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hsBAFF promotes proliferation and survival in cultured B lymphocytes via calcium signaling activation of mTOR pathway

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ABSTRACT

B-cell activating factor of the TNF family (BAFF, also called BLyS, TALL-1, THANK, or zTNF4) has revealed its critical function in B lymphocyte proliferation and survival, as well as the pathogenesis of autoimmune disease. However, the molecular mechanisms of excess BAFF-extended aggressive B lymphocytes have not been completely defined. Here we show that excessive hsBAFF-elevated $[Ca^{2+}]_i$ activated mammalian target of rapamycin (mTOR) signaling pathway, leading to proliferation and survival in B lymphocytes. This is supported by the findings that intracellular Ca^{2+} chelator (BAPTA/AM) or mTOR inhibitor (rapamycin) abolished the events. Sequentially, we observed that preventing $[Ca^{2+}]_i$ elevation using EGTA or 2-APB dramatically inhibited hsBAFF activation of mTOR signaling, as well as cell growth and survival, suggesting that hsBAFF-induced extracellular Ca^{2+} influx and ER Ca^{2+} release elevates $[Ca^{2+}]_i$ contributing to B lymphocyte proliferation and survival via activation of mTOR signaling. Further, we noticed that pretreatment with BAPTA/AM, EGTA or 2-APB blocked hsBAFF-increased phosphorylation of calcium/calmodulin-dependent protein kinase II (CaMKII), and inhibiting CaMKII with KN93 attenuated hsBAFF-activated mTOR signaling, as well as cell growth and survival, revealing that the effects of hsBAFF-elevated $[Ca^{2+}]_i$ on mTOR signaling as well as proliferation and survival in B lymphocytes is through stimulating phosphorylation of CaMKII. The results indicate that hsBAFF activates mTOR pathway triggering B lymphocyte proliferation and survival by calcium signaling. Our findings suggest that manipulation of intracellular Ca^{2+} level or CaMKII and mTOR activity may be exploited for the prevention of excessive BAFF-induced aggressive B lymphocyte disorders and autoimmune diseases.

1. Introduction

B-cell activating factor of the TNF family (BAFF), also known as BLyS, TALL-1, THANK, and zTNF4, is a ligand for three TNF-receptor-family members: BAFF-R (BR3), BCMA, and TACI [1–6]. BAFF and its receptors have revealed their critical function in development and homeostasis of normal B lymphocytes, and in cell growth and survival of neoplastic B lymphocytes [7–10]. BAFF allow peripheral B lymphocytes to complete their maturation and accumulate in the peripheral lymphoid organs [11]. In BAFF-deficient mice, the mature B lymphocyte population was severely reduced although T and B lymphocyte development in the thymus and bone marrow appeared to be normal [8]. Lack of BAFF leads to B lymphocyte death and immunodeficiency syndromes such as single IgA, IgG, or IgM deficiency, as well as common variable immunodeficiency (CVID) [12,13]. Conversely, increased levels of BAFF in the serum of mice that express both endogenous and transgenic BAFF result in an increased number of peripheral B lymphocytes, and

Abbreviations: 2-APB, 2-aminoethoxydiphenyl borate; 4E-BP1, eukaryotic initiation factor 4E binding protein 1; Akt, protein kinase B (PKB); BAFF, B-cell activating factor of the TNF family; BAPTA/AM, 1,2-bis(o-aminophenoxy) ethane-N,N,N′,N′-tetraacetic acid tetra(acetoxymethyl) ester; BLyS, B lymphocyte stimulator; BCMA, B cell maturation antigen; CaM, calmodulin; CaMKII, calcium/calmodulin-dependent protein kinase II; CRAC, Ca^{2+}-release activated Ca^{2+}; EGTA, ethylene glycol tetra-acetic acid; ER, endoplasmic reticula; Erk1/2, extracellular signal-related kinase ½; FBS, fetal bovine serum; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; MTT, 3-(4,5-dimethylazol-2-yl)-2,5-diphenyltetrazolim bromide; PBS, phosphate buffered saline; PI3K, phosphatidylinositol 3′-kinase; S6K1, S6 kinase 1; TACI, transmembrane activator and cyclophilin ligand interactor; TALL-1, TNF and apoptosis ligand-related leukocyte-expressed ligand1; THANK, TNF homologue that activates apoptosis, nuclear factor κB and c-Jun NH2-terminal kinase.

