Xth International Congress for Veterinary Virology

9th Annual Meeting of EPIZONE

Changing Viruses in a Changing World

August 31st - September 3rd 2015

Le Corum, Montpellier, France
**Conclusion:** The approach described in our work is a contribution to the development of an alternative and universal vaccine against influenza virus omitting time-consuming and cost-intensive immunogen purification process and use of adjuvants.

**Antiviral activity of type I, II and III porcine interferons against classical swine fever virus**

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Classical swine fever (CSF), caused by classical swine fever virus (CSFV), substantially impacts swine production industries worldwide. An important consideration for CSF control strategies in free areas is the ability to demonstrate freedom from disease serologically, thus allowing rapid resumption of trade after disease outbreaks. Biotherapeutics that stimulate the host’s immune response to restrict infection, but without impeding serological detection of disease, represent a novel disease control option. Interferons (IFN) are integral components of the host’s innate defense against viral infections and are able to protect against virus infections. Three types of IFN have been described which vary in their specific mode of action and target cell populations. Type I interferons (eg IFNα, β) induce a protective antiviral state in diverse cell types, whilst type II interferon (IFNλ) stimulates cell mediated responses and amplifies the effects of IFNα, and β. Type III IFNs (IFNλ) demonstrate antiviral properties on a variety of cells, although the distribution of the IFN receptor (IFNLR) is mostly limited to cells of epithelial origin. Porcine IFNλs may thus provide a useful protection against CSFV at the primary sites of infection.

**Objective:** To assess the antiviral activity of type III, as well as type I and II, porcine interferons, when expressed as both recombinant proteins and via an adenoviral delivery vector, against CSFV.

**Methods:** Porcine cell lines were screened for expression of the interferon λ receptor, IFNLR, by RT-PCR. Genes encoding porcine IFNs (PoIFN) (type I (α12, β), type II (γ) and type III (λ1, 3)) were cloned into the pcDNA3.1 V5-HisB mammalian expression vector and the proteins expressed by transfection into newborn porcine tracheal (NpTr) cells. The antiviral activity of the recombinant proteins, within the transfected cell supernatants, was assessed by treatment of NpTr cells prior to infection with vesicular stomatitis virus (VSV) or CSFV. Antiviral activity, compared to treatment with mock transfected cell supernatants, was assessed in a CPE reduction assay (VSV) or by assessing the percentage of cells expressing the E2 viral envelope protein (CSFV) by flow cytometry. The five poIFN genes were subsequently cloned into an adenovirus delivery/expression vector. The anti-VSV and CSFV activity was similarly assessed after inoculation of NpTr cells with the IFN expressing adenovirus constructs, or control constructs expressing GFP alone.

**Results:** Screening of a range of porcine cell lines identified that NpTr cells express the IFNLR. All of the recombinant porcine interferons had antiviral activity against VSV in this cell line. At the concentrations tested, recombinant PoIFN λ1, as well as poIFN α12 and β, significantly reduced the percentage of NpTR cells from subsequent infection with CSFV. When delivered via the adenovirus vectors all five porcine interferons significantly protected NpTR cells against CSFV infection compared to controls inoculated with adenovirus vectors expressing GFP alone.

**Conclusion:** Both interferon λ1 and λ3, as well as type I (IFNα12, β) and type II (IFNγ) interferon have antiviral activity against CSFV in-vitro. The use of adenovirus vectors to deliver interferons to pigs has potential as an intervention to assist in control of CSFV.

**RNA interference as antiviral therapy against Peste des Petits Ruminants: proof of concept of in vivo efficiency using a small animal model**

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Objective: Morbilliviruses are responsible of important diseases in human beings and animals with economical impact in affected countries. “Peste des petits ruminants” is one of these diseases affecting goats and sheep with high mortality and morbidity. Efficient vaccines exist but they are often used in emergency situation in animals and ten days are necessary to induce a sufficient immune protection.

Co-administration of an antiviral treatment with the vaccine could limit the disease impact while conferring a long-lasting protection. CIRAD has explored a biological antiviral therapy based on RNA interference. The identification of siRNA against morbilliviruses has been previously published and patented. The objective of this work was to validate the in vivo efficiency of these siRNAs.

Methods: The proof of concept for an efficient in vivo delivery of anti-PPRV siRNAs was developed in a mouse model. Briefly, it is based on the siRNA dynamic extinction of a luciferase reporter gene in mice measured by bioimaging. The originality is also based on the use of a double-reporter expression plasmid allowing standardization within and between the trials. The plasmid is made of a firefly gene placed downstream of one of our morbillivirus siRNA target sequence and a renilla gene used as a constant gene-expression system. In the initial phase, mice received a co-injection (double reporter plasmid + relevant or irrelevant siRNA-PPRV) in the tibialis muscle, followed by an electroporation to promote cellular uptake of DNA. The firefly and renilla signals were measured daily using a bio-imager. The firefly expression was normalized using renilla signal. The specificity of RNA interference was checked by comparison with an unrelated siRNA. Once this initial phase validated, a second phase consisted in testing a delivery system for siRNA based on a cell membrane penetrating peptide.

Results: The model was validated. In absence of any siRNA treatment, a good correlation was observed between the firefly and renilla luminescence activities. When the irrelevant siRNA was co-administrated, no incidence on these activities was detected. In contrast, mice treated with siRNA-PPRV showed a strong inhibition of about 99% of the firefly signal. This mouse model system is a proof of concept of in vivo siRNA efficiency and a very useful tool to assess in vivo siRNA delivery systems. Several candidates for in vivo delivery systems were investigated in our laboratory. Preliminary results showed that a cell membrane penetrating peptide could efficiently deliver a siRNA and inhibit the expression of firefly when tested in this mouse model.

Conclusion: This mouse model system is a very useful tool that can be applied to test siRNA delivery systems in vivo. In this model, a cell penetrating peptide showed encouraging performances for systemic delivery of siRNA but extensive confirmation will be necessary. The model is now available for the screening of alternative delivery systems, including viral expression vectors that might represent a better cost-effective strategy for small ruminant’s treatments in emerging countries.

Use of in silico prediction models to predict T-cell epitopes for the development of vaccines against African swine fever virus

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Objective: African swine fever virus (ASFV) causes a haemorrhagic and often lethal viral disease in pigs. With the exception of Sardinia, Europe was free of ASFV since the disease was eradicated from the Iberian Peninsula in 1995. In 2007, ASFV was introduced in Georgia, and spread from there to East-European countries where it has become endemic in several regions. The control of ASFV outbreaks in commercial pigs has been accomplished thus far by stamping out-methods. In regions where ASFV is endemic in wildlife, the use of a vaccine could make an important contribution to control of this disease. There have been several attempts to develop a vaccine for ASF, but until now this has not resulted in an efficient and safe vaccine. Studies have shown that effi-