The Strategy of Picornavirus Evading Host Antiviral Responses: Non-structural Proteins Suppress the Production of IFNs

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Viral infections trigger the innate immune system to produce interferons (IFNs), which play important role in host antiviral responses. Co-evolution of viruses with their hosts has favored development of various strategies to evade the effects of IFNs, enabling viruses to survive inside host cells. One such strategy involves inhibition of IFN signaling pathways by non-structural proteins. In this review, we provide a brief overview of host signaling pathways inducing IFN production and their suppression by picornavirus non-structural proteins. Using this strategy, picornaviruses can evade the host immune response and replicate inside host cells.

Keywords: IFNs, picornaviruses, non-structural proteins, immune evasion, signaling pathways

INTRODUCTION

Picornaviruses are small, non-enveloped, positive-strand RNA viruses that infect diverse animal and human hosts (Ehrenfeld et al., 2010; Feng et al., 2014b). As one of the largest viral families, picornaviruses contain 31 genera and 54 species, including cardioviruses [e.g., encephalomyocarditis virus (EMCV) and Theiler's virus (TEV)], enteroviruses [e.g., enterovirus 71 (EV71); poliovirus (PV); coxsackievirus (CV); and rhinovirus (RV)], hepatitis A virus (HAV), and foot-and-mouth disease virus (FMDV) (Feng et al., 2014b). Picornavirus genomes are single-stranded RNAs (7,000 to 9,000 nucleotides in length) which consist (from 5' to 3') of a 5' untranslated region (UTR), a single open-reading frame (ORF), a 3' UTR, and a poly(A) tail (Figure 1; Feng et al., 2014b). The ORF is translated into a polyprotein, which is processed by viral proteases into structural proteins (VP1–VP4) and non-structural proteins (2A, 2B, 2C, 3A, 3B, 3C, and 3D pro, and in some genera, L pro). Structural proteins are used to assemble viral capsids whereas non-structural proteins replicate the genomic RNA in conjunction with cell proteins (Argos et al., 1984; Buenz and Howe, 2006; Ehrenfeld et al., 2010).

Interferons (IFNs) which play important roles in regulation and activation of host immune responses, were first discovered by Isaacs and Lindenmann in 1950s (Isaacs and Lindenmann, 2015; Klotz et al., 2017). IFNs are classified into three categories according to their antiviral activities, genetic, structural and functional features and their cognate receptors (Nagano and Kojima, 1954): type I (IFN-α, IFN-β, IFN-γ, IFN-ε, IFN-ζ, IFN-τ, and IFN-ω), type II (IFN-γ) (Klotz et al., 2017), and type III (IFN-λ1 or IL-29, IFN-λ-2 or IL-28A, IFN-λ-3 or IL-28B, and IFN-λ-4)
(Schroder et al., 2004; González Navajas et al., 2012). Type I IFNs typically have antiviral effects and are the most broadly expressed, well-known antiviral IFNs. Although type I IFNs can be secreted by most parenchymal cells, the main type I IFN producer is plasmacytoid dendritic cell (pDC) (Coccia and Battistini, 2015; Kindler et al., 2016). Type II IFN is produced by activated T cells and NK cells and predominantly induce macrophage activation stimulating their activity against ingested intracellular non-viral pathogens (Coccia and Battistini, 2015). Type III IFNs are produced by epithelial cells, leukocytes, intestinal eosinophils and pDCs (Ank et al., 2006; Hillyer et al., 2012; Raki et al., 2013; Hernandez et al., 2015; Mahlakoiv et al., 2015; Pervolaraki et al., 2017). Type III IFNs are similar to type I IFNs, and also play roles in regulating the host antiviral response (Reid and Charleston, 2014; Kindler et al., 2016).

