Dexamethasone inhibits BAFF expression in fibroblast-like synoviocytes from patients with rheumatoid arthritis

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Abstract

Fibroblast-like synoviocytes (FLS) play a major role in the pathogenesis of rheumatoid arthritis (RA). FLS isolated from patients with RA (FLS-RA) express B cell-activating factor belonging to the TNF family (BAFF), a cytokine that has been associated with the onset and progression of RA. Glucocorticoids are powerful anti-inflammatory drugs used in the treatment of RA. In the present study, we examined the effect of dexamethasone (Dex) on constitutive and TNF-α- and IFN-γ-induced BAFF expression in FLS-RA. BAFF mRNA expression and soluble BAFF were determined by RT-PCR and ELISA, respectively. The results showed that constitutive BAFF mRNA expression was inhibited by Dex in a dose- and time-dependent manner. Also, Dex inhibited the secretion of BAFF in a time-dependent manner reaching 76% of inhibition 72 h after treatment. Moreover, Dex suppressed both mRNA and protein BAFF expression induced by TNF-α but had no effect on IFN-γ-induced BAFF expression. In comparison with B cells cultured alone, B cells co-cultured with FLS-RA exhibited a higher survival, which was inhibited when FLS-RA were pretreated with Dex. However, the enhanced B cell survival was reestablished by the addition of rhBAFF. Therefore, Dex is a potent inhibitor of constitutive and TNF-α-induced BAFF expression in FLS-RA.

Keywords: Glucocorticoid; BAFF; Rheumatoid arthritis; Synoviocytes; Inflammation

1. Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease characterized by hyperplasia of the synovial lining, composed of fibroblast-like synoviocytes (FLS) and macrophage-like synoviocytes. Growing evidence suggests that FLS play a major role in the pathogenesis of RA. FLS modulate the joint inflammation through the expression of cytokines and chemokines, such as interleukin (IL)-6; IL-8 and monocyte chemoattractant protein (MCP)-1; support myeloid and lymphoid cell growth and angiogenesis by releasing various growth factors including epidermal growth factor (EGF); and promote matrix degradation through the production of matrix metalloproteinases and cathepsins (for review see [1–3]).

B cell-activating factor belonging to the TNF family (BAFF; also called BlyS, TALL-1, THANK, zTNF4) is a recently described member of the TNF family ligands [4,5]. BAFF exists as a soluble and a cell surface molecule. Several studies indicate that it is involved both in the maturation and survival of B cells in the periphery (for review

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see [6–8]) and the activation and effector functions of T cells [9–12]. Initially, it was described that the expression of BAFF was highly restricted to cells of the myeloid lineage such as monocytes, macrophages and dendritic cells [13,14]. However, recently it has been shown that bone marrow stromal cells [15] and follicular dendritic cells [16] express BAFF constitutively. The expression of BAFF can be up-regulated in myeloid cells [14], astrocytes [17] and salivary gland epithelial cells [18] by pro-inflammatory cytokines such as IFN-α, IFN-γ, TNF-α and a combination of them, while that BAFF can be induced in neutrophils by G-CSF [19]. In contrast, stimuli inhibiting BAFF expression are less well known.

Elevated levels of BAFF have been detected in the serum of patients with autoimmune and chronic inflammatory diseases, such as systemic lupus erythematosus (SLE) [20,21]; Sjögren's syndrome (SS) [20,22]; bullous pemphigoid [23]; asthma [24] and chronic hepatitis C virus infection [25,26].

Evidence suggests that BAFF plays an important role in the pathogenesis of RA. Increased levels of BAFF have been detected in the serum of patients with RA [27] and a positive correlation was established between the intensity of the local inflammatory response and the levels of BAFF in the joint of patients with RA [28]. Moreover, concentrations of BAFF were higher in the synovial fluid that in the serum of RA patients [21,27,28], suggesting local production of BAFF by the inflamed synovium. Recently, it has been shown that macrophages [29] and FLS from patients with RA (FLS-RA) [30] express BAFF constitutively and that its expression was up-regulated in FLS-RA upon stimulation by TNF-α and IFN-γ [30]. Besides, increased BAFF production has been related to the onset and progression of disease in a collagen-induced arthritis (CIA) mouse model [31]. In these mice, splenic macrophages and dendritic cells expressed high levels of BAFF during the progression of RA [31]. Interestingly, the treatment with TACI-Fc, a soluble decoy receptor for BAFF, inhibited the production of autoantibodies and delayed the progression of disease in CIA mice [32]. In addition, using a xenogeneic model generated by implanting synovial tissue from RA patients into NOD-SCID mice, it was demonstrated that TACI-Fc abrogated the development of ectopic germinal centers, inhibited the human mRNA production of IgG, IFN-γ and TNF-α and reduced the number of infiltrating B and T lymphocytes in the transplanted tissues graft [29].

