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Karen Mossman *Editor*

Innate Antiviral Immunity

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Innate Antiviral Immunity

Methods and Protocols

Edited by

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Preface

This book on *Innate Antiviral Immunity* explores methods to study the complex and evolving interplay between a virus and its host that range from model systems to the detection of chemical molecules. The collection starts with the application of humanized mice and zebrafish as model organisms to study virus-host interactions and induction of innate immune responses. Subsequent chapters outline diverse methods to detect small interfering RNAs, microRNAs, and virus-derived dsRNA from a variety of cells, tissues, and organisms. Several chapters are dedicated to interrogating the cytosolic RNA and DNA sensing pathways, including using RNA PAMPs as molecular tools, purification of cGAMP from virus particles and infected cells, and mechanisms to visualize the subcellular localization and activation of the adaptor proteins MAVS and STING. Cutting-edge methods, including high-throughput and genome-wide CRISPR/Cas9 screens, chromosome conformation capture, and whole-exome sequencing, are described to identify novel mediators, pathways, and variants underlying host susceptibility. Given the importance of studying these pathways and players under physiologic conditions, methods describing the isolation of primary mouse sensory neurons and group 2 innate lymphoid cells are also provided. Finally, this collection comes full circle back to the whole organism level and concludes with epidemiological methods to investigate virus-host interactions and the induction of innate immunity. Thus, this collection in *Methods in Molecular Biology* spans a diverse array of approaches to study and elucidate the intricacies of innate antiviral immunity.

Hamilton, ON, Canada ***Karen Mossman***

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Chapter 1

The Application of Humanized Mouse Models for the Study of Human Exclusive Viruses

Fatemeh Vahedi, Elizabeth C. Giles, and Ali A. Ashkar

Abstract

The symbiosis between humans and viruses has allowed human tropic pathogens to evolve intricate means of modulating the human immune response to ensure its survival among the human population. In doing so, these viruses have developed profound mechanisms that mesh closely with our human biology. The establishment of this intimate relationship has created a species-specific barrier to infection, restricting the virus-associated pathologies to humans. This specificity diminishes the utility of traditional animal models. Humanized mice offer a model unique to all other means of study, providing an in vivo platform for the careful examination of human tropic viruses and their interaction with human cells and tissues. These types of animal models have provided a reliable medium for the study of human-virus interactions, a relationship that could otherwise not be investigated without questionable relevance to humans.

Key words Animal models, Disease Models, Human, Humanized mice, Immune system, Viruses

1 Introduction

1.1 Why Do We Need the Humanized Mouse Model for the Study of Human Tropic Viruses?

As viruses progress through the process of infection, environmental pressures from within the host demand the virus develop adaptive strategies to ensure its survival. The most fit viral particles are selected, which then produce incredible amounts of viral descendants proficient in manipulating the susceptible host for continued viral replication, survival, and transmission [1]. The immune responses to a pathogen contain distinct mechanisms unique to the infected host species, creating successful viral progeny highly skilled in manipulating the host through which selection occurred. These species differences create a profoundly specific interaction between the pathogen and its co-evolved host [1, 2]. The scientific journey to fully comprehend the relationship developed between human and virus has been incredibly strenuous. Following the formal recognition of the ethical concerns behind the use of

Fatemeh Vahedi and Elizabeth C. Giles contributed equally to this work.

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human subjects [3], the severe restrictions placed on human experimentation necessitate alternative models to study human disease [4].

In vitro models serve as fundamental tools to study the viral life cycle. Cell culture systems provide a carefully controlled platform for the examination of how the virus enters its tropic cell lines, replicates, assembles, and secretes budding viral progeny [5]. However, in vitro systems are highly isolated conditions unable to recreate the dynamic features present within an in vivo environment [6]. The absence of these features sources discrepancy between results derived from the use of an in vitro system and results obtained from in vivo investigation. Furthermore, features highly influential on viral infection are commonly altered within cell lines. Oftentimes genes and cell cycle profiles are expressed in a way unorthodox to the cells present within a living organism [7]. Alternatively, in vivo models resolve this problem presented in in vitro cultures. The quintessential model for the investigation of human and virus interactions would permit invasive examination into an infected host with an internal environment capable of manifesting disease outcome as if it were a human [8, 9]. However, the co-evolutionary history established between virus and human has created a relationship of intimacy between pathogen and host that cannot be truly replicated in traditional in vitro or in vivo models [9]. The value of this model is placed in its ability to provide a means for investigating the biological activity that occurs within a host throughout the process of viral infection [8, 9].

