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Coronavirus virulence genes with main focus on SARS-CoV envelope gene

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ABSTRACT

Coronavirus (CoV) infection is usually detected by cellular sensors, which trigger the activation of the innate immune system. Nevertheless, CoVs have evolved viral proteins that target different signaling pathways to counteract innate immune responses. Some CoV proteins act as antagonists of interferon (IFN) by inhibiting IFN production or signaling, aspects that are briefly addressed in this review. After CoV infection, potent cytokines relevant in controlling virus infections and priming adaptive immune responses are also generated. However, an uncontrolled induction of these proinflammatory cytokines can lead to pathogenesis and disease severity as described for SARS-CoV and MERS-CoV. The cellular pathways mediated by interferon regulatory factor (IRF)-3 and -7, activating transcription factor (ATF)-2/jun, activator protein (AP)-1, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), and nuclear factor of activated T cells (NF-AT), are the main drivers of the inflammatory response triggered after viral infections, with NF-κB pathway the most frequently activated. Key CoV proteins involved in the regulation of these pathways and the proinflammatory immune response are revisited in this manuscript.

It has been shown that the envelope (E) protein plays a variable role in CoV morphogenesis, depending on the CoV genus, being absolutely essential in some cases (genus α CoVs such as TGEV, and genus β CoVs such as MERS-CoV), but not in others (genus β CoVs such as MHV or SARS-CoV). A comprehensive accumulation of data has shown that the relatively small E protein elicits a strong influence on the interaction of SARS-CoV with the host. In fact, after infection with viruses in which this protein has been deleted, increased cellular stress and unfolded protein responses, apoptosis, and augmented host immune responses were observed. In contrast, the presence of E protein activated a pathogenic inflammatory response that may cause death in animal models and in humans.

The modification or deletion of different motifs within E protein, including the transmembrane domain that harbors an ion channel activity, small sequences within the middle region of the carboxy-terminus of E protein, and its most carboxy-terminal end, which contains a PDZ domain-binding motif (PBM), is sufficient to attenuate the virus. Interestingly, a comprehensive collection of SARS-CoVs in which these motifs have been modified elicited full and long-term protection even in old mice, making those deletion mutants promising vaccine candidates. These data indicate that despite its small size, E protein drastically influences the replication of CoVs and their pathogenicity. Although E protein is not essential for CoV genome replication or subgenomic mRNA synthesis, it affects virus morphogenesis, budding, assembly, intracellular trafficking, and virulence. In fact, E protein is responsible in a significant proportion of the inflammasome activation and the associated inflammation elicited by SARS-CoV in the lung parenchyma. This exacerbated inflammation causes edema accumulation leading to acute respiratory distress syndrome (ARDS) and, frequently, to the death of infected animal models or human patients.

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1. Introduction

An overview of the sensors detecting virus infection is presented first, followed by a description of the mechanisms elicited by CoV proteins to counteract innate immune responses. Some CoV proteins act as antagonists of interferon (IFN) production, whereas others inhibit IFN signaling. As a consequence, a collection of potent cytokines relevant in controlling virus infections and priming adaptive immune responses are generated (Le Bon and Tough, 2002).

Virus pathogenesis is frequently associated with an exacerbated induction of proinflammatory cytokines that is mainly driven by the activation of at least one of the following five pathways: IRF-3 and -7, ATF-2/jun, jun/fos (AP-1), NF-κB and NF-AT. Among them, the NF-κB pathway is the most frequently activated (Hatada et al., 2000; Mogensen and Paludan, 2001). NF-κB is a heterogeneous collection of dimers, composed of various combinations of members of the Rel family, which in eukaryotes include p50 (NF-κB1), p52 (NF-κB2), Rel (c-Rel), p65 (RelA) and RelB. An exacerbated immune response and a weak IFN response have been associated with virulent CoVs such as SARS-CoV and MERS-CoV (Baas et al., 2008; Lau et al., 2013; Smits et al., 2010).

The main focus of this review is the analysis of the role of the CoV envelope (E) protein in virus pathogenesis. E protein contains several active motifs despite its small size, between 76 and 109 amino acids depending on the CoV. The modification or deletion of E protein in different CoVs has led to viruses with different phenotypes and unique alteration of virus–host interactions, such as the induction of stress and unfolded protein responses, or changes in cellular ion concentrations due to the ion channel activity of E protein. All these activities have high impact on CoV pathogenesis (DeDiego et al., 2011; Nieto-Torres et al., 2014).

E protein PDZ-binding motif (PBM), which during SARS-CoV infection could potentially target more than 400 cellular PDZ motifs present within cellular proteins, confers to E protein virus pathogenicity modulating properties. Interestingly, deletion or modification of E protein PBM and internal regions within the carboxy-terminus of E protein most frequently results in attenuated CoVs that are good vaccine candidates (Jimenez-Guardeño et al., 2014; Regla-Nava et al., 2014). In addition, the identification of signaling pathways, such as NF-κB-mediated signaling, responsible for CoV pathogenicity has led to the selection of antivirals that considerably increase the survival of infected animal models (DeDiego et al., 2014).