Glucocorticoids (GCs) are powerful drugs with strong, albeit non-specific, anti-inflammatory and immunomodulatory effects used in the treatment of RA and many other inflammatory diseases [33,34]. This effect is mediated in part through down-regulation of inflammatory cytokines expression such as TNF-α and IFN-γ [33,35]. Previous reports have described that the treatment with GCs induces a marked decrease in BAFF levels in the serum of patients with SLE, asthma and bullous pemphigoid [23,24,36]. However, from these studies it is not clear if BAFF expression is directly affected by GCs or if attenuation of BAFF levels could be a consequence of the action of GCs upon others factors regulating BAFF expression, such as TNF-α or IFN-γ.

In this study, we were interested in analyzing the effect of dexamethasone (Dex), a synthetic glucocorticoid, on the constitutive and TNF-α- and IFN-γ-induced BAFF expression in FLS-RA.

2. Materials and methods

2.1. Cell isolation and culture

FLS were obtained from seven RA patients fulfilling the American College of Rheumathology 1987 criteria for the diagnosis of RA [37]. All samples were obtained after the patients had given their informed consent according to the Declaration of Helsinki and the study was approved by Ethics Committee of the Clínica Alemana Universidad del Desarrollo. Samples of synovial fluid (SF) were obtained by aspiration from the knee joints and biopsies of synovial membrane (SM) were obtained by arthroscopic biopsy. Three samples of SM from normal individuals were used as control. FLS were isolated from both SF and SM according to Neidhart et al. [38] and Zimmermann et al. [39], respectively. Upon reaching confluence, the cells isolated were split at a dilution 1:2 by tripsinization (trypsin/EDTA, Life Technologies, NY, USA) and grown in DMEM supplemented with 10% fetal bovine serum (FBS), L-glutamine and antibiotics (all from Life Technologies). Dermal fibroblasts (DF) from healthy children foreskin were maintained in DMEM supplemented with 20% FBS, L-glutamine and antibiotics. The human promyeloblastic cell line HL-60 was obtained from American Type Culture Collection (Rockville, MD, USA), and they were grown in RPMI 1640 (Life Technologies) supplemented with 10% FBS, L-glutamine and antibiotics.

FLS and DF, between the third and seventh passages, were grown in 60 mm tissue-cultured plates at a density of 0.3×10^6 cells/plate. For experiments, the cells were first washed with phosphate buffered saline (PBS) and then cultured in 2 mL of DMEM supplemented with 5% FBS in the absence or presence of either different doses of human recombinant IFN-γ (R&D Systems, Minneapolis, MN, USA) during 24 h or with 100 U/mL of IFN-γ for different time periods. In order to determine the effect of Dex on the BAFF expression, FLS were either incubated with different doses of Dex (Sigma, St. Louis, MO, USA) for 24 h or with 1 μM of Dex for different time periods. In other experiments, FLS were pre-incubated with 1 μM of Dex for 2 h and then treated with 100 U/mL of IFN-γ or with 10 ng/mL of human recombinant TNF-α (R&D Systems, Minneapolis, MN, USA) for another 24 h.

Peripheral blood mononuclear cells were isolated from healthy donors by density gradient centrifugation using Ficoll-Hypaque 1077 (Sigma–Aldrich, St. Louis, MO, USA). Then, B cells were stained with a mouse monoclonal

anti-human CD20 antibody conjugated with fluorescein isothiocyanate (FITC) (BD Pharmingen, San Diego, CA, USA) and purified using anti-FITC microbeads (Miltenyi Biotec, Germany) following manufacturer's instruction. The purity of the isolated B cells was >90% as assessed by flow cytometry.

2.2. Flow cytometry analysis

FLS and DF were harvested, washed with PBS supplemented with 2% FBS (PBS/2% FBS) and incubated with mouse monoclonal antibodies anti-human Thy-1 (CD90) (Dianova, Hamburg, Germany) or anti-human CD14 (BD Pharmingen, San Diego, CA, USA) on ice for 30 min. After washing in PBS/2% FBS, cells were incubated with a goat anti-mouse IgG antibody (F(ab′)2 fragment) conjugated with fluorescein isothiocyanate (FITC) (Dako, Carpinteria, CA, USA) on ice for 30 min. After washing once with PBS/2% FBS, cells were resuspended at a density of 1×10^6 cells/mL in PBS. In order to determine cell viability, propidium iodide was added at a final concentration of 10 μg/mL. Fluorescence analysis was performed with a FACScan flow cytometer and CellQuest software (Becton Dickinson, San Jose, CA, USA).

