Concentrations of BAFF Correlate with Autoantibody Levels, Clinical Disease Activity, and Response to Treatment in Early Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** To determine whether levels of B cell activating factor (BAFF), a member of the tumor necrosis factor family, relate to autoantibody levels, disease activity, and response to treatment in patients with early rheumatoid arthritis (ERA).

Methods. BAFF was measured by ELISA in 48 early RA patients; 21 were examined serially. These data were compared with 49 controls with longstanding RA (LSRA), 48 disease controls (DC), and 50 healthy controls (HC).

Results. BAFF levels were higher in ERA, compared with DC and HC [median 4.3 ng/ml (5th-95th: 0.8–38.8) vs 0.9 ng/ml (5th-95th: 0.7–4.5) and 2.0 ng/ml (5th-95th: 0.7–5.68), respectively; $p < 10^{-4}$ both comparisons], but not with LSRA controls [median 8.7 ng/ml (5th-95th: 0.8–46.1); p = non-significant]. BAFF correlated with the titers of IgM rheumatoid factor and anti-cyclic citrullinated peptide autoantibody ($r = 0.76$ and $r = 0.49$; $p < 0.00001$, $p = 0.0001$ for the 2 correlations), and with the number of swollen joints ($r = 0.37$; $p = 0.01$). The followup study of 21 methotrexate-treated ERA patients revealed reduced levels of BAFF, with parallel improvement in clinical activity and decrease in autoantibody titers.

Conclusion. Elevated BAFF in a subset of ERA patients is related to autoantibody levels and synovitis. BAFF level diminished with treatment, along with autoantibody titers, suggesting a rationale to treat ERA patients with BAFF-targeted agents. (First Release June 1 2008; J Rheumatol 2008; 35:1256–64)

Key Indexing Terms:
B CELL ACTIVATING FACTOR EARLY RHEUMATOID ARTHRITIS DISEASE ACTIVITY
RHEUMATOID FACTOR ANTI-CYCLIC CITRULLINATED PEPTIDE

Rheumatoid arthritis (RA) is characterized by the production of a vast array of autoantibodies, and the ensuing synovitis causes irreversible destruction of joints[1], making it necessary to treat patients with RA as soon as possible; this requires early diagnosis[2,3]. Studies of pathophysiology have determined that B cells play multiple roles in the rheumatoid disease process[4]. In addition to their classical role as producers of autoantibodies, B cells are highly efficient antigen-presenting cells and producers of proinflammatory cytokines; they are central to the activation of CD4+ T cells in synovial tissue[5], which in turn can produce their own proinflammatory cytokines. Finally, depletion of B cells disrupts the production of proinflammatory cytokines by T cells and macrophages, switching off the inflammatory process[5-7].

This interpretation has been strengthened by the emergence of mediators maintaining survival of autoreactive B lymphocyte activation. Two are dominant: BAFF [B cell activating factor belonging to tumor necrosis factor (TNF) family] and APRIL (a proliferation-inducing ligand[8]). These are generated not only by myeloid cells, but also by fibroblast-like synoviocytes (FLS)[9]. After cleavage from the plasma membrane, BAFF is shed as a 17-kDa active soluble product in the form of homotrimers of BAFF, or heterotrimers of BAFF and APRIL[10].

BAFF is specific for B cells, in that it promotes their survival[11] and induces their differentiation[12]. Three receptors for BAFF have been identified: the B cell maturation antigen, the transmembrane activator and calcium-modulator and cyclophilin ligand-interactor, and the third BAFF receptor[13]. BAFF-transgenic mice present with an autoimmune B cell phenotype, experience a systemic lupus erythematosus-like state[14], and develop a Sjögren's syndrome-like disease[15].

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Measures of BAFF are elevated in the serum[16-18], synovial fluid (SF)[9,19], synovial tissue[9,20], and saliva[21] of patients with longstanding RA (LSRA). It has been shown that rheumatoid FLS produce BAFF, especially in response to interferon-γ (IFN-γ) and TNF-α[9]. Support for these findings comes from the increased production of BAFF in collagen-induced arthritis[22], and the reduced inflammation resulting from use of BAFF receptor-targeting agents[23].

There is, however, a great deal of variation in the numbers of RA patients with increased concentrations of BAFF, with percentages ranging from 19% to 40%[17-19]. It has also been claimed that the levels of BAFF are greater in SF than in paired sera, and greater in SF samples from RA patients than in those from controls with osteoarthritis, probably due to the production of BAFF at the site of inflammation[19]. If correct, the latter interpretation implies that the level of BAFF should be elevated in patients with early RA (ERA). Therefore, we decided to focus on patients with ERA, and to employ patients with LSRA as RA controls, in search of evidence to support BAFF as a target for therapy in ERA.

