Journal of Autoimmunity 51 (2014) 30–37

Contents lists available at ScienceDirect

Journal of Autoimmunity

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

Specific forms of BAFF favor BAFF receptor-mediated epithelial cell survival

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ARTICLE INFO

Article history:
Received 4 February 2014
Accepted 5 February 2014

Keywords:
BAFF
BR3
Epithelial cells
Salivary glands
Sjögren's syndrome
Protein kinase C δ

ABSTRACT

Although B cell activating factor (BAFF) and its receptor BR3 are produced and expressed by many cells, their role has been restricted to the lymphocyte lineage. Using various techniques (RT-PCR, indirect immunofluorescence, flow cytometry analysis), we observed the expression of BR3 and the production of BAFF by the human salivary gland cell line, by epithelial cells from biopsies of Sjögren's syndrome patients and their controls, but also by salivary gland epithelial cells in culture. To decipher the role of BAFF and BR3 on epithelial cells, BAFF and BR3 were neutralized by blocking antibodies or RNA specific inhibitor (siBR3) and epithelial cell survival was analyzed. Blocking BR3 promotes epithelial cell apoptosis *in vitro*. This apoptosis resulted in the nuclear translocation of PKCδ. BAFF neutralization by various anti-BAFF antibodies leads to different effects depending on the antibody used suggesting that only some forms of BAFF are required for epithelial cell survival. Our study demonstrates that BR3 is involved in the survival of cultured epithelial cells due to an autocrine effect of BAFF. It also suggests that epithelial cells produce different forms of BAFF and that only some of them are responsible for this effect.

1. Introduction

Moutsopoulos defines SS as an auto-immune epithelitis [1] and considers the activation of epithelial cell (EC) as the main immune-pathologic process in the development of SS. ECs express type II HLA in the presence of INFγ [2], thus constituting a pool of antigen-presenting cells [3] that are able to present auto-antigens to T and B cells. In SS, ECs produce IFNγ [4], which in turn has been described to promote the B cell activating factor belonging to the TNF family (BAFF) production [5]. BAFF was identified as essential for the development and differentiation of B cells [6,7]. It interacts with three different receptors, BR3 (or BAFF-R), the transmembrane activator and CAML interactor (TACI) and the B-cell maturation antigen (BCMA) [8].

BAFF is produced in lymphoid organs by several cell types [6] such as monocytes, macrophages, dendritic cells, T cells and neutrophil polynuclear cells [7,9]. BAFF synthesis can be induced by cytokines such as interferon (IFN)α, IFNγ, interleukin (IL)-10 and CD40L [5]. Follicular dendritic cells are also a potential source of BAFF [10] and a weak production of BAFF was observed by activated T cells [7] providing co-stimulatory signals for B-cell selection. Furthermore, bone marrow stromal cells massively express BAFF in order to maintain B cell homeostasis [11]. Finally, production of BAFF is up-regulated in several pathologies. Indeed, BAFF is highly produced by B cells from chronic lymphocytic leukemia patients [12] and increased amounts of soluble BAFF have been detected in the serum of patients with myeloma [13]. Likewise, increased concentrations of BAFF were observed in the serum of patients with auto-immune diseases (AID), such as systemic lupus erythematosus, rheumatoid arthritis and Sjögren's syndrome (SS) [14]. In SS, infiltrating B and T cells produce BAFF [15], and more surprisingly, astrocytes in multiple sclerosis secrete BAFF [16].

Clearly, BAFF is essential for the survival of B cells because of its interaction with BR3 [17]. BR3 signaling activates PI3K as well as non-canonical NF-κB signaling in B cells. Following BAFF engagement, BR3 induces the recruitment of TNFα-associated factor (TRAF)2 and TRAF3, leading to the release of NF-κB-inducing kinase (NIK). Consequently, NIK phosphorylates IKK1 leading to the processing of p100 into p52 and to the activation of non-canonical NF-κB pathway which then results in B cell survival [18]. BR3 pathway is negatively regulated by Act-1 that has binding sites to TRAF molecules and inhibits TRAF

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http://dx.doi.org/10.1016/j.jaut.2014.02.004
0896-8411/

recruitment [19]. BR3 stimulation has also been linked to the negative regulation of PKCδ [20]. PKCδ is a target for caspase 3 cleavage that generates an active form of the kinase that operates in the nucleus and contributes to apoptosis [21]. However, nuclear substrates for this pro-apoptotic function of PKCδ have not been identified.