Viruses develop various strategies to inhibit secretion of IFNs and promote viral replication inside host cells. Mounting evidence shows that infecting viruses can evade IFN response either by suppressing IFN production or by blocking IFN induction of interferon-stimulated gene factors (ISGs) (Zinzula and Battistini, 2015). Type III IFNs are produced by epithelial cells, leukocytes, intestinal eosinophils and pDCs (Ank et al., 2006; Hillyer et al., 2012; Raki et al., 2013; Hernandez et al., 2015; Mahlakoiv et al., 2015; Pervolaraki et al., 2017). Type III IFNs are similar to type I IFNs, and also play roles in regulating the host antiviral response (Reid and Charleston, 2014; Kindler et al., 2016).

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**SIGNALING PATHWAYS INDUCING IFN PRODUCTION**

When viruses infect organisms, the host innate immune system detects the presence of pathogen-associated molecular patterns via host pattern recognition receptors (PRRs) (Vaccari et al., 2014; Coccia and Battistini, 2015). These include transmembrane PRRs such as Toll-like receptors (TLRs), cytosolic RIG-like RNA helicases such as melanoma differentiation-associated gene (MDA-5), retinoic acid induced gene-I (RIG-I), and other molecules (Barbé et al., 2014; Wu and Chen, 2014). PRRs recruit a number of specific adpoter proteins to trigger a downstream signaling cascade and activate three major pathways to produce IFNs: the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) (Coccia and Battistini, 2015), the mitogen-activated protein kinase (MAPK), and the IFN regulatory factor (IRF) pathways (Akira et al., 2006; Honda and Taniguchi, 2006). IFNs can signal in an autocrine or paracrine manner to induce hundreds of ISGs that fortify host defenses (Figure 2; Pham et al., 2016).
interferons are produced mainly by antigen-presenting cells and epithelial cells (Chasset and Arnaud, 2017), while the major cell type responsible for type I IFN production is the pDC (Mackern-Oberti et al., 2015; Chasset and Arnaud, 2017). Type I IFNs are induced by mitochondrial-associated MAVS, whereas type III IFNs are stimulated by peroxisome-associated MAVS (Odendall et al., 2014). In addition, IRF1 plays a unique role in type III IFN induction while IRF3 and IRF7 play vital roles in type I IFN production (Österlund et al., 2007).

PICORNAVIRUS NON-STRUCTURAL PROTEINS INHIBIT IFN PRODUCTION TO COUNTERACT HOST ANTIVIRAL RESPONSES

While the host secretes IFNs to defend against viral infection, viruses have also developed effective immune evasion mechanisms to counteract the host’s antiviral responses. Numerous studies have demonstrated that picornavirus proteases can cleave adaptors, receptors and regulators involved in the signaling pathways controlling IFN induction to inhibit production of type I IFNs (Wang et al., 2012; Lei et al., 2013). Besides, picornavirus non-structural proteins play important roles in the suppression of IFNs by down-regulating host gene expression and blocking the secretory pathway (Table 1).

However, in some picornaviruses, cooperation between non-structural proteins leads to inhibition of IFN induction (Sim et al., 2005; Chase and Semler, 2012).

