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Effect of interleukin-2 on synthesis of B cell activating factor belonging to the tumor necrosis factor family (BAFF) in human peripheral blood mononuclear cells

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ABSTRACT

B cell activating factor belonging to the tumor necrosis factor family (BAFF) is a cytokine, indispensable for B cell survival, maturation, and activation. Over-expression of BAFF leads to lupus like disease in mice and the serum level of BAFF is elevated in human lupus. However, little is known about BAFF synthesis and its regulation. In this study, we examined the effects of a series of inflammatory cytokines on BAFF production in human peripheral blood mononuclear cells (PBMCs) *in vitro*. We found interleukin-2 (IL-2) strongly and dose-dependently stimulated BAFF synthesis in PBMCs, and an anti-IL-2 antibody neutralized the effect. Furthermore, T and NK cells produced BAFF with IL-2 stimulation. From these observations, IL-2 is one of the regulatory cytokines having a positive effect on BAFF synthesis in human peripheral T and NK cells. Persistent over-production of IL-2 might lead to up-regulation of BAFF synthesis in PBMCs in pathological conditions such as lupus.

1. Introduction

B cell activating factor belonging to the tumor necrosis factor family (BAFF) is an important cytokine for B cell survival, maturation and activation. BAFF is a trimeric type II membrane-bound, 285 amino acid protein. It is cleaved by furin protease, secreted, and binds to three receptors, B cell maturation antigen (BCMA), transmembrane activator and calcium-modulator and cyclophilin ligand (CAML) interactor (TACI), and BAFF receptor (BAFF-R) [1–9].

BAFF transgenic mice develop lupus-like disease. The serum level of BAFF is elevated in human systemic lupus erythematosus (SLE) and other autoimmune diseases and it is correlated with clinical and immunological parameters, associated with disease activity. These results introduce a hypothesis that BAFF over-production may play a key role in pathogenetic processes such as inappropriate continuous B cell activation in SLE [10–15].

Initial reports have shown that BAFFs were mainly synthesized in macrophages, dendritic cells, and T cells. Besides haematopoietic cells, BAFF mRNA expression has been detected in various human tissues, such as brain, lung, and spleen. BAFF is produced by lipopolysaccharide (LPS) and interferon-γ in monocytes and by anti-CD3 antibody crosslinking in T cells [1–4].

However, little is currently known regarding BAFF synthesis and its physiological regulation. The present study thus investigated the effect of various cytokines on BAFF production in human peripheral blood mononuclear cells (PBMCs).

2. Materials and methods

2.1. Cell culture

The complete medium used for PBMC culture consisted of RPMI-1640 medium (Sigma Chemical, St. Louis, MO) supplemented with 10% (vol/vol) heat-inactivated fetal bovine serum (FBS) (Sigma), 2 M L-glutamine, sodium pyruvate, and HEPES.

2.2. Cell isolation and purification

Peripheral blood from healthy human volunteers was collected in a pre-heparinized tube, and PBMCs were isolated by Ficoll–Hypaque density-gradient centrifugation (Pharmacia Biotechnology, Uppsala, Sweden). Subpopulations of PBMCs (T cells, B cells, NK cells, and monocytes) were directly purified with RosetteSep™ (Stemcell Technologies Inc.). T cell subpopulations were sorted with a MACS column using specific antibodies, namely anti-human CD3-fluorescein isothiocyanate (FITC) antibody (UCHT-1, BD Bioscience), anti-human CD19-FITC antibody (HIB19, BD Bioscience), anti-humanCD56 antibody (B159, BD Bioscience), and anti-human CD14 antibody (RM052, Beckman Coulter (BC)). Subpopulations of T cells were purified with MACS (Miltenyi Biotech, Germany) and the abs used for flow cytometry were anti-CD4-FITC (13B8.2, BC),

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CD4-RD1 (T4, BC), CD8-FITC (T8, BC), CD8-RD1 (T8, BC), CD45RA-RD1 (2H4, BC), and CD45RO-RD1 (UCHL1, BC). Briefly, PBMCs ($0.5–1 \times 10^7$ cells) were stained with each fluorescence-conjugated antibody. The cells were washed in PBS twice, and stained with anti-fluorescence microbeads, Multisort anti-FITC or anti-phycoerythrin (PE) conjugated microbeads, (Miltenyi Biotech). The cells were rewashed twice. Cells attached to beads were purified with the column and washed. The purity in each subpopulation was ascertained cytometrically, FACS can (BD Bioscience) following staining with each antibody.