Nonhuman primates have become an incredibly valued tool for enriching our understanding of the mechanisms underlying the pathogenic process of viral infection and revealing the potential clinical efficacy of antiviral therapeutics [10, 11]. Their value is a result of their close phylogenetic relation to humans [10, 11], providing a model capable of closely resembling human biology [11]. However, this close phylogenetic relationship is what brings ethical concerns, in which the complexity of debate with regards to their use continues to grow [12, 13]. The difficulty in using non-human primates stems from their tight regulation [13, 14], demanding requirements for proper care [14], and high cost which limits cohort size [2, 9]. Even with their close phylogenetic relationship, the representation of disease pathogenesis remains inaccurate. Several diseases impact humans in a more severe manner than they do in nonhuman primates, discrepancies likely attributed to the inter-species immune system differences [2, 15]. Considering these growing limitations, it is ill advised to remain dependent on nonhuman primate experimentation [13].

The mouse model offers an abundant resource of genetic diversity and permits the creation of unique strains of transgenic mice since they can tolerate extensive genetic manipulation [9, 16]. Additionally, mice are capable of achieving pure strains, allowing

for the generation of reproducible results during experimentation [9]. These factors have established the mouse as the dominating animal in research [16]. However, even such a fundamental model is not suited to recreate the pathogenic process of every virus, especially those with highly species specific cellular tropism [16]. Human tropic pathogens have developed specific molecules and factors that have been established to interact with and manipulate the specific components of its co-evolved human host [1]. Traditional animal models, no matter the species, if they are other than human, will not show the interaction of human cells with the virus [9, 13]. Additionally, within the environment of these surrogate models, the replication and progression of disease is often unable to occur as a result of the species barrier. Humanized mice remove this barrier to infection and disease progression, thus offering a unique means of investigating viral pathogenesis [13, 17].

1.2 Humanized Mice: A Practical Solution for the In Vivo Study of Human-Specific Viral Infection

Several types of humanized mouse models exist, each displaying unique features of the pathogenic process of human infection. To "humanize" a mouse, human cells or tissues are engrafted into a recipient mouse with an injury in the murine equivalent organ you wish to examine. In attempts to remove the occurrence of xenograft rejection, mice with an immunodeficient background are used. Depending on the cellular and tissue tropism of the virus, various human organs would be implanted accordingly [9, 18]. Cells engrafted into the recipient mice retain their functional capacity, occupy their respective murine niches, and offer the virus its susceptible and permissive cells for viral infection and spread [9, 13]. In an ideally constructed humanized mouse model, the viral pathogen goes about infection as it would if it were in its human host, and the mouse responds as if it were a human [8, 9, 13].

A highly involved component in human viral infection is the human immune system. Thus, the study of the immune system and its interaction with the virus is an important component in understanding viral pathogenesis. Appropriately, the types of humanized mice frequently used for the study of viral infection are often reconstituted with human immune cells. Although the term "humanized mice" extends beyond the implantation of human immune cells, this is a primary method of humanization in the study of infectious disease. In fact, the study of major human pathogens such as Dengue (DENV), Ebola (EBOV), Epstein-Barr (EBV), human cytomegalovirus (HCMV), Human T-cell Leukemia Type-1 (HTLV-1), Human Immunodeficiency virus (HIV), and Hepatitis C (HCV) often involves the use of this type of humanized mouse model.

For simplicity's sake, human immune system (HIS) mice can be categorized into two types: mice created by the transplantation human hematopoietic stem cells (HSCs) or peripheral blood mononuclear cells (PBMCs) into a recipient immunodeficient

