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Current Opinion in Virology

Humanized mouse models of human cytomegalovirus infection

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The generation of humanized mouse models in which immune deficient mice are engrafted with human tissues allows for the direct *in vivo* investigation of human-restricted viruses. These humanized mouse models have been developed and improved over the past 30 years. It is now possible to achieve high levels of human cell engraftment producing human myeloid and lymphoid lineage cells. Humanized mouse models have been increasingly utilized in the study of human cytomegalovirus (HCMV), a human-specific beta-herpesvirus that infects myeloprogenitor cells and establishes a life-long latency in the infected host. This review focuses on the strengths and limitations of the current humanized mouse models used to study HCMV replication, pathogenesis and treatment.

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Current Opinion in Virology 2015, **13**:86–92

This review comes from a themed issue on **Animal models for viral diseases**

Edited by **Alexander Ploss** and **Christopher Walker**

For a complete overview see the Issue and the Editorial

Available online 25th June 2015

http://dx.doi.org/10.1016/j.coviro.2015.06.006

Introduction

Although most human cytomegalovirus (HCMV) infections are asymptomatic in immune competent individuals, the virus remains a significant cause of morbidity and mortality in bone marrow and solid organ transplant recipients. The strict species specificity of HCMV and the lack of a suitable animal model system have impeded our understanding of viral pathogenesis and the development of antiviral therapies. Over the last two decades, humanized mouse models in which immune deficient mice are engrafted with human tissues has opened the door for the direct *in vivo* investigation of viruses with growth restricted to human cells. Advancements relating to xenograft tolerance and xenograft tissue function have allowed high levels of human chimerism, especially with respect to immune cells and liver tissue. Due to the critical role that immune cells play in the latency, persistence, and/or in the pathobiology of many human herpesviruses, the field of herpesvirus research has benefited tremendously over the last decade from the continued improvements in human immune system (HIS) mouse technology. HIS mice are generated from immunodeficient mice in which the murine immune cell compartments, most notably the bone marrow, are depleted, typically by irradiation, and reconstituted with human hematopoietic progenitor cells (HPCs). This review will focus on the use of humanized mouse models to study mechanisms of HCMV latency, reactivation and treatment.

Overview on HCMV

Human cytomegalovirus is the prototypical betaherpesvirus and a ubiquitous opportunistic pathogen infecting the majority of the world's population. HCMV infection is usually asymptomatic in healthy individuals, but viral infection causes severe disease in immunocompromised adults and birth defects in newborns (Table 1) [1]. Additionally, HCMV has been implicated as a possible cofactor in the development of vascular diseases such as atherosclerosis, transplant vascular sclerosis, and coronary restenosis after angioplasty surgery [2] (Table 1).

A characteristic of HCMV infection is the ability of the virus to spread to and persist within multiple host organs [1]. HCMV infects a variety of cells types, including hematopoietic and stromal cells of the bone marrow, endothelial cells, epithelial cells, fibroblasts, neuronal cells, and smooth muscle cells [3,4]. Of the hematopoietic lineage cells, which comprise all hematopoietic stem cell-derived myeloid and lymphoid lineages, the myeloid cell lineage is the most important with respect to HCMV latency, reactivation and persistence [5,6]. Monocytes are the primary targets for infection in the blood and are non-permissive for viral gene expression [7–9]. Macrophages, however, are productively infected in patients with HCMV disease [3], and *in vitro* studies have confirmed that macrophages and monocyte-derived dendritic cells are permissive for HCMV replication [3,10]. HCMV dissemination is proposed to occur, therefore, after infected monocytes migrate into tissues and differentiate into permissive macrophages [11]. Significant evidence indicates that latently infected peripheral blood monocytes

Table 1

Human cytomegalovirus-associated diseases

Category	Patient	Diseases
Infection in normal host	Healthy, adult	Mononucleosis-like syndrome
Congenital infection	Healthy, fetal	Intrauterine growth retardation, hepatosplenomegaly, thrombocytopenia, petechiae, chorioretinitis, and hepatitis; CNS involvement (microcephaly, encephalitis, seizures, and focal neurological signs)
Infection in the immunocompromised host	Solid organ and hematopoieticstem cell transplant	CMV syndrome, gastrointestinal disease (esophagitis, colitis), pneumonitis, hepatitis, allograft vasculopathy (heart transplant)
	AIDS	Encephalitis, radiculopathy, pneumonitis, gastrointestinal disease, retinitis
Contribution to chronic vascular disease		Atherosclerotic vascular disease and autoimmune vasculitides

