EXTENDED REPORT

Decreased B cell activating factor receptor expression on peripheral lymphocytes associated with increased disease activity in primary Sjögren's syndrome and systemic lupus erythematosus

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Objective: To analyse B cell activating factor (BAFF) receptor (BAFF-R) expression on peripheral lymphocytes from patients with primary Sjögren's syndrome (pSS) and systemic lupus erythematosus (SLE).
Patients and methods: Peripheral blood mononuclear cells from 20 patients with pSS, 19 patients with SLE and 15 controls were examined by flow cytometry to investigate BAFF-R mean fluorescence intensity (MFI) on lymphocytes. BAFF-R mRNA level from isolated blood B cells of nine patients with pSS and eight controls was assessed by real-time quantitative reverse transcription-PCR. BAFF serum level was determined by ELISA.
Results: In all subjects, BAFF-R was expressed on all naïve CD27− and memory CD27+ B-cells and was present on <0.5% of T cells. The expression of BAFF-R on B cells was significantly decreased in patients with pSS as compared with controls (MFI = 7.8 vs 10.6, p = 0.001), and was intermediate in patients with SLE (MFI = 9.5). Serum BAFF level was inversely correlated with BAFF-R MFI (p = 0.007), but not because of competition between endogenous BAFF (at observed concentrations in patients) and the monoclonal antibody (11C1) detecting BAFF-R. BAFF-R mRNA levels did not differ between patients with pSS and controls (p = 0.48). BAFF-R MFI decreased after overnight culture with recombinant human BAFF (from 32.5 to 25.4, p = 0.03). Contrary to the serum BAFF level, BAFF-R expression was correlated with extraglandular involvement in pSS and SLE Disease Activity Index.
Conclusions: BAFF-R expression is reduced on peripheral B cells of patients with pSS and SLE. This down-regulation occurs through a post-transcriptional mechanism and could be the consequence of chronic increase in BAFF. BAFF-R levels on B cells could be a novel activity biomarker in autoimmune diseases.

B cells play a key role in the pathogenesis of autoimmune diseases (AID), by secreting autoantibodies, presenting antigens and secreting cytokines.[1–3] Activation of autoreactive B cells is largely mediated by a cytokine called B cell activating factor of the tumour necrosis factor (TNF) family (BAFF; also called BLyS (B lymphocyte stimulator), TALL-1 (TNF- and ApoL-related leucocyte-expressed ligand), zTNF4, THANK (TNF homologue that activate apoptosis, NF-κB and c-jun N-terminal kinase) and TNFSF-13B (tumour necrosis factor (ligand) superfamily member 13B)).[4–6] BAFF has been shown to be critical for the maintenance of normal B cell development, homeostasis and autoreactivity.[4] BAFF binds to three receptors present on B cells: BAFF receptor (BAFF-R, BR-3, TNFRSF13C); transmembrane activator and calcium modulator, and cyclophilin ligand (TACI, TNFRSF13B); and B cell maturation antigen (TNFRSF17).[7 8] BAFF-R is the main BAFF receptor transmitting the BAFF signal to B cells.[9] BAFF-R is expressed on all peripheral B cells and some T cells, especially after their activation.[9 10]

Autoreactive B cell activation is one of the hallmarks of primary Sjögren's syndrome (pSS) and systemic lupus erythematosus (SLE).[11 12] In both diseases, BAFF is considered as an essential factor of this activation, as indicated by an increased level of BAFF in the sera of patients with pSS and SLE and the presence of BAFF in inflamed salivary glands of patients with pSS.[13–19] In AID, specific blockade of BAFF might be sufficient to suppress systemic autoimmunity, and preliminary data seem promising in clinical trials on SLE and rheumatoid arthritis.[20]

To date, the data concerning BAFF receptors in human AID are scarce. Moreover, it is not clear whether B cell activation in AID is only due to increase in BAFF or could also be modulated by the expression of BAFF-R. Recently, Carter *et al*[21] reported that the expression of free available BAFF-R in blood B cells was decreased in patients with SLE and was associated more with disease activity than the serum BAFF level.

Therefore, we analysed the expression of BAFF-R on blood B and T cells of patients with SLE or pSS. BAFF-R expression was markedly decreased in diseased blood as compared with controls, and, interestingly, this decrease was significantly associated with increased disease activity.

PATIENTS AND METHODS

Patients and control population

After approval by the local ethics committee and after obtaining informed consent from all subjects, blood was collected from 20 patients (19 women; median age 58 years; range 35–79 years) with pSS fulfilling the European–American Consensus Group criteria (10 had positive anti-Sjogren's syndrom A/Ro and anti-Sjogren's syndrom B/La antibodies; 17 had a focus score ⩾1 on

Abbreviations: AID, autoimmune disease; BAFF, B cell activating factor of the tumour necrosis factor family; BAFF-R, BAFF receptor; CE, cell equivalence; FACS, fluorescence-activated cell sorter; mAb, monoclonal antibodies; MFI, mean fluorescence intensity; PBMC, peripheral blood mononuclear cell; pSS, primary Sjögren's syndrome; rhBAFF, recombinant human BAFF; SLE, systemic lupus erythematosus; SLEDAI, SLE Disease Activity Index