2.3. Reverse transcriptase-polymerase chain reaction (RT-PCR)

RNA was extracted by using Trizol reagent as suggested by the manufacturer (Life Technologies). cDNA synthesis was carried out for 1 h at 42 °C by incubating 1 μg of RNA, 500 ng of oligo$(dT)_{18}$ (TIB Molbiol, Berlin, Germany), 200 U MMLV-reverse transcriptase (Promega, Madison, USA), 24 U RNasin (Promega, Madison, USA) and 0.5 mM dNTP (Life Technologies) dissolved in MMLV-RT buffer to 20 μL final volume. PCRs were performed in a Mastercycler personal thermocycler (Eppendorf, Hamburg, Germany) on a reaction mix containing 2 μL of the resulting cDNAs, 200 ng of the sense and antisense primers, 200 μM dNTP, 2.5 mM $MgCl_2$ and 5 U *Taq* DNA polymerase (Life Technologies) in AmpliTaq buffer to 25 μL final volume. Primers were designed to span intron/exon junctions to differentiate between cDNA and genomic DNA. The sequences for primers were as follows: sense 5′-TCACGCCTTACTTCTTGC-3′ and antisense 5′-CACCAGACTCAATTCATCC-3′ for BAFF (654 bp) and sense 5′-CCGATCCACACGGAGTACTT-3′ and antisense 5′-AAATCGTGCGTGACATTAAGG-3′ for β-actin (406 bp). The amplification profile were: 30 cycles of 60 s at 94 °C, 60 s at 60 °C and 90 s at 72 °C for BAFF; and 25 cycles of 30 s at 94 °C, 45 s at 60 °C and 90 s at 72 °C for β-actin. All profiles included a first cycle of 5 min at 94 °C and a final extension cycle of 10 min at 72 °C. Ten microliters of the PCR product were electrophoresed on a 1.2% agarose gel and stained with ethidium bromide to visualize the amplification product. Quantification of the PCR bands was performed by densitometric analysis using image analysis software (Scion Image for windows). The data were normalized to β-actin expression.

2.4. ELISA

Supernatants of cultures were recollected, centrifuged at 600*g* for 7 min and stored at −80 °C. The concentration of soluble BAFF in the supernatants was assayed by ELISA following the manufacturer's recommendations (R&D Systems). The sensitivity of ELISA kit was 2.43 pg/mL. Data are expressed as means ± SEM cytokine concentration (pg/mL). In order to compare the secretion of BAFF between different types of cells, BAFF concentration was normalized by the number of cells (pg/mL/1×10^6 cells).

2.5. Viability assay

FLS-RA (1.5×10^6 cells/well) were cultured into 24-well plates for 4 days. Then, the medium was discarded and the cells were washed with fresh serum-free medium and cultured in the presence of either 1 μM of Dex or medium alone for 24 h. FLS-RA were further washed and incubated with 0.5×10^6 B cells in RPMI 1640 medium supplemented with 10% FBS and antibiotics. The cells were co-cultured with either 0.5 μg/mL of recombinant human BAFF (PeproTech Inc., New Jersey, USA) or medium alone for 4 days. Then, the B cells were collected by gentle washings and the remaining B cells and FLS-RA recovered from wells by treatment for 10 min at 37 °C with 1.3 mM EDTA in PBS (Invitrogen Life Technologies, NY, USA). The cells were further mixed and stained with a mouse monoclonal anti-human CD20 antibody conjugated with fluorescein isothiocyanate (FITC) (BD Pharmingen, San Diego, CA, USA) on ice for 30 min. After washing once with PBS/2% FBS, cells were resuspended at a density of 1×10^6 cells/mL in PBS. The absolute number of vital B cells was determined according to Bono et al. [40]. Briefly, cells were transferred into flow cytometer tubes and stained with 1 μL of propidium iodide (1 mg/mL)/0.1×10^6 cells. To obtain the absolute number of B cells, 5×10^5 Calibrate beads (BD Pharmingen, San Diego, CA, USA) were added to each cell suspension as an internal standard and cells and beads were counted on a cytometer. The number of beads acquired for each experimental point is obtained from a region (R1) designed around the bead population in an ungated FSC/SSC dot plot. In the same dot plot, a second region (R2) was defined around the lymphocyte population. Next, a region for live cells (R3) was defined on a FL3/FSC dot plot excluding propidium iodide stained cells and debris. Finally, the numbers of live B cells was obtained from FL1/FL3 dot plot gated on R2 and R3 ($R2 * R3$). The absolute number of live B cells was calculated as follows: (average number of labeled live B cells in a given gate/average number of beads of ungated data) × number of beads added to the test. Percentage of B cell survival was determined by the formula: (number of live B cells × 100)/initial number of added B cells.

2.6. Statistical analyses

For statistical analyses, Mann–Whitney U test or Bonferroni t-test were used for comparisons between multiple groups. Analyses were performed using GraphPad Prism Software.

3. Results

3.1. Constitutive expression of BAFF in FLS-RA

FLS isolated from patients with RA (FLS-RA) or with patellar dislocation (Normal FLS) were grown *in vitro* to obtain an enriched population of fibroblast. Next, the cells were analyzed for the expression of cell surface markers of fibroblasts (Thy-1) and macrophages (CD14) by flow cytometry. Human normal primary dermal fibroblasts (DF) were used as positive control of Thy-1 expression. Homogeneous populations of FLS (>95% Thy-1 and <1% CD14 positive) (Fig. 1), were then used for the subsequent experiments. First, we analyzed the expression of BAFF mRNA in FLS-RA by RT-PCR. HL-60, a human promyeloblastic cell line known to express BAFF constitutively was used as positive control. FLS and DF from healthy donors were used as negative control. Consistent with a previous report [30], FLS-RA expressed constitutive levels of BAFF mRNA. The pattern of expression was variable between the samples derived from different patients (Fig. 2A). This variability was independent of whether FLS-RA was isolated from synovial fluid or membrane and independent of the number of culture passages. As expected, normal FLS and DF expressed either none or very low levels of BAFF mRNA. We next analyzed whether the soluble form of BAFF is constitutively secreted by FLS-RA and DF. Therefore, the supernatants of unstimulated cells were assayed by ELISA after 72 h of culture. Significantly higher amounts of BAFF were

