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Human immune system mouse models of Ebola virus infection

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Abstract

Human immune system (HIS) mice, immunodeficient mice engrafted with human cells (with or without donor-matched tissue), offer a unique opportunity to study pathogens that cause disease predominantly or exclusively in humans. Several HIS mouse models have recently been used to study Ebola virus (EBOV) infection and disease. The results of these studies are encouraging and support further development and use of these models in Ebola research. HIS mice provide a small animal model to study EBOV isolates, investigate early viral interactions with human immune cells, screen vaccines and therapeutics that modulate the immune system, and investigate sequelae in survivors. Here we review existing models, discuss their use in pathogenesis studies and therapeutic screening, and highlight considerations for study design and analysis. Finally, we point out caveats to current models, and recommend future efforts for modeling EBOV infection in HIS mice.

Keywords

Ebola virus; hemorrhagic fever; humanized mice

Introduction

Ebola virus (EBOV; species *Zaire ebolavirus*; family *Filoviridae*) is the primary etiologic agent of Ebola virus disease (EVD), a zoonotic viral hemorrhagic fever. Current animal models of EVD include rodents (mice, hamsters, guinea pigs), ferrets, and several non-human primate (NHP) species. Wild-type (WT) EBOV causes lethal disease in ferrets and NHPs [1–3]. NHPs remain the gold standard model for studies of EVD. Ferrets are new and promising models, but are currently not well established, lack tools for immunological

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analyses and present additional challenges compared to other small animal models, including handling and housing requirements. Rodents exhibit no, or very mild clinical signs despite viral replication, and require viral serial adaptation to cause severe disease [4–6]. In rodents, disease from WT-EBOV infection is restricted to immunocompromised mice, such as $STAT^{-/-}$, $IFNAR^{-/-}$, A129, and SCID strains [7–9]. Despite the limitations, rodent models have been important in early investigations of viral pathogenesis and for preliminary therapeutic and vaccine screening studies, including for the development of viral-vectored EBOV vaccines that are currently in clinical evaluation [10,11].

The discrepancies between mouse and human immune systems [12] are of considerable concern, especially for diseases in which pathogenesis is, at least partially, immune-mediated. Advanced mouse models attempt to address these discrepancies. “Humanized mice” is a general designation for mice that express human genes, or contain human cells and/or tissues (e.g., transgenic mice or xenograft mouse models). Included in the term “humanized mice” are human immune system (HIS) mice, mice engrafted by a variety of approaches, including the use of human hematopoietic stem cells (HSC) or induced pluripotent stem cells (iPSC), resulting in reconstitution of human immune lymphoid and myeloid cells that are both present and functional to varying degrees [13,14]. HIS mice are increasingly being used to study human cancer and tumor biology, immunity, infections, autoimmunity, allergies, organ transplantation, vaccine development, and immune regulation [15].

EVD involves a complex combination of virus-mediated and immune-mediated alterations in organ and vascular function. The initial targets of filoviral infection and replication are immune cells, including macrophages and myeloid dendritic cells, which contribute to viral dissemination and immune dysregulation [16,17]. Using HIS mice to study EVD is promising for several reasons. The human immune cells in HIS mice provide an abundance of susceptible targets for filovirus replication in a rodent model that would otherwise efficiently control WT virus replication [18]. With this unique human immune system milieu, HIS mice are a platform to investigate early events in the infection of immune cells, to study the development of adaptive immune responses, and to test vaccines and immunomodulatory therapies.