Table 1 Characteristics of the lymphocyte population and subpopulations, B cell activating factor (BAFF) receptor expression and serum BAFF level in patients with primary Sjögren's syndrome, systemic lupus erythematosus and controls.

	pSS (n = 20)	SLE (n = 19)	Controls (n = 15)	p Value (SLE vs controls)	p Value (pSS vs controls)	p Value (pSS vs SLE)
Leucocyte number	6150 (2400–9570)	5845 (3100–14 810)	7390 (4290–15 300)	0.16	0.1	0.89
Lymphocyte number	1333 (200–2500)	1460 (800–3140)	2390 (830–3400)	0.006	0.002	0.49
B cell number	123 (37–570)	206 (67–597)	221 (64–1462)	0.58	0.04	0.016
Memory B cell number	8.2 (1.7–54.1)	16 (3.2–36.1)	42 (20–529)	0.003	<0.001	0.11
Memory B cell frequency	7.3 (4.5–26)	8.3 (2.7–19.7)	24.4 (8.6–43.3)	0.001	0.001	0.99
T cell number	974 (143–1810)	962 (228–2402)	1838 (1090–2698)	0.001	<0.001	0.86
Frequency of BAFF-R+ T cells	0.2 (0.05–0.8)	0.3 (0.05–0.65)	0.15 (0.1–0.6)	0.19	0.68	0.37
BAFF-R+ T cell number	1.6 (0.6–11)	2.6 (0.3–7.5)	2.4 (0–10.8)	0.13	0.1	0.42
BAFF-R MFI on B cells	7.8 (4.2–14.8)	9.5 (3.2–16.1)	10.6 (5.6–16.5)	0.19	0.001	0.14
BAFF-R MFI on CD27− B cells	8.1 (4.1–15.8)	9.7(3.2–16.1)	10.9 (5.8–16.8)	0.14	<0.001	0.09
BAFF-R MFI on CD27+ B cells	6.6 (4.1–12.3)	9.7 (3.7–16.2)	10.0 (5.3–17.9)	0.36	0.001	0.06
BAFF-R MFI on T cells	7.2 (3.3–12.9)	8.1 (3.4–16.1)	9.7 (3.4–15.3)	0.06	0.002	0.3
Serum BAFF level (ng/ml)	1.2 (0.8–2.2)	0.9 (0.3–5.0)	NA	NA	NA	0.33

BAFF, B cell activating factor; BAFF-R, BAFF receptor; MFI, mean fluorescence intensity; NA, not available; pSS, primary Sjögren's syndrome; SLE, systemic lupus erythematosus.
Values are shown as median (range).
Absolute numbers of cells are expressed as number of cells/mm^3; frequencies are expressed in percentages.

labial salivary gland), and from 19 female patients (median age 38 years; range 25–73 years) with SLE according to the American College of Rheumatology criteria.[22 23] The control population consisted of 15 subjects (9 women; median age 54 years; range 24–87 years) including 5 healthy staff members and 10 patients with chronic low back pain or lumbosciatic pain receiving no corticosteroids and without inflammatory or neoplastic disorders.

For patients with pSS, we distinguished those with and without extraglandular manifestations at the time of blood examination as described previously.[24] In six patients, extraglandular involvement was defined as lung involvement (n = 2), neurological involvement (n = 1), active synovitis (n = 1), myositis (n = 1) or vasculitis (n = 1). For patients with SLE, disease activity was measured by the SLE Disease Activity Index (SLEDAI) on the day of blood testing.[25] The median SLEDAI score was 4.0 (range 0–16). Immunosuppressive drugs were given to five patients with pSS (mycophenolate mofetyl (n = 2), methotrexate (n = 3)) and to eight patients with SLE (mycophenolate mofetyl (n = 7), azathioprine (n = 1)). None of the patients had received B cell-targeted therapy.

For mRNA analysis, we investigated a second group of nine other patients with pSS (seven women; median age 54 years; range 31–69 years; six with anti-SSA/Ro and two with anti-SSB/La antibodies, all with a focus score ⩾1 on labial salivary gland biopsy, five with extraglandular involvement) and eight other controls (six women; median age 42 years; range 24–53 years). None of these patients with pSS received immunosuppressive drugs or had received B cell-targeted therapy.

Antibodies and reagents

Direct fluorochrome-conjugated monoclonal antibodies (mAb) to CD22 (PC5), CD27 (fluorescein isothiocyanate), CD3 (ECD) and conjugated isotype controls were used (Becton Dickinson, Le Pont le Claix, France). The use of mAb to BAFF-R (11C1) conjugated to phycoerythrin was in accordance with the manufacturer's instructions (Becton Dickinson). The isotype control was from eBioscience (San Diego, California, USA).