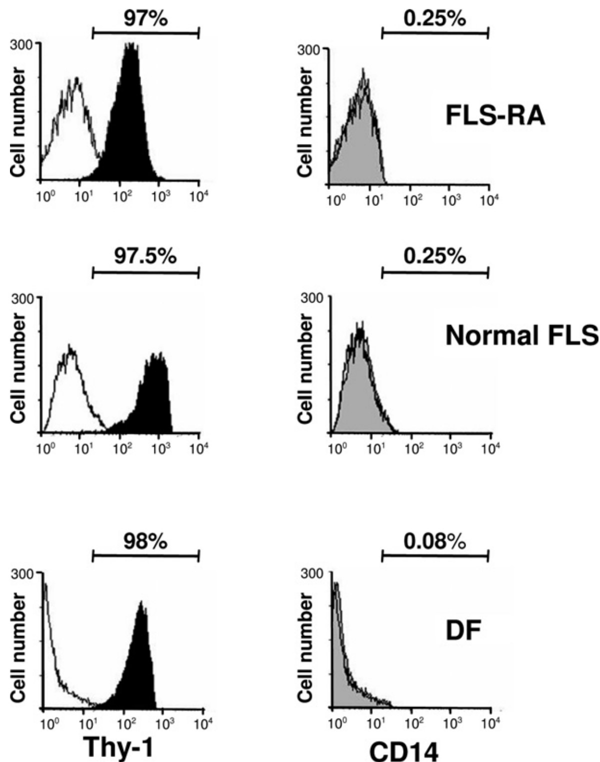

Fig. 1. Phenotype of primary culture of FLS-RA. FLS-RA, normal FLS and dermal fibroblast (DF) were incubated with either anti-Thy-1 (black histograms) or anti-CD14 (grey histograms) primary antibodies followed by incubation with FITC-conjugated secondary antibody. As specificity control, FLS-RA, normal FLS and DF were incubated with only FITC-conjugated secondary antibody (white histograms). Cells were analyzed by flow cytometry. Bars indicate the percentage of positive cells for the respective cell surface marker. A representative staining pattern is shown.

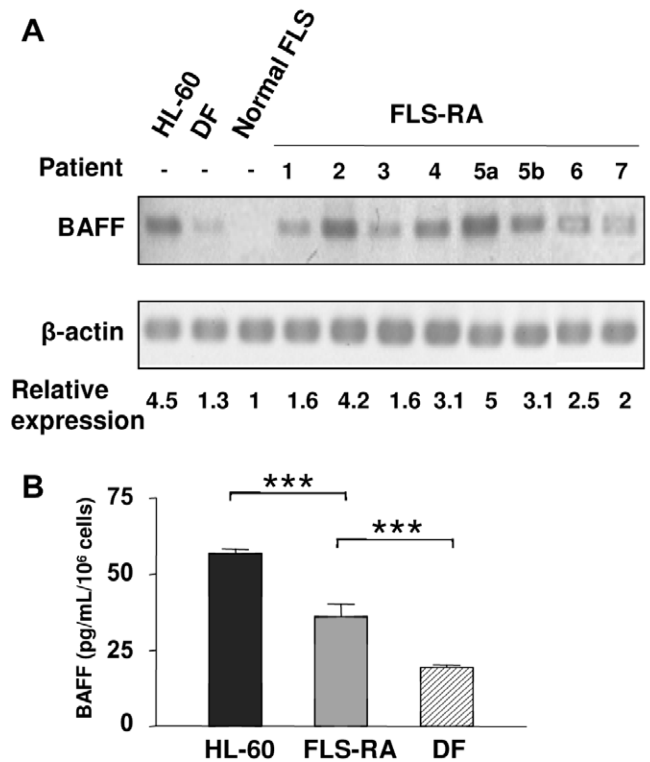

Fig. 2. BAFF mRNA expression and secretion in FLS-RA. (A) FLS-RA, normal FLS, dermal fibroblast (DF) and HL-60 promyeloblastic cell line were cultured for 72 h. Patient 5a and 5b correspond to FLS obtained from two joints with synovitis of a same RA patient. Total RNA isolated from these cells was utilized for synthesis of cDNA by reverse transcription. BAFF mRNA was amplified by PCR. β-Actin mRNA expression was used as expression and loading control. One of three similar independent experiments using FLS-RA from seven RA patients and FLS from three normal donors is presented. PCR amplifications were quantified by densitometric analysis. Relative expression represents the BAFF expression normalized to β-actin (BAFF/β-actin) of each cell culture with respect to the expression of BAFF/β-actin measured in normal FLS. (B) HL-60 (black bar; 5×10^6 cells), FLS-RA (grey bar; 0.3×10^6 cells) and DF (shaded bar; 0.3×10^6 cells) were grown for 72 h. Then, the cells were counted and cell-free supernatants, obtained by centrifugation, were analyzed for the presence of BAFF by ELISA. Data were normalized by cell number and they are expressed as means ± SEM of two independent experiments performed in duplicate and include FLS from seven RA patients and DF from two individuals. Asterisks represent statistically significant differences between samples ($p < 0.001$, by Bonferroni t-test).

detected in the conditioned media from FLS-RA and HL-60 when compared with DF (Fig. 2B).