Lpro

Lpro of FMDV

Foot-and-mouth disease virus Lpro is a kind of papain-like cysteine protease, which was first identified by Strebel and Beck (1986). FMDV’s RNA genome encodes a polypeptide, Lpro is located near its N-terminus (residue ~2330) and there are two forms of FMDV Lpro, Laboratory (201 aa) and Lb (173 aa) (Kirchweger et al., 1994). FMDV infection selectively induces IFN-α1 mRNA, and IFN-β mRNA levels become elevated only after a significant duration of infection (24 h) (de Los Santos et al., 2006). By inhibiting IFN production (including type I and type III IFNs) at the transcriptional and translational levels, FMDV Lpro down regulates the host innate immune response to FMDV infection. FMDV Lpro can repress IFN-β transcription by reducing IFN-stimulated gene products (Li D. et al., 2016) decreasing IFN-β mRNA levels during early infection and inhibiting activation of NF-κB via degradation of the NF-κB subunit p65/RelA and ubiquitination of RIG-I, TBK1, and TRAF3/6, resulting decreased IRF-3/7 protein expression (de Los Santos et al., 2007; Wang et al., 2011). Additionally, Lpro contributes to induce the cleavage of host eukaryotic translation initiation factor 4γ (eIF4G) (Devaney et al., 1988; Belsham et al., 2000), shutting off host cap-dependent mRNA translation, thus
| Non-structural Proteases | Virus | Involved signaling pathways/structure | Type of IFN | Reference |
|-------------------------|-------|--------------------------------------|-------------|-----------|
| Lpro                    | FMDV  | Cleaving eIF4G, shutting off host cap-dependent mRNA translation, limiting the synthesis of host proteins | Type I IFNs | Devaney et al., 1988; Belsham et al., 2000; de Los Santos et al., 2006 |
|                         |       | Degrading NF-κB subunit p65/RelA, ubiquitinating RIG-I, TBK1, and TRAF3/6, decreasing IRF-3/7, inhibiting NF-κB | IFN-β | de Los Santos et al., 2007; Wang et al., 2011 |
|                         |       | Via Lpro’s catalytic activity and SAP domain | IFN-λ1 | Wang et al., 2011 |
|                         |       | Disrupting NF-κB and IRF via RIG-I/MDA5 | IFN-λ1 | Shi et al., 2011; Wang et al., 2011 |
| EMCV                    |       | Lpro hinge domain interacting with Ran and disrupting the Ran GDP-GTP gradient, inhibiting nucleocytoplasmic transport | Unclear | Porter et al., 2006; Ma, 2007; Bacoldtav and Palmenberg, 2013 |
| TMEV                    |       | Interfering IRF3 | IFN-α/β | Hato et al., 2010 |
| 2A                      | EV71  | Cleaving MAVS and MDA5, preventing IRF3 phosphorylation | Type I IFNs | Feng et al., 2014a |
|                         |       | Inhibiting induction of downstream IFN-stimulated genes, the detailed mechanism is controversial | Unclear | Lu et al., 2012; Liu et al., 2014 |
|                         |       | Downregulating KPNA1, reducing formation of the STAT/karyopherin-α1 (KPNA1) complex | Unclear | Wang et al., 2017 |
|                         |       | Reducing serine phosphorylation of STAT1 and inactivating extracellular signal-regulated kinases | IFN-γ | Morrison and Racaniello, 2009 |
| RV                      |       | Cleaving MAVS | Unclear | Mukherjee et al., 2011 |
| CVB3/PV                 |       | Cleaving MAVS and MDA5 | Type I IFNs | Feng et al., 2014a |
| 2B                      | HAV   | Influencing MAVS function | IFN-β | Ashutosh et al., 2015 |
|                         |       | Interfering TBK1/IKKε kinase complex, inhibiting RIG-I/MDA-5 and IRF3 | IFN-β | Paulmann et al., 2008 |
| 2C                      | EV71  | Inhibiting IKKβ phosphorylation and NF-κB activation via PP1 binding NF-κB | Unclear | Zheng et al., 2011; Li Q. et al., 2016 |
|                         |       | Suppressing p65/p50 dimerization by competing p65 IPT domain, suppressing the activation of NF-κB | Unclear | Du et al., 2015 |
|                         | CVA16/CVB3 | Inhibiting IKKβ phosphorylation and NF-κB activation via PP1 binding | Unclear | Paulmann et al., 2008; Du et al., 2015 |
| 3A                      | FMDV  | Reducing expression of MDA5, RIG-I and VISA by decreasing their mRNA levels, inhibiting RLR pathway | IFN-β | Li D. et al., 2016 |
|                         | EV71  | Cleaving TRIF and TBK1, inhibiting TLR3 and RIG-I, preventing activation of IRF3 and IRF7 | IFN-β | Lei et al., 2010 |
|                         |       | Inhibiting IRF7 and IRF9 | Type I IFNs | Hung et al., 2011; Lei et al., 2013 |
|                         |       | Cleaving TAK1/TAB1/TAB2/TAB3 complex, NF-κB | Unclear | Lei et al., 2014 |
|                         |       | Binding with RIG-I, impairing RIG-I’s interaction with MAVS | Type I IFNs | Xu et al., 2014 |
|                         |       | Cleaving TAK1 to inhibit the NF-κB response | Unclear | Rui et al., 2017 |
|                         | CV-A16, CV-A6, EV-D68 | Binding with MDA5, inhibiting the interaction with MAVS | Type I IFNs | Rui et al., 2017 |
|                         | CVB3  | Cleaving MAVS and TRIF | Type I IFNs | Mukherjee et al., 2011 |
|                         | EMCV  | Cleaving TANK, disrupting the formation of the TANK–TBK1–IKKε–IRF3 tetramer, decreasing TBK1- and IKKε-mediated IRF3 phosphorylation, impeding the ability of TANK to inhibit TRAF6-mediated NF-κB signaling | Type I IFNs | Huang et al., 2015, 2017 |
|                         |       | Blocking formation of SG | Unclear | |
|                         |       | Cleaving IRF3-5D, inhibits JAK-STAT signaling | Type I IFNs | |
|                         |       | Suppressing STAT1 or IRF3 binding to the IFN-β promoter | Type I IFNs | |
|                         | FMDV  | Cleaving NEMO | Unclear | Zhao et al., 2007 |
|                         |       | Cleaving TANK, generating a 15-kDa N-terminal fragment and impeding TANK’s ability to suppress TRAF6-mediated NF-κB signaling | Unclear | Fan et al., 2017 |
αIRF3-mediated IFN-α interferes with the transactivation function of IRF3 suppressing GTPase (Bacotdavis and Palmenberg, 2013). EMCV Lpro hinge domain plays a major role in the interaction with Ran (Ma, 2007), suppressing the production of IFN. EMCV Lpro inhibition of nucleocytoplasmic transport (Porter et al., 2006; Ran and disrupts the RanGDP-GTP gradient leading to the Ran-GTPase system. EMCV Lpro directly interacts with nucleocytoplasmic transport of RNA and protein relies on translation whose function is quite similar to Lpro in FMDV.