Flow cytometric analysis of B and T cells

Peripheral blood mononuclear cells (PBMCs) were prepared according to standard procedures on Ficoll-Hypaque (Pharmacia Biotech, Uppsala, Sweden), and stained with anti-BAFF-R, anti-CD27, anti-CD3, anti-CD22 mAb or isotype-matched controls. The cells were incubated for 30 min at 4°C, then with fluorescence-activated cell sorter (FACS) lysing solution (Becton Dickinson). Flow cytometry analysis was performed directly after sample preparation. All experiments were analysed by gating on lymphocytes according to forward side scatter/side light scatter, excluding dead or dying cells or monocytes. Flow cytometry was performed with an EPICS XL device (Coulter, Brea, California, USA) and analyses were performed by RXP-software analysis (Beckman Coulter). The frequency of cells stained for BAFF-R, their mean fluorescence intensity (MFI), and the frequency and absolute numbers of each lymphocyte subpopulation were determined. Leucocyte subsets in the peripheral blood were analysed by standard haematological procedures.

Isolation of peripheral blood B cell

PBMCs of subjects of the second group of nine patients with pSS and eight controls were isolated by Ficoll-Hypaque centrifugation. The resulting cell population was highly enriched for B cells by positive selection with magnetic microbeads coupled to anti-CD19 (magnetic-activated cell sorting direct isolation kit (Miltenyi Biotec, Bergisch Gladbach, Germany)). For each sample, B cell purity was >95% on CD22 staining during flow cytometry.

Effect of short-and long-term incubation with BAFF on BAFF-R expression

To investigate a potential competition mechanism between 11C1 mAb and endogenous BAFF, PBMCs from four healthy controls were incubated at 37°C with or without recombinant human BAFF (rhBAFF; Alexis Biochemicals, Lausanne, Switzerland) at different concentrations (0, 1, 10, 100, 1000 ng/ml) during 30 min in RPMI1640-supplemented 10% fetal calf serum, penicillin and streptomycin.

To investigate a potential modulation of BAFF-R expression by long exposure to BAFF, PBMCs from six healthy subjects were cultured overnight with or without rhBAFF at concentrations similar to the range observed in the sera of patients with AID and not competing with 11C1 (0, 1, 10 ng/ml). After overnight culture, BAFF-R expression on B cells was analysed by FACS gating on live lymphocytes.

Serum BAFF determination

Serum BAFF concentration was measured by using ELISA kit from R&D systems (Lille, France). The results of this kit are not influenced by the presence of rheumatoid factor in the patient