3.2. IFN-γ induces the expression and secretion of BAFF in FLS-RA

A previous study has shown that IFN-γ, TNF-α or a combination of both were potent inducers of BAFF expression in FLS-RA [30]. In order to determine whether our FLS-RA cultures were able to up-regulate the expression of BAFF in response to inflammatory stimuli, we analyzed the expression of BAFF in FLS-RA treated with IFN-γ by RT-PCR. The results showed that IFN-γ increased the expression of BAFF mRNA in FLS-RA in a dose- and time-dependent manner (Fig. 3A and B). Similar experiments were then performed to investigate whether IFN-γ also regulates BAFF expression in DF. As in FLS-RA, IFN-γ was also able to induce the expression of BAFF in DF in a dose- and time-dependent manner (Fig. 3C and D). Next, we determined the effect of IFN-γ on the secretion of BAFF in FLS-RA and DF by ELISA. The results showed that IFN-γ strongly induced secretion of BAFF in FLS-RA as well as in DF (Fig. 3E). In comparison with unstimulated cells, IFN-γ treatment induced a 21.3- and 27.9-fold increase in the secretion of BAFF on FLS-RA and DF, respectively.

3.3. Constitutive expression of BAFF in FLS-RA is inhibited by Dex

Because FLS obtained from RA synovium, a chronic inflammatory microenvironment, exhibit constitutive expression of BAFF and as pro-inflammatory cytokines up-regulate the BAFF expression in these cells, we investi-

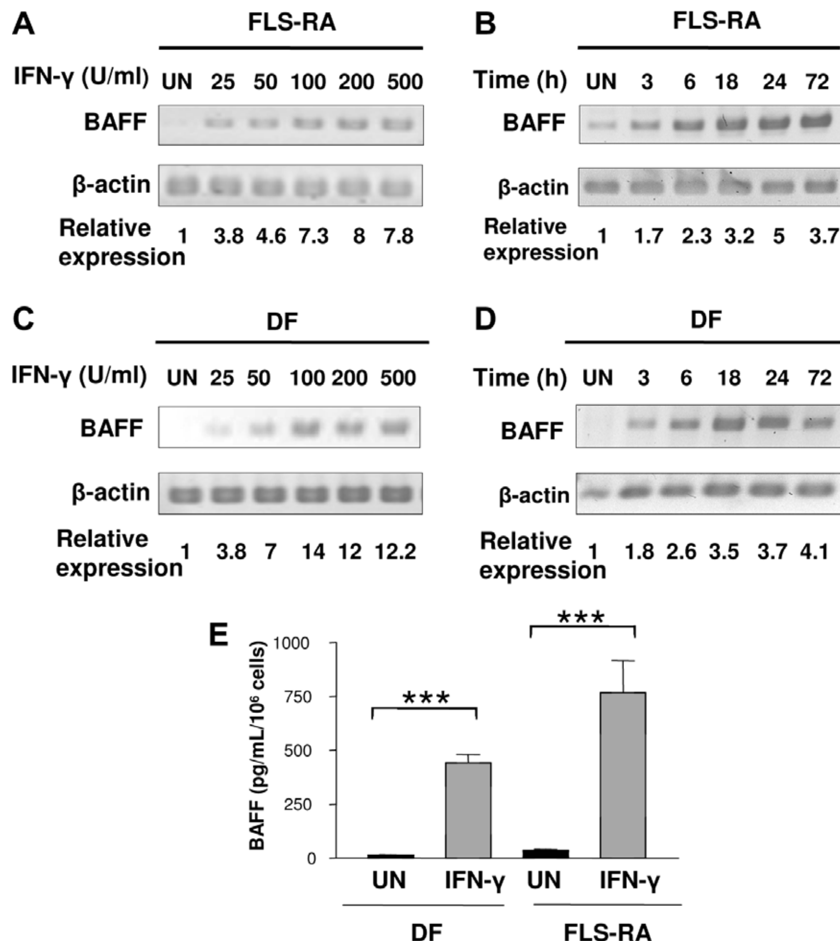

Fig. 3. BAFF mRNA expression and secretion in FLS-RA and DF induced by IFN-γ. FLS-RA and DF were either untreated (UN) or treated with different doses of IFN-γ during 24 h (A and C) or with 100 U/mL of IFN-γ for different time periods (B and D). Then, BAFF mRNA expression was determined by RT-PCR. β-Actin mRNA expression was used as expression and loading control. Relative expression represents the BAFF expression normalized to β-actin (BAFF/β-actin) in each cell culture with respect to the expression of BAFF/β-actin measured in untreated cells. A representative result of two independent experiments using FLS from three RA patients and DF from two individuals is shown. (E) FLS-RA and DF (0.3×10^6 cells) were grown in the absence (UN, black bar) or presence of 100 U/mL of IFN-γ (grey bar). After 72 h, the number of cells was counted and cell-free supernatants were prepared by centrifugation and processed for the detection of BAFF by ELISA. Data were normalized by number of cells and are expressed as means ± SEM of two independent experiments performed in duplicate and include FLS-RA from four patients and DF from two individuals. Asterisks represent statistically significant differences between untreated and IFN-γ treated samples ($p < 0.001$, by Bonferroni *t*-test).

