneath the kidney capsules or back of C.B-17 SCID mice. Within eight weeks, the mice develop mature lung structures that function similarly to normal human lung tissues. The model can be applied in various *in vivo* studies of viruses that target the lung, including VZV, HCMV and NiV. These viruses are able to infect the lung xenografts of the SCID-hu mice, and immune responses can be observed within the mice over a period of time. After infection, the models display viral responses similar to that of humans', including effective viral replication in alveolar epithelial and mesenchymal cells, as well as inflammatory cytokine production. In a study of VZV infection in SCID-hu mice, it was found that viral growth did not cease in response to the pro-inflammatory response; in fact, it continued for a longer period of time until the fetal lung tissues from the implantation sites completely collapsed⁵. Furthermore, anti-viral treatments and their effects can be tested in SCID-hu mice, allowing for the developments of novel drug targets. The immunostaining and histopathological analysis of infected lung xenograft shows that viruses can replicate efficiently and cause syncytia formation and loss of alveolar structures, recapitulating natural infection. It has also been demonstrated that active viral replication leads to the robust necrosis of infected cells in the lung xenograft¹⁹. This evidence strongly suggests that a SCID-hu model infection with diverse set of human pathogens recapitulate the natural infection in terms of pathogenesis, replication, inflammatory response and generate similar histopathological data as with human infection.

However, there are several limitations to this model. SCID-hu models lack human immune cells and does not have the adaptive immunity needed for precise studies on host immune response to viral infection. Moreover, a SCID mouse model may not be suitable for studying viral latency because viruses such as VZV establish latency in neurons and HCMV in lymphocytes. Most of the time, active viral replication leads to the infection of other organs such as the liver, kidney, and brain, as in case of HCMV, fetal brain, in the case of ZIKV, which leads to microcephaly; therefore, the SCID model may not be used for complete pathogenesis study of a virus.

To overcome this limitation to an extent, a novel humanized SCID mouse model, BLT-lung mouse model (BLT-L), has been developed. The BLT-L model contains up to 40 cell types, including non-hematopoietic cells, and it contains xenografts from the human lung, thymus, liver and bone marrow. This allows for the model to have a systemic autologous human innate and adaptive immune response, permitting studies of immune responses to viral infections in SCID mice. BLT-L mice have also been used to study HCMV pathogenesis, where specific B and T cell reactions could be generated in response.

With the usage of SCID-hu mouse models, disease pathology can be further studied without putting human patients at risk. The SCID-hu lung mouse model will help identify viral or host factors that cause the development of diseases caused by lung-related viruses, including RSV, MERS-CoV, HCMV, and NiV. Furthermore, the addition of the BLT-L mouse model allows for the number of human pathogens that can be studied *in vivo* to be substantially increased. Because of its ability to be generated in large numbers from small tissue samples, the SCID-hu lung model is also able to meet the demand for research on human-specific viruses and

their pathogenesis, increasing its usability and relevance in the field. In the future, these humanized mouse models may be the key to develop novel antiviral compounds and treatments for human autoimmune diseases involving the lungs.

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