EBOV infection in human immune system mice

All of the current HIS mouse models of EBOV infection are derived from the highly immunodeficient NOD-scid/IL2R$\gamma^{-/-}$ (NSG) mouse background (Figure 1). The humanization process increases susceptibility of the mice to disease from EBOV infection. Unengrafted NSG mice do not develop disease until 3 to > 6 weeks post-infection [19,20] [Spengler & Prescott, unpublished data], similar to SCID mice that are deficient in adaptive immune responses, lacking both B and T cells, but capable of type I IFN responses [4]. Although current HIS models are from the NSG background, they differ in engraftment approach, expression of human transgenes, and potential for human HLA/MHC compatibility, differences that result in complex variation in human and mouse immune system functionality. Early investigations of EBOV infection in humanized mice were challenging due to inherent limitations of the existing models. The NSG-huPBL model,

engrafted with peripheral blood lymphocytes, is primarily reconstituted with T cells that have a mature phenotype, and is plagued with a rapid onset of graft-versus-host disease (GVHD), which limited studies to about a month post-engraftment. The NSG-huPBL model was used to investigate human lymphocyte apoptosis in mice inoculated with mouse-adapted EBOV (MA-EBOV) or WT-EBOV (Table 1). Increased levels of several human cytokines and significant lymphocyte apoptosis were reported in MA-EBOV compared to WT-EBOV infection, and only MA-EBOV infection resulted in a lethal disease [21], similar to other rodent models.

The first lethal humanized mouse model of EVD was reported in hu-NSG-A2, humanized mice [19] (Table 1, Figure 1). Hu-NSG-A2 have functional human HLA-A2-restricted T-cell responses [22,23], which is beneficial for investigating A2-restricted antigens involved in the immune response to EBOV infection [24]. In hu-NSG-A2 mice engrafted with HSC, EBOV was lethal in an engraftment level-dependent manner; lethality was uniform in mice with high peripheral engraftment (>40% human $CD45^+$ of total peripheral blood leukocytes), but sub-lethal in mice with low peripheral engraftment (20–40% $CD45^+$). In hu-NSG-A2 mice, EBOV infection was disseminated, and liver damage and hemorrhage were observed [19]. EBOV studies in hu-NSG-A2 concluded that the association between disease severity and engraftment levels might be a result of immunopathology rather than the availability of human immune target cells of infection to overcome restricted viral replication in traditional mouse models [19]. However, support for these conclusions would require further investigation of immunopathogenic processes in this model.

Subsequent studies emphasized that contributors to EVD in HIS models are complex and still unclear. Hu-NSG-SGM3 mice are derived from the triple transgenic NSG-SGM3 (NSGS) mice expressing human IL-3, GM-CSF, and SCF cytokines that support the stable engraftment of myeloid lineages and result in high levels of regulatory T cell (Treg) populations [25,26] (Table 1, Figure 1). Hu-NSG-SGM3 studies were the first to demonstrate susceptibility of humanized mice to EBOV infection after intramuscular (IM) inoculation. This is notable, as rodents only develop disease when adapted virus is administered intraperitoneally (IP), whereas peripheral inoculation routes result in vaccination and protection against subsequent lethal challenge [4]. In hu-NSG-SGM3, despite high myeloid and lymphoid reconstitution, infection with EBOV-Makona did not result in uniform lethality; lethality was ~50% in IP-inoculated mice (10^3 FFU) and ~70% in IM-inoculated mice (10 or 10^3 FFU). In NHPs, EVD is characterized in part by hepatocyte necrosis and apoptosis [27], a result of high levels of cytokine expression leading to immunopathogenesis [28]. Interestingly, in hu-NSG-SGM3 mice with severe or lethal disease hepatocytes did not appear to be responding to inflammatory cells, as the typical pathology seen in NHPs was not observed in the mice, despite the presence of higher levels of mononuclear cells. This may be due to a lack of epitope-restricted responses in hu-NSG-SGM3. Alternatively, this may be due to high levels of Tregs that may modulate an anti-inflammatory response to EBOV, as transfer of human $CD34^+$ HSC into NSG-SGM3 also significantly increases the $CD4^+CD25^+Foxp3^+$ Treg population. In these models, Tregs have been shown to suppress polyclonal T cell proliferation [26], and to prevent virus-induced liver fibrosis [29], potentially explaining the absence of severe hepatic pathology in this EBOV model.

