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Cytokine and immunoglobulin isotype profiles during CP7_E2alf vaccination against a challenge with the highly virulent Koslov strain of classical swine fever virus

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ABSTRACT

CP7_E2alf is a promising marker vaccine candidate against classical swine fever (CSF). To better understand the mechanisms of protection, cytokine and isotype-specific antibody profiles were investigated in CP7_E2alf vaccinated pigs before and after challenge with the highly virulent CSFV strain "Koslov" at 14 days or 6 months post-vaccination. The interference of vaccination with CSFV pathogeny-related cytokine responses, previously described following a moderately virulent challenge, was confirmed. However, the levels of additional cytokines, TNF-α and IL-6, were significantly attenuated by vaccination following highly virulent challenge. This vaccine interference with cytokine response was not dependent on the immunization route or the consequence of competition between vaccine and challenge strain. Interestingly, IFN-γ enhancement and persistent high IgG2 levels suggested an important role of cell-mediated immunity in long-term protection against CSFV induced by CP7_E2alf vaccination. IgA production also revealed a stimulation of mucosal immunity, especially after oral administration of the vaccine.

1. Introduction

Classical swine fever (CSF) is a highly contagious viral disease in swine. Highly virulent CSFV strains can lead to acute haemorrhages, severe immune suppression and high mortality. CSFV is a small enveloped *Pestivirus* in the *Flaviviridae* family. Its RNA genome encodes 12 proteins, including the E2 glycoprotein, which is the most immunogenic one (Wensvoort et al., 1990).

The CP7_E2alf vaccine, a live chimera of Bovine viral diarrhoea virus (BVDV) expressing the CSFV E2 protein shows a strong potential to control CSF with the possibility to differentiate infected from vaccinated animals (Reimann et al., 2004). The CP7_E2alf vaccine is also the first efficient and safe marker vaccine candidate for oral immunization of wild boar against CSFV (Koenig et al., 2007). The CP7_E2alf marker vaccine conferred full clinical protection against a challenge with the highly virulent Koslov CSFV strain 7 days after intramuscular or 14 days after oral immunization (Leifer et al., 2009). Complete protection against a Koslov challenge was also demonstrated 21 days after oral CP7_E2alf vaccination (Blome et al., 2012). Moreover, the CP7_E2alf vaccine induces long-term immunity against a Koslov challenge, for at least 6 months after intramuscular or oral administration (Gabriel et al., 2012).

Protection from the disease is commonly associated with a humoral (Th2) immune response with the production of CSFV-specific antibodies or neutralizing antibodies (NAb) that usually appear about 2 weeks post-vaccination (pv) (van Oirschot, 2003). The duration of immunity induced by the CP7_E2alf vaccine is also attributed to stable antibody titres (Gabriel et al., 2012). Information about isotype differentiation is currently lacking for the early or late systemic antibody response (IgM/IgG differentiation), the balance of adaptive immunity toward Th1 or Th2 response (IgG1/IgG2 differentiation) or mucosal immunity (IgA detection). Partial investigation of IgG sub-class differentiation revealed that CSFV-specific IgG2 levels induced by early challenge with the moderately virulent CSFV strain were higher in CP7_E2alf orally vaccinated pigs than in unvaccinated pigs (Renson et al., 2013). No data is as yet available for IgA or IgM.

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A previous study involving early challenge with a moderately virulent strain of CSFV at 2 days pv had revealed that oral CP7_E2alf vaccination interfered with pathogeny-related cytokine responses, e.g. attenuated the increase of IFN-α and IL-12 levels or the reduction of TGF-β1 level in serum (Renson et al., 2013). In this study, cytokine responses that might be influenced by CSFV infection were further investigated and immunoglobulin (Ig) G isotype profiles were completed with those for IgM and IgA, on samples obtained from different experiments, in which challenges with the highly virulent Koslov strain of CSFV had been applied later i.e., at 14 days or 6 months post-vaccination. The immune profiles obtained after oral or intramuscular immunizations were also compared. This study not only confirmed our initial results but also provided new elements about the responses induced by CP7_E2alf vaccination during the onset and duration of immunity.

2. Materials and methods

2.1. Samples origin

2.1.1. OOI (onset of immunity) study – challenge at 14 days post-vaccination (dpv)

Serum samples, collected at 0, 4, 7, 10, 14 and 21 days post-infection or post-challenge (dpi) were kindly provided by NFCSO-DVMP from their efficacy study (unpublished study). Samples were obtained from five 6-week-old Kahyd pigs orally vaccinated with a single dose of the CP7_E2alf vaccine solution (1.6 ml at $10^{5.6}$ $TCID_{50}$/ml) then oronasally challenged at 14 dpv with the highly virulent Koslov strain (2 ml at $10^{5.5}$ $TCID_{50}$/ml). Three sera from an unvaccinated positive control group were also provided.

2.1.2. DOI (duration of immunity) study – challenge at 6 months post-vaccination (mpv)

Serum samples collected at 0, 4, 7, 10, 14 and 21 dpi were kindly provided by FLI from their duration of immunity study (Gabriel et al., 2012). Samples were obtained from two groups of five 8-week-old Landrace × Pietrain pigs vaccinated either orally or intramuscularly (i.m.) with a single dose of the CP7_E2alf vaccine solution (1.6 ml at $10^{5.5}$ $TCID_{50}$/ml for oral vaccination or 1 ml at 10^{4} $TCID_{50}$/ml for i.m. vaccination) and then oronasally challenged 6 months later with the Koslov strain (2 ml at $10^{5.5}$ $TCID_{50}$/ml). Six sera from an unvaccinated positive control group were also provided. Samples were collected at 6 dpi from some pigs in the control group, before these animals were compassionately euthanized.