Enterovirus 71 2A cleaves MAVS from the outer membrane of mitochondria. The cleaved fragments are released into the cytoplasm where they effectively inactivate downstream signaling and cleave MDA5, thus preventing IRF3 phosphorylation, down regulating production of type I IFNs and increasing viral replication (as it was shown in Figure 2; Wang et al., 2013). During this process, EV71 2A cleaves at MAVS residues Gly209, Gly251, and Gly265, with a strong preference for cleavage at Gly251 (Wang et al., 2013). Similarly, Influenza A virus 2A inhibits the production of IFN by cleaving MAVS (Mukherjee et al., 2011). CVB3 (coxackievirus B3) 2A and poliovirus (PV) also mediate the cleavage of MAVS and MDA5, exerting the same functions in inhibiting type I IFNs (Feng et al., 2014a).

Foot-and-mouth disease virus Lpro also antagonizes IFN-λ-1: Lpro’s catalytic activity and SAP domain are involved in the suppression of IFN-λ-1 induction (Wang et al., 2011). In addition, by disrupting activation of NF-κB and IRFs and inhibiting IFN-λ-1 expression induced by RIG-I/MDA5, FMDV Lpro inhibits IFN-λ-1 promoter activation (Wang et al., 2011).

**Lpro of Cardiovirus**
Cardiovirus polyproteins begin with short N-terminal Leader (L) sequences, EMCV Lpro (~67 residues) and TMEV Lpro (~76 residues) contains common zinc-finger and acidic domains. Although cardiovirus Lpro is different from FMDV Lpro and does not function as a protease, it represses IFN-α/β synthesis during viral infection. In eukaryotes, nucleocytoplasmic transport of RNA and protein relies on the Ran-GTPase system. EMCV Lpro directly interacts with Ran and disrupts the RanGDP-GTP gradient leading to inhibition of nucleocytoplasmic transport (Porter et al., 2006; Ma, 2007), suppressing the production of IFN. EMCV Lpro hinge domain plays a major role in the interaction with Ran GTPase (Bacotdavis and Palmenberg, 2013). EMCV Lpro interferes with the transactivation function of IRF3 suppressing IRF3-mediated IFN-α/β production (Hato et al., 2010). Studies have demonstrated that TMEV Lpro can block the production of type I IFN at the transcriptional level (Van et al., 2001), this transcriptional inhibition is correlated with inhibition of IRF-3 dimerization (Ricour et al., 2009).