NSG-huBLT (bone marrow/liver/thymus) mice are prepared by co-transplanting human fetal liver, thymus, and autologous HSC (Table 1, Figure 1). A subset of cellular responses (Th1, Th2, and CTL), and of antigen-specific antibody responses that generate consistent IgM, but variable IgG [30–32] are functional in NSG-huBLT. Advantages of the hu-NSG mice with donor-matched thymic and liver tissue, such as the NSG-huBLT, include multilineage hematopoiesis (myeloid and lymphoid), T cell maturation in autologous thymus, HLA restriction, and mucosal human cell reconstitution [33]. In this model, human antigen presenting cells can present antigen to T cells in an MHC I- and II-restricted manner. Disadvantages to the model include the development of a wasting disease and the requirement of donor-matched tissue for implantation. Studies of EBOV infection in NSG-huBLT highlighted several advantages to humanized mouse studies, including the ability to lower the challenge dose to reduce lethality, and were the first to demonstrate the ability to use EBOV strains other than the prototypic Mayinga isolate from the first EVD outbreak in 1976, to which all adapted rodent models have previously been restricted [20]. EBOV infection in NSG-huBLT mice resulted in disease and pathology consistent with that seen in hu-NSG-A2, suggesting that HLA restriction may contribute to altered pathogenesis in the model. Although disease in NSG-huBLT was consistent with the findings in hu-NSG-A2, lethality in NSG-huBLT did not appear to be associated with engraftment levels in peripheral blood; engraftment ranged from ~25% to >50% total human CD45+ in peripheral blood, and no association with outcome was observed.

What do human immune system mice offer to the field of EBOV research?

There are many fundamental differences between mouse and human immune systems, including differences in blood cell populations; in MHC class expression by endothelial cells; in genomic inflammatory responses; and in innate immune molecules resulting in the functional loss or complete absence of homologous receptors [34]. These differences, coupled with the necessary use of rodent-adapted virus, present fundamental challenges for the use of mice in EBOV research. HIS mice offer several advances in rodent modeling of EBOV infection, including the study of (1) clinical isolates of EBOV; (2) early interactions of EBOV with human immune cells; (3) therapeutic interventions that modulate the human immune response to EBOV; and (4) EVD survivors.

The most valuable aspect of existing humanized mouse models of EVD is the ability to investigate early viral interactions with host cells and the resultant human immune responses. Early targets of EBOV infection include monocytes, macrophages, and dendritic cells, and EBOV's ability to modulate the activation and maturation of these initial target cells correlates with its pathogenic potential [35,36]. Previously, studies of human immune cell responses were limited to ex vivo and in vitro experimental approaches. In humanized mice, the immune response to peripheral inoculation in resident immune cell populations can be investigated in an in vivo context, and humanized mice offer significant benefits for evaluating interventions that modulate these responses [37,38]. HIS models are particularly useful for studies of immunomodulatory therapies, including monoclonal antibodies that target specific immune pathways (e.g., anti-type II IFN).

NHPs develop acute, uniformly lethal disease and recapitulate many of the clinical signs and abnormalities noted in severe human disease. However, there are still several differences in the disease observed in NHPs versus humans, and studies of protracted or mild disease and of survivors can be challenging in these and other highly sensitive models of EVD. The 2013–2016 EVD outbreak in West Africa generated reports of ocular deficits, hearing loss, difficulty sleeping, arthralgias, and the potential for viral persistence in immunologically protected sites in survivors [39–41], strongly supporting the need for additional animal models in which EBOV infection is not uniformly lethal to study disease sequelae. There are currently no NHP models for mild and protracted Ebola disease or to study disease sequelae in survivors. The establishment of those models would take huge effort and expense, as high numbers of animals are needed to study such scenarios. In HIS mice, EBOV infection causes disease, but subsets of animals survive, permitting the study of protracted disease and investigation of sequelae (e.g., examining humanized mice for histological evidence of damage to the auditory system and CNS). While studies of survivors are an important and promising application of HIS mice, extrapolating observations to human disease will require further evaluation of the models based on current knowledge from EVD survivors to determine characteristics that mirror human disease and those that are dissimilar. Although some aspects may differ between HIS mice and human disease, we can still learn from these models. For example, virus appears to persist longer in surviving HIS mice than in humans. This may simply reflect poor humoral responses in current HIS mouse models, supporting the association between antibody levels and viral clearance in EVD patients [42].