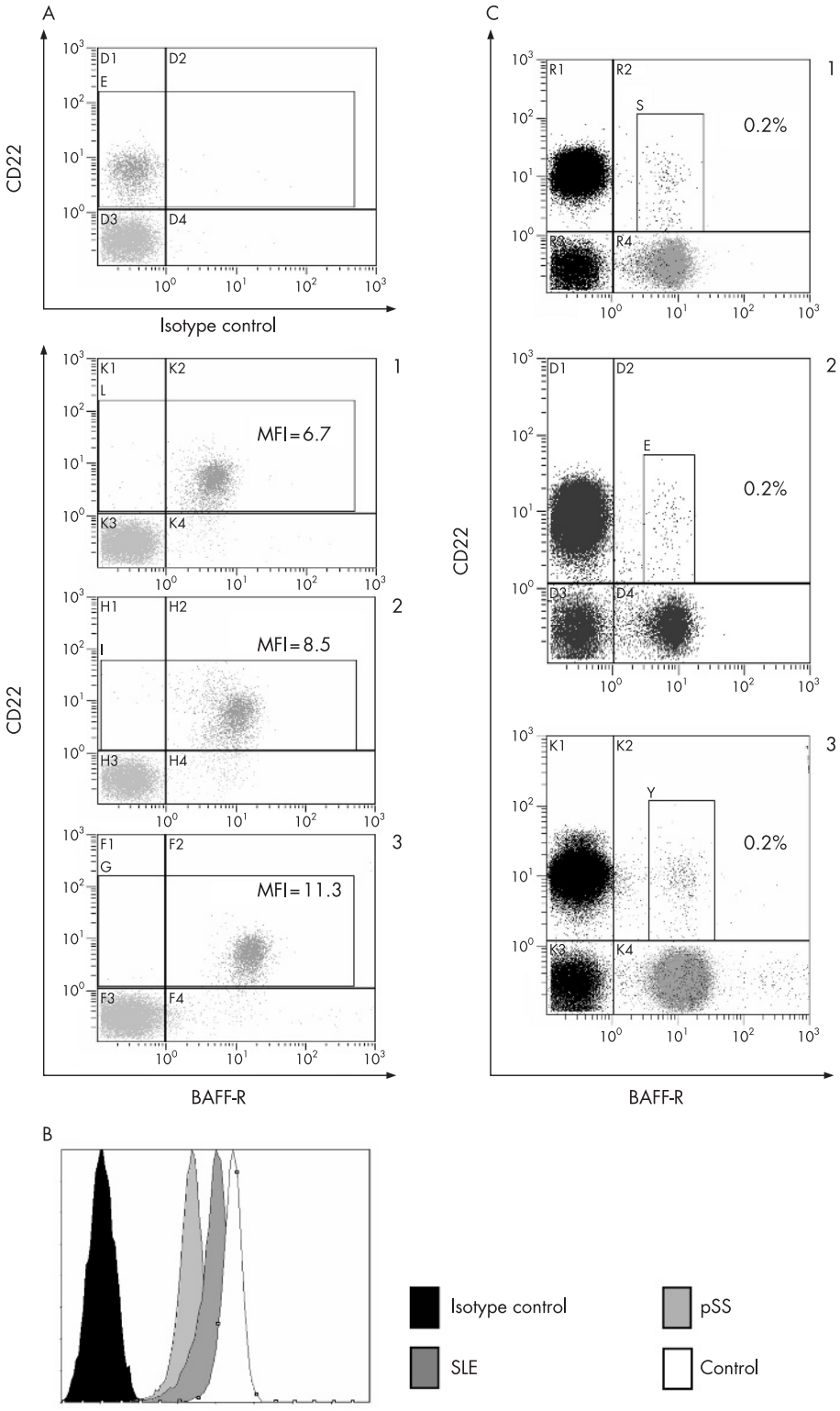

Figure 1 Analysis of the expression of B cell activating factor receptor (BAFF-R) by flow cytometry. (A, B) CD22 B cells in the blood of a representative patient with primary Sjögren's syndrome (pSS),[1] patient with systemic lupus erythematosus (SLE)[2] and a control subject.[3] (C) CD3 T cells in the blood of the same subjects. MFI, mean fluorescence intensity.

serum.[26] The serum sample was collected on the same day as the sample for flow cytometry in 34 patients with pSS or SLE.

Real-time quantitative reverse transcription-PCR for BAFF-R mRNA measurement

Total RNA was isolated from isolated B cells by using RNeasy Mini kit Qiagen (Courtaboeuf, France). Complementary DNA synthesis involved the use of Enhanced Avian HS reverse transcription-PCR (Sigma-Aldrich, Saint Quentin Fallavier, France). Quantitative PCR was performed using conditions described elsewhere.[27] Amplification primers for BAFF-R were 5′-AGACAAGGACGCCCCAGAGCCC-3′ and 5′-GGCCGTCTTG-GTGGTCAC-3′. Quantitative PCR runs were considered only if amplification efficiencies were high (slopes ranging from −3.2 to −3.8). Each sample was processed in duplicate. For each run, serially diluted complementary DNA of human B cells infected

with Epstein–Barr virus were used for quantitative standards. We determined the cell equivalence (CE) number of BAFF-R and β-actin mRNA in each sample according to the standard curve generated from values obtained for human B cells infected with Epstein–Barr virus. The unit number showing the relative BAFF-R mRNA level in each sample was calculated as a value of BAFF-R CE normalised with β-actin CE.

Serologic tests

Anti-Ro/SSA and anti-La/SSB antibodies and anti-DNA antibodies were determined by counterimmunoelectrophoresis and ELISA, respectively, as described previously.[24]

Statistical analysis

For statistical analysis, Analyse-it (V 7.13) for Microsoft Excel (Leeds, England, UK) was used.

Results are expressed as median and range. Qualitative expression involved the χ^2 test. Quantitative comparisons involved the Mann–Whitney U test for unpaired data and the Wilcoxon test for paired data. Correlations were established with the Spearman's test. $p \leqslant 0.05$ was considered significant.

RESULTS

Characteristics of peripheral lymphocytes in patients with pSS and SLE

Lymphocyte count, number of T cells, and absolute number and frequency of CD27 memory B cells were reduced in patients with pSS and SLE as compared with controls. Cell features did not differ between patients with pSS and SLE, except for reduced number of total B cells in patients with pSS (table 1).

Reduced BAFF-R expression in peripheral B cells

In all subjects, the entire peripheral blood B cell population expressed BAFF-R (fig 1). The level of BAFF-R on B cells was significantly decreased in patients with pSS compared with controls (MFI 7.8 (4.2–11.8) vs 10.6 (5.6–16.5); $p = 0.001$; table 1, figs 1 and 2) but was intermediate in patients with SLE (MFI 9.5 (3.2–16.1)), with a trend for a lower level than in controls ($p = 0.19$; fig 1 and table 1).

Because B cell subpopulation homeostasis is disturbed in pSS and SLE, with a decreased percentage of CD27+ memory peripheral B cells, we analysed BAFF-R expression in CD22+ CD27− naïve and CD22+ CD27+ memory B cells (fig 3). BAFF-R level did not differ between naïve and memory B cells in patients with pSS, those with SLE and controls; the level decreased to the same extent in both B cell subpopulations in all subjects (table 1 and fig 3). The BAFF-R level on B cells was not different in the control group between the healthy staff members and the other controls (data not shown).

Reduced BAFF-R expression on peripheral T cells in pSS

BAFF-R was expressed on a very small subset of blood T cells in patients with pSS, those with SLE and controls (0.2%, 0.3% and 0.15% of T cells, respectively). Because of the small size of BAFF-R-positive T cells, the maximum number of events was acquired (until 500 000 events). As on B cells, the BAFF-R levels on T cells was decreased in patients with pSS compared with controls (MFI 7.2 (3.3–12.9) vs 9.7 (3.4–15.3); $p = 0.002$), whereas the level was intermediate in patients with SLE (MFI 8.1 (3.4–16.1), $p = 0.3$ vs patients with pSS and $p = 0.06$ vs controls; table 1).

