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Enter at your own risk: How enteroviruses navigate the dangerous world of pattern recognition receptor signaling

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ABSTRACT

Enteroviruses are the most common human viral pathogens worldwide. This genus of small, non-enveloped, single stranded RNA viruses includes coxsackievirus, rhinovirus, echovirus, and poliovirus species. Infection with these viruses can induce mild symptoms that resemble the common cold, but can also be associated with more severe syndromes such as poliomyelitis, neurological diseases including aseptic meningitis and encephalitis, myocarditis, and the onset of type I diabetes. In humans, polarized epithelial cells lining the respiratory and/or digestive tracts represent the initial sites of infection by enteroviruses. Control of infection in the host is initiated through the engagement of a variety of pattern recognition receptors (PRRs). PRRs act as the sentinels of the innate immune system and serve to alert the host to the presence of a viral invader. This review assembles the available data annotating the role of PRRs in the response to enteroviral infection as well as the myriad ways by which enteroviruses both interrupt and manipulate PRR signaling to enhance their own replication, thereby inducing human disease.

1. Introduction

1.1. Enteroviruses

Enteroviruses (EVs), which include coxsackievirus, rhinovirus, echovirus, and poliovirus species, are members of the picornavirus family. These small (~30 nm), non-enveloped, single stranded RNA viruses consisting of a genome of ~7 kB are the most common human viral pathogens worldwide [1,2]. EVs, excluding rhinoviruses, are responsible for as many as 15 million symptomatic infections in the United States every year [3] and are commonly associated with neurological disease. As many as 10–15% of encephalitis cases in the United States and worldwide have been associated with non-poliovirus EV infections [4–7] and they are the leading causative agents of aseptic meningitis worldwide [8]. Although EV-induced CNS complications are more commonly associated with mortality in neonates and children, adult infections can also lead to severe complications (particularly when the adult has not been exposed to the EV serotype previously) [9]. Enterovirus 71 (EV71) has become an important public health concern in recent years, especially in Asia, as its incidence has increased in the region and the illness it causes is often associated with severe neurological complications and/or death [10]. Importantly, EVs, particularly coxsackievirus B (CVB), are also linked to the development of myocarditis with up to 50% of patients with myocarditis displaying evidence of an EV infection [11–13]. Finally, EV infections, specifically CVB4, have also been linked to the onset of type I diabetes [14–16]. In contrast, rhinoviruses are the causative agent of over 50% of human viral-induced acute respiratory tract infections [17], which are associated with nearly $40 billion in direct and indirect costs annually in the United States alone [18].

Studies detailing how these medically relevant viruses interact with the host immune system are described in this review, with a specific focus on how the innate immune system alerts the body to the presence of an enteroviral invader and how enteroviruses have evolved to attenuate this system in order to enhance their replication. In this review, we focus on the non-rhinovirus EVs.

1.2. Pattern recognition receptor signaling

It has long been appreciated that the innate immune response is necessary for the induction of the subsequent adaptive immune response [19,20]. Innate immunity to pathogens is largely mediated by pattern recognition receptors (PRRs), which recognize a variety of pathogen associated molecular patterns (PAMPs) that are highly conserved amongst classes of pathogens [21]. During a viral infection, PRRs induce an intracellular signaling cascade resulting in the alteration of the host cell's transcription profile in response to recognition of their cognate PAMP. Two major classes of transcription factors are activated in response to this signaling: Interferon Regulatory Factors (IRFs) and NF-κB family members. These transcription factors act in concert to induce the expression of type I interferons (IFN) [22]. These auto- and

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paracrine signaling molecules serve to upregulate a cadre of genes, known as interferon stimulated genes (ISGs). The effects of type I IFNs and ISGs are legion; they are pro-inflammatory [23], enhance adaptive immunity [24], and are directly antiviral [25]. Additionally, NF-κB activation induces a host of pro-inflammatory and pro-survival genes independently of type I IFN induction [26–29] and may be required for full induction of type I IFNs [27,30].

Toll-like receptors (TLRs) 1–13 are transmembrane PRRs that recognize a diverse range of PAMPs. TLRs can be divided into two broad categories—those that are localized to the cell surface and those that are localized to the endosomal lumen. TLRs that are present on the cell surface are important in recognition of bacterial pathogens. In contrast, TLRs that are localized to the lumen of endosomes, TLRs 3, 7, 8, and 9, serve to recognize nucleic acids and are thus traditionally thought to be the most important in the promotion of an antiviral response. TLR3 recognizes dsRNA and the synthetic dsRNA structural homolog poly(I:C) [31]. TLR7 and TLR8 recognize ssRNA and imidazoquinoline compounds [32–35]. TLR9 recognizes unmethylated deoxycytidylate-phosphate-deoxyguanylate (CpG) DNA, found almost exclusively in bacteria [36,37].

In addition to TLRs, cytoplasmic PRRs exist and are divided into two main groups—the NOD-like receptors (NLRs) and the RIG-I-like receptors (RLRs). There are three RLRs: RIG-I, MDA5, and LGP2. RIG-I recognizes cytoplasmic short dsRNA and 5′ppp-ssRNA [38–41]. MDA5 binds the internal duplex structure of cytoplasmic long dsRNA and cooperatively assembles into a filamentous oligomer composed of MDA5 dimers [41–47]. The role of LGP2 has not been thoroughly elucidated. Early studies suggested that it acted as a negative regulator of RIG-I and MDA5 [48–50]. However, further studies revealed that LGP2 was essential for type I IFN response to picornavirus infections in mice and that LGP2 with active helicase activity is required for IFNβ production in response to various RNA viruses in dendritic cells (DCs) and mouse embryonic fibroblasts (MEFs) [51]. Further studies of LGP2 have yielded equally disparate results, as both overexpression of chicken LGP2 and knockdown of endogenous LGP2 in chicken cells resulted in reduced IFNβ production in response to avian influenza infection [52].