Conclusions and future considerations

Characterization of HIS mouse models for the study of EVD is currently in its infancy. Many questions remain, including, and most importantly, what events lead to EVD manifestations, and what factors in the humanized milieu are unique in these processes. For example, in contrast to human cases, in which inflammation is a result of cytokine expression in response to viral infection, NSG-huBLT mice have a high residual mouse myeloid background, which could account for much of the inflammation seen in these animals. Thus, while NSG-huBLT demonstrated gross human cell-specific immunological alterations consistent with human disease, the mechanism leading to this result may not mirror the process in humans. As contributors to disease in these mice are better understood, the current complexity in interpreting findings in HIS mice and translating them to human disease will also significantly improve.

The studies described above represent significant breakthroughs in mouse modeling of EVD, but each presents its own caveats. Conclusions drawn from HIS models are highly dependent on knowledge of the particular model independently of challenge agent; the quantity of human and mouse immune cells (% and absolute), and the functional ability of reconstituted immune cell populations must be known. Without this information it is difficult to draw conclusions, such as those regarding the association of engraftment levels in HIS mice. Importantly, to date, engraftment comparisons have only been based on relative human and mouse contributions to cell populations in the blood, which may not reflect the potential impact of mouse cells in the leukocyte population. Further studies should examine this

impact by assessing association of infection outcome with absolute cell counts of both species, which will vary by model.

Other caveats involving immune function are illustrated in hu-NSG-SGM3 mice, in which selection of T cells on a murine thymic scaffold may result in rather modest adaptive immune responses [43]. Even in models with human thymic education, such as the NSG-huBLT, some T cells still develop on the mouse thymus, leading to a mix of mouse and human MHC-compatible cells. Furthermore, most models lack human cytokines critical for hematopoiesis and/or have insufficient interactions between cells of the murine stroma and human hematopoietic cells. This less than optimal lymphoid architecture can also affect adaptive immune responses. Alternatively, as in hu-NSG-SGM3 mice, cell populations under the influence of overexpressed transgenic human cytokines can lead to increased baseline inflammatory responses or an artificial cellular maturing environment, which may limit the cells' ability to respond appropriately to infection.

Another factor in HIS models of EVD is the function of the humoral immune response. Currently, evaluation of the humoral immune response in EBOV-infected HIS mice is lacking. HIS mice can generate humoral responses upon immunization or viral infection [44], but IgM production has been the predominant response observed, with only low frequency production of specific IgG. Poor humoral responses can result from the inability to efficiently recognize antigens presented by human APCs and B cells in HIS mice, where the murine MHC positively selects human T cells during the intrathymic development process [45]. However, T-cell function does not appear to be the only factor important in B-cell maturation and class switching. In the BLT model, with enhanced T-cell engraftment and human MHC-restricted T-cell function, IgM remains the predominant antibody response [46]. Despite limitations in some models, vaccine studies are currently being performed using HIS mice [47,48]. Furthermore, recent reports demonstrating virus-specific and neutralization-capable IgM and IgG responses supports the use of huBLT models in vaccines studies [49,50]. Development of HIS mouse models with advanced humoral immune function is a focus in the field. As the availability of these models expands, future efforts should concentrate on characterizing them for EBOV vaccine studies.