(PBMCs) are generated from latently infected HPCs of the bone marrow [6]. HCMV-infected HPCs transiently expressed a subset of viral genes that largely become undetectable by 10 days after infection [5]. Nevertheless, viral genomes are maintained at approximately 5–10 copies per cell in the absence of viral replication during long-term culture. HCMV replication can be reactivated by co-culture of both $CD34^+$ and $CD33^+$ progenitor cells with human fibroblasts [5,6,12]. Although these primitive HPCs have the capacity to mature into a number of cell lineages, latent HCMV DNA is strictly associated with myelomonocytic lineage cells in healthy hosts [13]. This suggests that either latent infection of myeloid stem cells promotes maturation into the myelomonocytic lineage or that only cells of the myelomonocytic lineage are capable of maintaining the latent viral genome.

Development of humanized mouse models to study HCMV

The strict species specificity of HCMV and the lack of surrogate CMV animal models have driven the development of humanized mouse models in which mice are engrafted with human cells or tissues capable of supporting local HCMV infection. The original humanized mouse models, first reported in 1988, involved SCID (severe combined immunodeficient) mice engrafted with either human peripheral blood leukocytes (SCID-hu-PBL model) [14] or with human fetal thymic and liver tissues (SCID-huThy/Livmodel) [15]. Mocarski *et al.* utilized a SCID-huThy/Livmouse model to assess the ability of the Toledo strain of HCMV to replicate within human fetal tissue implants [16]. In a separate study, Brown *et al.* utilized a SCID-huThy/Livmouse model to evaluate and compare the replicative capacity of a low-passage Toledo strain of HCMV and high-passage, laboratory-adapted HCMV strains AD169 and Towne [17]. These early humanized mouse models had several limitations including lack of long-term human cell engraftment, low diversity in types of cells engrafted, lack of distribution of human cells in the mouse and inability to generate human immune responses.

Over the past decade, a second generation of humanized mouse models has been developed in which immune deficient mice have been engrafted with primary HPCs with the goal of recapitulating a functional human immune system. The biggest breakthrough occurred with the development of immune deficient mice with a mutation ininterleukin-2 receptor γ-chain locus (*IL-2* $\gamma c^{-/-}$). These mice exhibited a severe impairment of mouse B, T, and NK cell development allowing greater retention of HPC allografts [18,19]. Three main mouse strains have been developed with the *IL-2* $\gamma c^{-/-}$ mutation including NOD. Cg-*Prkdc*^{scid}*Il2rg*^{tm1Wjl}(NSG mice), NOD. Cg-*Prkdc*^{scid}*Il2rg*^{tm1Sug}(NOG mice) and strains based on C;129S4-*Rag2*^{tm1Flv}*Il2rg*^{tm1Flv}(RG mice). Each of these mouse strains exhibit differences in human immune system cell development in which NSG mice support higher levels of HSC engraftment and T cell development in comparison to RG mice and greater HSC engraftment in the bone marrow in comparison to NOG mice [19–21]. Analysis of human hematopoietic cells demonstrated that these mice reconstituted monocytes, macrophages and B cells as well as limited T cells. The limited maturation of the T cells is believed to be due to education of these cells in the mouse thymus in the context of mouse MHC-I and II. This limitation was overcome with the development of humanized mice that have been reconstituted with human fetal bone marrow/liver/thymus tissue (BLT) [22]. BLT mice exhibit improved systemic reconstitution of human hematopoietic cells including myeloid lineage cells, NK cells and CD4+ and CD8+ T cells due, in part, to the presence of human thymic epithelium (Figure 1). NSG mice reconstituted with human CD34+ HPCs have been used to examine HCMV latency and reactivation as described below. Newer models using BLT mice are being developed to examine not only HCMV latency and reactivation but also immune responses to the virus.

HCMV latency and reactivation in humanized mice

As discussed above, myeloid lineage cells play an integral role in viral latency, persistence, dissemination to organ