Serum BAFF concentration and correlation with BAFF-R expression

Serum BAFF concentration was determined by ELISA in 16 patients with pSS and in 18 patients with SLE, and the mean concentration did not differ between the two groups (1.2 (0.8–2.2) and 0.9 (0.3–5.0) ng/ml, respectively, $p = 0.33$).

In the 34 patients with AID for whom the serum BAFF level was available, serum BAFF concentration and BAFF-R level on B cells ($r = -0.46$, $p = 0.007$; fig 4) and T cells ($r = -0.34$, $p = 0.04$; data not shown)were negatively correlated.

No relevant competition between endogenous BAFF and mAb anti-BAFF-R (11C1)

Because the serum BAFF level is usually increased in pSS and SLE, the decreased expression of BAFF-R on peripheral lymphocytes in the diseases could be linked to a spatial competition between the anti-BAFF-R antibody (11C1) and endogenous BAFF already bound to membranous BAFF-R. To explore this possibility, PBMCs from four healthy subjects were incubated with different concentrations of rhBAFF (0, 1, 10, 100, 1000 ng/ml). The expression of BAFF-R on B cells did not

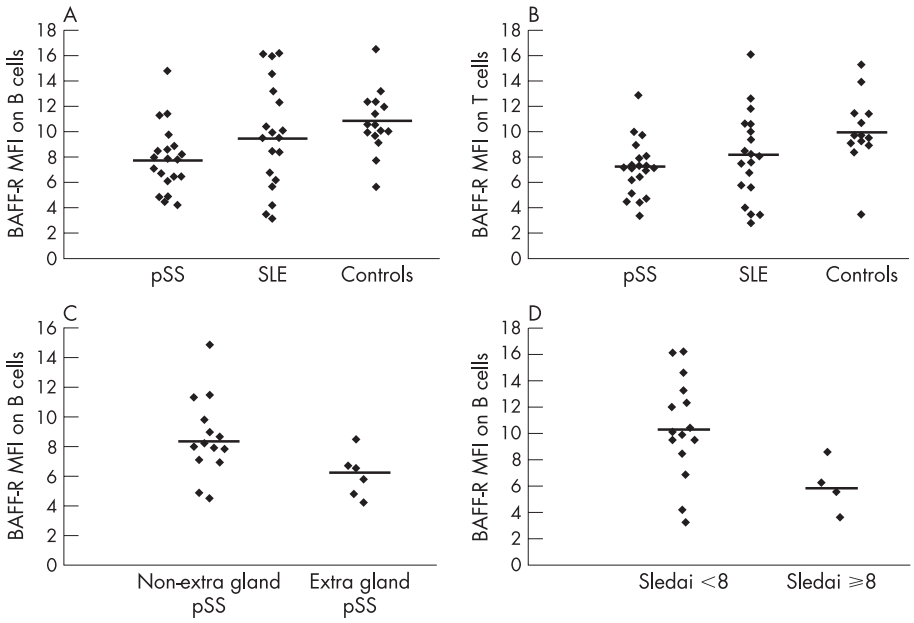

Figure 2 Cytometric expression of B cell activating factor receptor (BAFF-R) in 20 patients with primary Sjögren's syndrome (pSS), 19 patients with systemic lupus erythematosus (SLE) and 15 controls. (A) B cells. (B) T cells. (C) B cells in the blood of patients with pSS with non-extraglandular involvement (non-extragland pSS; n = 14) and extraglandular involvement (extragland pSS; n = 6). (D) BAFF-R expression in patients with SLE by SLE Disease Activity Index above and below 8. Solid black bars represent the median values.

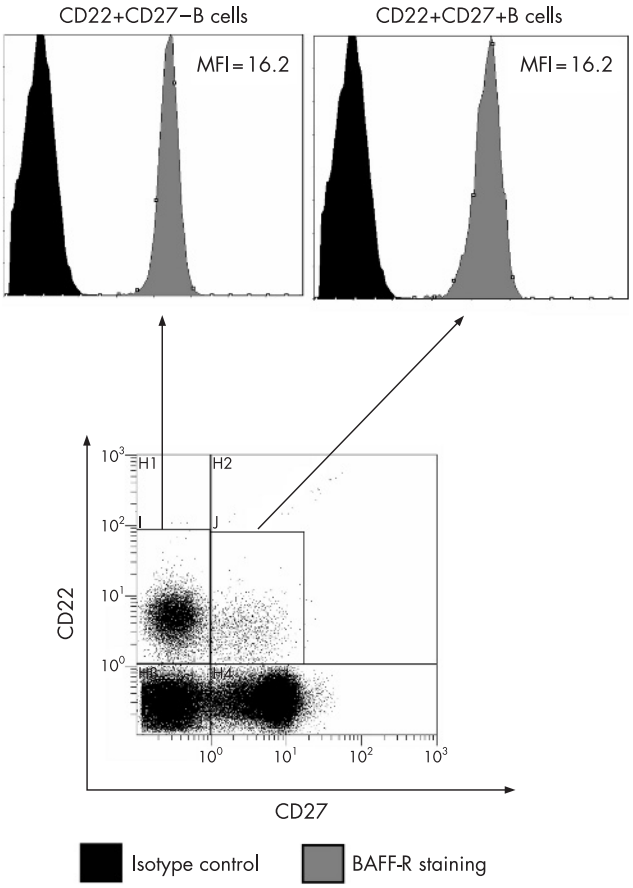

Figure 3 Analysis of the expression of B cell activating factor receptor (BAFF-R) by flow cytometry on memory CD27+ and naïve CD27− B cells in blood. MFI, mean fluorescence intensity.