Advances in HIS models also aim to ameliorate reconstitution of specific cell populations. EBOV targets myeloid cells, which historically were poorly reconstituted in HIS mice. Newer models, such as hu-NSG-SGM3 mice, demonstrate improved development of human myeloid-lineage cells. Monocyte, DC, granulocyte, and mast cell reconstitution can be selectively enhanced with expression of human transgenes, including IL-3, IL-4, and IL-15 with Flt-3 ligand (Flt3L) or macrophage CSF; or by replacing murine genes with human homologs [51–53]. In addition, other humanized mouse models offer unique opportunities to study EBOV. Humanized liver models, mice in which the mouse liver is replaced by xenografts of human primary hepatocytes (some at > 80% replacement) [52,54], have been successfully applied in studies of other hepatotropic viruses [55]. Efforts to combine technical advances in HIS mice with tissue engraftment may offer novel opportunities to further model EBOV pathogenesis.

EVD studies in HIS mice to date have emphasized the need for particular considerations in study design and data interpretation. Donor variability may affect the results depending on the model. A subset of NSG-huBLT studies that investigated this effect, although small in number, supported the presence of donor-associated outcome. However, this affect was not apparent in larger hu-NSG-SGM3 studies. The relative contribution of donor variability in HIS models may reflect differences in model characteristics, namely the presence or absence of HLA-restriction, but remains to be further investigated. Route of inoculation can also play a critical role in outcome, and IP is not always the preferred route, as is described in HIS mouse models of *Plasmodium falciparum* infection [56]. This was indicated in the EBOV studies in hu-NSG-SGM3, as increased lethality and reproducibility was seen with IM inoculation versus IP inoculation [25]. Finally, HIS mice are a dynamic milieu of human cell populations; the time post-engraftment that animals are inoculated can lead to different outcomes due to temporal variation in the relative presence of immune cell populations in both tissue and blood. Considerations in data analysis and reporting of age post-engraftment, engraftment levels, and donors' HLA types when applicable, are essential to improve our knowledge of how and when these variables affect study outcome, and to further understand the EVD process in these models.

Humanized mice are highly promising for research of EVD and other hemorrhagic fever viruses, especially in light of continued advancement in HIS models. An ideal HIS mouse model would reconstitute all human immune cell populations in relative frequencies observed in humans, and be engineered to maintain natural cellular function and recapitulate both innate and adaptive immune responses. However, the complex dynamic between reconstituted cell populations and murine parenchymal cells will always be present, and should be characterized to aid in interpretation of experimental infection data. For future studies, the selection of which HIS mouse model to use will be pivotal and highly dependent on the questions being addressed. Appropriate model selection will rely on continued efforts to characterize and determine which factors, immune and non-immune, contribute to EVD susceptibility in these models, and how this compares to the human disease process.

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Highlights

- Human immune system mice are useful tools for Ebola virus research.
- Human lymphoid and myeloid immune cells are reconstituted in human immune system mice.
- Wild-type Ebola virus causes disease in humanized mice.
- Disease outcome varies with model, dose, route, donor, and time post-engraftment.
- Knowledge of human immune cell levels and function in each model is key in interpreting studies.