2.3. Inflammatory cytokine stimulation assay

Whole PBMCs (5×10^5/well) or purified subpopulation cells in 2 ml medium were cultured under 5% CO_2 at 37 °C in 24 well flat bottom plates (Multiwell, BD). For T cell stimulation, the wells were previously pre-courted for 3 h with an anti-human CD3 antibody (BD Bioscience) (0.1–10 ug/ml) and/or an anti-human CD28 antibody (BD Bioscience) (1–10 ug/ml) in 1 ml PBS. The wells were rinsed with fresh PBS three times before the addition of the cells.

For cytokine stimulation, each cytokine was premixed with the cells at the determined concentration. In the IL-2 blocking assay, a rat anti-human IL-2 antibody (BD Bioscience) was pre-bound to the wells in a similar manner as for the T cell stimulation assay. After 3 days' incubation, cells were harvested in microtubes and prepared for the protein assay. A rat IgG (BD Bioscience) was used for the negative controls. The inflammatory recombinant human (rh) cytokines used were as follows: rhTNF-α (Sigma), rhIFN-γ (BD Biosciences), rhIL-2 (BD Biosciences), rhIL-4 (BD Biosciences), rhIL-10 (BD Biosciences), and rhIL-18 (MBL, Nagoya, Japan).

2.4. Western blotting and quantification of the BAFF and beta-actin expression

Cells were lysed with 1 ml of lysis buffer (10 mM Tris–HCl (pH 8.0), 150 mM NaCl, 1% Nonidet P-40, 10 mM EDTA, 1 mM sodium orthovanadate, 1 mm PMSF, 10 μg/ml aprotinin, and 10 μg/ml leupeptin) at 4 °C for 15 min and were disrupted with sonication. After centrifuging at 10,000g for 5 min, the supernatant was loaded onto a 15% SDS–PAGE gel. The proteins were electrophoretically blotted onto polyvinylidene difluoride (PVDF) membranes (Millipore, Bedford, MA), and the membranes were soaked at 37 °C for 1 h in blocking agents (Blockace; Dainippon Pharmaceuticals, Tokyo, Japan). The blots were then probed with a rabbit anti-human BAFF antibody (Chemicon International, Temecula, CA) or a rabbit anti-human beta actin antibody (Sigma) at 16 °C for 1 h. The antibody was visualized using peroxidase-conjugated anti-rabbit IgG (Amersham Bioscience). Biotinylated proteins were detected using streptavidin-peroxidase (Southern Biotechnology Associates, Birmingham, AL). After washing three times, the signals were detected with chemiluminescence-enhancing reagents (Amersham Pharmacia Biotech). The treated membranes were visualized on ECL X-ray film (Amersham Pharmacia Biotech). The density of the specific bands was quantified by scanning with a Scan Jet II (Hewlett Packard) and National Institutes of Health Image software (version 1.56) and protein expression levels were semiquantified. We defined freshly isolated PBMC (Fig. 1–3c), monocytes (Fig. 3d) and T cells (Fig. 4) samples with no treatment as the absolute baseline and calculated fold induction values compared as baseline in each experiment.

2.5. Real-time PCR

Messenger RNA was isolated from PBMCs with an mRNA Isolation Kit (Pharmacia Biotechnology) immediately after separated from peripheral blood. The mRNA was converted to whole cDNA by reverse transcriptase. Using 5 μl of the whole cDNA derived from the PBMCs as the template, the cDNA was quantified by detection of TaqMan probe fluorescence using the ABI PRISM 7700 sequence detector system (Applied Biosystems). The relative expression level was determined by normalization to GAPDH. Specific primers and TaqMan probe sequences were as follows: BAFF, 5′-cgcgggactgaaaatctttg and 5′-cacgcttatttctgctgttctga; probe: 5′-ccaccagctccaggagaaggcaact; and GAPDH, TaqMan control reagent (Applied Biosystems). Amplification conditions were as follows: 15 sec at 95 °C for denaturation and 60 sec at 60 °C for annealing and primer extension (40 cycles).