change with rhBAFF added at 0, 1 or 10 ng/ml in all experiments (MFI 22.0, 21.7 and 21.4 at 0, 1 and 10 ng/ml, respectively). The level of expression decreased only at very high levels of rhBAFF, which are not achieved in AID (MFI 14 and 9.5 at 100 and 1000 ng/ml, respectively; fig 5A).

Decreased expression of BAFF-R after long exposure to BAFF

As the decrease of BAFF-R in AID could be explained by a chronic BAFF exposure, we investigated BAFF-R expression on B cells of six healthy controls after a long incubation without or with rhBAFF at similar concentrations as those observed in the sera of patients with AID and which cannot be responsible for a spatial competition with 11C1 mAb (1 and 10 ng/ml). After 24 h, in all experiments, we found a decreased expression of BAFF-R on B cells cultured with 1 ng/ml, and further decrease with 10 ng/ml of rhBAFF (MFI 30.9 (16.5–34.9)) and 25.4 (15.4–30.7), respectively), compared with that in culture without rhBAFF at the same time (MFI 32.5 (16.6–33.8); p = 0.40 and 0.03, respectively; fig 5B, C).

Variation of BAFF-R expression according to activity of pSS or SLE

The modulation of BAFF-R expression in pSS and SLE might be related to disease activity. Thus, BAFF-R expression was analysed according to the presence (n = 6) or absence (n = 14) of extraglandular involvement in pSS. The decreased expression of BAFF-R on B cells in pSS was greater in the six patients with extraglandular involvement on blood testing

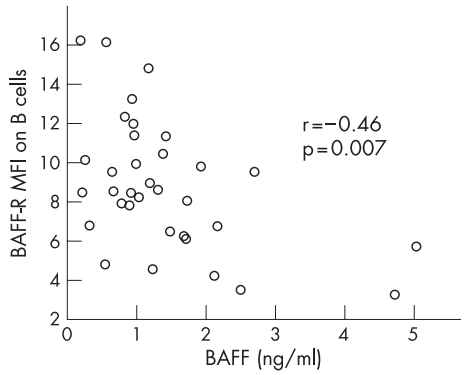

Figure 4 Correlation between B cell activating factor receptor (BAFF-R) level as assessed by mean fluorescence intensity (MFI) and serum B cell activating factor (BAFF) level (ng/ml).

(MFI 6.3 (4.2–8.5)) than in the 14 patients with glandular involvement only (MFI 8.1 (4.5–14.8); p = 0.05; fig 2), and involved both naïve and memory B cells (p = 0.05 and 0.07, respectively). Likewise, in patients with SLE with more active disease (SLEDAI ⩾8; n = 4), the BAFF-R level on B cells was lower than in patients with less active disease (SLEDAI <8; n = 15; MFI 5.9 (3.5–8.5) vs 10.1 (3.2–16.1), p = 0.04; fig 2). The activity of the AID was not correlated with serum BAFF level. In patients with pSS the median serum level of BAFF in patients with and without extraglandular involvement was 1.5 (0.2–2.2) and 1.2 (0.8–1.9) ng/ml, respectively (p = 0.96), and in patients with SLE the level of BAFF was not statistically different between patients with SLEDAI above and below 8 (2.1 (0.7–5.0) and 0.9 (0.3–4.7) ng/ml, respectively, p = 0.13).

No transcriptional regulation of BAFF-R on B cells in pSS

To investigate a potential transcriptional regulation of BAFF-R mRNA in pSS, BAFF-R mRNA level on isolated peripheral B cells was assessed with real-time quantitative PCR in nine patients with pSS and eight controls. BAFF-R mRNA levels did not differ between pSS and controls (ratio of BAFF-R to β-actin 22.1 (2.5–39.5) and 9.5 (2.6–81), respectively, p = 0.48). BAFF-R mRNA levels did not differ between patients with pSS with and those without extraglandular features (data not shown).

DISCUSSION

Because BAFF is involved in B cell activation in AID and mediates its signal mainly through BAFF-R, it is important to investigate the pattern of BAFF-R expression in AID. To date, the variation in BAFF-R expression has been determined during B cell ontogeny,[8–10] but the data concerning BAFF-R in human AID are very limited.[21 26 28] Our study revealed that BAFF-R expression was reduced on peripheral B and T cells in patients with pSS and SLE as compared with controls. The decrease in BAFF-R level was inversely correlated with serum BAFF level and associated with disease activity.

