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Short communication

cDNA cloning, expression and bioactivity of porcine BAFF

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Abstract

B cell activating factor belonging to the tumor necrosis factor (TNF) family (BAFF) is critical for B cell survival, maturation and T cell activation by acting through its three receptors, BAFF-R, BCMA and TACI. In the present study, a porcine BAFF cDNA, designated pBAFF, was cloned by RT–PCR and rapid amplification of cDNA ends (RACE) strategies. The full-length cDNA of pBAFF consists of 805 bp with a 702 bp open reading frame, encoding 233 amino acids. The deduced amino acid sequence contains a predicted transmembrane domain and a putative furin protease cleavage site corresponding to other identified BAFF homologues. The amino acid similarity between the functional soluble parts of pBAFF and human BAFF (hBAFF) or chicken BAFF (cBAFF) is 93% and 85%, respectively, with identity at the amino acid level was 88% and 76%, respectively. The characteristic of the three-cysteine residues of BAFF is conserved in pBAFF. RT-PCR showed that BAFF is expressed in many tissues in the pig, including spleen, liver, lung, heart, intestine, kidney, thymus and PBLs. Recombinant soluble pBAFF (psBAFF) fused with His_6 tag was efficiently expressed in *Escherichia coli* BL21 (DE3) and its expression was confirmed by sodium dodecyl sulfate polyacrylamide gel electropheresis (SDS-PAGE) and Western blotting. In vitro, purified psBAFF co-stimulates the proliferation of not only porcine B cells but also human B cells. In addition, hsBAFF binds to porcine B cells and has a positive effect on their proliferation. These findings indicate pBAFF plays an important role in proliferation of porcine B cells and functional cross-reactivity occurs between porcine and human BAFF. In vitro expression of bioactive psBAFF provides the basis for further investigation of its potential to be used as an immunoadjuvant for enhancing vaccine efficacy and an immunotherapeutic in pig. It also provides the basis for investigations on the role of BAFF in this important domestic species and an animal model for human diseases.

Keywords: RACE; Molecular cloning; mRNA expression; B cell proliferation

Abbreviations: PBLs, peripheral blood leukocytes; SAC, *Staphylococcus aureus* Cowan 1; GAPDH, glyceraldehyde phosphate dehydrogenase; IPTG, isopropy-β-D-thiogalactoside; TNF, tumor necrosis factor; IgG, immunoglobulin G; IgM, immunoglobulin M; PBS, phosphate-buffered saline; RNA, ribonucleic acid; cDNA, complementary DNA; RT-PCR, reverse transcription-PCR; BAFF-R, BAFF-receptor; BCMA, B cell maturation antigen; TACI, transmembrane activator and calcium modulator and cyclophilin ligand; *E. coli*, *Escherichia coli*

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1. Introduction

Members of the TNF family and their receptors are important regulators of the immune system [1]. B cell activating factor (BAFF, also known as BLyS, TALL-1, THANK, zTNF4 or TNFSF13b) is a novel member of the human TNF family of ligands that plays a major role in B cell survival, proliferation and differentiation [2]. BAFF is a homotrimer that is found either on the cell surface as a type II transmembrane protein or is released in a soluble form after cleavage by a furin-like protease [3,4]. This process is regulated at both a stimulus and cell type level [5,6]. The crystal structure of soluble BAFF (sBAFF) revealed that it has an unusual long D–E loop compared to other TNF-family members, which forms a region that might be important for receptor binding and the virus-like assembly [7,8]. BAFF binds to three cell-surface receptors: BAFF-receptor (BAFF-R or BR3), B cell maturation antigen (BCMA, or TNFRSF17), and transmembrane activator and calcium modulator and cyclophilin ligand (TACI), all of which are expressed primarily by B cells. BAFF is the sole ligand for BAFF-R [9], while it shares receptor specificity for TACI and BCMA with a proliferation-inducing ligand (APRIL) [10]. BAFF-R, TACI and BCMA display unique but overlapping expression patterns, and functional analysis has revealed distinct roles for these three receptors in mediating BAFF (and APRIL) signals [11].

BAFF-transgenic mice display increased numbers of mature B cells in the circulation, hyperglobulinemia, and lupus-like nephritis, secondary to enhanced survival of auto-reactive B cells [12–14], consistent with in vitro observations. Agents that block BAFF have proven to be highly effective in the treatment of certain autoimmune conditions in mice [15,16]. In contrast, BAFF-deficient mice exhibit dramatically reduced B cell numbers peripherally and impaired development of germinal-center responses [17,18]. BAFF is also a survival factor for certain B cell lymphomas [19–21].

In vitro and in vivo, BAFF induces peripheral B-lymphocyte survival, proliferation and Ig secretion, suggesting that BAFF is important for maintaining peripheral B-lymphocyte homeostasis and enhancing antigen-specific humoral immunity. BAFF also acts as a T-cell co-stimulatory factor in vitro [22]. These activities indicate that BAFF might have an adjuvant-like effect on the immune system to boost immunity.

In this study, our objectives were to clone porcine BAFF (pBAFF) full-length cDNA, to investigate its expression profile, to produce bioactive psBAFF (the functional soluble form of pBAFF, aa 82-233) in prokaryotic expression system and to determine the bioactivity of recombinant psBAFF. This is the first report on in vitro characterization of the porcine BAFF molecule and raises the possibility that psBAFF could be used as an immunoadjuvant in raising the immunity of pigs against infectious diseases.

2. Materials and methods

2.1. Cell preparations

Pigs were maintained in Red-sun breed cultivation farm, Jiangsu, China. Porcine splenocytes were prepared by disruption of fragments of freshly dissected pig spleen under sterile conditions followed by filtering of cell suspensions through a 0.45-mm nylon mesh, lysis of erythrocytes, and re-suspension in RPMI1640/10% fetal calf serum (FCS). Purified porcine B cells were prepared from erythrocyte-depleted splenocyte suspensions by incubation at room temperature for 30 min in 10-cm tissue culture dishes that were coated with mouse anti-pig IgG (BD Pharmingen, USA), as described by Kanaan et al. [23]. Non-adherent cells were removed by repeated gentle washing with PBS following which adherent B cells were removed by flushing the plates with culture medium. Human B cells were isolated from peripheral blood mononuclear cells (PBMCs) using magnetic activated cell sorter (MACS) super-paramagnetic MicroBeads conjugated to a monoclonal mouse anti-human CD19 antibody (Miltenyi Biotec, Germany) as described previously [24]. All cells were maintained in RPMI1640 medium with penicillin/streptomycin (Gibco-BRL, USA) supplemented with 10% FCS at 37 °C in a CO_2 incubator.

2.2. Tissue sampling, RNA isolation and RT-PCR

Tissue samples of pig were collected, immediately placed in liquid nitrogen, and stored at −85 °C until use. Total RNA was extracted using TRIzol reagent (Gibco-BRL, USA) according to the manufacturer's protocol. A first-strand cDNA was synthesized from 1 μg of RNA isolated from spleen using Reverse Transcriptase XL (Takara, Japan) according to the manufacturer's protocol. A pair of