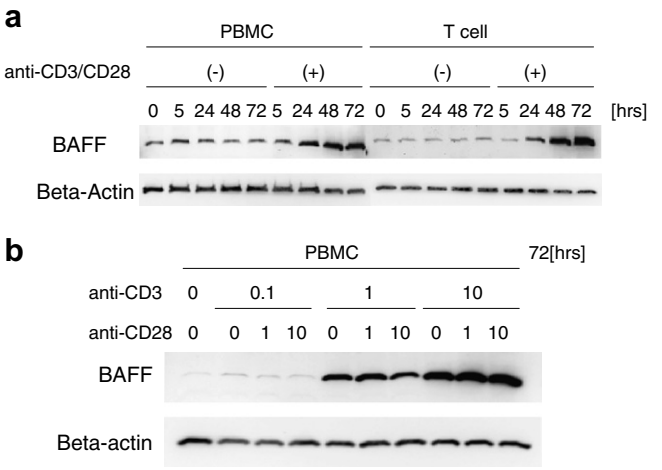

Fig. 1. BAFF synthesis up-regulation by anti-CD3/CD28 stimulation in PBMC. (a) Time course of BAFF synthesis in PBMC and T cells by *in vitro* anti-CD3/CD28 stimulation. After PBMC and T cells were purified, 5×10^5 cells/well were cultured in 1 ml of 10% FCS/RPMI and were pre-courted with or without anti-CD3 and anti-CD28 antibody plating under the conditions of 5% CO_2 at 37 °C. After incubation for 5, 12, 24 48, and 72 h, cells were harvested, and BAFF protein was measured with Western blot analysis. (b) Effect on BAFF synthesis by various doses of anti-CD3/CD28 stimulation. Various dose combinations of the stimulating antibody (anti-CD3 [0, 0.1, 1, 10 μg/ml] and CD28 [0.1, 1, 10 μg/ml]) were determined and cultures were performed in PBMCs in the same way as the experiments in (a). β-Actin is shown as the loading control.

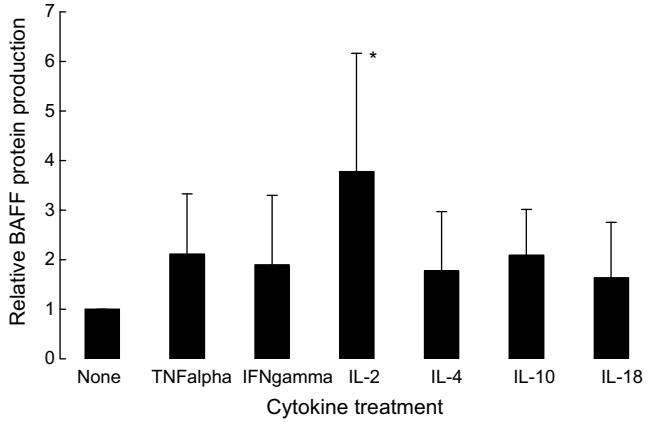

Fig. 2. Screening of the effect on BAFF synthesis by stimulation with various inflammatory cytokines in PBMCs. PBMCs were cultured for 72 h with none, rhTNF-α (10 ng/ml), rhIFN-γ (10 ng/ml), rhIL-2 (10 ng/ml), rhIL-4 (10 ng/ml), rhIL-10 (10 ng/ml), and rhIL-18 (10 ng/ml). BAFF protein expression was quantified with Western blot analysis. Relative BAFF expression and standard deviation in 7 healthy donors are shown compared for each donor without treatment after calibration by β-actin expression. Statistical significance was calculated with a non-repeated measure of ANOVA. p value < 0.05 is indicated by an asterisk (*).