Because peripheral B cell homeostasis is disturbed in patients with pSS and SLE, we investigated naïve (CD27−) and memory (CD27+) B cells using CD27 staining, which is a well-recognised marker for somatically mutated memory B –cells,[29 30] and found a similar BAFF-R level in both cell types in each group. Interestingly, the absolute number and frequency of CD27+ B cells in patients with pSS was significantly decreased as compared with controls, as reported previously.[31 32] However, our results in SLE conflict with those of other reports,[33 34] which suggest an increase of CD27+ B cells and a decrease of CD27− B cells, but agree with other reports.[35–37] A very small subset of blood T cells expressed BAFF-R in all subjects, with no difference

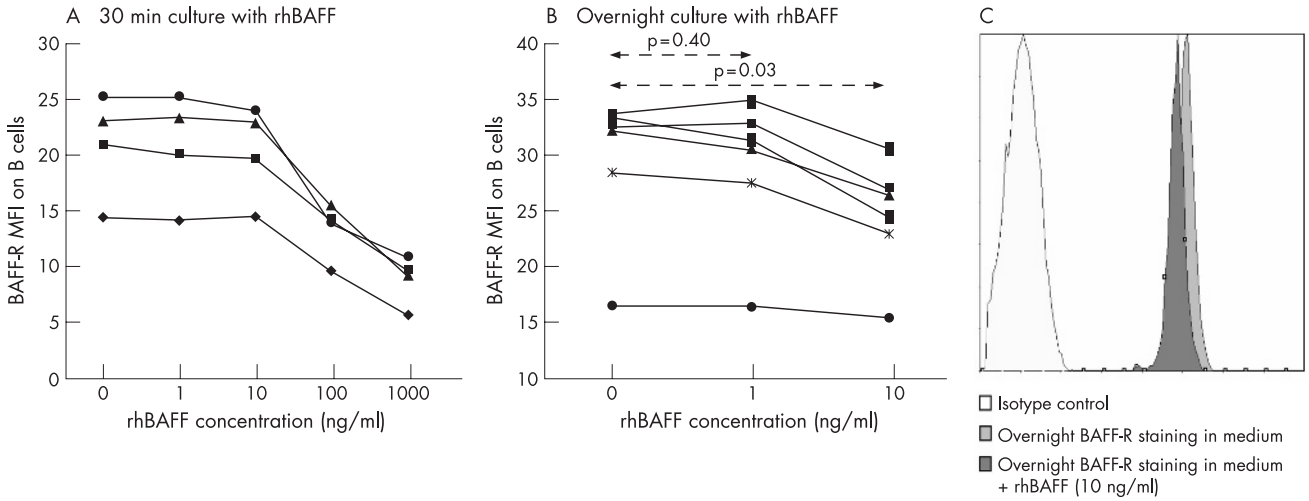

Figure 5 (A) Determination of B cell activating factor receptor (BAFF-R) level by mean fluorescence intensity (MFI) on B cells from four healthy subjects after incubation with recombinant human BAFF (rhBAFF) during 30 min at the indicated concentrations. The anti-BAFF-R antibody (11C1) and rhBAFF compete only at very high concentrations of BAFF (100–1000 ng/ml) and not at concentrations typically obtained in serum of patients with autoimmune diseases (1–10 ng/ml). (B) BAFF-R MFI on B cells from six healthy subjects after overnight culture with/without rhBAFF (0, 1 and 10 ng/ml). Compared with culture without rhBAFF (median MFI = 32.5), BAFF-R MFI decreases in the presence of 1 ng/ml (median MFI = 30.9) and 10 ng/ml (median MFI = 25.4; p = 0.40 and p = 0.03, respectively) of rhBAFF. (C) One representative example of decrease of BAFF-R expression on B cells by flow cytometry after overnight culture in the presence of rhBAFF (10 ng/ml).

between patients and controls. We therefore performed a maximum acquisition of events during FACS acquisition to ensure the validity of results. Of note, the presence of BAFF-R on T cells has already been reported to have the same proportion and seems to increase after in vitro activation.[9] Although the size of the BAFF-R-positive T cells is small, we observed that the level of BAFF-R on T cells was decreased in patients with pSS and SLE as compared with that observed on B cells in controls. This finding has not been reported before, and could suggest a global regulation of BAFF-R in AID independently of the cells exhibiting this receptor. The precise phenotype of the T cells expressing BAFF-R in AID remains to be elucidated. A previous report suggested the presence of BAFF-R on central and effector memory, but not on naïve T cells.[9]

The decreased BAFF-R expression observed on B and T cells in patients with pSS and SLE was an unanticipated finding. Although we used an anti-BAFF-R mAb from the clone 11C1, which is the most largely used to detect BAFF-R in flow cytometry and which is not known to be in competition with endogenous BAFF,[21] our first hypothesis to explain this phenomenon was spatial inhibition of access to BAFF-R by endogenous BAFF. The negative correlation between serum BAFF level and BAFF-R expression could argue in favour of this mechanism. However, results of our in vitro binding assay of 11C1 in the presence or absence of rhBAFF did not confirm this hypothesis. Indeed, MFI of BAFF-R on B cells decreased only at very high rhBAFF concentrations (100 and 1000 ng/ml), which are much higher than maximal values observed in the sera of patients with AID. At rhBAFF concentrations of 1–10 ng/ml corresponding to the range of serum BAFF level observed in patients with pSS and SLE, no decrease of 11C1 binding was observed. Of note, a concentration of 10 ng/ml of BAFF was largely above the maximum concentration observed in the serum of our patients.