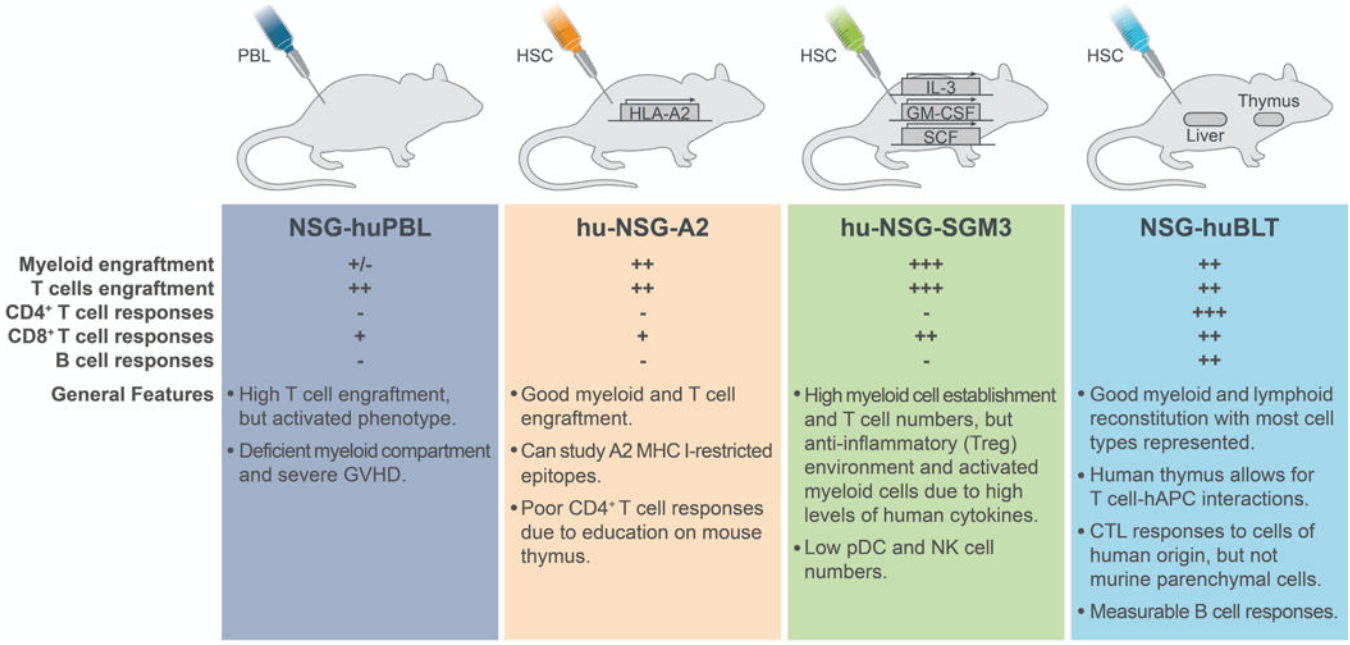

Figure 1.

Characteristics of human immune system mouse models used in Ebola virus infection studies. All strains are derived from a NOD-scid/IL2R$\gamma^{-/-}$ (NSG) background and are injected intravascularly with either human peripheral blood lymphocytes (PBL) or hematopoietic stem cells (HSC). Strains vary in human transgene expression, and in presence or absence of co-engrafted, donor-matched human liver and thymic tissue. Human cell engraftment and responses are indicated as (−) absent, (+) present, or (+/−) may only be present in low numbers and may not be functional. CTL: cytotoxic T lymphocyte; GVHD: graft versus host disease; NK: natural killer cells; pDC: plasmacytoid dendritic cells; Treg: regulatory T cells.

Table 1

EBOV-infection in human immune system mouse models.

Model	EBOV Strain	Route/Dose	Survival	Pathology/Immune response	Ref.
NSG-huPBL	MA-EBOV	IP, 1000 PFU	0%	Human lymphocyte apoptosis	[21]
	Mayinga	IP, 1000 PFU	100%	Absence of human lymphocyte apoptosis	
Hu-NSG-A2	Mayinga	IP, 1000 FFU	0% – 25%	Focal hemorrhage in liver, splenomegaly, steatosis; elevated hepatic enzymes	[19]
Hu-NSG-SGM3	Makona (C07)	IP, 10 PFU	100%	NR	[25]
		IP, 1000 PFU	50%	NR	
		IM, 10 PFU	33%	NR	
		IM, 1000 PFU	33%	Macrophage infiltration, lymphocytes in liver and spleen; increased T cell frequency in blood, T cell activation in the spleen	
NSG-huBLT	Mayinga	IP, 100–100000 $TCID_{50}$	0%*	Hepatic viral inclusion bodies and cellular necrosis; elevated hepatic enzymes; upregulation of human cytokines and chemokines	[20]
	Makona (LBR/2014)	IP, 100 $TCID_{50}$	50%	NR	

*Excludes one survivor that was most likely a result of infection failure; unlike other survivors, there was no evidence of viral RNA in any tissues at time of euthanasia. MA, mouse adapted; NR, not reported.