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Review

Studying the immune response to human viral infections using zebrafish

Michelle F. Goody [a], Con Sullivan [a,b], Carol H. Kim [a,b,*]

[a] Department of Molecular and Biomedical Sciences, University of Maine, Orono, ME 04469, USA
[b] Graduate School of Biomedical Sciences and Engineering, University of Maine, Orono, ME 04469, USA

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ABSTRACT

Humans and viruses have a long co-evolutionary history. Viral illnesses have and will continue to shape human history: from smallpox, to influenza, to HIV, and beyond. Animal models of human viral illnesses are needed in order to generate safe and effective antiviral medicines, adjuvant therapies, and vaccines. These animal models must support the replication of human viruses, recapitulate aspects of human viral illnesses, and respond with conserved immune signaling cascades. The zebrafish is perhaps the simplest, most commonly used laboratory model organism in which innate and/or adaptive immunity can be studied. Herein, we will discuss the current zebrafish models of human viral illnesses and the insights they have provided. We will highlight advantages of early life stage zebrafish and the importance of innate immunity in human viral illnesses. We will also discuss viral characteristics to consider before infecting zebrafish with human viruses as well as predict other human viruses that may be able to infect zebrafish.

Contents

Abbreviations: HSV-1, herpes simplex virus type 1; CHIKV, chikungunya virus; IAV, influenza A virus; HCV, hepatitis C virus; (q)PCR, (quantitative) polymerase chain reaction; ISH, *in situ* hybridization; IHC, immunohistochemistry; CNS, central nervous system; TCID50, 50% tissue culture infectious dose; EID50, 50% embryo infectious dose; MDCK, Madin-Darby canine kidney; IFN, interferon; Hpi/dpi, hours post-infection/days post-infection; Hpf/dpf, hours post-fertilization/days post-fertilization; MAVS, mitochondrial antiviral signaling protein; CRFB1/CRFB2, cytokine receptor family member b1/b2; PRR, pattern recognition receptor; PHB, prohibitin; PFU, plaque forming units; NS1-GFP, strain of human IAV engineered to express GFP fused to the non-structural NS1 gene product; mpx, myeloid-specific peroxidase; mpeg1, macrophage expressed 1; fli1, friend leukemia integration 1; mxa, myxovirus (influenza) resistance a.

* Corresponding author at: 360 Hitchner Hall, Orono, ME 04469, USA. Tel.: +1 207 581 2803.
E-mail address: carolkim@maine.edu (C.H. Kim).

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1. Introduction

It has been estimated that 90% of the infectious diseases that afflict human beings are caused by viruses (Norkin, 2010), and over 200 different viruses have been isolated from the human upper respiratory tract alone (Mackie, 2003). Many viral infections are asymptomatic and may go unnoticed, but others cause severe or even life threatening diseases in humans. The immune system has evolved in many species, including humans, to recognize and clear foreign infectious agents from the body. In vertebrates, there are two branches of the immune system that work in concert: innate and adaptive. Innate immunity is more evolutionarily ancient and functions to initially recognize the threat and limit the spread of infection. In jawed vertebrates, adaptive immunity triggers cell-mediated and antibody-mediated responses that can curtail existing infections and provide the host with long-term immunological memory. The adaptive immune response has been exploited in the development of vaccines. Through immunizations, some viral illnesses have been essentially eradicated from the human population. Due to the success of some vaccination programs, the importance of adaptive immunity in virus infections is widely recognized; however, the important role of the innate immune response in viral diseases is only now being appreciated.

Viruses are recognized by the innate immune system upon binding to pattern recognition receptors (PRRs) on or within host cells. PRRs that recognize viruses include Toll-like receptors, retinoic acid-inducible gene I-like receptors, nucleotide oligomerization domain-like receptors, and receptors that detect DNA in the cytosol of cells. Several reviews provide detailed information on viral detection by PRRs and the signaling pathways elicited (Kawai and Akira, 2006; Pichlmair and Reis e Sousa, 2007; Saito and Gale, 2007; McCartney and Colonna, 2009; Shayakhmetov et al., 2009; Takeuchi and Akira, 2009; Pedraza et al., 2010; Drutskaya et al., 2011; Thompson et al., 2011). Signaling by PRRs through distinct or overlapping signal transduction pathways culminates in the production of cytokines, such as interferon (IFN), and chemokines. Through paracrine signaling, IFN is secreted by infected cells and binds to receptors on nearby uninfected cells in an attempt to wall off the spread of infection by instructing cells to suspend transcription and translation. Other cytokines and chemokines are involved in pro-inflammatory signaling cascades that recruit phagocytes to sites of infection. A better understanding of the innate immune response to viral infections will be beneficial in developing preventative and adjuvant therapies for viral illnesses because activation of innate immune signaling pathways and phagocytic cells is common to all viral infections, potentiates a robust adaptive immune response, has both positive and negative effects on the health of the host, and can be thwarted by certain viral adaptations.