A second possible explanation for the decreased expression of BAFF-R could be an internalisation of the BAFF-R/BAFF complex similar to the rapid internalisation of TNF receptor 1 10–30 min after the binding of TNF or shedding of this complex from the membrane.[38] The absence of downregulation of BAFF-R after 30 min of incubation with rhBAFF at 1 or 10 ng/ml (corresponding to the level observed in serum) does not support this hypothesis. Likewise, Carter *et al*[21] did not find any evidence of internalisation or shedding of the BAFF/BAFF-R complex. However, an in vitro overnight exposure to rhBAFF, at not competing concentrations, is responsible for a significant decrease expression of BAFF-R on B cells, suggesting an internalisation or shedding of BAFF-R in vivo—for example, by formation of microparticles, after long-term exposure to BAFF. Finally, to investigate whether the decrease in BAFF-R level resulted from downregulation of BAFF-R mRNA, we assessed the BAFF-R mRNA levels in B cells. The level of BAFF-R mRNA was similar between controls and patients with pSS, even in patients with extraglandular involvement, which suggests a post-transcriptional downregulation of BAFF-R related to the serum BAFF increase in AID.

Thus, chronic elevated overproduction of BAFF, as observed in pSS or SLE, could downregulate BAFF-R expression on the cell surface through post-transcriptional regulation. Such a post-transcriptional regulation, consistent with the coordinate down-regulation of BAFF-R on both B and T cells observed in the present study, was recently suggested in mice by Lesley *et al*,[39] who found a downregulation of BAFF-R by BAFF in autoreactive B cells in an immunoglobulin/hen egg lysozyme transgenic mouse found to have a high serum BAFF level, without any change in BAFF-R mRNA levels. Interestingly, in that study, BAFF-R downregulation was observed in vivo only after a long incubation of B cells with BAFF (12 h or more). Similarly, we have found a downexpression of BAFFR after overnight incubation with rhBAFF at concentrations in the range of those observed in the sera of patients and not competing with 11C1 staining. This result argues in favour of a direct effect of BAFF on BAFF-R regulation, explaining the correlation we have found between BAFF serum level and BAFF-R MFI. However, the precise implicated mechanism (post-transcriptional mechanism and especially internalisation or shedding) needs further investigations. Such a downregulation of the receptor by the ligand has been reported on B cells bearing the inducible costimulator ligand, which is decreased in level in patients with SLE.[40] This observation could be explained by a chronic exposure to inducible costimulator, which is upregulated on T cells from patients with SLE. Likewise, downregulation of receptors is a

classical feature of hormonal regulation. Carter *et al* have reported that BAFF-R was present but occupied in SLE.[21] This is not contradictory with our results of downregulation of BAFF-R, and probably both mechanisms occur in AID and can account for the decrease in availability of BAFF-R on the surface of lymphocytes.

Interestingly, the BAFF-R level, but not the serum BAFF level, was associated with disease activity in the present study. We have previously shown that serum BAFF concentration is correlated with the presence of anti-SSA or anti-SSB antibodies, rheumatoid factor or gammaglobulin levels, but not with activity of pSS.[13 24] In patients with SLE, correlation between serum BAFF level and SLEDAI is conflicting and seems to be weak if it exists.[41] Collins *et al*[42] reported that the mRNA BAFF level was better correlated with SLEDAI than the serum BAFF level. We observed a decreased level of BAFF-R, particularly in patients with pSS or SLE with active disease. Surprisingly, Carter *et al*[21] found that BAFF-R expression evaluated by 11C1 immunostaining was similar between healthy controls and patients with SLE. This discrepancy might be explained by differences in characteristics of patients between the two studies (eg, concerning disease activity). BAFF-R expression could be a more relevant and global biomarker than serum BAFF level, which would not reflect exactly BAFF production because efficient BAFF bound to its receptor is not measurable on ELISA and because the serum BAFF level depends on other parameters such as the total number of B cells.[43] In pSS and SLE, the chronic increase in BAFF level could lead to a downregulation of BAFF-R. Thus, the decreased level of BAFF-R expression might be the consequence of time-integrated increased BAFF secretion, and could be a signature of recent interaction with BAFF in vivo.

In conclusion, BAFF-R expression is reduced on peripheral B and T cells of patients with pSS and SLE. This downregulation of BAFF-R occurs through a post-transcriptional mechanism and could be a reflection of chronic BAFF overproduction over time. Because reduced BAFF-R expression is inversely correlated with activity of the AID, BAFF-R levels on B cells could be a novel activity biomarker in AID. It will be interesting to assess the possible implications of this downregulation of BAFF-R on the efficacy of B cell or BAFF-targeted therapy in these diseases.

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