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Treatment with interferon-alpha delays disease in swine infected with a highly virulent CSFV strain

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ABSTRACT

Interferon-alpha (IFNα) can effectively inhibit or abort a viral infection within the host. It has been reported that IFN induction and production is hindered during classical swine fever virus (CSFV) infection. Most of those studies have been performed *in vitro*, making it difficult to elucidate the actual role of IFNs during CSFV infection in swine. Here, we report the effect of IFNα treatment (delivered by a replication defective recombinant human adenovirus type 5, Ad5) in swine experimentally infected with highly virulent CSFV strain Brescia. Treatment with two different subtypes of IFNα delayed the appearance of CSF-related clinical signs and virus replication although it did not prevent lethal disease. This is the first report describing the effect of IFNα treatment during CSFV infection in swine.

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Introduction

Classical swine fever (CSF) is an economically significant, highly contagious swine disease. The etiological agent, CSF virus (CSFV), is an enveloped virus with a positive-sense, single-stranded RNA genome, classified as a member of the genus *Pestivirus* within the family *Flaviviridae* (Becher et al., 2003). The 12.5 kb CSFV genome contains a single open reading frame that encodes a 3898-amino acid polyprotein and ultimately yields 11–12 final cleavage products (NH_2-N^{pro}-C-E^{rns}-E1-E2-p7-NS2-NS3-NS4A-NS4B-NS5A-NS5B-COOH) through co- and post-translational processing of the polyprotein by cellular and viral proteases (Leifer et al., 2013).

Prophylactic vaccination with live attenuated viruses (LAVs) can induce effective protection against CSF, often earlier than protection afforded by the adaptive immune response (van Oirschot, 2003); however, the host mechanisms mediating this innate response remain unknown.

The ability of the host's innate immune system to interact with CSFV replication has been studied *in vitro* by several groups (Bensaude et al., 2004; Choi et al., 2004; Dong et al., 2013; Fernandez-Sainz et al., 2010), and included evaluation of the inhibitory effects induced by Mx1 (He et al., 2014) and MxA proteins (Zhao et al., 2011) as well as by IFN-α and IFN-γ (Graham et al., 2012; Xia et al., 2005). It has been reported that CSFV possesses mechanisms that hinder the induction and production of IFNs during infection. This inhibitory effect has been associated with two viral proteins, N^{pro} and E^{rns}. For instance, the dsRNA-binding and RNase activities of pestivirus E^{rns} has been shown to inhibit IFN synthesis induced by extracellular dsRNA (Magkouras et al., 2008) and/or pestiviral single- and double-stranded RNAs (Iqbal et al., 2004; Luo et al., 2009; Mätzener et al., 2009). In addition, IFN regulatory factor 7 (IRF7) is deregulated by directly interacting with CSFV Npro, thus inhibiting the production of IFNα and decreasing the anti-viral cellular response (Fiebach et al., 2011). Also, N^{pro} promotes proteosomal degradation of IFN regulatory factor 3 (IRF3) (Bauhofer et al., 2007; La Rocca et al., 2005; Seago et al., 2007) and recombinant CSFV lacking N^{pro} is attenuated in swine (Mayer et al., 2004). However, replacement of N^{pro} in a virulent CSFV strain by the corresponding gene of an attenuated strain does not diminish the virulent phenotype (Mayer et al., 2004). Moreover, recombinant viruses, developed from a highly virulent parental virus, harboring N^{pro} mutations abrogating the ability to degrade IRF3 and thus preventing IFN-α/β induction were not attenuated in swine (Ruggli et al., 2009).

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Therefore, the actual role of IFN during CSFV infection in swine is poorly understood.

Here, we report the effect of IFNα treatment (delivered by a replication defective human adenovirus type 5, Ad5) on the course of experimental infection of swine infected with the highly virulent CSFV Brescia strain. Our results show that IFNα treatment significantly delays the appearance of CSF disease and systemic virus replication. To our knowledge, this is the first report describing the effect of IFN-α during CSFV infection in swine.

Results

Anti-CSFV activity of swine IFNα

Similar to other mammals, the swine genome encodes for several IFN genes (Cheng et al., 2006; Sang et al., 2010). The antiviral effect of 18 swine IFNα genes was assessed in CSFV-infected cultures. IBRS2 cells were transfected with the same amount of individual plasmids representing each of the 18 swine IFNα genes described in *Material and methods*. IFNs produced in the supernatant of the transfected cell cultures were harvested and assessed for their anti-CSFV activity in primary cell cultures of fetal porcine kidney (EPK). A wide range, from 3 to 10 $\log_n$, of anti-viral activity was observed depending on the subtype of IFNα tested (Fig. 1). The subtype IFNα-D2 exhibited the greatest activity ($\sim$9 $\log_2$ U/ml) and therefore it was selected to be cloned in the recombinant replication defective Ad5 vector for further studies.