**2A of Enteroviruses**
Enteroviruses 2A has protease activity (Racaniello, 2007), which can not only process the viral polyprotein (Toyoda et al., 1986), but also cleave a variety of host proteins and inhibit the host translation whose function is quite similar to Lpro in FMDV.

| Non-structural Proteases | Virus | Involved signaling pathways/structure | Type of IFN | Reference |
|-------------------------|-------|--------------------------------------|-------------|-----------|
| SVV                     |       | Suppressing IRF3 by degrading autophagy-related protein   | Unclear     | Fan et al., 2017 |
|                         |       | Degrading KPNA1, blocking STAT1/STAT2 nuclear translocation | Unclear     | Du et al., 2014 |
|                         |       | Reducing the expression of IRF3 and IRF7 and phosphorylating them | Type I IFNs | Qian et al., 2017 |
| HAV                     |       | Cleaving MAVS, TRIF, and TANK          | IFN-α1, IFN-α4, and IFN-β | Xue et al., 2018 |
|                         |       | Reducing the expression of IRF3 and IRF7 and phosphorylating them | IFN-α1, IFN-α4, and IFN-β | Xue et al., 2018 |
| 3ABC                    |       | Cleaving MAVS and disrupting activation of IRF3 through the RLR pathway | Unclear     | Lei et al., 2013, 2014 |
| 3CD                     |       | Disrupting RIGI/MDA5, inhibiting dimerization of IRF-3 and translocation of IRF-3 to the nucleus | IFN-β       | Qu et al., 2011 |
| 3D                      | EV71  | Attenuating STAT1 tyrosine phosphorylation | IFN-γ       | Wang et al., 2015 |

limiting the synthesis of host proteins (de Los Santos et al., 2006), which may possibly include type I IFNs.

**TABLE 1 | Continued**
unable to replicate in IFN-pretreated cells. Their 2A might not have function on evading the host immune response (Morrison and Racaniello, 2009).

2B
2B of HAV
Hepatitis A virus 2B is a peripheral membrane protein, its coding region has variants (Emerson et al., 1991, 1992, 1993), which makes it significantly larger than 2B in other picornaviruses. HAV 2B was found in close vicinity to the tubular interconnected network of mitochondrial membranes through its ability induce membrane rearrangements resulting in the influence of the production of IFNs (Gosert et al., 2000). HAV 2B suppresses MAVS signaling more effectively with the cooperation of HAV 3A (Ashutosh et al., 2015). HAV 2B appears to influence MAVS function without directly affecting the antigenic structure of MAVS (Paulmann et al., 2008); it also interferes with the TBK1/IKKe kinase complex. Consequently, RIG-I/MDA-5-mediated activation is inhibited and inhibition of IRF-3 signaling results in efficient suppression of IFN-β synthesis (Paulmann et al., 2008).

2C
2C of Enteroviruses
Enteroviruses 2C (329 aa and 37.5 kDa), such as EV71 and CVA16 2C ATPase, is not only an RNA helicase but also an ATP-independent RNA chaperone, which is critical for RNA replication and viability of enteroviruses (Xia et al., 2015; Guan et al., 2017). EV71 2C is localized both to the cytoplasm and the nucleus. EV71 2C interacts with protein phosphatase 1 (PP1) catalytic subunit through PP1-docking motifs (residues 1 to 47) located near the N-terminus of EV71 2C. Interactions with IKKβ are formed through a motif (residues 105 to 121) located within N-terminal region of EV71 2C, resulting in formation of a complex between PP1 and IKKβ (Li Q. et al., 2016). PP1 binding is crucial for EV71 2C-mediated inhibition of IKKβ phosphorylation. EV71 2C-mediated PP1 recruitment inhibits IKKβ phosphorylation, NF-κB activation and NF-κB signaling pathway-induced IFN production (Zheng et al., 2011; Li Q. et al., 2016). Other enteroviruses, such as PV, coxsackie A virus 16 (CVA16), and coxsackie B virus 3 (CVB3) also exploit this mechanism to inhibit the production of IFN (Li Q. et al., 2016).

