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Porcine B-cell activating factor promotes anti-FMDV antibodies *in vitro* but not *in vivo* after DNA vaccination of pigs

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Abstract

'B-cell activating factor belonging to the TNF family' (BAFF) represents a cytokine produced by antigen presenting cells promoting B-cell maturation, activation and immunoglobulin class switching. In the present study, we demonstrate expression of BAFF on cultured monocyte-derived dendritic cells, which is further enhanced by interferon-α or interferon-γ treatment. From these cells, porcine BAFF was cloned and the recombinant protein was expressed in mammalian cells with and without a FLAG tag at the carboxyl terminus. Only the protein without the FLAG tag was bioactive *in vitro*, and promoted B-cell survival and the differentiation of foot-and-mouth disease virus (FMDV)-specific memory B cells into antibody producing cells. Based on this result it was tested whether BAFF can enhance FMDV antibody responses in the context of a DNA vaccination. To this end, pigs were immunised with the anti-FMDV DNA vaccine plasmid pcDNA3.1/P1-2A3C3D and a pCI plasmid expressing porcine BAFF. Using a needle-free transdermal application method, also referred to as 'jet injection', pigs were vaccinated three times and their humoral response quantified by ELISA and a virus neutralisation test. After the third vaccination, three out of six animals vaccinated with the pcDNA3.1/P1-2A3C3D alone but none of the animals that also received the BAFF expressing plasmid had seroconverted. These data suggest that BAFF is not appropriate as a genetic adjuvant when applied as a simple co-injection with the antigen-encoding plasmid.

Keywords: BAFF; Antibody response; Foot-and-mouth disease virus; DNA vaccination; Genetic adjuvant

1. Introduction

Foot-and-mouth disease virus (FMDV) is a highly infective member of the Picornaviridae family, affecting all cloven-hoofed animals. Foot-and-mouth disease outbreaks may lead to explosive epidemics with a major financial impact in agriculture worldwide. For immunity against FMDV, a high level of neutralizing antibodies correlates with protection against challenge infection (Doel, 2003; McCullough et al., 1992; Sutmoller and Vieira, 1980; van Bekkum, 1969). Consequently, the design of vaccines should primarily ensure an induction of potent antibody responses. With this respect the cytokine 'B-cell activating factor belonging to the TNF family' (BAFF) targeting B-lymphocytes has the potential to enhance antibody response. This cytokine, also referred to as B-lymphocyte stimulator (BLyS) and CD 257, is expressed by cells of the myeloid origin (Litinskiy

Abbreviations: aa, amino acid; BAFF, B-cell activating factor belonging to the TNF family; CMV, cytomegalovirus; DC, dendritic cell; FMDV, foot-and-mouth disease virus; GM-CSF, granulocyte-macrophage colony-stimulating factor; hBAFF, human BAFF; HEK, human embryonic kidney; MoDC, monocyte-derived dendritic cells; poBAFF, porcine BAFF; SWC, swine workshop cluster

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et al., 2002; Nardelli et al., 2001; Schneider et al., 1999) and plays an important role in B-cell maturation, activation and immunoglobulin class switching. In immune responses, BAFF expands the maturing and differentiating clones of antigen-reactive B cells leading to larger number of plasma cells and consequently higher levels of secreted immunoglobulin (reviewed in Rolink and Melchers, 2002). Despite these promising properties in promoting antibody responses, nothing has been reported on the potential use of BAFF as a cytokine adjuvant in the context of vaccination.