We utilized ELISA investigations to verify whether levels of BAFF are increased early in the development of RA, whether these levels correlate with the titers of IgM-rheumatoid factor (RF), IgA-RF, and anti-cyclic citrullinated peptide (CCP) autoantibody, and whether their fluctuations parallel the disease activity and treatment.

MATERIALS AND METHODS

Patients and controls. Forty-eight patients with ERA were recruited from the Department of Rheumatology at the Catholic University Hospital in Rome, all fulfilling the American College of Rheumatology classification criteria for RA[24] and having a disease duration ≤ 1 year. At the time of enrollment, they were using no steroids or disease modifying antirheumatic drugs (DMARD). All provided informed consent, and ethical approval was granted by our institutional committee.

We selected 3 groups of controls: (1) 49 LSRA patients with disease duration > 1 year were enrolled as RA controls [35 receiving methotrexate (MTX) 15–20 mg/wk, 5 receiving cyclosporin A 3–5 mg/kg/day, 2 hydroxychloroquine 6 mg/kg/day, 3 sulfasalazine 4–6 g/day, and 4 leflunomide 20 mg/day]; (2) 48 patients with rheumatic diseases other than RA served as RF-negative disease controls (DC): 12 had psoriatic arthritis, 12 seronegative spondyloarthritis, 12 crystal-induced arthritis, and 12 polymyalgia rheumatica; and (3) 50 blood donors served as healthy controls (HC).

On the day of blood sampling, medications were recorded and disease activity assessed with the Health Assessment Questionnaire and by clinical examination. Tender and swollen joint counts of 28 and 44 joints were evaluated by 2 observers, and their results were averaged. The Disease Activity Score (DAS) results using 28-joint counts were computerized[25].

Clinical and biological characteristics of 21 of the 48 ERA patients were followed over 6.7 ± 2.8 months of treatment. There were 17 women and 4 men, with a mean age of 56.9 ± 2.8 years and mean disease duration of 5.9 ± 0.7 months. Following their first referral, they were treated with MTX 10–20 mg/week, with or without prednisone 5 mg/day. Their response was considered positive when the DAS28 improved by ≥ 1.2 or they presented with a DAS28 < 3.2[26].

ELISA for BAFF. Serum levels of BAFF were assessed using an in-house ELISA[27], and validated by titration of recombinant BAFF (PeproTech, Rocky Hills, NJ, USA) and multiple analyses of reference sera. To obviate interassay variability, all samples were stored at –70°C and thawed on the same day. Briefly, the protein was accumulated by a mouse anti-BAFF monoclonal antibody (mAb) coated in the test wells, with an irrelevant IgG1 mAb coated in paired wells to counterbalance the confounding effects of RF. Absorbance values of control IgG1 were subtracted from those of anti-BAFF IgG1. To confirm that RF did not bind to the anti-BAFF IgG1, immunoglobulins were depleted from 10 randomly selected sera from autoimmune patients and from controls with protein A-sepharose. ELISA showed that levels of BAFF did not differ before and after absorption. We decided to adopt an internal assay, because all the commercial anti-BAFF mAb have been raised in the mouse towards synthetic peptides (not the molecule). Another problem would be that the reference BAFF purchased to construct the dose/optical density standard curve was recombinant and made in prokaryotic cells (MW 17 kDa); therefore it is not glycosylated. To overcome this we carried out affinity-purification of the human BAFF, making an anti-BAFF column with a panel of several monoclonals and loading the column with supernatant from U937 cell lines. The result was that some, but not all, of the commercial monoclonals recognized the human glycosylated form of BAFF (by SDS-polyacrylamide gel electrophoresis; MW 28 kDa instead of 17 kDa)[28,29]. After 4-hour incubation of the sera diluted 1:20, the binding of BAFF was developed by sequential incubations with rabbit anti-BAFF antibody, biotinylated goat anti-rabbit antibody, and horseradish peroxidase-conjugated streptavidin. Values > 8.6 ng/ml, i.e., the mean plus 2 standard deviations (SD) of 50 normal sera, scored positive.

Concentrations of other biological markers. IgM-RF, IgA-RF (Orgentec Diagnostika, Mainz, Germany), and anti-CCP antibody (Axis Shield Diagnostics, Kimbolton, UK) were measured using commercial ELISA, according to the manufacturers' instructions, with the proposed cutoff levels: 20 U/ml for IgM-RF and IgA-RF, and 5 U/ml for anti-CCP. Nephelometric IgM-RF, directed against the human IgG-Fc, was considered within the normal levels when the value was < 20 U/ml. Measurements of erythrocyte sedimentation rate, C-reactive protein, and total IgG, IgM, and IgA were part of the routine clinical care of each patient.

