Developmental and Comparative Immunology 33 (2009) 697–708

Contents lists available at ScienceDirect

Developmental and Comparative Immunology

journal homepage: www.elsevier.com/locate/dci

Expression and localization of rabbit B-cell activating factor (BAFF) and its specific receptor BR3 in cells and tissues of the rabbit immune system

Jiahui Yang, Richard Pospisil, Rose G. Mage *

Laboratory of Immunology, NIAID, NIH, Bldg 10, 11N311, 10 Center Drive, Bethesda, MD 20892-1892, USA

ARTICLE INFO

Article history:
Received 6 October 2008
Received in revised form 1 December 2008
Accepted 7 December 2008
Available online 3 January 2009

Keywords:
MiniBR3
Appendix
Gut-associated lymphoid tissue
Autoimmune diseases
Vaccine development

ABSTRACT

Rabbits are widely used for vaccine development, and investigations of human infectious and autoimmune diseases such as Systemic Lupus Erythematosus (SLE). For these applications, we cloned, sequenced and expressed rabbit B-cell Activating Factor (BAFF), and localized BAFF in cells and tissues of the rabbit immune system. The rabbit homolog of the human BAFF binding site (miniBR3 peptide) within the BAFF-specific receptor BR3 was synthesized. This 26-residue core domain binds to recombinant rabbit BAFF protein. Flow cytometric analyses using purified recombinant rabbit BAFF combined with real-time PCR findings revealed that BAFF detected on peripheral blood B-cells from normal rabbits is probably complexed to BAFF receptors rather than produced by the B-cells. BAFF was detected in developing appendix of young rabbits by immunohistochemical staining suggesting that BAFF plays a role during the period following birth when rabbit B-cell development and pre-immune antibody repertoire diversification and selection is occurring.

Published by Elsevier Ltd.

1. Introduction

Members of the TNF/TNF-R superfamily have been shown to play significant roles in B-cell differentiation. B-cell activating factor of the TNF family (BAFF; also termed TNFSF13b, BLyS, TALL-1, and zTNF4) is a TNF-like cytokine that is essential for the survival and homeostasis of B lymphocytes [1–3] (reviewed in [4] and cited articles). BAFF is a type II transmembrane protein but also becomes a soluble ligand after cleavage at the cell surface by a furin-like protease. BAFF was initially found to be expressed and secreted by cells of the myeloid lineage [5], but there have been recent reports that BAFF is also expressed by neutrophils [6], neoplastic B-cells [7,8], activated mouse B-cells [9], T-cells from patients with autoimmune disorders [10], as well as by non-hematopoietic cells including astrocytes [11] and fibroblast-like synoviocytes of mesenchymal origin [12]. These mesenchymal-derived cells were shown to express functional BAFF in vitro after induction with proinflammatory cytokines such as IFN-γ and TNF-α [12]. Although BAFF is essential for development and maintenance of most B-cells, CD5-positive B-1 cells develop in mice lacking either BAFF or BAFF-receptor (BR3) [13,14]. Since BAFF was dispensable for B-1 B-cell development in mice, we investigated whether functional BAFF and it receptors are present in rabbits where the majority of B-cells are CD5 positive [15]. We report here, comparative sequence and expression analyses of rabbit BAFF, and BR3 and production of recombinant BAFF (rBAFF) protein.

BAFF can bind to three distinct receptors, the BAFF receptor (BR3, BAFF-R), the transmembrane activator and calcium-modulator and cyclophilin ligand interactor (TACI) and the B-cell maturation Ag (BCMA)[16,17]. BR3 binds BAFF with high affinity and is considered the only BAFF-specific receptor [18]. Failure of survival of peripheral B-cells from immature transitional to mature naïve stages is observed in mice lacking BAFF or with defective BR3 [19]. TACI and BCMA bind BAFF with intermediate and low affinity, respectively. TACI plays a key role in negatively regulating mature B-cell homeostasis, but TACI also plays an important role in T-cell independent B-cell responses and class switch recombination [20,21]. Much less is known about BCMA, but recent reports suggest a role of BCMA in controlling the lifespan of long-lived plasma cells [22]. All three receptors are mainly expressed on B-cells, but their expression levels change with B-cell maturation.