The currently used traditional vaccines against FMDV, based on chemically inactivated virus preparations, are potent but costly in production due to safety precautions. In addition, they are fraught with the risks of virus escape from vaccine production units or use of improperly inactivated vaccines. DNA vaccines have been proposed as a safe and cost-effective alternative. However, contrasting with the data obtained with mouse models, in pigs the potency of unformulated naked plasmid DNA is generally limited and requires multiple vaccinations. As one possible strategy to improve their immunogenicity, the addition of genes encoding for certain cytokines has been applied. For example co-administration of a plasmid encoding for granulocyte-macrophage colony-stimulating factor (GM-CSF) together with a plasmid encoding for FMDV P1 results in a significantly enhanced B-cell response (Cedillo-Barrón et al., 2001). On the other hand, not all cytokines used in this context have been equally effective. Positive effects have been reported for porcine IL-2 (Rompato et al., 2006; Wong et al., 2002), IL-3 (Andrew et al., 2006), IL-18 and CD40L (Wienhold et al., 2005), but not for IL-6 (Larsen, 2002) and IL-12 (Wienhold et al., 2005).

In the present study porcine BAFF (poBAFF) expression was demonstrated on monocyte-derived dendritic cells (MoDC), the BAFF gene cloned and the recombinant protein expressed. The latter was demonstrated to enhance B-cell survival and FMDV-specific antibody responses induced *in vitro*. *In vivo*, the effects of BAFF were tested in the context of a DNA vaccination against FMDV.

2. Materials and methods

2.1. Cloning of poBAFF

Porcine monocyte-derived dendritic cells (MoDC) were prepared as described by Carrasco et al. (2001) with modifications described by Guzylack-Piriou et al. (2004). After 7 days of culture in presence of GM-CSF and IL-4, they were stimulated with 5 ng/ml IFN-γ (kindly provided by Novartis, Basel, Switzerland) for additional 3 days. Total RNA was extracted from harvested cells using RNeasy (Qiagen) and reverse transcribed using Omniscript RT (Qiagen) and an oligo d(T) primer according to the manufacturer's instruction. poBAFF DNA was amplified by PCR using the *Taq* DNA polymerase (Sigma) and the primers 5′-AAG-GYCCMAACCTTCAAAGT-3′ (based on a homologue stretch of the human and murine BAFF DNA sequences) and 5′-GCCTCTTTCAATCCTGTGCT-3′ (derived from a porcine EST, GenBank accession no. BI337275). The PCR conditions were 30 s 94 °C, 30 s 58 °C, 1 min 72 °C with 35 cycles. The resulting 1053 bp PCR product was cloned into pCR 2.1-TOPO (Invitrogen) and sequenced using the Thermo Sequenase DYEnamic direct cycle sequencing kit (Amersham Biosciences), the LI-COR Global IR^2 DNA sequencer and e-Seq and AlignIR software (LI-COR Biosciences).

Predicted BAFF protein sequences were analysed using the web-based programs CLUSTAL W for multiple alignment and HMMTOP for prediction of transmembrane domains.

2.2. Expression of recombinant full-length poBAFF

Two versions of recombinant full-length poBAFF, with and without FLAG tag, respectively, were cloned by PCR amplification with *Taq* DNA polymerase using cDNA from IFN-γ simulated porcine MoDC as template and the sense primer 5′-AATAATCTCGAGCCAC-CATGGATGACTCCACGGGGG-3′ (introducing a XhoI restriction site and a Kozak sequence) and the antisense primers 5′-TTTATTTAATGCGGCCGCT-CACTTATCGTCATCGTCTTTATAGTCGCTGCCTC-CGCTTCCGC-3′ (introducing linker sequence, a FLAG tag and a NotI restriction site) or 5′-AAA-TATGCGGCCGCTCACAGAAGTTTCAATGCAC-CAAAAAATG-3′ (without the linker and FLAG sequence). PCR conditions were as described before. The resulting PCR product was cloned into pCR 2.1-TOPO (Invitrogen), digested with the restriction endonucleases NotI and XhoI (New England Biolabs) and cloned into pCI mammalian expression vector (Promega) driven by a cytomegalovirus (CMV) promoter. The resulting plasmids were called pCI/BAFF and pCI/BAFF-FLAG, respectively. Expression of BAFF was verified by transfection of human embryonic kidney (HEK) 293T (American Type Culture Collection, ATCC) and SK6 swine kidney cells (kindly provided by Prof. M. Pensaert, Faculty of Veterinary Medicine, Ghent University, Belgium) using the calcium–phosphate method.