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Short Communication

Antiviral activity of type I and type III interferons against porcine reproductive and respiratory syndrome virus (PRRSV)

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ABSTRACT

The newly identified type III interferons (IFNs), also known as IFN-λ1/IL-29, IFN-λ2/IL-28A and IFN-λ3/IL-28B, like type I IFNs, have antiviral activity against a broad spectrum of viruses. We therefore examined whether type III IFNs, as well as type I IFNs, has the ability to inhibit porcine reproductive and respiratory syndrome virus (PRRSV) replication in MARC-145 cells. We found that replication of PRRSV in MARC-145 cells was significantly reduced following treatment with IFN-λ1, IFN-λ2 and IFN-λ3, respectively, and such inhibition was dose-dependent. However, type III IFNs (IFN-λ1, IFN-λ2 and IFN-λ3) was less effective than type I IFNs (IFN-α and IFN-β) in antiviral activity against PRRSV. Mixture of two types of IFNs could not improve the antiviral activity of each type alone. In addition, all types of IFNs in our study were able to induce the expression of ISG56, 2′,5′-OAS and MxA in MARC-145 cells. These data demonstrate that type III IFNs had antiviral activity against PRRSV and may serve as useful antiviral agents against infectious swine diseases.

Interferons (IFNs) form an important group of cytokines that are now recognized as key components of the innate immune response and the first line of defense against virus infection (Sadler and Williams, 2008). IFNs belong to the class II cytokine receptor family, and are divided into three classes according to the receptor complex, designated types I–III (Kotenko et al., 2003; Sheppard et al., 2003). The group of type I IFNs are composed of IFN-α, β, -ω, -ε, and -κ, which all bind to the IFNα receptor (IFNAR) complex. The binding of a type I IFNs to the receptor, with ensuing signal transduction, leads to the expression of a distinct set of IFN-stimulated genes (ISGs) (Der et al., 1998). These ISGs mediate the biological effects of IFN, such as inhibition of viral replication, cellular growth inhibition, and apoptosis (Maher et al., 2008). Type II IFN consists of a single type, IFN-γ, which binds to the IFNGR receptor complex. IFN-γ plays important roles in adaptive immune response, since this cytokine is crucial in the activation of natural killer (NK) cells and macrophages (Schroder et al., 2004). Type III IFNs, also noted as IFN-λ, were recently identified as a member of the IL-10-related cytokine family but displays type I IFN-like antiviral activity and induction of typical IFN-inducible genes (Ank et al., 2006; Uze and Monneron, 2007). Three distinct forms of IFN-λs were identified, which have been named IFN-λ1/IL-29, IFN-λ2/IL-28A and IFN-λ3/IL-28B (Sheppard et al., 2003). IFN-λ exerts its action through a distinct receptor complex, composed of IFN-λR1 (also known as IL-28R) and IL-10R2 which is shared with the IL-10 receptor and other IL-10-related cytokine receptors (Kotenko et al., 2003). The binding of IFN-λs to its receptor results in the phosphorylation of STAT1, STAT2 and STAT3 and the subsequent formation of the interferon-stimulated gene factor 3 (ISGF3) complex and then the induction of typical ISGs such as 2′,5′-oligoadenylate synthetase (2′5′-OAS) and MxA genes (Kotenko et al., 2003).

Similar to type I IFNs, IFN-λs expression are also induced by viral infection or treatment with poly(I:C) or lipopolysaccharide (Coccia et al., 2004). Earlier studies have demonstrated that IFN-λs have the ability to inhibit the replication of many viruses, such as hepatitis B virus and hepatitis C virus (Robek et al., 2005), human immunodeficiency virus 1 (Hou et al., 2009), murine cytomegalovirus (Brand et al., 2005) and murine herpes simplex virus 2 (Ank et al., 2006).