The early development of B-cells in the rabbit initiates in sites including fetal liver, omentum and bone marrow (reviewed in Mage et al.) [23]. After birth, the pre-immune antibody (Ab) repertoire is expanded and diversified in gut-associated lymphoid tissue (GALT).

* Corresponding author at: LI, NIAID, National Institutes of Health, Bldg 10, Rm lIN3ll, 10 Center Drive, Bethesda, MD, USA. Tel.: +1 301 496 6113; fax: +1 301 496 0222.
E-mail address: rmage@niaid.nih.gov (R.G. Mage).

Abbreviations: Ab, antibody; AID, activation induced deaminase; BAFF, B-cell activating factor; BR3, BAFF receptor; GALT, gut-associated lymphoid tissue; HRP, horse radish peroxidase; mAb, monoclonal antibody; MFI, median fluorescent intensity (or intensities); pAb, polyclonal antibody; PBMC, peripheral blood mononuclear cells; PE, phycoerythrin; POD, streptavidin-β-peroxidase conjugate; rBAFF, recombinant BAFF; SLE, systemic lupus erythematosus; TBS, Tris buffered saline; TMB, 5,5′-tetramethylbenzidine; TNF, tumor necrosis factor.

0145-305X/$ – see front matter. Published by Elsevier Ltd.
doi:10.1016/j.dci.2008.12.001

The molecular events that occur during Ab diversification have been intensively studied in rabbit, but less is known about the control at functional checkpoints and B-cell survival.

The present report provides the basis for further studies of the function of BAFF and its receptors in rabbit immune responses that are particularly needed now because in addition to being a valuable resource for development of diagnostic and therapeutic antibodies, rabbits are important for vaccine development and as animal models of human diseases. Our laboratory has described a model of the autoimmune disease SLE in allotype-defined pedigreed rabbits [24,25] and (Yang J, et al., ms in preparation). Before we could investigate the specific roles of BAFF and its receptors in rabbit B-cell development, maturation and Ab production in normal and autoimmune animals, it was necessary to first determine their cDNA and encoded protein sequences and their expression patterns in hematopoietic cells. In this study, we used a cross-species comparison strategy to design PCR primers, amplify, clone, and sequence rabbit BAFF and BR3. We found BAFF protein in developing appendix of young rabbits suggesting that BAFF plays a role during the period following birth when B-cell development, pre-immune Ab repertoire diversification, selection and expansion is occurring. We quantitated BAFF and BR3 mRNA expression and detected staining patterns on peripheral blood mononuclear (PBMC) and spleen cells of adult rabbits, expressed recombinant rabbit BAFF protein and utilized the protein for further investigations of BAFF and BR3 interactions.

2. Materials and methods

2.1. Animals

Rabbits were obtained from the allotype-defined pedigreed colony maintained at the National Institute of Allergy and Infectious Diseases. The animal studies described here were reviewed and approved by the animal care and use committees of NIAID/NIH (ASP LI6) and of the Spring Valley Laboratories, Inc. where the NIAID allotype-defined rabbit colony was housed. Normal adult rabbits of known Ig allotypes from our breeding colony, provided whole blood and spleens for flow cytometry, immunohistochemistry, and mRNA detection. In addition, one spleen from a 7-week old rabbit and appendix tissues of young rabbits at 4, 8 and 10 days of age were studied by immunohistochemistry.

2.2. Abs and reagents for flow cytometry, cell isolation and ELISA

BAFF was detected using biotin-conjugated goat anti-human BAFF polyclonal Ab (pAb) (Antigenix America Inc.) and rat anti-mouse BAFF monoclonal antibody (mAb) (clone 121809 R&D Systems) that we found to cross-react with rabbit BAFF. BR3 was detected using purified goat anti-human BR3 pAb (R&D Systems) that also cross-reacts with rabbit BR3. Also used were FITC-labeled mouse anti-rabbit CD14 (clone K4), FITC-labeled mouse anti-rabbit CD4 (clone KEN-4), FITC-labeled mouse anti-rabbit CD8 (clone C7) (Antigenix America Inc.), FITC-labeled goat anti-rabbit IgM, FITC-labeled goat anti-rabbit IgG (Southern Biotechnology Associates), biotin-labeled donkey anti-goat IgG, biotin-labeled goat IgG, normal goat IgG (Jackson ImmunoResearch Laboratories, Inc.), and FITC-labeled mouse IgG2a (BD Pharmingen). Biotinylated Abs were visualized by phycoerythrin-(PE)-conjugated streptavidin (Jackson ImmunoResearch Laboratories, Inc.).