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especially extend B lymphocyte survival beyond physiological limits; this prolonged B lymphocyte life span causes BAFF-transgenic mice to develop lupus-like autoimmune diseases [8,14–16]. High levels of BAFF may drive continued production of plasma cells producing pathogenic autoantibodies, which exerts an important action in the SLE pathogenesis [14,17]. In humans, increased serum BAFF levels are found in a number of different autoimmune diseases, such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), Sjögren's syndrome (SS) [14,18,19]. These data suggest that excessive BAFF is a possible etiological factor of aggressive or neoplastic B lymphocyte disorders and autoimmune diseases. The purpose of our study was to elucidate how excessive BAFF promotes the proliferation and survival of B lymphocytes.

Multiple studies have shown that BAFF-derived intracellular signaling pathways play a critical role in development and homeostasis of normal B lymphocytes, and in cell growth and survival of neoplastic B lymphocytes [10,20]. In this regard, BAFF regulates several antiapoptotic Bcl-2 family members, including Bcl-x_L, Mcl-1, A1/Bfl-1, Bcl-2, and Bim, via survival-promoting kinase systems such as Pim 1/2 or extracellular signal-related kinase 1/2 (Erk1/2) [10]. Mammalian target of rapamycin (mTOR), a ubiquitously expressed, 289-kDa serine/threonine (Ser/Thr) kinase, lies downstream of phosphatidylinositol 3′-kinase (PI3K) and protein kinase B (PKB/Akt) [21,22], and senses mitogenic stimuli, nutrient conditions [23,24] and ATP [25]. Activated PI3K results in activation of Akt, which may positively regulate mTOR, thereby leading to increased phosphorylation of ribosomal p70 S6 kinase (S6K1) and eukaryotic initiation factor 4E (eIF4E) binding protein 1 (4E-BP1), the two best-characterized downstream effector molecules of mTOR [26]. mTOR has been widely recognized as a central controller for cell proliferation/growth and survival [22]. Ongoing studies have highlighted that mTOR is required for the maturation and differentiation of multiple immune cell lineages [27,28]. Recent evidence has begun to emerge demonstrating a role for the PI3K/Akt signaling pathway in response to BAFF [1]. BAFF stimulation of B lymphocyte growth and survival is involved in activation of Akt and mTOR-mediated 4E-BP1 pathway [20]. However, it is still incompletely defined how BAFF activates Akt/mTOR signaling pathway in B lymphocytes.

Calcium ion (Ca^{2+}) is a ubiquitous intracellular signal responsible for controlling numerous cellular processes including effector functions, gene expression, cell activation and differentiation in various cells of the immune system [29,30]. Studies have demonstrated that the elevation in intracellular free Ca^{2+} ($[Ca^{2+}]_i$) level activates PI3K-Akt signaling pathway [31]. The activation of mitogen extracellular kinase (MEK) and Erk1/2 in B lymphocytes is dependent on calcium flux [32,33]. Ca^{2+} is also critical for amino acid-mediated activation of mTOR [34]. Recently, we have demonstrated that human soluble BAFF (hsBAFF) elicits higher but homeostatic $[Ca^{2+}]_i$, which activates Erk1/2 pathway contributing to the proliferation and survival of cultured mouse splenic B lymphocytes [35]. However, the underlying mechanism remains elusive. Calcium/calmodulin-dependent protein kinase II (CaMKII), a Ser/Thr specific protein kinase, is a general integrator of Ca^{2+} signaling [36]. CaMKII is activated in the presence of Ca^{2+} and calmodulin (CaM), which leads to autophosphorylation, generating a Ca^{2+}/CaM-independent form of the enzyme [36,37]. It has been reported that CaMKII transduces signals to MAPKs and mTOR involved in cell proliferation, survival or apoptosis [38–40]. This prompted us to study whether BAFF activates mTOR pathway, triggering B lymphocyte proliferation and survival, through Ca^{2+} signaling.