Among BAFF receptors, BR3 is the most specific, mainly expressed by transitional and mature B cells. In SS, we previously observed that BR3 was present on infiltrating B cells but not on T cells [15]. Regulation of the BAFF/BR3 axis in B cells is crucial to prevent autoimmune manifestations [22]. BAFF overexpression in mice transgenic for BAFF, promotes autoimmune-like manifestations such as systemic lupus erythematosus and SS in the presence of high levels of anti-ssDNA and anti-dsDNA autoantibodies, circulating immune complexes, and immunoglobulin deposition in the kidneys. These mice have also vastly increased numbers of mature B cells with high proportion of marginal zone B cells [6].

Act-1 functions as a negative regulator of CD40- and BAFF-mediated B cell survival [19]. Mice deficient in Act-1 developed also systemic autoimmune disease with histological and serological features of human SS, in association with systemic lupus erythematosus-like nephritis [23]. Histological analyses revealed profound lymphocyte infiltration in lacrimal, parotid and submaxillary glands. The majority of the infiltrated B cells displayed a phenotype resembling marginal zone-like B cells. High titers of anti-SSA/Ro and anti-SSB/La in association with anti-ssDNA and anti-dsDNA were detected in sera of Act-1 deficient mice. These two mouse models emphasize the pivotal role of the BAFF/BR3 axis in B cell tolerance.

Meanwhile, we also demonstrated that BR3 was expressed by ECs without being able to explain the reason for this expression. The aim of the present study was to decipher the role of BAFF and BR3 on ECs in SS.

2. Material and methods

2.1. Patients and cell line

Salivary gland (SG) biopsies were obtained from 18 patients (3 men and 15 women; ages 32–77 years) fulfilling the American-European Consensus Group criteria [24] for SS. All had a focus score $\geq$1. Control samples consisted of 15 SG specimens from patients who did not meet the criteria for primary SS (4 men and 11 women: ages 39–74 years), but they had presented sicca symptoms and, as such, had undergone an SG biopsy. All SS patients and controls gave their consent and the study was approved by the Brest CHRU Ethics Committee. To summarize, sections were cut into small fragments and incubated in Supplemented Basal Epithelial Medium (SBEM). SBEM medium contains three volumes of Ham's F12 medium (Invitrogen), one volume of Dulbecco's Modified Eagle Medium (DMEM) (Lonza, Verviers, Belgium), 2.5% of Fetal Calf Serum (FCS) (Eurobio, Courtaboeuf, France), 2 mM of L-glutamine, 10 ng/ml of EGF (Epidermal Growth Factor) (Promega, Madison, WI, USA), 0.5 μg/ml of insulin (Novo-Nordisk, Küsnacht, Switzerland) and 0.4 μg/ml of hydrocortisone (Sigma–Aldrich, Saint-Quentin-Fallavier, France). Cells were incubated at 37 °C with 5% CO_2. The HSG cell line was incubated in DMEM, supplemented with 10% FCS, 2 mM L-glutamine (Gibco, Invitrogen, Auckland, New Zealand), 1% non-essential amino acids (Sigma–Aldrich, St Louis, MO), 100 IU/ml penicillin (Panpharma, Fougères, France) and 100 μg/ml streptomycin (Panpharma).