Additionally, EV71 2C (residues 105–125 and 126–263) is capable to suppress p65/p50 dimerization by competing p65 IPT domain in association with p50, suppressing the activation of NF-κB and IFN (Du et al., 2015).

3A
3A of FMDV
Foot-and-mouth disease virus 3A is a partially conserved protein, it has no homologous sequence to any other known proteases, which is unique among the picornaviruses. A recent study revealed that FMDV 3A down regulates FMDV-associated IFN-β induction via FMDV 3A inhibition of RLR-mediated IFN-β induction (Li D. et al., 2016). Residues 103–153 near 3A's N-terminus interact with MDA5, RIG-I and VISA, and a 102-residue region near the N-terminus mediates inhibition of the IFN-β signaling pathway (Li D. et al., 2016). FMDV 3A reduces expression of MDA5, RIG-I and VISA by decreasing their mRNA levels (Li D. et al., 2016). This finding not only reveals a novel mechanism of FMDV 3A-mediated evasion of host innate immunity but also provide a new thought to explore this kind of non-structural proteins in other picornaviruses.

3C
Picornavirus 3C is a unique cysteine protease that combines features of both serine and cysteine proteases (Di et al., 2016). Although 3C has similar spatial structures among all picornviruses, and can inhibit IFN expression through similar pathways, including the NF-κB, Jak/STAT and IRF pathways, its specific sites of action are different.

3C of Enteroviruses
Enterovirus 71 3C is one of the most common functional proteins, which has been most widely studied in enteroviruses. EV71 3C inhibits induction of IFN by RIG-I or TLR3 and prevents activation of IRF3 and IRF7. Upon viral infection, TLR3 recruits TRIF (TIR domain-containing adaptor inducing IFN-β) and TBK1, which phosphorylate IRF3 and IRF7 (Lei et al., 2010). The TRIF Q312–S313 junction is critical for its cleavage by EV71 3C. EV71 3C-induced TRIF cleavage blocks IFN-β and NF-κB activation by TRIF (Lei et al., 2011). EV 71 3C can directly inhibit IRF7 and IRF9, repressing type I IFN production (Hung et al., 2011; Lei et al., 2013). EV71 3C protease activity is necessary to cleave IRF7. EV71 3C cleaves IRF7 at the Q189–S190 junction, yielding two fragments that are unable to stimulate IFN production (Lei et al., 2013).

Likewise, EV71 3C reduces IFN production by inhibiting activation of NF-κB (Lei et al., 2014). Transforming growth factor-β-activated kinase 1 (TAK1), TAK1-binding protein (TAB)1, TAB2, and TAB3 are all required for activation of downstream NF-κB. In mammalian cells, TAB1 binds to TAB1, forming TAB1-TAB1 complex. Thereafter, TAB2 and TAB3 are recruited to TAB1-TAB1 complex forming TAB1/TAB1/TAB2/TAB3 complex. This complex activates p38, IKKα/β and c-Jun N-terminal kinase (JNK), thus inducing IFN production (Li et al., 2014). EV71 3C cleaves TAB1 at the Q360–S361 junction yielding smaller products of about 30 kDa. The TAB1 Q414–G415 and Q451–S452 junctions are EV71 3C cleavage sites; cleavage results in about 45 kDa and 50 kDa products. EV71 3C cleaves TAB2 at the Q113–S114 junction. EV71 3C cleaves TAB3 at the Q173–G174 and Q343–G344 junctions, resulting in about 45 kDa and 60 kDa products. Cleavage disrupts the TAB1/TAB1/TAB2/TAB3 complex and reduces IFN production. It should be noted that TAB2 has NF-κB-activating function, but cleavage by EV71 3C impairs this activity (Lei et al., 2014). On the other hand, CVA-16, CV-A6, and EV-D68 3C cleave TAB1 to inhibit the NF-κB response (Rui et al., 2017).