There are 22 human NLRs that can be further subdivided into five families: NLR families A, B, C, P, and X. These families are structurally related. All NLRs have three domains: an N-terminal domain involved in signaling, a nucleotide-binding NOD domain, and a C-terminal leucine rich region (LRR) important for ligand recognition (reviewed in [53,54]). The NLR most traditionally associated with response to viral infection is NALP3, a member of the NLRP family. NALP3, also known as cryopyrin, is a member of the NALP3 inflammasome, which is responsible for the processing of the proinflammatory cytokine IL-1β to its mature form [55]. NALP3 has been shown to be a sensor for bacterial peptidoglycans [56], endogenous uric acid crystals (associated with gout) [57], bacterial RNA [58], and, importantly, imidazoquinolines and viral RNA [58,59]. Recent data has shown that NOD2, a member of the NLRC family traditionally viewed as a sensor for bacterial muramyl dipeptide [60,61], also serves to sense viral ssRNA [62]. Finally, there has been conflicting data published on the role of NLRX1 in the negative regulation of RLR antiviral signaling, with initial studies showing that the presence of NLRX1 dampens RLR signaling [63,64], but subsequent studies showing no role for NLRX1 in RLR signaling [65,66].

As summarized above, the activation of various PRRs by PAMPs produced by viral infection leads to an altered transcription profile of the infected cell. The induction of type I IFN signaling is important for the control of EV infections *in vivo*, as evidenced by enhanced EV-induced lethality in type I IFN receptor (IFN-αβ R) null mice [67–69] and increased viral susceptibility in IFNβ-deficient mice [70]. In addition, purified IFNβ treatment of patients diagnosed with EV-induced myocarditis significantly improves cardiac function [71], underscoring the role of this cytokine in the control of human EV infections. Below we review what is known regarding the sensing of non-rhinovirus EVs and how these viruses target a variety of components within both the TLR and RLR pathways to promote their replication and/or spread.

2. Recognition of enteroviral infections

The literature shows that TLRs, RLRs, and NLRs, the three broad categories of PRRs described above, all play an important role in the sensing of EV infections. Below we summarize these studies based upon the subtype of PRRs responsible for this sensing.

2.1. Toll-like receptors

TLR3 has been shown to play an essential and non-redundant role in the response to EVs, and may be considered the TLR identified as most critical in the control of EV infections. TLR3-deficient mice exhibit significantly increased mortality in response to a dose of coxsackievirus B4 (CVB4) that is sublethal in TLR3-expressing mice [72]. In addition, mice deficient in TLR3 or TIR-domain-containing adaptor-inducing IFNβ (TRIF), a key adaptor in TLR3 signaling, are more susceptible to poliovirus (PV) infection, displaying increased mortality and viral load which were correlated with an inability to produce type I IFNs [73,74]. TLR3 also plays a protective role in restricting CVB3 infection in the heart as $TLR3^{-/-}$ mice infected with CVB3 display increased mortality and myocarditis [75] due at least in part to an increase in IL-4 in $TLR3^{-/-}$ mice upon CVB3 infection and a subsequent shift from a protective Th1 response to a Th2 response in the hearts of these mice [76,77]. $TRIF^{-/-}$ mice infected with CVB3 display increased viral replication in cardiomyocytes, decreased left ventricular functioning, and increased cardiac fibrosis [78]. Further supporting a role for TLR3 in EV innate immune signaling, human patients diagnosed with EV-induced myocarditis have increased frequencies of two single-nucleotide polymorphisms (SNPs) in TLR3 which result in variants that exhibit a reduced capacity to promote type I IFN and NF-κB signaling *in vitro* in response to poly(I:C) or CVB3 infection [79]. This suggests that a reduced ability to sense viral invasion through TLR3 results in an increased risk of developing virally induced cardiac inflammation.

In addition to TLR3, several other TLRs have been shown to be important in the sensing of EV infections. TLR4, which is localized to the cell surface, has also been shown to be important in the detection of EVs, although it is mainly studied in the context of bacterial pathogens. Infection with CVB4 is implicated in the development of type I diabetes, and the damage to the pancreatic beta cells is thought to be mediated by pro-inflammatory cytokines. It has been shown that TLR4 mediates the production of TNFα and IL-6 in pancreatic cells infected with CVB4, suggesting a role for TLR4 in recognizing not only bacterial LPS, but viral proteins as well [80]. Additionally, the level of TLR4 expression and the level of EV RNA present in endomyocardial tissue of patients with myocarditis have been shown to be positively correlated [81]. However, in contrast to the studies described above related to TLR3 signaling, much less is known regarding the consequences of TLR4 signaling on EV infection *in vivo*.

The ssRNA sensors TLR7 and TLR8 have also been shown to play some role in the induction of antiviral signaling in response to CVB3 infection, although their precise function remains largely unclear [82–84]. TLR7 has been shown to be required in plasmacytoid dendritic cells (pDCs), also known as interferon-producing cells because of their role in producing copious amounts of type I IFNs [85], for the production of IFNα and IL-12p40 in response to