2.2. Detection of BAFF and its receptors

Total RNA was extracted by the RNAble® method (Eurobio, Les Ulis, France) according to the supplier's instructions. One μg of total isolated RNA was converted to cDNA using SuperScript II reverse transcriptase (Invitrogen), according to the manufacturer's instructions. PCR was performed using GoTaq polymerase (Promega) under the following conditions: initial denaturation at 94 °C for 5 min, followed by 5 cycles at 94 °C for 30 s, 1 min at 61 °C and 1 min at 72 °C, then 40 cycles at 94 °C for 30 s, 40 s at 56 °C and 1 min at 72 °C, and finally a final extension at 72 °C for 10 min. PCR products were separated on a 2% agarose gel (Interchim, Montluçon, France) containing GelRed™ Nucleic Acid gel Stain (Interchim) and analyzed using Quantity One® software (version 4.6.3, Biorad, Marnes-la-Coquette, France). Primers used for PCR are: GAPDH (5′-CTTAGCACCCCTGGCCAAGG-3′ and 5′-CTTACTCCTTGGAGGCCATG-3′), BAFF (5′-TTGCAGACAGTGAAACA-CCAACT-3′and 5′TTCATCTCCTTCTTCCAGTTTTGC-3′), BR3 (5′-CTGGTCCTGGTGGGTCTG-3′and 5′-TCTTGGTGGTCACCAGTTCA-3′), TACI (5′-AGTGGCCTGGGCCGGAG-3′ and 5′-CTCCTTGCGGCAGC-TGAGTGAC-3′), BCMA (5′-CTCCTCTAACATGTCAGCGTTATTGT A-ATG-3′and 5′-GTCAATGTTAGCCATGCCCAGGGA-3′).

OCT-embedded (Miles, Naperville, IL) SG biopsies were snap-frozen in isopentane (Sigma–Aldrich). 4 μm-thick cryostat sections were cut from the blocks and mounted onto poly-L lysine-coated slides (Thermo Scientific, St Herblain, France). The slides were then incubated for 40 min at room temperature with a rabbit anti-BAFF Ab (Upstate Lake Placid, NY) alone and with fluorescein isothiocyanate (FITC)-conjugated mouse anti-cytokeratin (CK) 18 Ab (Sigma–Aldrich), in combination with either a rabbit anti-BR3 Ab (ProSci, Poway, CA) or a rabbit anti-CD20 Ab (Thermo Scientific). After three washes in PBS, the slides were incubated for another 40 min with FITC-conjugated polyclonal donkey anti-rabbit IgG Ab (Jackson ImmunoResearch, West Grove, PA) or tetramethylrhodamine isothiocyanate (TRITC)-conjugated donkey anti-rabbit IgG Ab (Jackson ImmunoReserarch), in PBS supplemented with 2% donkey serum (Sigma–Aldrich). After five washes, the sections were fixed with 4% cold paraformaldehyde (Sigma–Aldrich) and analyzed with the TCS-NT Leica confocal imaging system (Leica Microsystems, Wetzlar, Germany). FITC-conjugated donkey anti-rabbit IgG Ab and TRITC-conjugated donkey anti-rabbit IgG served as negative controls and did not show any fluorescence.

HSG cells were incubated with a mouse anti-BAFF mAb (R&D System, Minneapolis, MN), a rabbit anti-BR3 Ab, a goat anti-TACI Ab (Peprotech, Rocky Hill, NJ) or a goat anti-BCMA Ab (R&D Systems) for 40 min at room temperature. After 3 washes in PBS, the cells were incubated with a FITC-conjugated donkey anti-mouse IgG Ab, a TRITC-conjugated donkey anti-rabbit IgG Ab or a TRITC-conjugated donkey anti-goat IgG Ab (Jackson ImmunoResearch) for 30 min at room temperature. Cells were then analyzed by confocal microscopy. For PKCδ localization, HSG cells were stained with a mouse anti-PKCδ mAb (BD Biosciences, Franklin Lakes, NJ) revealed by a FITC-conjugated donkey anti-mouse IgG Ab (Jackson ImmunoResearch). Cell nuclei were labeled with propidium iodide (PI) for 20 min at 4 °C. After 3 washes in PBS, HSG cells were observed by confocal microscopy.

After trypsination and washing at 1200 rpm for 10 min, HSG cells or ECs purified from SG biopsies were incubated with a mouse anti-BAFF mAb, a rabbit anti-BR3 Ab, a goat anti-TACI Ab or a goat anti-BCMA Ab, for 30 min at 4 °C. After 3 washes in PBS, stainings were revealed with a FITC-conjugated donkey anti-mouse IgG Ab, a FITC-conjugated donkey anti-rabbit IgG or with a FITC-conjugated donkey anti-goat IgG (all from Jackson ImmunoResearch) for 30 min at 4 °C. Corresponding FITC-conjugated isotypes were used as controls and cells were analyzed by flow cytometry (EPICS® XL-MCL, Coulter).

2.3. Cell stimulation

HSG cells were removed from the flask using trypsin (PAN-biotech GmbH, Aidenbach, Germany), washed in PBS and incubated