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Review

Innate immune evasion by filoviruses

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ABSTRACT

Ebola viruses and Marburg viruses, members of the filovirus family, cause severe hemorrhagic fever. The ability of these viruses to potently counteract host innate immune responses is thought to be an important component of viral pathogenesis. Several mechanisms of filoviral innate immune evasion have been defined and are reviewed here. These mechanisms include suppression of type I interferon (IFN) production; inhibition of IFN-signaling and mechanisms that either prevent cell stress responses or allow the virus to replicate in the face of such responses. A greater understanding of these innate immune evasion mechanisms may suggest novel therapeutic approaches for these deadly pathogens.

Contents

Introduction

The filovirus family includes the Ebola viruses and the Marburg viruses (Sanchez et al., 2007). The family is divided into the ebolavirus genus which has five species, Zaire ebolavirus, Sudan ebolavirus (SUDV), Bundibugyo ebolavirus (BDBV), Tai Forrest ebolavirus (TAFV) and Reston ebolavirus (RESTV). The marburgvirus genus consists of a single species, Marburg marburgvirus (MARV), but is divided into two clades. These are zoonotic pathogens that likely used bats as reservoir hosts (Amman et al., 2012; Pourrut et al., 2009; Towner et al., 2009). Among the various species, Reston virus is unique in that it has not been associated with human illness. Of the pathogenic members, filoviruses have been associated with repeated outbreaks of viral hemorrhagic fever with high fatality rates (Feldmann and Geisbert, 2010). Before 2014, outbreaks in human populations had been recognized in equatorial regions of Africa or arose due to export of non-human primates from this region of the continent. However, in March of 2014 an Ebola virus outbreak was recognized in the West African country of Guinea (Baize et al., 2014). This outbreak spread to the neighboring countries of Sierra Leone and Liberia, becoming the largest filovirus outbreak on record, having caused, according to World Health Organization (2015) numbers, 22,092 cases of Ebola virus

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Fig. 1. *Genome organization of filoviruses*. The names of genes, designated according to proteins encoded by each, are indicated. NP, nucleoprotein; VP35, viral protein 35; VP40, viral protein 40; GP/sGP, glycoprotein, soluble glycoprotein; VP30, viral protein 30; VP24, viral protein 24; L, Large protein (the viral polymerase). Note that Marburg virus encodes GP but not sGP. The spacing between genes is variable and is not drawn to scale.

disease and 8810 deaths as of January 21, 2015. Infected individuals also brought the virus to the United States, the United Kingdom and Europe, highlighting the global public health importance of these viruses.

Many aspects of the structure and molecular biology of filoviruses are well-described (Sanchez et al., 2007). They are filamentous, enveloped, negative-sense RNA viruses. The surface of the virus has a single virus-encoded glycoprotein (GP) that mediates virus attachment and entry. Underlying the viral membrane is a viral matrix comprised mainly of viral protein 40 (VP40). Within the particle is the uncapped, single-stranded RNA genome which is coated by the viral nucleoprotein (NP). Also associated with the encapsidated genomic RNA are the virus encoded proteins VP35, VP30, VP24 and the large protein (L). The filoviral genome is approximately 19 kb in length and has 7 distinct transcriptional units (Fig. 1). Viral genome replication and transcription, resulting in the production of 5′-capped, 3′-polyadenylated mRNAs encoding viral proteins, are carried out in the cell cytoplasm by a virus encoded RNA-dependent RNA polymerase (RDRP) complex comprised of NP, VP35, VP30 and L, the enzymatic component of the RDRP complex.

The connection between filovirus disease and immune evasion mechanisms

The severe disease associated with filoviral infection is characterized by systemic virus replication, which results in very high titers in the blood (Feldmann and Geisbert, 2010). A presumed consequence of this robust virus replication is the appearance of damaging host responses. These include excessive cytokine production, release of tissue factor and other mediators that contribute to a severe disease featuring liver damage, vascular leakage and bleeding (Feldmann and Geisbert, 2010). The excessive replication reflects an ability of Ebola and Marburg viruses to very effectively counteract host antiviral defenses, particularly interferon (IFN) responses, which serve as critical innate immune responses toward virus infection (Basler and Amarasinghe, 2009; Bray and Geisbert, 2005). An overview of filoviral mechanisms of innate immune evasion, including several recent developments, is provided below.