Statistical analysis. All analyses were carried out using SPSS 13.0 (SPSS, Chicago, IL, USA). Categorical variables were expressed as numbers, and quantitative variables as mean ± SD and median plus 5th and 95th percentiles. Non-normally distributed data were compared by Mann-Whitney test, and Wilcoxon's test for paired data. Odds ratios (OR) and confidence intervals (CI) were calculated. Correlations were determined by the Spearman rank order correlation for ordinal data and for non-normally distributed interval data.

RESULTS

BAFF levels in early RA. Levels of BAFF were elevated in ERA patients, compared with DC and HC [median 4.3 ng/ml (5th-95th 0.8–38.8) vs median 0.9 ng/ml (5th-95th 0.7–4.5) and median 2.0 ng/ml (5th-95th 0.7–5.68); $p < 10^{-4}$ for both comparisons, and nonsignificant (NS) between the 2 groups of controls; Figure 1]. Interestingly, they were similar to those of LSRA controls [median 8.7 ng/ml (5th-95th 0.8–46.1); p = NS]. They exceeded 8.6 ng/ml, the upper cutoff limit, in 17 of the 48 ERA patients (35.4%) and 25 of 49 LSRA controls (51.0%) (p = NS), but in none of the DC or HC. At baseline, ERA patients and LSRA controls presented comparable autoantibody levels and clinical indices (Table 1).

BAFF levels and autoantibodies in ERA. With regard to ERA patients, the levels of BAFF were higher in the presence than in the absence of RF [median 12.5 ng/ml (5th-95th 2.1–50.9) vs median 1.8 ng/ml (5th-95th 0.8–5.9); p < 0.0001]. These levels correlated with nephelometric levels of RF (r = 0.69; p < 0.00001), but also with IgA-RF (r = 0.74;

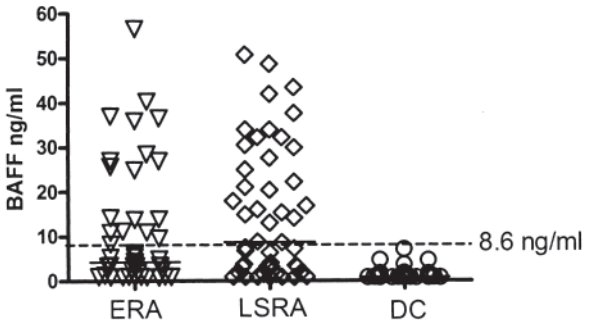

Figure 1. BAFF levels in 48 patients with early RA [ERA; median 4.3 ng/ml (5th-95th 0.8–38.8)], 49 control patients with longstanding RA [LSRA; median 8.7 ng/ml (5th-95th 0.8–46.1)], and 48 controls with rheumatic diseases other than RA [DC; median 0.9 ng/ml (5th-95th 0.7–4.54)]. Each point indicates an individual blood sample. Lines represent medians. Broken line indicates the cutoff value of 8.6 ng/ml that corresponds to mean + 2 SD of 50 normal sera.

$p < 0.00001$) and IgM-RF titers ($r = 0.76$; $p < 0.00001$) (Figure 2A, 2B, 2C). They were higher in the presence than in the absence of anti-CCP antibody [median 8.9 ng/ml (5th-95th 0.8–46.0) vs median 2.9 ng/ml (5th-95th 0.8–25.7); $p = 0.001$], and there was a correlation between the levels of BAFF and titers of anti-CCP ($r = 0.49$; $p = 0.0001$) (Figure 2D).

Similarly, the LSRA controls presented higher levels of BAFF in the presence than in the absence of RF [median 16.9 ng/ml (5th-95th 1.3–49.3) vs median 6.5 ng/ml (5th-95th 0.8–37.6); $p = 0.001$], and in the presence than in the absence of anti-CCP antibody [median 16.0 ng/ml (5th-95th 1.3–49.3) vs median 2.4 ng/ml (5th-95th 0.8–21.1); $p < 0.0001$]. As in ERA, BAFF levels correlated with nephelometric levels of RF ($r = 0.66$; $p < 0.0001$), but also with IgA-RF ($r = 0.62$; $p < 0.0001$) and IgM-RF ($r = 0.66$; $p < 0.0001$), and with anti-CCP antibody titers ($r = 0.54$; $p < 0.0001$) (Figure 3). Clearly, these results indicate that irrespective of the disease duration, increased levels of BAFF are associated with high titers of autoantibody in RA sera.

Further analysis of ERA patients with increased levels of

Table 1. Demographic, biological, and clinical characteristics of patients with early RA (ERA) and longstanding RA (LSRA), and patients with elevated levels and normal levels of BAFF.