gated whether Dex, a glucocorticoid with potent anti-inflammatory effects, is able to inhibit the constitutive expression of BAFF in FLS-RA. The results showed that Dex suppressed the constitutive expression of BAFF mRNA in a dose- and time-dependent manner (Fig. 4A and B). The Fig. 4A shows that even low doses of Dex (0.1 μM) inhibited the expression of BAFF in FLS-RA, reaching maximum suppression at 1 μM of Dex. Kinetic analysis showed an early inhibition of BAFF mRNA 3 h after Dex treatment, which reached a maximum suppression at 24 h of stimulation (Fig. 4B). Moreover, the secretion of BAFF was strongly down-regulated by Dex in a time-dependent manner reaching 76% of inhibition 72 h after of treatment (Fig. 4C).

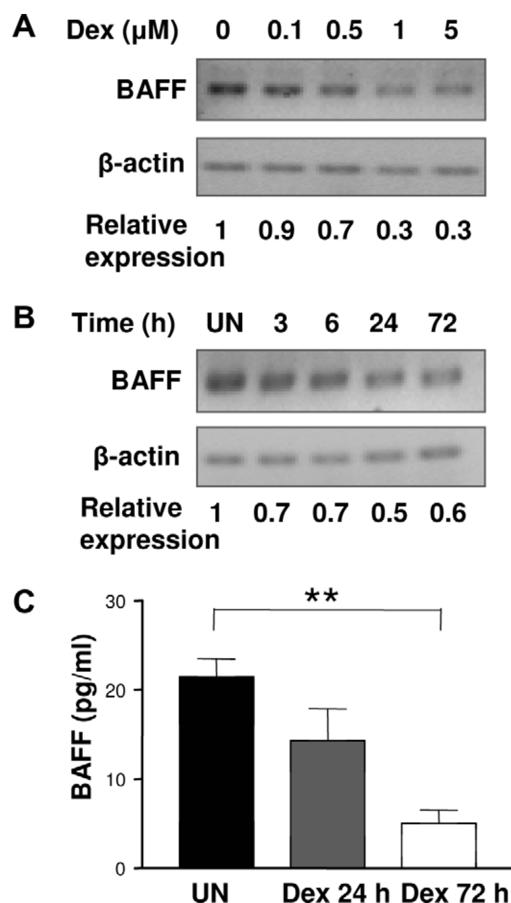

Fig. 4. Dex inhibits constitutive BAFF mRNA expression and secretion in FLS-RA. FLS-RA were incubated either with different doses of Dex for 24 h (A) or in the absence (UN) or presence of Dex (1 μM) for different time periods (B). Then, BAFF mRNA expression was determined by RT-PCR. β-Actin mRNA expression was used as expression and loading control. Relative expression represents the BAFF expression normalized by β-actin (BAFF/β-actin) in each cell culture with respect to the expression of BAFF/β-actin measured in untreated cells. A representative result of two independent experiments using FLS from three RA patients is shown. (C) FLS-RA were incubated with media alone (UN; black bar) or with Dex (1 μM) for 24 (grey bar) or 72 h (white bar). Supernatants were collected and BAFF concentration was assayed by ELISA. Results are expressed as means ± SEM of two independent experiments performed in duplicate using FLS-RA from four patients. Asterisks represent statistically significant differences between untreated and Dex-treated samples ($p < 0.01$, by Bonferroni t-test).

3.4. Effect of Dex on the expression of BAFF induced by IFN-γ and TNF-α

We next analyzed the effect of Dex on the BAFF expression induced by IFN-γ and TNF-α in FLS-RA. Fig. 5A shows that, as expected, FLS-RA up-regulated the expression of BAFF mRNA 24 h after the treatment with TNF-α or IFN-γ. However, 2 h pre-treatment with Dex inhibited the expression of BAFF mRNA stimulated by TNF-α, while a slight decrease in IFN-γ-induced BAFF expression was observed. ELISAs performed with the supernatants of the same cultures used for RT-PCR analysis showed that Dex significantly inhibited TNF-α-induced BAFF secre-