IFN responses

Type I IFNs are critical components of the innate response to viral infection (Ivashkiv and Donlin, 2014). These are a family of proteins encoded by a single IFNβ gene and multiple IFNα genes. The interplay between the type I IFN response, called IFN-α/β hereafter, and filoviruses has been studied relatively intensively. When expressed, IFN-α/β are secreted from producing cells and can signal in an autocrine or paracrine manner by binding to a heterodimeric receptor, the IFN-α/β receptor, found on the cell surface. This triggers a JAK–STAT signaling cascade that upregulates hundreds of genes that cumulatively render cells resistant to virus infection and better able to block virus replication. IFN-α/β are encoded in humans and in mice by a single IFNβ gene and multiple IFNα genes. IFN-α/β gene expression is inducible following activation of several different pattern recognition receptor pathways, including the RIG-I-like receptor (RLR) pathways, select Toll-like receptor (TLR) pathways and the STING/cGAS pathway (Brubaker et al., 2015). Most likely, two RIG-I-like receptors, RIG-I and MDA5, have the most relevance to filoviruses. This reflects the facts that the RLRs reside in the cytoplasm of cells, where filoviruses replicate, and that they detect and signal in response to RNA products of virus replication. RIG-I senses RNA molecules with features such as 5′ triphosphates and dsRNA features, while MDA5 appears to recognize longer dsRNAs. These are features that characterize or may characterize the products of filovirus replication and purified Ebola virus genomic RNA has in fact been demonstrated to activate RIG-I.

Filovirus VP35 proteins block IFN-α/β production

One major mechanism by which filoviruses evade innate antiviral defenses is by blocking the RLR pathways that would otherwise trigger IFN-α/β production. This mechanism is carried out by the VP35 proteins of both Ebola viruses and Marburg viruses. That Ebola virus VP35 can block IFN-α/β was first suggested by the observation that VP35 expression could complement the growth of a mutant influenza A virus that was unable to counteract the IFN-α/β response (Basler et al., 2000). VP35 expression also prevented activation of the IFN-β promoter following infection by Sendai virus, a potent IFN-α/β inducer, or following transfection of the IFN-inducing mimic of virus, polyI:C (Basler et al., 2000). VP35 was subsequently demonstrated to prevent phosphorylation of interferon regulatory factor 3 (IRF-3), a transcription factor critical for induction of the IFN-β promoter (Basler et al., 2003). VP35 was also shown to impair RIG-I signaling and this inhibition correlated with the capacity of VP35 to bind to dsRNA (Cardenas et al., 2006; Hartman et al., 2004) (Fig. 2). In examining the mechanisms by which VP35 carries out these immune suppressive functions, several non-mutually exclusive models are supported by existing data. Because VP35 could impair activation of the IFN-β promoter in the presence of over-expressed IKKε or TBK1, the kinases that phosphorylate and activate IRF-3, the impact of VP35 on these kinases was assessed. VP35 was demonstrated to interact in co-immunoprecipitation studies with either IKKε or TBK1 *via* their more conserved kinase domains. The interaction of VP35 with the kinases was sufficient to block kinase interaction with, and phosphorylation of, either IRF-3 or IRF-7 and, *in vitro*, resulted in the phosphorylation of VP35 (Prins et al., 2009). While the functional consequence of VP35 phosphorylation is unclear, the ability of VP35 to prevent kinase phosphorylation of IRF-3 or IRF-7 would be expected to disrupt induction of IFN-α/β gene expression. A second inhibitory activity of VP35 that would act downstream of IKKε and TBK1 was also described. This mechanism was first suggested by yeast two-hybrid assay results, where use of VP35 as bait identified Ubc9, the E2 enzyme for SUMOylation, and protein inhibitor of activated STAT (PIAS1, a SUMO E3 ligase) as interactors. Through this interaction, VP35 enhanced SUMOylation of IRF-7 and IRF-3, likely contributing to suppression of IFN-α/β gene transcription (Chang et al., 2009).

As noted above, VP35 is a dsRNA binding protein and point mutations that disrupt VP35 inhibition of virus or dsRNA-induced IFN-α/β responses have been described (Cardenas et al., 2006; Leung et al., 2010a). These mutations do not significantly impact VP35 function as part of the Ebola virus RDRP complex, indicating that they do not promote the misfolding of the protein (Leung et al., 2010a; Prins et al., 2010). In addition, when IKKε or TBK1 are over-expressed, the over-expression is sufficient to trigger IFN-β gene transcription. Expression of VP35 is sufficient to inhibit this activation and VP35 mutants unable to bind to dsRNA were as effective as wild-type VP35 in this assay. These same mutants are severely impaired in blocking IFN-α/β induction by Sendai virus or transfected dsRNA. Therefore,