Concise Report

Expression of B-cell activating factor of the tumour necrosis factor family (BAFF) in T cells in active systemic lupus erythematosus: the role of BAFF in T cell-dependent B cell pathogenic autoantibody production


Introduction

B cell activating factor of the tumour necrosis factor (TNF) family (BAFF; also known as BlyS, TALL-1, THANK, TNFSF13B and zTNF-4) is a 285-amino-acid member of the TNF ligand superfamily [1–6]. It is expressed as a type II transmembrane protein which is cleaved at the cell surface by a furin protease, resulting in release of a soluble, biologically active 17-kDa molecule [7]. Expression of BAFF is highly restricted to myeloid lineage cells (e.g. monocytes, macrophages, dendritic cells, neutrophils), and levels of BAFF mRNA and protein are up-regulated by interferon (IFN) γ, interleukin (IL)-10 and CD40L. Expression of the three known BAFF receptors (BCMA, TACI and BAFF-R) is also highly restricted. TACI and BCMA bind both BAFF and APRIL, another TNF superfamily member, and their roles are more controversial. The agonist effects of BAFF on B cells are mediated mainly via BAFF-R [8–10]. Systemic lupus erythematosus (SLE) is characterized by loss of B cell tolerance and the presence of polyclonal B cell activation [11–13]. Recent studies have shown that the serum levels of BAFF are elevated in patients with SLE and Sjögren’s syndrome and in the synovial fluid of patients with rheumatoid arthritis [14–18]. The association of each of these diseases with autoantibody production suggests a potential role of increased BAFF in the disease process. Moreover, cross-sectional studies have demonstrated elevated levels of circulating BAFF in SLE [15, 16]. However, the role of T cell-dependent B cell autoantibody production by the BAFF system in SLE is still unclear.

In the present study, we examined whether BAFF is involved in T cell-dependent B cell pathogenic autoantibody production in SLE.

Materials and methods

Subjects

Twenty-three patients with SLE who had been admitted to Juntendo University Hospital were recruited for this study. The clinical diagnosis in all patients was made in accordance with the American College of Rheumatology 1982 revised criteria for the SLE [17]. In order to be enrolled, each patient had to be suffering from active SLE as assessed subjectively by the patient’s physician, and was required to provide informed consent. Disease activity was assessed by the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) [18]. Eighteen patients had nephritis [CH50: 19.3 ± 10.2 U/ml, DNA/Ria: 58.3 ± 69.5 IU/ml, SLEDAI: 23.0 ± 6.8, prednisone (median): 21.25 mg/day (range: 0–55)] and five had neuropsychiatric involvement [CH50: 39.2 ± 6.9 U/ml, DNA/Ria: 7.35 ± 4.2 IU/ml, SLEDAI: 19.5 ± 4.9, prednisone (median): 8 mg/day (range: 5–10)]. Twenty-three healthy controls were recruited from personnel at Juntendo University School of Medicine. Ethical approval was not required under the present rules of our university when using and investigating the peripheral blood of patients or healthy donors. All the patients and healthy donors were fully informed and gave their consent to participate in our study. All information and data about patients or healthy donors is kept confidential and the data are fully available to patients or donors upon request.

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Antibodies and reagents
Fluorescein isothiocyanate-conjugated anti-CD45RA, anti-CD45RO, anti-CD27, anti-BAFF, phycoerythrin/Cy5-conjugated anti-CD14, anti-CD20, anti-CD38, anti-BAFF-R, anti-TACI and allophycocyanin-conjugated anti-CD4, anti-CD8 and anti-CD20 monoclonal Ab (mAb) were purchased from BD Biosciences (San Jose, CA). Unconjugated antibodies against BAFF (1D6; mouse IgG1) and BAFF-R (8A7; mouse IgG2a) and the matched isotype control were conjugated to biotin, and the specificities of mAbs 1D6 for BAFF and 8A7 for BAFF-R have been documented previously [19, 20]. Recombinant human TAC1/Fc chimera (R&D systems, MN) and recombinant human Fas/Fc chimera (Bender Medsystems GmbH, Vienna, Austria). Assays were performed according to the manufacturer’s instructions.

Flow cytometric analysis
In order to prepare peripheral lymphocytes, venous blood samples were collected from SLE patients and healthy controls after obtaining informed consent. Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized venous blood by Ficoll density-gradient centrifugation and were triple-stained with fluorescein isothiocyanate-conjugated anti-CD45RA, anti-CD45RO, anti-CD20, anti-CD27, anti-BAFF and phycoerythrin/Cy5-conjugated anti-CD14, anti-CD20, anti-CD38, anti-BAFF-R, anti-TACI and allophycocyanin-conjugated anti-CD4, anti-CD8 and anti-CD20 mAb. For intracellular staining of BAFF, we used Intraprep (Beckman Coulter, Miami, FL) for fixation and membrization according to their manufacturer instruction. Flow cytometric analysis was performed using FACSARia (Becton Dickinson, San Jose, CA), and data were processed using the Cell Quest program (Becton Dickinson).

