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Review

Nucleic acid-induced antiviral immunity in invertebrates: An evolutionary perspective

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ABSTRACT

Nucleic acids derived from viral pathogens are typical pathogen associated molecular patterns (PAMPs). In mammals, the recognition of viral nucleic acids by pattern recognition receptors (PRRs), which include Toll-like receptors (TLRs) and retinoic acid-inducible gene (RIG)-I-like receptors (RLRs), induces the release of inflammatory cytokines and type I interferons (IFNs) through the activation of nuclear factor κB (NF-κB) and interferon regulatory factor (IRF) 3/7 pathways, triggering the host antiviral state. However, whether nucleic acids can induce similar antiviral immunity in invertebrates remains ambiguous. Several studies have reported that nucleic acid mimics, especially dsRNA mimic poly(I:C), can strongly induce non-specific antiviral immune responses in insects, shrimp, and oyster. This behavior shows multiple similarities to the hallmarks of mammalian IFN responses. In this review, we highlight the current understanding of nucleic acid-induced antiviral immunity in invertebrates. We also discuss the potential recognition and regulatory mechanisms that confer non-specific antiviral immunity on invertebrate hosts.

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1. Introduction

Pathogenic microbes pose constant threats to all metazoans. To combat infections, animals have developed powerful innate immune systems to recognize and target the invaders from self-cells (Hoffmann and Reichhart, 2002). Innate immunity is the paramount antimicrobial response of metazoans that is achieved through pattern recognition by germline-encoded pattern-recogni-

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tion receptors (PRRs). After microbial infections, PRRs can recognize microbial components known as pathogen-associated molecular patterns (PAMPs), which include lipopolysaccharides (LPS), lipoproteins, flagellin, peptidoglycan, single-stranded RNA (ssRNA), double-stranded RNA (dsRNA), unmethylated CpG-containing DNA, etc. (Akira et al., 2006). PRR-mediated recognition of PAMPs allows a finite set of receptors to recognize an enormous amount of diverse potential pathogens. Nucleic acids including ssRNA, dsRNA, and DNA, are shared by all viruses, not easily mutated to avoid recognition, and one of the few viral features suitable for innate immune recognition (Barbalat et al., 2011).

PRR-mediated PAMP recognition is the first step of host innate immune response. These innate immune recognition mechanisms are evolutionarily conserved in arthropods, and probably even in nematodes (Ausubel, 2005). Thus, the innate immune systems of mammals and their invertebrate predecessors, e.g., arthropods, are perceived to share similar elements. Therefore, the study of invertebrate (and, possibly, more ancient) immunity is reasonable to obtain a comprehensive understanding of the evolution and function of human innate immunity (Irazoqui et al., 2010). Similarly, the study of invertebrate pathogenesis models (e.g., insect-dengue virus infection model) can provide new insight into conserved virulence strategies that have been successful for pathogens, irrespective of the host (Irazoqui et al., 2010; Sessions et al., 2009). In *Drosophila*, Toll-7 can activate autophages to limit Rift Valley fever virus (RVFV) replication and mortality. RVFV infection also elicits autophagy in mouse and human cells, and viral replication increases in the absence of autophagy genes. The mammalian Toll-like receptor (TLR) adaptor, MyD88, is required for anti-RVFV autophagy, revealing an evolutionarily conserved requirement for PRRs in antiviral autophagy (Moy et al., 2013). Studies on *Drosophila* innate immune responses to systemic bacterial and fungal infections have resulted in numerous seminal discoveries, e.g., the discovery of *Drosophila* Toll and mammalian TLRs. Toll or TLRs, the key regulators of the immune response, were first studied in cultured *Drosophila* cells, then in *Drosophila in vivo* and finally in mammalian cells and in mice *in vivo* (Rämet and Hultmark, 2014). The recognition of fungi and bacteria by TLRs-NF-κB pathways are well studied in *Drosophila* and mammals (Akira et al., 2006). In mammals, viruses can be sensed by TLRs and retinoic acid-inducible gene (RIG)-I-like receptors (RLRs) to activate the IFN system through the sensing of nucleic acids (e.g., ssRNA, dsRNA, and DNA), which are derived from viruses However, less data are available on how *Drosophila* or other invertebrates recognize and resist viruses, especially the recognition of viral nucleic acid-induced non-specific antiviral responses (Rämet and Hultmark, 2014).