mouse [8, 19]. In using HSCs, human T and B cell progenitors are able to go through the maturation process in the environment of the mouse recipient. As a result, negative and positive selection of the human immune cells occurs within the mouse, giving the immune cells an opportunity to develop a tolerance toward the murine host [19]. The PBMC model does not provide this process however, the transplantation of functionally mature leukocytes allows for human immune cell function to be examined more immediately [19]. Reservoir sources of HSCs often used in mouse humanization include umbilical cord blood, mobilized peripheral blood [19, 20], bone marrow, and fetal liver [19, 21]. Studies have also utilized thymus, lymph node, and skin [22]. Each source allows for the subsequent reconstitution of human immune cell components [19]. These sources have been used in combination, such as the bone marrow, liver, thymus (BLT) model [23], and the SCID-hu thymus and liver (SCID-hu Thy/Liv) mouse, in which SCID (severe combined immunodeficient) mice are engrafted with human thymus and liver tissue [24], or used individually. Comparatively, PBMCs can be obtained in a simple process from either whole blood samples or spleens [19]. An incredible aspect of "humanizing" immunodeficient mice is how with different sources of human immune cells, the reconstitution of human immune cell populations, and subsequently, the display of infection, can be presented in very different ways depending on the source [13].

1.3 The Gradual Transformation of the Humanized Mouse Model

The evolution of the humanized mouse has coincided with the advancements in the immunodeficient mouse models. The development of a more sophisticated immunodeficient strain of recipient mice has allowed for enhanced engraftment and reconstitution of human components within their respective murine biological niches [8, 9, 13]. Attempts to construct a human hematolymphoid system within a mouse model began with athymic (nude) mice [25]. The significant depletion of T cell maturation and T cell activity gave them promise [9, 26, 27]. Unfortunately, despite extensive efforts, results were continually unsatisfactory and the successful engraftment of normal human tissues appeared impossible [25]. It was the remaining components of the murine immune system, functional B cells and natural killer (NK) cells that created significant obstacles to achieving adequate humanization. The presence of these cells leads to the gradual rejection of transplanted human cells and tissues [9].

In 1983 [28], the discovery of the severe combined immunodeficient (SCID) mouse greatly enhanced the humanization process [8]. A spontaneous mutation within the Prkdc (protein kinase, DNA activated, catalytic polypeptide) gene of C.B-17 mice was found to produce mice with serious depletions in the functional capacity of the murine B and T lymphocytes. The reconstitution of the human immune system is enhanced within SCID mice in

comparison to athymic mice; however, they do not come without limitations. SCID mice undergo a phenomenon termed "leakiness" in which murine T and B cells are spontaneously generated throughout the natural aging process of the mouse. This sporadic production of functional murine T and B cells interferes with the engrafted human cells, eventually causing rejection of the human graft. An additional factor at play is the presence of high functioning NK cells and other innate immune cell types of mouse origin, recognizing the human cells as foreign, thus disrupting successful engraftment [8, 29].

In 1992, in efforts to remove the problem of "leakiness" and enhance the ability of recipient mice to accept human tissues and cells, Mombaerts et al. [30] and Shinkai et al. [31] created mice with targeted mutations in the V(D)J recombination-activating gene 1 and 2 (Rag 1/2) loci, respectively [8]. The presence of these mutations removes the natural process of T and B lymphocyte maturation [8, 30, 31]. These mutant mice retain high levels of NK cell activity, restricting their engraftment potential [8, 31]. In 1995, Shultz et al. were able to mitigate the problem of the persisting murine innate immune response. Through a process of backcrossing the SCID mutation onto the non-obese diabetic (NOD/Lt) mouse background, Shultz et al. created the NOD/SCID mouse, containing several functional deficiencies in the murine innate and adaptive immune response [8, 9, 31, 32]. Accordingly, these mice have allowed for improved reconstitution of human hematopoietic stem cells. However, this improved model remains to have several faults, complicating its use for accepting human cells and tissues, and studying virally induced pathologies [8, 32]. NOD/SCID mice contain residual NK cell and innate immune cell function and possess a fairly limited life span. The presence of these interfering factors enables the problem of impaired engraftment to persist in the NOD/SCID mouse model [32]. In the mid-2000s, the introduction of a targeted mutation in the interleukin (IL)-2 receptor gamma chain loci (IL-2rg) caused mice to develop severe impairments in the maturation process and functional capacity of B and T cells, and eradicated NK cell development [8, 9, 13, 33]. These immunodeficient mutations have been combined to recreate numerous types of immunodeficient mice, often enhancing the immune depletions and thus the engraftment success within the mice [8, 9].

To recreate a functional human immune system within a mouse, the process requires more than just the immune cells itself. For the development, survival, and function of human hematolymphoid cells, there are a number of hormones, growth factors, and cytokines essential to ensure optimal health and function of human cells [25]. The presence of residual immune system components