Determination of BAFF and BAFF-R mRNA levels in peripheral blood
For isolation of peripheral blood CD4+ , CD8+ T cells or CD20+ B cells, 5 ml of peripheral blood was labelled with 40 μl of anti-human CD4, CD8 or CD20 antibody coupled to colloidal paramagnetic microbeads (Miltenyi Biotech, Bergisch-Gladbach, Germany) and isolated using AutoMACS (Miltenyi Biotech), respectively. CD4+ , CD8+ or CD20+ cells were isolated at a purity of more than 93% and the resulting cell population was <2% CD14+ and <2% CD57+. All cultures were conducted in RPMI 1640 medium supplemented with 10% FCS, 2 mM l-glutamine, penicillin G (200 U/ml) and gentamicin (10 μg/ml). Lymphocytes (2 × 10^6/well) were cultured in 96-well round-bottom plates in 0.2 ml of culture medium for 10 days at 37°C in a humidified atmosphere with 5% CO2. The culture supernatant was harvested and soluble (s) BAFF titres were determined using human BAFF ELISA kit (Bender Medsystems GmbH, Vienna, Austria). Assays were performed according to the manufacturer’s instructions.

Suppression of anti-dsDNA antibodies by TAC1-Ig
PBMCs were depleted of monocytes by adhesion to the plastic culture dishes and further purified into lymphocytes by complement (Cedarlane, Ontario, Canada) lysis with HNK-1 and 63D3 plus rat anti-mouse IgG1 mAb (PharMingen). The resulting cell population was <2% CD14+ and <2% CD57+. All cultures were conducted in RPMI 1640 medium supplemented with 10% FCS, 2 mM l-glutamine, penicillin G (200 U/ml) and gentamicin (10 μg/ml). Lymphocytes (2 × 10^6/well) were cultured in 96-well round-bottom plates in 0.2 ml of culture medium for 10 days at 37°C in a humidified atmosphere with 5% CO2. Nil, TAC1-Ig or control-Ig was added at the beginning of the experiment. The culture supernatants were harvested and anti-dsDNA titres were determined using an ELISA kit (Bio-Rad, CA, USA). Assays were performed according to the manufacturer’s instructions.

Statistical analysis
Statistical analysis was performed using non-parametric test for comparison of population samples. A value of P < 0.05 was used to reject the null hypothesis.

Results
Expression of BAFF or BAFF-R on circulating lymphocytes
To determine whether the increased BAFF antigen is produced by circulating T cells in patients with active SLE, we first examined its surface expression by flow cytometric analysis. Monocyte from active SLE was highly expressed BAFF antigen (CD14+; 28.6 ± 3.4) [mean fluorescence intensity (MFI)]. However, we did not detect any cell surface expression of BAFF on T cells from either control subjects or patients with active SLE (data not shown). We then looked for intracellular expression of BAFF in circulating T cells. A striking finding was that CD4+ T cells from patients with active SLE showed the intracellular BAFF expression, whereas those from normal controls did not (Fig. 1A). This CD4+ T cell population comprised almost entirely memory (CD45RO+) T cells (data not shown). Another unexpected finding was that CD8+ T cells from patients with active SLE also expressed the BAFF antigen (Fig. 1B), whereas circulating CD8+ T cells from normal controls did not.

In particular, patients with kidney involvement had significantly higher MFI of intracellular BAFF expression on CD4+ T cells (23.4 ± 8.74) and CD8+ T cells (20.1 ± 4.80) in comparison with non-kidney involvement (CD4+: 6.40 ± 0.57, CD8+: 19.7 ± 11.46) and normal controls (CD4+: 4.19 ± 0.74, CD8+: 6.01 ± 1.56), respectively (P < 0.01).

We next examined the expression of BAFF receptors on B cells. BAFF-R and TACI were expressed on B cells from both normal controls and patients with active SLE and the expression levels in the two groups did not differ significantly (Fig. 1C and D). Within the sensitivity limits of flow cytometric analysis, the expression density-gradient centrifugation. PBMCs were separated by the E rosette-positive and E rosette-negative populations with 5% sheep erythrocytes. The E rosette-positive cells were depleted of monocytes by adherence to the plastic surface of culture dishes and further purified T cells by complement (Cedarlane, Ontario, Canada) lysis with anti-CD57 (HNK-1; mouse IgM), anti-CD14 (63D3; mouse IgG1) plus rat anti-mouse IgG1 mAb (Becton Dickinson). The resultant T cell population was <2% CD19 and CD14, <2% CD57 and >93% CD3. T cells (2 × 10^6/well) were cultured in 96-well round-bottom plates in 0.2 ml of culture medium for 10 days at 37°C in a humidified atmosphere with 5% CO2. The culture supernatant was harvested and soluble (s) BAFF titres were determined using human BAFF ELISA kit (Bender Medsystems GmbH, Vienna, Austria). Assays were performed according to the manufacturer’s instructions.