RNA interference (RNAi) is believed to be one of the most robust antiviral responses in plants and invertebrates. The RNAi pathway can utilize virus-generated dsRNA to produce small, interfering RNAs (siRNAs) to target viral RNA for degradation and hence inhibit virus replication (Karlikow et al., 2014; Kingsolver et al., 2013; Sabin et al., 2010). However, mammals have been suggested to supplant the RNA-based antiviral RNAi pathway with protein-based antiviral IFN response (Sagan and Sarnow, 2013). Recently, two groups have provided strong support for an antiviral role of RNAi in mammals (Li et al., 2013; Maillard et al., 2013). The existence of a functional antiviral RNAi pathway in mammals suggests that both RNA and protein-based antiviral mechanisms possibly operate simultaneously in mammalian tissues (Sagan and Sarnow, 2013). RNAi-mediated sequence-specific antiviral mechanisms and IFN system-mediated non-specific antiviral responses are parallel antiviral pathways involved in the recognition of nucleic acids generated by viral replication in mammals (Sagan and Sarnow, 2013). Thus, the RNAi-mediated antiviral mechanisms are evolutionarily conserved from plants and invertebrates to mammals. However, whether IFN system-mediated non-specific antiviral responses or the protein-based antiviral mechanisms exist in invertebrates should be clarified.

In mammals, nucleic acids, include ssRNA, dsRNA, and DNA, generated by viral replication can be recognized by PRRs and subsequently activate the IFN system and induce antiviral responses (Akira et al., 2006). Information on the PRRs of nucleic acids and most of the core elements of the IFN system in invertebrates has not been updated (Wang et al., 2013c). However, several groups have found that viral infections or nucleic acid stimulations can indeed induce non-specific antiviral immunity in some invertebrate species, which differ from RNAi-mediated sequence-specific antiviral mechanisms, but shows high similarities to the hallmarks of mammalian IFN responses (Deddouche et al., 2008; Green and Montagnani, 2013; Paradkar et al., 2012; Pitaluga et al., 2008; Sun et al., 2014, 2013; Takeuchi and Akira, 2008; Wang et al., 2013c; Zhang et al., 2010). Recent findings related to nucleic acid-induced antiviral immunity in invertebrates will be summarized and the potential mechanisms will be discussed in this review.

2. Mammalian innate antiviral immune system

2.1. IFN-mediated antiviral immunity

The IFN system is an extremely powerful antiviral response that can control most, if not all, virus infections in the absence of adaptive immunity (Randall and Goodbourn, 2008). This system predominates the mammalian innate antiviral immunity. After viral infections, viral nucleic acids can be recognized by various PRRs, such as TLRs and RLRs. Recognition of viral nucleic acids induces the production of proinflammatory cytokines, chemokines, and IFNs through the activation of NF-κB and IRF3/7 pathways, triggering inflammation and IFN responses, which are the hallmarks of host innate antiviral immunity (Akira et al., 2006). IFNs can bind their cognate receptors and induce the expression of hundreds of interferon-stimulated genes (ISGs) through the JAK-STAT pathway. ISGs including PKR, MX1, OAS1, APOBEC3G, TRIM5, ZAP, ISG15, ADAR, IFITM1/2/3, tetherin, viperin, etc., can target multiple stages in the virus life cycle and mediate the inhibition of viral replication and clearance of virus-infected cells (Schoggins and Rice, 2011).

2.2. Nucleic acid receptors and signaling pathways

PRRs for nucleic acids include the transmembrane and cytosolic receptor families. Four TLRs, namely, TLR3, TLR7, TLR8, and TLR9, that are localized on endosomes and lysosomes have been implicated in nucleic acid recognition (Barbalat et al., 2011). The dsRNA can be generated during viral infection as a replication intermediate for ssRNA viruses or as a by-product of symmetrical transcription in DNA viruses (Akira et al., 2006). The dsRNA is a universal viral PAMP and a potent inducer of type I IFNs. The dsRNA and its synthetic analog, poly(I:C) are recognized by TLR3. TLR3 has been implicated in the host response to ssRNA, dsRNA, and DNA viruses (Akira et al., 2006; Barbalat et al., 2011). The ssRNA released from the damaged viral particles can be recognized by TLR7 or TLR8. However, TLR7 and TLR8 can recognize distinct sequence motifs in ssRNA (Akira et al., 2006; Barbalat et al., 2011). DNA viruses, including herpes simplex virus 1 (HSV-1), HSV-2, and murine cytomegalovirus (MCMV), contain genomes that are rich in CpG-DNA motifs and can activate inflammatory cytokines and type I IFN secretion through the stimulation of TLR9 (Akira et al., 2006; Barbalat et al., 2011). TLR9 has been identified as the receptor that recognizes nonmethylated cytosine-guanosine (CpG) motifs in DNA. Although the stimulatory capacity of CpG