The dynamics between host immune responses and human viral pathogens can only be studied in animal models where tissue interactions are intact and where genetics, cell types, and signaling cascades are homologous to those in humans. Commonly used animal models of human viral infections include small mammals and non-human primates. In an effort to reduce, replace, and refine the animal models used in scientific research, the zebrafish infectious disease model has become an attractive option. Zebrafish are vertebrates that possess both innate and adaptive immune systems. The conservation of innate and adaptive immunity between zebrafish and humans has been well-characterized (reviewed in Traver et al., 2003; Trede et al., 2004; van der Sar et al., 2004; Phelps and Neely, 2005; Jakovlic et al., 2006; Sullivan and Kim, 2008; Cui et al., 2011; Meijer and Spaink, 2011; Novoa and Figueras, 2012; Crim and Riley, 2012; van der Vaart et al., 2012). This review will focus on the use of zebrafish to study human viral illnesses and the host's innate immune response, largely because this represents the bulk of current research. Zebrafish phagocytic macrophages and neutrophils are similar to those in mammals in terms of their morphology, molecular signatures, and functionality (Bennett et al., 2001). Certain other innate immune cell types found in mammals, including monocytes, NK cells, dendritic cells, eosinophils, and basophils, have been identified in zebrafish and are beginning to be characterized (Dobson et al., 2008; Moss et al., 2009; Balla et al., 2010; Lugo-Villarino et al., 2010; Da'as et al., 2011). In addition, innate immune signal transduction pathways downstream from pathogen receptors, such as IFN signaling, are well-conserved between mammals and zebrafish (Stein et al., 2007; Langevin et al., 2013). Thus, the zebrafish is a useful model for the study of the innate immune response to human infectious diseases. Moreover, adult zebrafish have adaptive immunity and could be used to study the adaptive immune response to human viruses that are able to infect adult zebrafish. In this review we will discuss the studies utilizing the four known zebrafish models of human viral illnesses. Roles for zebrafish neutrophils, macrophages, and IFN signaling in response to these human virus infections will be described. Given the involvement of other innate immune cell types (e.g. NK cells, dendritic cells) in these four specific virus infections in mammals and the recent identification of similar cell types in zebrafish, the zebrafish infection model will likely contribute additional important insights regarding roles for these cell types in *in vivo* infections in the near future. We will also propose additional human viral pathogens that may be able to infect zebrafish and describe the insights that the zebrafish infectious disease model can provide due to the unique research opportunities possible in the zebrafish system.

2. Zebrafish models of human viral illnesses

The zebrafish is rapidly gaining in popularity as an infectious disease model. Zebrafish have been used to study fish-specific infectious diseases that afflict economically important fish species (reviewed in Trede et al., 2004; van der Sar et al., 2004; Phelps and Neely, 2005; Sullivan and Kim, 2008; Meijer and Spaink, 2011; Milligan-Myhre et al., 2011; Novoa and Figueras, 2012; Crim and Riley, 2012). It has recently been shown that zebrafish can be good models in which to study human infectious diseases as well. It has been suggested that to be able to preserve and study the complexities of host–pathogen co-evolution when using animals to model human infectious diseases, it is important to employ the most closely related pathogen that naturally infects the model species (Baker, 1998; Crim and Riley, 2012; Keebaugh and Schlenke, 2014). However, the natural pathogens of zebrafish are currently unknown (Crim and Riley, 2012). A different approach, that potentially has more direct translational impact, is to use an animal model with an immune response similar to humans that can be infected by human isolates of a pathogen. The first reported human pathogens that could infect and cause disease in zebrafish were bacteria (reviewed in Trede et al., 2004; van der Sar et al., 2004; Phelps and Neely, 2005; Sullivan and Kim, 2008; Meijer and Spaink, 2011; Milligan-Myhre et al., 2011; Novoa and Figueras, 2012). There are now reports of zebrafish models of human fungal (Chao et al., 2010; Brothers and Newman, 2011; Brothers et al., 2013; Chen et al., 2013; Gratacap et al., 2013; Kuo et al., 2013; Wang et al., 2013) and human viral pathogen infections (Burgos et al., 2008; Ding et al., 2011; Antoine et al., 2013; Palha et al., 2013; Gabor and Kim, personal communication). We will describe the human viral illnesses for which there are currently zebrafish infection models and then discuss the findings and insights obtained thus far from these zebrafish models of human viral infections.