Upon viral infection, EV71 3C can directly bind to RIG-I, impairing RIG-I interaction with MAVS and inhibiting RIG-I-mediated type I IFN responses. It has been reported that...
ubiquitination of RIG-I is controlled by a tumor suppressor called CYLD (Xu et al., 2014). CYLD is a target of miR-526a, a potent IFN-β inducer, and miR-526a upregulation during viral infection is partially mediated by IRF7. By suppressing CYLD expression, miR-526a positively regulates VSV-associated type I IFN production. EV71 3C inhibits production of type I IFN by blocking miR-526a upregulation and CYLD downregulation.

CV-A16, CV-A6, and EV-D68 3C can bind to MDA5 and inhibit the interaction with MAVS, thus blocking the production of type I IFN (Rui et al., 2017). CVB3 3C also cleaves MAVS and Toll/IL-1 receptor domain-containing adaptor inducing interferon-beta (TRIF) at specific sites and inhibits the induction of type I IFN (Mukherjee et al., 2011).

3C of EMCV
*Encephalomyocarditis virus* 3C is the only cysteine protease encoded by the viral genome, and it has a high degree of substrate specificity, besides Lpro, EMCV 3C is another antagonist. TANK is an NF-κB activator, TRAF6 serves as a platform to recruit the IKK complex and kinase TAK1, and TANK negatively regulates this function (Papon et al., 2009). EMCV 3C can cleave TANK at Gln291 and Gln197 (Huang et al., 2015), disrupting formation of the TANK–TBK1–IKKe–IRF3 tetramer, decreasing TBK1- and IKKe-mediated IRF3 phosphorylation, impairing the ability of TANK to inhibit TRAF6-mediated NF-κB signaling, and reducing type I IFN production (Huang et al., 2015, 2017). SG is the location for efficient interaction between viral RNA and RLRs; EMCV 3C can also block formation of SG to inhibit activation of IFN genes (Huang et al., 2017). By cleaving IRF3-5D and other key proteins, EMCV 3C inhibits JAK-STAT signaling, suppressing type I IFN production (Huang et al., 2017). EMCV 3C may also suppress STAT1 or IRF3 binding to the IFN-β promoter to inhibit type I IFN production (Huang et al., 2017).

3C of FMDV
Foot-and-mouth disease virus 3C plays important roles in disrupting the translational system of the host and can negatively regulate innate immune signaling by degrading essential molecules in different pathways (Ma et al., 2018a). FMDV 3C has the ability to cleave NEMO at Gln383 (Zhao et al., 2007); cleavage impairs NEMO-mediated IFN production and its ability to act as a signaling adaptor in the RIG-I/MDA5 pathway (Wang et al., 2012). Moreover, FMDV 3C cleaves TANK, generating a 15-kDa N-terminal fragment and impairing TANK's ability to suppress TRAF6-mediated NF-κB signaling (Fan et al., 2017).

Under normal conditions, ATG5-ATG12 promotes activation of IRF3 and phosphorylation of TBK1 by preventing TRAF3 degradation, resulting in enhanced expression of IFN-β (Fan et al., 2017). FMDV suppresses IRF3 by degradation of autophagy-related protein ATG5-ATG12 to attenuate production of IFN via 3C (Fan et al., 2017).

Karyopherin α1 (KPNα1) is the nuclear localization signal receptor for STAT1. FMDV 3C interferes with the JAK-STAT signaling pathway by degrading KPNα1, blocking STAT1/STAT2 nuclear translocation and inhibiting IFN signaling (Du et al., 2014).

