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Comparison of immune responses in parenteral FaeG DNA primed pigs boosted orally with F4 protein or reimmunized with the DNA vaccine

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Abstract

We previously showed that an intradermal (i.d.) FaeG DNA prime (2×)-oral F4 protein boost immunization induces a systemic response and weakly primes a mucosal IgG response in pigs, especially when plasmid vectors encoding the A and B subunit of the *E. coli* thermo-labile enterotoxin (LT) are added to the DNA vaccine. In the present study, we evaluated whether addition of 1α,25-dihydroxyvitamin D_3 (vitD_3) to the DNA vaccine could further enhance this mucosal priming and/or modulate the antibody response towards IgA. To further clarify priming of systemic and mucosal responses by the i.d. DNA vaccination, we firstly compared the localization of the F4-specific antibody response in pigs that were orally boosted with F4 to that in pigs that received a third i.d. DNA immunization and secondly evaluated cytokine mRNA expression profiles after i.d. DNA vaccination. The i.d. DNA prime (2×)-oral F4 boost immunization as well as the 3 i.d. DNA vaccinations induced mainly a systemic response, with a higher response observed following the heterologous protocol. Co-administration of vitD_3, and especially of the LT vectors, enhanced this response. Furthermore, only the heterologous immunization resulted in a weak mucosal priming, which appeared to require the presence of the LT vectors or vitD_3 as adjuvants. In addition, the LT vectors strongly enhanced the FaeG-specific lymphocyte proliferation and this was accompanied by the absence of a clear IL-10 response. However, despite two DNA immunizations in the presence of these adjuvants and an oral F4 boost, we failed to demonstrate the secretory IgA response needed to be protective against enterotoxigenic *E. coli*.

Keywords: Pig; DNA vaccination; Mucosal IgG

1. Introduction

Enterotoxigenic *E. coli* (ETEC) that express F4 (K88) fimbriae are an important cause of diarrhea in recently weaned piglets. The F4 fimbriae are long proteinaceous appendages mainly composed of several hundreds identical FaeG subunits. They enable the bacteria to adhere to F4-specific receptors (F4R) on the intestinal epithelium and subsequently to colonize the small intestine (Nagy et al., 1985). Presence of the F4R is genetically determined and F4R negative ($F4R^-$) pigs are resistant to an F4 positive ($F4^+$) ETEC infection (Rutter et al., 1975). It was previously shown that oral immunization of $F4R^+$ weaned pigs with F4 fimbriae resulted in an antigen-specific secretory IgA (sIgA) response at the intestinal mucosa, protecting these pigs against a subsequent $F4^+$ ETEC challenge (Van den Broeck et al., 1999a). However, to prevent post-weaning

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diarrhea, an F4-specific intestinal mucosal immune response should already be primed during the suckling period, often in the presence of F4-specific maternal antibodies. These lactogenic antibodies will hamper the use of F4 as a vaccine. Several studies have shown that DNA vaccines, in contrast to conventional vaccines, can successfully prime immune responses in the presence of maternal antibodies (Hassett et al., 1997; Fischer et al., 2003; Van Loock et al., 2004). Therefore, we hypothesized that priming with an FaeG DNA vaccine during the suckling period combined with an oral F4 protein boost immediately after weaning could be an interesting approach. However, in a previous study a parenteral DNA prime-oral F4 boost induced a good systemic response, but was weak in priming mucosal immunity, failing to completely prevent $F4^+$ *E. coli* colonization. Furthermore, addition of plasmid vectors encoding the LTA and LTB subunits to the FaeG DNA vaccine enhanced the IgG response, but did not result in the sIgA response that is desired to completely prevent an $F4^+$ ETEC infection. Nevertheless, a significant reduction was obtained in the amount of $F4^+$ ETEC excreted as well as in the duration of faecal $F4^+$ ETEC excretion (Melkebeek et al., 2007). These data suggest that the systemic DNA immunization is weak in priming mucosal responses. Previous studies showed that a systemic immunization could induce an intestinal mucosal IgA response in mice (Enioutina et al., 1999, 2000) and in pigs (Van der Stede et al., 2001, 2004) if 1α,25-dihydroxyvitamin D_3 ($vitD_3$) was used as adjuvant.

Therefore, we examined in the present study whether addition of $vitD_3$ could enhance the mucosal priming by the FaeG DNA vaccination and/or whether it could modulate the antibody response towards IgA in the i.d. DNA prime-oral F4 boost immunization. Furthermore, to gain insight in the way systemic and mucosal responses are primed by the i.d. DNA vaccination, we compared the localization of the F4-specific antibody response in pigs that were orally boosted with F4 to that in pigs that were reimmunized i.d. with DNA and we evaluated cytokine mRNA expression profiles in the local draining lymph nodes after the i.d. DNA priming.

2. Materials and methods

2.1. Animals

Forty-nine conventionally bred pigs (Belgian Landrace x Piétrain), seronegative for antibodies against F4 as determined by ELISA, were weaned at the age of 4 weeks. Subsequently, they were housed in isolation units. From 1 day before weaning, all animals were orally treated with colistine (150,000 U/kg body weight; Colivet, Prodivet Pharmaceuticals, Eynatten, Belgium) during 5 successive days to prevent *E. coli* infections during the weaning period.

2.2. Isolation of F4 fimbriae

F4 fimbriae were isolated from the ETEC strains GIS26, serotype 0149:K91:F4ac, $LT^+STa^+STb^+$, and IMM01, serotype 0147:F4ac, LT^+STb^+ as described by Van den Broeck et al. (1999a). The protein concentrations of the fimbrial solutions were determined by the bicinchoninic acid reaction with bovine serum albumin as a standard (ICN Biomedicals, Belgium) and the purity was assessed using a Coomassie stained 15% SDS-PAGE and the Image Master 1D prime software (Amersham Pharmacia Biotech, Belgium). GIS26 F4 fimbriae were used for the oral immunization of pigs. The IMM01 strain carries F4 fimbriae with an FaeG sequence identical to the GIS26 strain, but fimbriae isolated from this strain have a higher purity, containing no flagellin (Verdonck et al., 2004). Therefore, IMM01 fimbriae were used for the FaeG-specific ELISA and ELIspot assays and, after sterilization by filtration through a 0.2 μm filter, in an FaeG-specific lymphocyte proliferation assay.

2.3. Plasmids and 1α,25(OH)$_2$D$_3$

The pcDNA3-rpGM-CSF plasmid consists of the cDNA encoding the porcine GM-CSF cloned in the pcDNA3.1zeo expression vector.

The pWRGFaeGopt vaccine was constructed as previously described (Melkebeek et al., 2007). This construct consists of a codon optimized *faeG* cloned into the pWRG7079 vector behind a tPA signal sequence, allowing the extracellular secretion of the encoded FaeG. The pJV2004 and pJV2005 plasmids consist of the pWRG7054 vector encoding the A and B subunit of the thermolabile enterotoxin of *E. coli* (LT), respectively, behind a tPA signal sequence to allow their extracellular secretion (Arrington et al., 2002). All plasmids were propagated in *E. coli* DH5α and large-scale purification of the plasmids was conducted by Qiagen Endofree plasmid kits (Qiagen GmbH, Germany). After determining purity and concentration by measuring the O.D. at 260 and 280 nm, the plasmids were stored at −20 °C.

1α,25(OH)$_2$D$_3$ ($vitD_3$) (Sigma), was dissolved in absolute ethanol at a concentration of 200 μg/ml and stored at 4 °C.