Porcine reproductive and respiratory syndrome virus (PRRSV) is a member of the family *Arteriviridae* in the order *Nidovirales*, and it causes severe reproductive failure in sows, and respiratory distress in piglets and growing pigs (Rossow, 1998). Previous studies have demonstrated that IFN-α, IFN-β and IFN-γ have an antiviral effect against PRRSV (Albina et al., 1998; Bautista and Molitor, 1999; Overend et al., 2007). However, it is still unclear whether IFN-λs have the ability to inhibit PRRSV replication. In the present study, we compared the antiviral activity of type I and type III IFNs against PRRSV and assessed the levels of ISGs expression induced by IFNs to elucidate the mechanism that underlies the different antiviral efficacy of various IFNs.

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For these studies, PRRSV strain CH-1a (kindly provided by Dr. Guangzhi Tong), the first field isolate in China, was used as a model virus to evaluate the antiviral activity of type I and type III IFNs against PRRSV. The virus was propagated and titrated in MARC-145 cells (Overend et al., 2007). Recombinant human IFN-α 2a and IFN-β 1a were from PBL Biomedical Laboratories and recombinant human IFN-λs were from R&D Systems. For the antiviral tests, MARC-145 cells were primed for 12 h with IFN-α, -β, -λ1, -λ2 or -λ3, respectively. The cells were then infected with PRRSV for 1–2 h, washed and replenished with fresh medium containing the indicated IFN. Then, at 12, 24, 36 or 48 h after infection, the cells were submitted to two freeze–thraw cycles and titrated by plaque assay. Plaque forming units were counted and the viral titer was determined as plaque forming units per ml (pfu/ml). As shown from Fig. 1A, treatment of Marc-145 cells with IFN-α, IFN-β or IFN-λs, respectively, could all inhibit the multiplication of PRRSV. When all IFNs were compared, IFN-α and IFN-β was more potent at inhibiting PRRSV replication than IFN-λs at 12, 24, 36 or 48 h postinfection. Treatment with 10, 100 or 1000 ng/ml doses of all IFNs protected cells from PRRSV infection when compared to the untreated cells (Fig. 1B). IFN beta was the most potent IFN when compared to other IFNs at the 10, 100 or 1000 ng/ml doses.

IFN-λs mediate their effects through distinct receptors than IFN-α or -β, therefore we wondered whether co-treatment with type I and type III IFNs could enhance the antiviral efficacy against PRRSV. As shown in the Fig. 2, treatment of cells with a low dose (10 ng/ml) of IFN-α or -β in combination with 100 ng/ml IFN-λs resulted in a greater inhibition of PRRSV replication than treatment of cells with 100 ng/ml IFN-λs alone. However, these additive effects have not been found when compared with 10 ng IFN-α/β alone, suggesting that 10 ng IFN-α or IFN-β enhance the antiviral activity of 100 ng IFN-λs owing to IFN-α/β possess greater antiviral activity than IFN-λs. Similarly, 100 ng of IFN-λ1, -λ2 or -λ3 along with 10 ng of another IFN-λ could not improve the antiviral activity