3C of SVV
Seneca Valley virus (SVV) is most closely related to *Cardiovirus* (Hales et al., 2008). SVV 3C has a conserved catalytic box with His and Cys residues (Qian et al., 2016), which is similar to FMDV Lpro. SVV 3C can inhibit the production of type I IFN by directly cleaving MAVS, TRIF, and TANK (Qian et al., 2016). In addition, a recent result indicates that SVV 3C reduces the expression of IRF3 and IRF7 and phosphorylates them and then blocks the transcription of IFN-β, IFN-α1, IFN-α4, and ISG54 (Xue et al., 2018).

3C of HAV
Hepatitis A virus 3C is a cysteine protease which is responsible for most cleavages within the viral polyprotein (Schultheiss et al., 1994, 1995). HAV 3C cleaves MAVS at Gln428 to inhibit type I IFN production (Yang et al., 2007). Similar to FMDV 3C, HAV 3C also cleaves NEMO, impairing NEMO-mediated IFN production and its ability to act as a signaling adaptor in the RIG-I/MDA5 pathway (Wang et al., 2014; Xu et al., 2014). Moreover, HAV 3C inhibits NF-κB activation through cleavage of the TAK1/TAB1/TAB2/TAB3 complex, inhibiting the induction of IFNs (Lei et al., 2013, 2014).

3ABC and 3CD of HAV
Processing intermediate HAV 3ABC and 3CD are both unique and have proteolytically activities in particle assembly (Probst et al., 1998). HAV 3ABC is a precursor cysteine protease. 3ABC cleaves MAVS and disrupts activation of IRF3 through the RLR pathway in mitochondria (Yang et al., 2007; Debing et al., 2014). With the help of the transmembrane domain of 3A, 3ABC localizes to mitochondria. MAVS cleavage also requires the protease activity of 3C (Yang et al., 2007). This feature of 3ABC is unique among picornviruses.

Hepatitis A virus 3CD is the processing intermediate of 3ABCD. HAV 3CD disrupts RIGI/MDA5, inhibits dimerization of IRF-3 and translocation of IRF-3 to the nucleus, and impairs IFN-β promoter activation (Qu et al., 2011).

3D

3D of EV71
*Enterovirus* 71 3D is a kind of RNA-dependent RNA polymerase (Jiang et al., 2011; Sun et al., 2012). Wang et al. (2015) found that without interfering with IFN-γ receptor expression, EV71 3D can attenuate STAT1 tyrosine phosphorylation resulting in defective IFN-γ signaling. The detailed signaling pathway how 3D regulate STAT1 need further investigation, Wang et al. (2015) guess that the function of EV71 3D may similar to EV71 2A, as a viral factor for immune-editing.

**OUTLOOK**

The interactions between picornviruses and host defenses are complex and diverse. Moreover, viruses have developed multiple strategies to evade the host's innate immune system. To date, some of these strategies have been uncovered and significant progress has been achieved in understanding signaling pathways...
related to immune evasion. For example, the mechanism underpinning inhibition by some non-structural proteins of IFN production in picornaviruses has been well studied. However, what we know today just represent a drop in the bucket, and we still need to understand the viral strategies involved in antagonizing the host's innate immune system. For example, SVV 3C has similar conserved catalytic box and similar function to FMDV Lpro in antagonizing the innate immune response and whether SVV 3C has other similar function to FMDV Lpro need further research. In addition, there are many similarities between different genera of picornaviruses. However, further efforts should be made to explore key mechanisms underlying inhibition by some non-structural proteins of IFN production, such as 2B, 2C, 3A, and 3D, across all picornavirus.

Recently, it has been discovered that some picornaviruses only cause an acute and self-limiting infection without major pathogenesis in hosts requiring more research on therapeutic approach (Weinberg and Morris, 2016; Ma et al., 2018b). The role of non-structural proteins in such picornaviruses may make contributions to better understand not only the therapeutic antiviral activity of IFNs, but also may reveal how these proteins (with or without protease activities) influence and control the IFN signaling transduction in vivo.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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