2.2. Cytokine quantification

Porcine cytokines in serum were measured using ready-to-use ELISA kits from different manufacturers: TNF-α, IL-8, IL-10 and IFN-γ (Life Technologies, Carlsbad, USA), IL-6, IL-12 and TGF-β1 (R&D systems, Minneapolis, USA) and carried out according to the accompanying manual. All these kits had been validated for serum as well as cell culture supernatant by the manufacturer. Porcine IFN-α was detected by an in-house ELISA test as previously described (Jamin et al., 2006).

2.3. Ig isotypes assessment

CSFV-specific IgG1, IgG2, IgM or IgA were measured in serum by indirect ELISA. IgG1 and IgG2 subclasses were detected as previously described (Renson et al., 2013). For IgM and IgA subclasses assessment, the protocol was adapted using anti-porcine IgM or anti-porcine IgA (AbD Serotec, Oxford, UK) as primary antibodies at the dilution of 1:100 or 1:250 respectively, for 1 h at room temperature. The reproducibility of the assay was checked by including positive and negative controls on each plate.

2.4. Statistical test

The variation measured between groups was analyzed by Mann–Whitney test using Systat 9 software (Systat Software Inc., Point Richmond, USA), with a limit of significance of $p < 0.05$.

3. Results

3.1. Effect of CP7_E2alf vaccine on cytokine responses induced after Koslov CSFV strain infection

In the OOI study, a challenge with the highly virulent Koslov strain at 14 dpv led to an increase in the serum levels of IFN-α, TNF-α, IL-6 and IL-12 in the unvaccinated controls (Fig. 1b–e) whereas the level of TGF-β1 was reduced at 7 and 10 dpi in the last surviving pig (Fig. 1a). Prior to the challenge, no significant differences were observed in the levels of any cytokine between vaccinated pigs and unvaccinated pigs.

After challenge, the CP7_E2alf vaccination prevented the reduction of TGF-β1 levels, or delayed it to 21 days pi for pig No. 29. IFN-α production at 4 dpi was reduced in vaccinated pigs despite the fact that two pigs (Nos. 36 and 40) were shown to be non-responders (Fig. 1b). More interestingly, none of the vaccinated pigs showed the increased levels of IL-12, TNF-α or IL-6 which were detected in unvaccinated pigs at 4 dpi (Fig. 1c–e). One vaccinated pig, as well as one unvaccinated pig, displayed high IL-8 levels at 4 days pi (1205 and 914 pg/ml respectively) (Fig. 1f). The cytokine IL-10 was only detected in two unvaccinated animals at 4 or 7 days pi (16 and 11 pg/ml respectively) (Fig. 1g). IFN-γ was never detected in serum from any pig during this experiment (Fig. 1h). IL-4 was never detected in serum from the OOI study (data not shown).

Values for TNF-α, IL-6 and IL-12 measured in the unvaccinated group from the DOI study at 4 days pi were 10–15 times lower than those measured in the OOI study. The highest values were apparent at 6 days pi but were still much lower than in the OOI study (highest levels for TNF-α: 345 and 1703 pg/ml, IL-6: 93 pg/ml and 641 pg/ml; IL-12: 3772 pg/ml and 12928 pg/ml, in the DOI and OOI studies respectively) (Fig. 2). At 4 days post-challenge, no decrease of TGF-β1 levels post-challenge was found in vaccinated pigs, as in the OOI study (Fig. 2a). IFN-α levels were significantly attenuated by CP7_E2alf vaccination, both by oral and i.m. routes ($p = 0.006$ and $p = 0.018$ respectively) (Fig. 2b). An obvious stimulation of IL-12 levels by CSFV infection was only observed in one unvaccinated animal (No. 8) (Fig. 2c). An increase in TNF-α and IL-6 levels was prevented by vaccination (Fig. 2d and e). No significant difference in the ability of vaccination routes to influence the levels of TGF-β1, IFN-α, IL-12, TNF-α and IL-6 was apparent. Oral vaccination, but not i.m. vaccination, significantly increased the IL-8 levels at 4 days pi compared to infection alone ($p = 0.009$ and $p = 0.602$ respectively) (Fig. 2f). As already observed in the OOI study, the IL-10 levels measured in unvaccinated pigs were low (around 15 pg/ml) and were undetectable in orally vaccinated pigs except for one pig (No. 11), which showed high levels (from 24 to 229 pg/ml) throughout the kinetic experiment (Fig. 2g). Similarly, the IL-10 protein was only detected in two i.m. vaccinated pigs, (Nos. 3 and 12) which exhibited low IL-10 levels. High levels of IFN-γ were only detected in purified sera from two orally vaccinated pigs (Nos. 5 and 11) (Fig. 2h). Pig No 5 had already displayed high levels of IFN-γ (826 pg/ml) before the challenge, at 6 mpv. Only very low levels of IL-4 were detected in serum from the last surviving unvaccinated pig (No. 29) at 14 dpi (3 pg/ml; data not shown).