2.3. Isolation of cell subpopulations

For quantitative analyses of mRNA expression in cell subpopulations, spleen tissues were chopped in small pieces in PBS and pressed through a stainless steel mesh screen with rubber-tipped syringe plunger. Debris and cell clumps were removed by passing the suspension through 70 μm nylon mesh. Freshly isolated spleen cells were stained with mouse anti-rabbit CD14 (Antigenix America) or mouse anti-rabbit CD4 (Serotec) Ab and $CD4^+$ or $CD14^+$ cell subpopulations were isolated with goat anti-mouse IgG covalently bound to Dynabeads (Dynal). To isolate IgM positive B-cell subpopulations, cells were stained with biotin-conjugated polyclonal μ-heavy chain specific goat anti-rabbit IgM (Southern Biotechnology Assoc.) followed by Streptavidin-Dynabeads. Viable peripheral blood mononuclear cells (PBMC) were isolated from heparinized blood by density gradient centrifugation on Lympholyte-Mammal (Cedarlane Laboratories Limited). This permitted recovery of lymphocytes and monocytes but eliminated most red cells and granulocytes. IgM positive subpopulations from PBMC were isolated as described above. IgM depleted PBMC (IgM^- cells) were then stained with mouse anti-rabbit CD14 mAb and separated into two populations, IgM^-CD14^+ and IgM^-CD14^- using anti-mouse IgG-Dynabeads.

Upon analyses by flow cytometry, fractions were shown to be at least 90% pure. After isolation, cells were immediately placed into TRIzol™ reagent (Invitrogen) for RNA isolation.

2.4. Rabbit BAFF and BR3 cloning

Total RNA was obtained from normal rabbit PBMCs that were immediately placed in TRIzol reagent. PBMC were homogenized and filtered with Qiashredder column (Qiagen). Total RNA was isolated by RNeasy spin column (Qiagen) and precipitated with ethanol. First-stand cDNA was synthesized using the SuperScript first-strand synthesis kit (Invitrogen). The BAFF primers (Table 1A) were designed after searching the rabbit whole genome shotgun (WGS) trace archives database and aligning the sequence with the highly conserved BAFF domains of multiple species. The cDNA was amplified by Platinum® pfx DNA polymerase (Invitrogen). PCR conditions were first melting at 94 °C for 2 min, then 30 cycles of amplification: 15 s at 94 °C, 30 s at 55 °C, and 1 min at 68 °C. A final extension was at 72 °C for 10 min. The rabbit BR3 primers (Table 1A), were designed based on the 'working draft' sequence of genomic DNA in *Oryctolagus cuniculus* clone LB1-145O11 (AC145540.1) positions 124760–123559 on the reverse strand, that appears to contain the sequence of the rabbit homolog of human BR3. Touchdown PCR conditions were melting at 94 °C for 30 s, annealing and elongation at 72 °C for 3 min. The annealing temperature was dropped from 72 °C at a rate of 2 °C for five cycles to 68 °C for the remaining 30 cycles. The rabbit BAFF PCR products were cloned into pCR-Blunt II-TOPO (Invitrogen), and sequenced from SP6 and T7 promoter sites with SP6 and T7 primers. The rabbit BR3 PCR products were cloned into pCR 2.1 vector (Invitrogen), and sequenced from the M13 reverse priming and T7 promoter sites with M13 reverse and T7 primers. At least three independent PCR cloning and sequencing experiments were conducted to rule out errors introduced by PCR.

2.5. Construction, expression, and purification of recombinant rabbit BAFF protein

A construct was designed with a signal sequence of human Ig heavy chain (VH1), a $(His)_6$ tag at the N-terminus, followed by the cDNA sequence of the predicted rabbit BAFF extracellular domain (amino acids 139–290) and a stop codon. The desired product was amplified by two-rounds of PCR from the pCR-Blunt II-TOPO-BAFF construct using primers shown in Table 1B. After the second-round overlapping PCR, the specific DNA fragment was cloned by KpnI/XhoI ligation into the mammalian cell expression vector pCEP4