Here we show that excessive hsBAFF-elevated $[Ca^{2+}]_i$ is associated with activation of mTOR signaling pathway in primary mouse splenic B lymphocytes. hsBAFF-induced extracellular Ca^{2+} influx and ER Ca^{2+} release elevates $[Ca^{2+}]_i$ contributing to B lymphocyte proliferation and survival via activation of mTOR signaling. We demonstrate that hsBAFF elicits CaMKII phosphorylation by elevated $[Ca^{2+}]_i$, thereby activating mTOR signaling, leading to the proliferation and survival in B lymphocytes. These findings suggest that manipulation of intracellular Ca^{2+} level or CaMKII and mTOR activity may be exploited for the prevention of excessive BAFF-induced aggressive B lymphocyte disorders and autoimmune diseases.

2. Materials and methods

2.1. Reagents

Anti-CD19 magnetic fluorobeads-B was purchased from One Lambda (Canoga Park, CA, USA). RPMI 1640 Medium was from Gibco (Rockville, MD, USA). Fetal bovine serum (FBS) was supplied by Hyclone (Logan, UT, USA). Refolded human soluble BAFF (hsBAFF) was a recombinant form of the extracellular domain of the BAFF synthesized in *Escherichia coli* from our group [41]. KN93 and rapamycin were from ALEXIS (San Diego, CA, USA). Fluo-3/AM was from Fluka (Buchs, SG, Switzerland). Enhanced chemiluminescence solution was from Millipore (Billerica, MA, USA). 1,2-bis(o-aminophenoxy) ethane-N,N,N′,N′-tetraacetic acid tetra(acetoxymethyl) ester (BAPTA/AM) and 2-aminoethoxydiphenyl borane (2-APB) were purchased from Calbiochem (San Diego, CA, USA), whereas ethylene glycol tetra-acetic acid (EGTA) and goat anti-mouse IgM were purchased from Sigma (St. Louis, MO, USA). The following antibodies were used: phospho-CaMKII (Thr286), phospho-Akt (Ser473), phospho-S6K1(Thr389), phospho-S6 ribosomal protein (Ser235/236), S6 ribosomal protein, 4E-BP1 (all from Cell Signaling Technology, Beverly, MA, USA), Akt, CaMKII, S6K1, β-actin (all from Santa Cruz Biotechnology, Santa Cruz, CA, USA), goat anti-rabbit IgG-horseradish peroxidase (HRP), goat anti-mouse IgG-HRP, and rabbit anti-goat IgG-HRP (Pierce, Rockford, IL, USA). Other chemicals were purchased from local commercial sources and were of analytical grade quality.

2.2. Preparation for purified mouse B lymphocytes

ICR mice, each weighing 20–25 g, were obtained from Experiment Animal Center of Nanjing Medical University, Nanjing, China. The animals were sacrificed via cervical dislocation to collect spleens under sterile conditions. Spleen was minced with dissecting scissors into pieces, followed by further grinding and filtering through sterilized nylon membranes and washed in Hank's solution (pH 7.2) via two centrifugations at 1500 r/min for 5 min after erythrocytes were dissolved with 0.85% amchlor solution. After that, the isolated cells are fresh splenic cells. B lymphocytes of >97% purity were isolated from splenic cell suspensions using anti-CD19 magnetic fluorobeads as described previously [35], then suspended in RPMI 1640 medium containing 10% FBS and 100 U/ml penicillin/streptomycin for further experiments.

2.3. Assay for proliferation and survival in B lymphocytes

Purified mouse B lymphocytes were seeded in 24-well plates (3×10^5 cells/well) or 6-well plates (2×10^6 cells/well) under standard culture conditions and kept overnight at 37 °C humidified incubator with 5% CO_2. The next day, the experiments were randomly divided into a normal control group, a treatment group with anti-IgM (2.5 μg/ml), four treatment groups with hsBAFF (1, 2.5, 5, 10 μg/ml, respectively), and four co-treatment groups with hsBAFF plus anti-IgM with a 3–6 replicates of each group. The cultures were maintained for 48 h. Then, the cell survival was monitored, respectively, by counting viable cells using trypan blue exclusive or by a fluorescence-activated cell sorter (FACS) Vantage SE flow