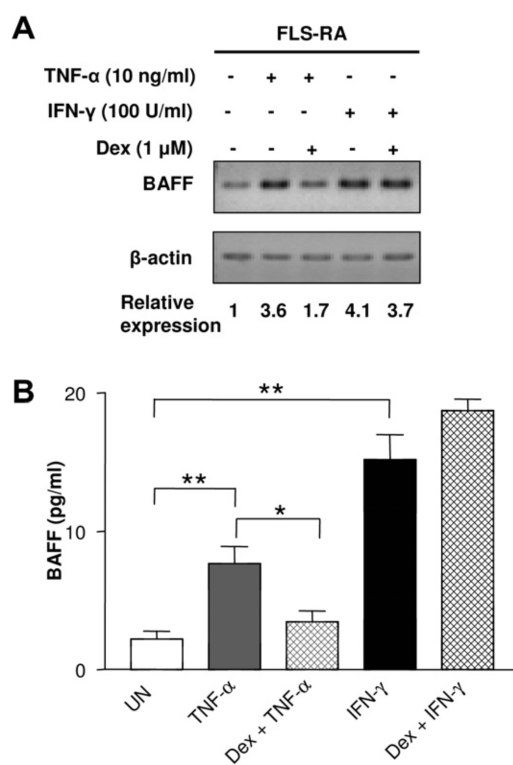

Fig. 5. Effect of Dex on mRNA expression and secretion of BAFF in FLS-RA stimulated by TNF-α and IFN-γ. FLS-RA were incubated in either media alone (UN, white bar); with TNF-α (10 ng/mL) (grey bar); with IFN-γ (100 U/mL) (black bar) for 24 h or pretreated with Dex (1 μM) for 2 h followed for the addition of TNF-α (10 ng/mL) (grey shaded bar) or IFN-γ (100 U/mL) (black shaded bar) for another 24 h. (A) BAFF mRNA expression was determined by RT-PCR. β-Actin mRNA expression was used as expression and loading control. Relative expression represents the BAFF expression normalized to β-actin (BAFF/β-actin) in each cell culture with respect to the expression of BAFF/β-actin measured in untreated cells. A representative result of two independent experiments using FLS from three RA patients is shown. (B) Supernatants from FLS-RA were collected and BAFF concentration was assayed by ELISA. Results are expressed as means ± SEM of two independent experiments performed in duplicate and include FLS-RA from four RA patients. Asterisks represent statistically significant differences between untreated and treated FLS-RA ($^{**}p < 0.01$, $^{*}p < 0.05$ by Bonferroni t-test).

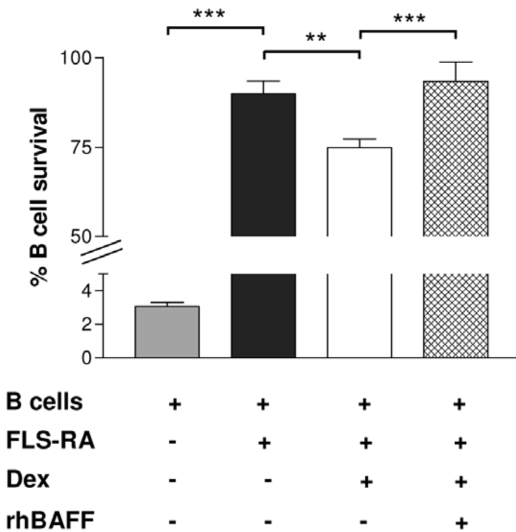

Fig. 6. Effect of Dex on the survival of B cells co-cultured with FLS-RA. Purified B cells were cultured alone (grey bar) or co-cultured with either untreated FLS-RA (black bar); with FLS-RA pre-treated with 1 μM of Dex for 24 h (conditioned, white bar) or with conditioned FLS-RA and 0.5 μg/mL of rhBAFF (shaded bar), for 4 days. Then, the percentage of live CD20–positive B cells was assessed by flow cytometry as described. Results are expressed as means ± SEM of two independent experiments performed in triplicate. Asterisks represent statistically significant differences ($^{***}p < 0.001$, $^{**}p < 0.05$ by Bonferroni t-test).

tion, but had not effect on the secretion induced by IFN-γ (Fig. 5B). In these experiments, untreated and Dex-treated cells were analyzed 24 h after stimulation, which could explain the lower amount of BAFF detected in the supernatants of untreated cells, in comparison to untreated cells cultured for 72 h (Figs. 2B, 3E and 4C).

3.5. Dex inhibits the enhanced survival of B cells induced by co-culturing with FLS-RA

In order to analyze functional effects derived from BAFF inhibition by Dex, we determined the viability of B cells co-cultured with FLS-RA. Consistent with a previous study, B cells cultured with FLS-RA during 4 days showed a significantly higher viability that B cells cultured alone (Fig. 6). However, the enhanced survival of B cells was significantly inhibited when FLS-RA were pretreated during 24 h with 1 μM of Dex. The improved B cell survival was reestablished by addition of recombinant human BAFF (Fig. 6), indicating that BAFF expression was selectively inhibited by Dex.

4. Discussion

Several data supports the pivotal role of BAFF in the pathogenesis of RA. Elevated levels of BAFF have been detected in the serum of RA patients [21,27,28] and studies using both CIA mouse model and human synovium-SCID mouse chimeras have suggested that increased production of BAFF may contribute to the onset and progression of RA [29,31,32]. Different stimuli such as cytokines, including IL-10, IFN-α, IFN-γ and TNF-α, as well as bacterial components like LPS and peptidoglycan or CD40 ligand augment BAFF expression in different cell types [7,8]. However, very little is known about the stimuli inhibiting BAFF expression.