Development of a recombinant Ad5 expressing swine IFNα

It had been reported that an Ad5 expressing pIFNα (Ad5-pIFNα-MG) effectively controls foot- and-mouth disease (FMD) in swine (Chinsangaram et al., 2003; Moraes et al., 2001, 2003). The clone IFNα-D2 was therefore selected and expressed using the same Ad5 vector (Ad5-Blue). Recombinant Ad5-pIFNα-MG and Ad5-pIFNα-D2 were used to infect IBRS2 cells and supernatants containing secreted proteins were analyzed for the presence of IFN protein and antiviral activity. Quantification was performed using an in-house developed ELISA as previously described (Moraes et al., 2003). While IFNα-MG was produced at a concentration of 6.59 μg/ml (SD=0.205), IFNα-D2 was produced at 4.45 μg/ml (SD=0.262) (Fig. 2A). These preparations consistently exhibited similar antiviral activity against CSFV (7.09 $\log_2$ IU/ml) and VSV (11.54 $\log_2$ IU/ml) (data not shown). Western blot analysis suggested that IFNα-MG had a significantly higher MW compared to IFNα-D2; however, treatment of the secreted proteins with a mix of deglycosylases (PDM) indicated that the heterogeneity in MW was mainly due to glycosylation. While IFNα–MG displayed a significant shift in its electrophoretic mobility, IFNα-D2 appeared unchanged after PDM treatment (Fig. 2B).

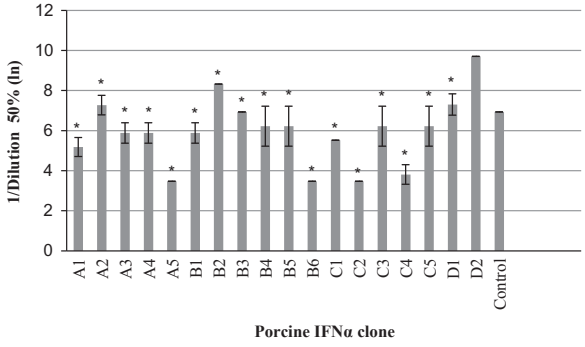

Fig. 1. Anti-CSFV activity of different swine IFNα genes transiently expressed in IBRS2 cells. Activity is detected in EPK cells pretreated with the different IFNα preparations and infected with CSFV Brescia strain (BICv). Control is an Ad5-pIFNα–MG derived IFN-α preparation. Asterisk on the top the bar indicates statistical significance compared with preparation D2 (*T*-test $p < 0.01$).

Effect of Ad5-pIFNα on the course of disease in animals experimentally infected with virulent CSFV Brescia strain.

The possible protective role of swine IFNα on the course of infection was assessed in susceptible animals treated with recombinant Ad5 viruses expressing each of the IFNα, and experimentally infected with virulent CSFV Brescia. Three groups of 30–40 pound pigs were SC inoculated, one day prior to CSFV infection, with 10^{10} pfu of either Ad5-pIFNα-MG or Ad5-pIFNα-D2, while a third group received the same dose of the empty vector, Ad5-Blue, as a control. Twenty-four hours later animals were intranasally infected with 10^5 $TCID_{50}$ of CSFV Brescia. Animals were monitored daily for increases in body temperature and the appearance of CSF-related clinical signs.

Detection and characterization of IFNα in serum of inoculated animals was determined by three methodologies: the IFN-dependent CAT assay in cells harboring the MxCAT reporter construct (Fray et al. 2001), in-house sandwich ELISA (Moraes et al., 2003), and by quantifying the anti-viral activity in cell cultures infected with VSV (Rubinstein et al. 1981).

Results, detected by the MxCAT assay (Fig. 3A), demonstrated that by 24 h postinoculation all animals treated with Ad5-pIFNα had significant levels of IFN activity in sera. Swine treated with Ad5-pIFNα-D2 displayed an average of 393.06 ± 52.72 IU/ml while animals treated with Ad5-pIFNα-MG had significantly higher levels, 674.34 ± 103.44 IU/ml. As expected, control animals inoculated with Ad5-Blue did not develop an IFN response one day after treatment.

The differences in IFN levels observed using the MxCAT ELISA at the time of CSFV challenge (0 dpc) in animals receiving Ad5-pIFNα-D2 or Ad5-pIFNα-MG were not detected when IFN levels were assayed with either anti-VSV replication or a quantitative IFN ELISA. Anti-VSV activity at 1 dpi in sera of animals inoculated with Ad5-pIFNα-D2 (average=5.94 $\log_2$ IU/ml; SD=0.35) did not significantly differ from those inoculated with Ad5-pIFNα-MG (average=5.77 $\log_2$ IU/ml; SD=0) (Fig. 3B). Similarly, levels of IFNα protein in

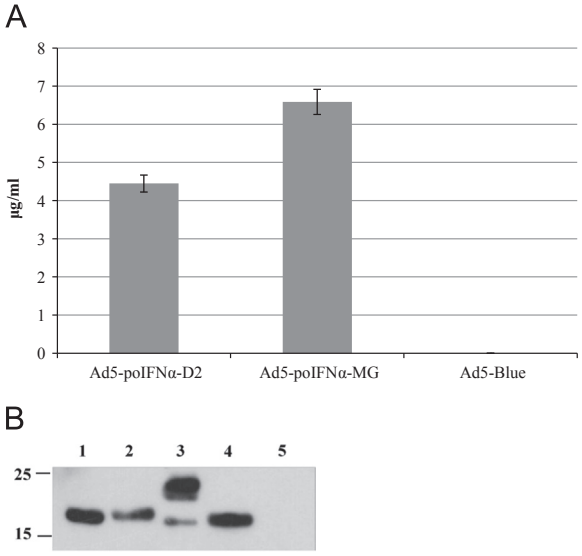

Fig. 2. Expression of swine IFNα in recombinant Ad5-pIFNα. Analysis by (A) quantitative ELISA and (B) Western blot of untreated (lanes 1 and 3) and PNGase treated (lanes 2 and 4) Ad5-pIFNα–D2 (lanes 1 and 2) and Ad5-pIFNα–MG (lanes 3 and 4) derived semi-purified IFNα preparations. Lane 5 contains Ad5-Blue.

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