BAFF secretion by T cells
PBMCs were isolated from active SLE and healthy controls by Ficoll–Hypaque (Pharmacia, Piscataway, NJ)
We then investigated the expression of mRNA for BAFF-R in CD20⁺ B cells from SLE and normal controls, and found that both groups expressed the BAFF-R mRNA, with no significant expression level between them (Fig. 1G and H).

**BAFF secretion by T cells from active SLE**

Then, we examined the BAFF secretion by T cells from active SLE. T cells from active SLE and normal controls produced 7.2 ± 1.1 and 1.3 ± 0.7 (ng/ml), respectively, of sBAFF in vitro without any stimulation (P < 0.05). We found T cells from active SLE produced higher amount of sBAFF than T cells from normal control under basal conditions.

In vitro suppression of anti-dsDNA antibodies production by TACI-Ig

To investigate the direct involvement of BAFF in T cell-dependent B cell autoantibody production, we then examined whether TACI-Ig inhibited spontaneous production of anti-dsDNA antibodies by cultured T and B cells from six patients with active SLE showing kidney involvement. Table 1 shows the characteristics of the individual SLE and the extent of suppression of anti-dsDNA antibody titres by TACI-Ig. The addition of TACI-Ig, but not control-Ig, significantly suppressed in vitro T cell-dependent anti-dsDNA antibodies production by B cells. These results strongly suggest that BAFF plays an important role in T-cell-dependent anti-dsDNA antibodies production in SLE patients through BAFF-R and/or TACI.

**Discussion**

In the present study, we have demonstrated abnormal production of BAFF in T cells from SLE (Fig. 1), especially in patients with kidney involvement. Furthermore, we showed that blocking of BAFF in T cell-B cell interaction reduced the production of autoantibody by TACI-Ig. These results suggest that another mechanism operates in the pathogenesis in SLE, i.e. autoantibody production driven by BAFF produced in part by T cells, supporting a previous study indicating expression of BAFF by T cells [4]. A recent study has also shown that BAFF is expressed in T cells infiltrating salivary glands in patients with Sjögren’s syndrome [14, 23]. Moreover, a very recent report has indicated that SLE T cells produce soluble BAFF upon stimulation and that the BAFF mRNA robustly induced by a human TE cell line, Loucy [24]. Therefore, we tried to stimulate T cells with anti-CD3 to study a possible increase of BAFF expression by intracellular cytometric assay (data not shown). The result was controversial. We speculate this reason why CD3/TCR-mediated response of purified T cells in SLE ranges normal to enhanced [25] and T cells from SLE produced higher amount of sBAFF than T cells from normal controls without any stimulation (P < 0.05). We found T cells from active SLE showed marked suppression of anti-dsDNA antibodies production in vitro by TACI-Ig.

We think that these results neglect the monocyte contamination by flow-cytometric analysis (CD14⁺ < 2%). However, CD4⁺ and CD8⁺ T cells did not express APRIL mRNA (data not shown).
CD8+ and CD4+ T cells for generation of pathogenic autoantibodies and that regulation of homoeostatic T cells is defective. Moreover, present study showed that blocking of BAFF in T cell–B cell interaction reduced the production of autoantibody by TACI-Ig which is a soluble decoy receptor for BAFF and APRIL. Patients with SLE have elevated serum levels of BAFF correlated with elevated levels of autoantibody Abs [15]. Therefore, BAFF may be an appropriate target for intervention in autoimmune diseases in which elevated levels of autoantibodies contribute to disease pathology. In NZB/W F1 mice, administration of TACI-Ig and/or BAFF-R-Ig prolongs the life span [28] and prevents the emergence of IgG anti-DNA antibodies [9]. That study has shown that treatment of NZB/W F1 mice with BAFF-R-Ig reduced the circulating levels of anti-dsDNA antibody titres in parallel with clinical improvement [9]. These results and our present data suggest that BAFF derived from T cells may also play a pathogenic role of SLE and blockade of BAFF as a promising therapeutic approach for SLE, especially in patients with kidney involvement.

Rheumatology key messages

- BAFF is present in intracellular T cells in active SLE patients.
- BAFF derived from T cells may play a pathogenic role of SLE, especially in patients with kidney involvement.
- BAFF is a therapeutic approach for SLE, especially in patients with kidney involvement.

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References