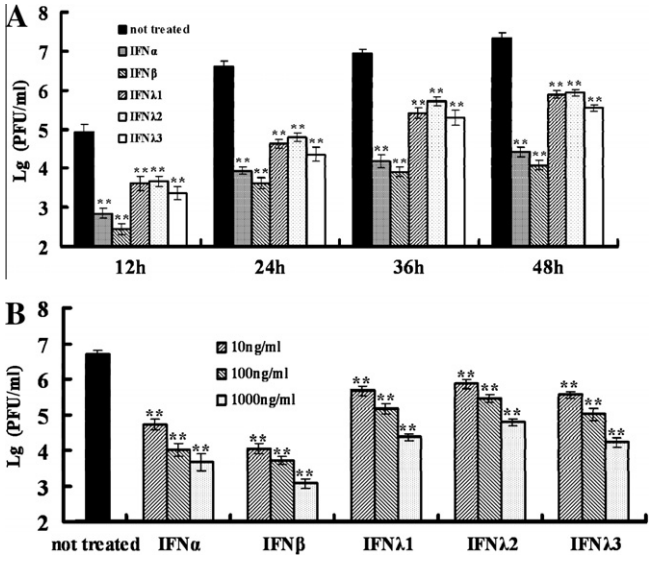

Fig. 1. Type I and type III IFNs have antiviral activity against PRRSV and such inhibition is dose-dependent. (A) MARC-145 cells were treated with the indicated various IFNs, respectively, at 100 ng/ml 12 h before PRRSV infection. The cells were then infected with PRRSV at a MOI of 0.1 for 1–2 h, washed and replenished with fresh medium containing the indicated IFN. Infected cells were frozen 12, 24, 36 and 48 h after infection and titrated. (B) MARC-145 cells were treated with different doses of IFNs 12 h before infection with PRRSV at a MOI of 0.1. MARC-145 cells were treated with DMEM before infection with 0.1 MOI PRRSV as control (not treated group). All infected cells were frozen 36 h after infection and titrated. Values are mean ± SEM of three independent tests. $^{**}P < 0.01$ compared with virus control (not treated group).

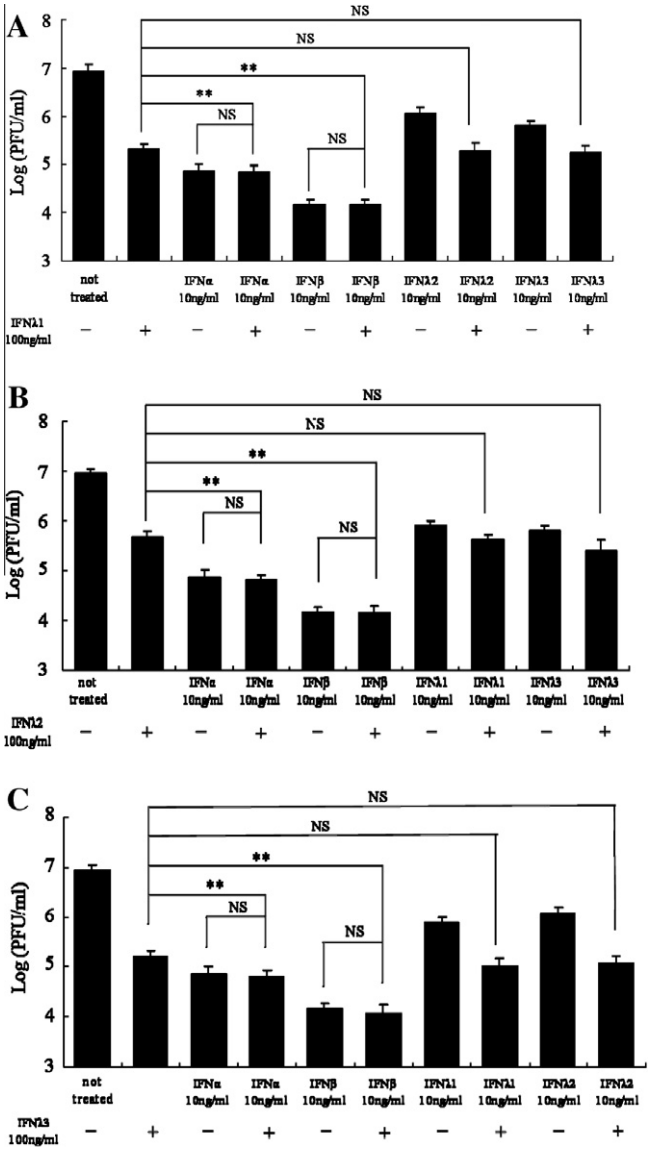

Fig. 2. Co-treatment with type I and III IFNs could not enhance the antiviral activity. MARC-145 cells were treated with a combination of type I and type III IFNs 12 h before infection with PRRSV at a MOI of 0.1. Infected cells were frozen 36 h after infection and titrated. Viral titer of cells treated with 100 ng/ml of IFN-λ1 alone or mixed with 10 ng/ml of IFN-α, -β, -λ2 or -λ3, respectively (A), with 100 ng/ml of IFN-λ2 alone or mixed with 10 ng/ml of IFN-α, -β, -λ1 or -λ3, respectively (B), with 100 ng/ml of IFN-λ3 alone or mixed with 10 ng/ml of IFN-α, -β, -λ1 or -λ2, respectively (C). Values are mean ± SD of three independent tests. $^{**}P < 0.01$ compared with the group treated with 100 ng/ml of IFN-λ1 (A), -λ2 (B) or -λ3 (C) alone. "NS" means no statistically significant ($P > 0.05$).

against PRRSV compared with 100 ng IFN-λ1, -λ2 or -λ3 alone. These results demonstrated that there was no additive effect when two types of IFNs were used together.