In the present study, we show for the first time an inhibitory effect of Dex on constitutive mRNA expression and protein secretion of BAFF in FLS-RA. Kinetic analysis demonstrated an early inhibition of BAFF mRNA expression 3 h after Dex treatment, suggesting a direct transcriptional effect. Moreover, we have found that Dex suppressed TNF-α-induced expression of BAFF but had not effect on IFN-γ-mediated expression of BAFF, suggesting a selective inhibitory effect of Dex on the TNF-α signaling pathway.

GCs have long been recognized to have beneficial effects in RA. Over the last decade several clinical trials have further documented the efficacy of GCs in relieving inflammation and in preventing erosions in patients with RA (for review see [33]). This effect is mediated in part through of down-regulation of inflammatory cytokines. In FLS-RA, it has been described that Dex is able to inhibit the gene expression and protein secretion of IL-8 [41], IL-6 and IL-1β [42]. In these same cells, Dex also substantially suppressed the TNF-α-stimulated production of IL-8, MCP-1 and IL-1 receptor antagonist (IL-1Ra) [41]. In this study, we show that gene and protein BAFF expression by FLS-RA is down-regulated by Dex, which may also contribute to explain the beneficial effects of GCs in the treatment of RA.

Although we did not analyze the mechanism by which Dex dramatically decreased constitutive and TNF-α-induced expression of BAFF, there is evidence that may suggest a possible mechanism of regulation. GCs action is mediated through their interaction with cytoplasmic glucocorticoid receptors (GR). Then, GC/GR complexes translocate to the cell nucleus where they bind to specific DNA motifs, called glucocorticoid-responsive elements (GRE). The mechanism of GC-mediated transcriptional repression involve either interaction direct between GC/GR and a negative GRE (nGRE) or via protein–protein interactions with others transcription factors such as nuclear factor-κB (NF-κB), activator protein 1 (AP-1) or nuclear factor of activated T cells (NFAT) leading to inhibition of the nuclear translocation and/or function of these transcription factors (for review see [35,43–45]). GC can also up-regulate the synthesis of IκBα, which binds NF-κB to sequester it in the cytoplasm by masking the nuclear localizing sequence [46]. Recently, it has been reported that NF-κB and NFAT transcription factors are involved in the constitutive BAFF expression by binding to one NF-κB and two NFAT sites in the BAFF promoter [47]. Thus, Dex may inhibit constitutive BAFF expression by interfering with the transcriptional activity of NF-κB and/or NFAT. As it is well known that the TNF-α signaling pathway involves NF-κB activation, Dex may also inhibit

TNF-α-induced expression of BAFF by interacting with NF-κB. Previous studies have shown that Dex had no effect either on the DNA-binding activity of NF-κB or on the synthesis of IκBα protein in FLS-RA stimulated by TNF-α [42,48,49]. However, further assays will be necessary in order to determine whether Dex interferes with the binding and transcriptional activity of NF-κB on the BAFF promoter.

Our results are in line with a previous study showing constitutive and IFN-γ and TNF-α-induced BAFF expression by FLS-RA [30]. We extended those data showing that these cells secrete a significant amount of BAFF protein, which could be strongly induced in response to treatment with IFN-γ and TNF-α (Figs. 2B, 3F and 5B). We also showed that DF were strongly induced to express and secrete BAFF in response to IFN-γ (Figs. 3 and 4). Likewise, it has been reported that myeloid cells [14], astrocytes [17] and salivary gland epithelial cells from controls and patients with SS are induced to express and secrete BAFF after stimulation with IFNs, TNF-α or a combination of them [18]. This evidence demonstrates that an inflammatory microenvironment could induce the expression of high levels of BAFF in different cell types. Although this event could be part of the normal immune response against foreign stimulus such as infections, there is the notion that a persistent BAFF production induced by chronic inflammatory conditions could mediate the onset and/or progression of autoimmune diseases.

In the RA synovium, BAFF could exert the effects formerly described, including the survival and maturation of B and plasma cells [50–52]. Our results corroborates prior study demonstrating that blood B cells co-cultured with FLS-RA exhibited increased survival [30]. Interestingly, the pre-treatment of FLS-RA with Dex by 24 h inhibited the enhanced survival of B cells. Although the inhibitory effect was partial, it could be due to de novo synthesis of BAFF by FLS-RA after pre-treatment with Dex. Alternatively, others survival factors not affected by Dex could be involved. The reestablishment of the enhanced survival of B cells co-cultured with Dex-treated FLS-RA by addition of rhBAFF, suggests that Dex selectively inhibited the BAFF expression.

Other effects and new cellular targets of BAFF in the RA synovium cannot be discarded. In this regard, current efforts are undertaken to evaluate the effect that BAFF could have on synoviocytes and their role in the synovial membrane hyperplasia.

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