1.1. Coronavirus proteins inhibiting type I interferon production

IFNs are potent cytokines relevant in the control of virus infections and in the priming of adaptive immune responses (Le Bon and Tough, 2002). Treatment with type I IFN inhibits CoV growth in tissue culture and in animal models such as cynomolgus macaques and mice (Barnard et al., 2006; Dahl et al., 2004; Fuchizaki et al., 2003; Haagmans et al., 2004; Kumaki et al., 2011; Mahlakoiv et al., 2012; Sainz et al., 2004; Stroher et al., 2004; Zheng et al., 2004). To circumvent the inhibition of virus replication, many viruses, including CoVs, encode viral proteins inhibiting IFN production or signaling (Table 1). However, most of the studies describing the IFN antagonist activity of coronavirus-encoded proteins have been conducted in cells transiently expressing the viral proteins. Therefore, additional analyses in the context of the virus infection are required.

Type I IFN production is controlled by two major pathways dependent on RNA helicases or toll-like receptors (TLRs) (Arpaia and Barton, 2011; Rathinam and Fitzgerald, 2011; Sen, 2001) (Fig. 1). RNA helicases containing the cytoplasmic CARD domain, retinoic acid-inducible gene 1 (RIG-I) and melanoma differentiation-associated protein 5 (MDA5), sense pathogen-associated molecular patterns (PAMPs) in the cell cytoplasm. On the other hand, toll like receptors detect PAMPs in the cell surface and in endosomal compartments.

The RNA helicases-dependent cytoplasmic IFN induction pathways use the adaptor molecule mitochondrial antiviral signaling protein (MAVS) (Fig. 1). MAVS promotes the activation of a complex comprising the proteins TNF receptor-associated factor 3 (TRAF-3), TRAF family member-associated NF-κB activator (TANK), TANK-binding kinase 1 (TBK-1) and IkappaB kinase ε (IKKε). Active TBK1 and IKKε directly phosphorylate the transcription factors IRF-3 and IRF-7, promoting homodimerization (Sharma et al., 2003). Then, the IRF-3 and IRF-7 dimers are imported into the nucleus, leading to IRF-3 and IRF-7-dependent transcription. In addition, MAVS triggers the NF-κB pathway through IKKα and IKKβ activation (Kawai and Akira, 2007).

The TLRs-dependent IFN induction pathways use the adaptor molecules TIR-domain-containing adapter-inducing IFN-β (TRIF) and myeloid-differentiation primary response 88 (MyD88) (Fig. 1) (Kawai and Akira, 2007). TRIF-dependent pathway leads to the activation of IRF-3 and -7, and NF-κB. The activation of IRF-3 and IRF-7 is mediated by the phosphorylation of these factors by TBK-1 and IKKε, which promote their activation, as described above. TRIF also mediates NF-κB activation through the activation of IKKα and IKKβ. MyD88-mediated pathway activates the transcription factors NF-κB, AP-1 and ATF-2/jun, through the activation of mitogen-activated protein kinases (MAPKs) (Herlaar and Brown, 1999; Whitmarsh and Davis, 1996). NF-κB is also activated in this pathway through IKKs (Kawai and Akira, 2007).

IRF-3 and IRF-7, with the help of other transcription factors like NF-κB, and AP-1, initiate the transcription of IFN-β and selected IFN-α genes. IFN-α and IFN-β proteins are then secreted from the cell and can act in either an autocrine or a paracrine fashion to amplify the IFN response (Fig. 1).

CoVs have devised a number of cell type-specific strategies to inhibit type I IFN production (Table 1; Fig. 1). These viruses encode a 2′-*O*-methylase (non-structural protein nsp16) that creates a 5′-cap structure analogous to the cellular mRNAs on the viral mRNAs, thereby escaping detection by MDA5 (Zust et al., 2011). MERS-CoV accessory protein 4a is a dsRNA binding protein that blocks IFN induction by suppressing PACT-induced activation of RIG-I and MDA5 (Niemeyer et al., 2013; Siu et al., 2014). The ORF4b encoded accessory proteins of MERS-CoV and two related bat CoVs localize to the cell nucleus and inhibit type I IFN production and NF-κB signaling pathway (Matthews et al., 2014). Interestingly, a MERS-CoV lacking 4a and 4b proteins grew about 10-fold lower than the parental virus in IFN competent infected-cells (Almazan et al., 2013). However, the specific effect of 4a and 4b proteins IFN antagonistic activity in virus growth and virulence still needs to be determined. SARS-CoV membrane (M) protein impairs the formation of TRAF3/TANK/TBK1/IKKε complex, inhibiting IFN-β production (Siu et al., 2009). SARS-CoV structural nucleocapsid (N) protein blocks IFN-β production after induction with Sendai virus and polyI:C, but not upstream of components such as RIG-I, MDA5, MAVS, IKKε, TBK1 or TRIF, indicating that N protein acts after these signaling mediators (Kopecky-Bromberg et al., 2007; Lu et al., 2011). SARS-CoV papain-like protease (PLP) domain of nsp3 inhibits RIG-I and TLR3-dependent IFN-β production, being this activity independent of the deubiquitinating and protease activities (Clementz et al., 2010), and most probably mediated by the interaction of PLP domain with the protein stimulator of IFN genes (STING), which is a protein that stimulates phosphorylation of IRF3 by the kinase TBK1 (Sun et al., 2012). The inhibition of IFN production has also been described for nsp3 PLP2 of HCoV-NL63 (Clementz et al., 2010; Sun et al., 2012), MHV (Wang et al., 2011; Zheng et al., 2008), and for the PLP domain of MERS-CoV, which blocks IFN production by inhibiting IRF3 phosphorylation and translocation into

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