We next assessed whether the different antiviral efficacy of two types of IFNs was caused by the levels of ISGs expression induced by IFNs. To this end, MARC-145 cells were stimulated with various IFNs and lysed for RNA extraction and quantitative PCR 24 h later. As seen in Fig. 3, all tested IFNs induced a significant mRNA increase of antiviral genes ISG56, 2′5′OAS and MxA, which have well-known antiviral properties and may affect PRRSV replication. Although type I IFNs were more efficient at inhibiting PRRSV multiplication than type III IFNs, type I IFNs had not exhibited higher ability in inducing 2′5′OAS and MxA expression than type III IFNs (Fig. 3B and C), suggesting that some additional effector ISGs

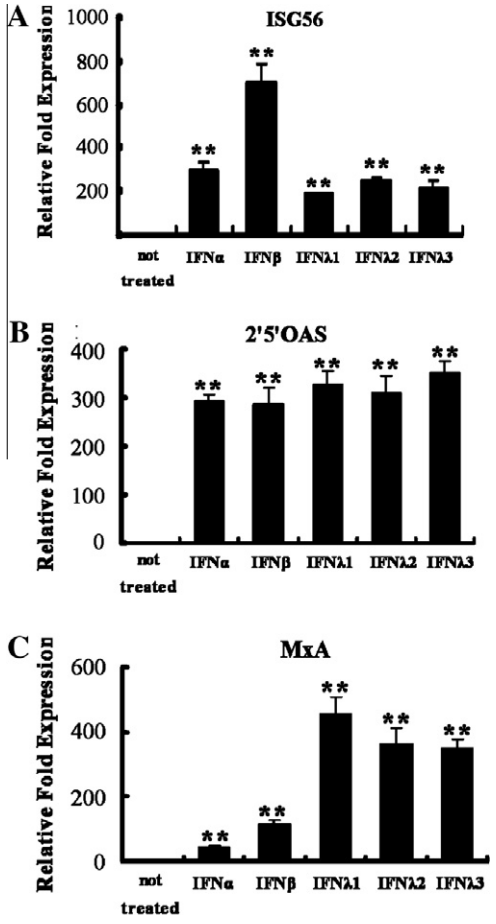

Fig. 3. ISG56, 2′5′OAS and MxA genes are induced after IFN treatment. MARC-145 cells were treated with IFNs at 100 ng/ml and total cellular RNA was extracted 24 h later. The levels of ISG56 (A), 2′5′OAS (B) and MxA (C) mRNAs were measured by quantitative PCR, and the results were normalized by the GAPDH levels of each sample. Values are mean ± SEM of three independent tests. $^{**}P < 0.01$ compared with untreated MARC-145 cells (not treated group).

against PRRSV specifically induced by IFNs should be responsible for the difference of antiviral activity between type I and III IFNs, although the special effector ISGs that mediate the antiviral effect against PRRSV have not been elucidated. Further studies should be performed to identify the effector ISGs against PRRSV and to determine its antiviral effects *in vivo* by animal experiments.

Collectively, our data demonstrated that IFN-λ, as well as IFN-α and IFN-β, has the ability to suppress PRRSV replication in MARC-145 cells and such inhibition is dose-dependent, although IFN-λs were less efficient than IFN-α and IFN-β in antiviral effect. Co-treatment of cells with both type I and III IFN could not enhance the antiviral efficacy of each type alone, and all tested IFNs could induce the expression of ISG56, 2′5′OAS and MxA. Additionally, the signaling pathway and the anti-PRRSV mechanism of two types IFNs remain to be fully elucidated. These studies pave the foundation to utilize type III IFNs as useful antiviral agents against infectious swine diseases.

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