	ERA, (n = 48)	LSRA, (n = 49)	Subgroup A 17 ERA pts (35.4%) BAFF ≥ 8.6 ng/ml	Subgroup B 31 ERA pts (64.6%) BAFF < 8.6 ng/ml	p**
Age, yrs (mean ± SD)	58.1 ± 13.7	58.2 ± 12.4	56.5 ± 12.3	59.0 ± 14.5	NS
F/M	38/10	34/15	12/5	26/5	NS
Disease duration, mo (mean ± SD)	5.5 ± 3.1	98.1 ± 102.7*	6.6 ± 3.3	4.9 ± 2.9	NS
RF-positive, %	54.2	67.3	100.0	29.0	< 0.0001
RF (nephelometric assay), median U/ml (5th–95th)	56.0 (10.0–1141.8)	158.5 (10.0–1572.2)	256.0 (45–1800.0)	10.0 (10.0–221.0)	< 0.0001
IgA-RF (ELISA), median U/ml (5th-95th)	14.1 (1.5–537.9)	25.3 (2.0–783.3)	116.9 (14.7–773.8)	6.7 (0.8–71.2)	< 0.0001
IgM-RF (ELISA), median U/ml (5th–95th)	30.7 (0.1–443.6)	43.3 (1.0–369.8)	132.4 (30.7–691.0)	5.8 (0.1–122.2)	< 0.0001
Anti-CCP-positive, %	66.7	67.3	94.1	51.6	< 0.0001
Anti-CCP, median U/ml (5th-95th)	14.0 (0.6–485.4)	26.5 (0.6–642.5)	81.6 (2.4–345.4)	5.7 (0.5–691.0)	0.009
BAFF levels, median ng/ml (5th-95th)	4.3 (0.8–38.8)	8.7 (0.8–64.1)	25.7 (3.7–56.6)	3.3 (0.8–7.1)	< 0.0001
BAFF ≥ 8.6 ng/ml, %	35.4	51.0	—	—	—
IgG, mg/dl (mean ± SD)	1164.2 ± 342.9	1109.2 ± 333.9	1117.0 ± 327.6	1190.7 ± 358.9	NS
IgA, mg/dl (mean ± SD)	227.2 ± 101.7	286.1 ± 142.7	265.1 ± 104.3	208.3 ± 98.2	NS
IgM, mg/dl (mean ± SD)	173.8 ± 240.7	122.4 ± 58.9	255.8 ± 399.9	127.6 ± 33.6	NS
ESR, mm/h (mean ± SD)	39.4 ± 28.7	29.2 ± 25.6	43.0 ± 32.5	37.4 ± 26.8	NS
CRP, mg/dl (mean ± SD)	25.9 ± 30.2	21.5 ± 31.5	28.0 ± 28.0	26.0 ± 31.7	NS
SJ28 (mean ± SD)	10.0 ± 5.7	9.8 ± 6.1	12.3 ± 6.1	8.7 ± 6.1	0.04
TJ28 (mean ± SD)	14.4 ± 6.3	14.0 ± 6.3	15.2 ± 6.9	14.0 ± 5.8	NS
SJ44 (mean ± SD)	13.5 ± 9.7	12.7 ± 6.1	17.2 ± 10.6	11.5 ± 8.3	0.04
TJ44 (mean ± SD)	18.3 ± 9.3	19.1 ± 8.1	20.0 ± 10.5	18.4 ± 8.5	NS
DAS28 (mean ± SD)	5.9 ± 1.6	5.8 ± 1.2	5.7 ± 1.7	5.7 ± 1.3	NS
HAQ (mean ± SD)	1.5 ± 0.7	1.4 ± 0.8	1.4 ± 0.8	1.4 ± 0.7	NS

Values are percentage or mean ± SD or median plus 5th-95th percentiles, per normal or non-normal distribution. RF: rheumatoid factor, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, anti-CCP: anti-cyclic citrullinated antibodies, SJ28: swollen joint count 28 joints, TJ28: tender joint count 28 joints, SJ44: swollen joint count 44 joints, TJ44: tender joint count 44 joints, DAS28: Disease Activity Score for 28 joints, HAQ: Health Assessment Questionnaire. ERA: ≤ 1 year disease duration; LSRA: > 1 year disease duration. Cutoff level for BAFF was set at 2 SD above the mean of 50 normal samples (BAFF ≥ 8.6 ng/ml). * $p < 0.05$ ERA vs LSRA, Mann-Whitney nonparametric test. ** p: patients with BAFF ≥ 8.6 ng/ml (subgroup A) vs patients with BAFF < 8.6 ng/ml (subgroup B).

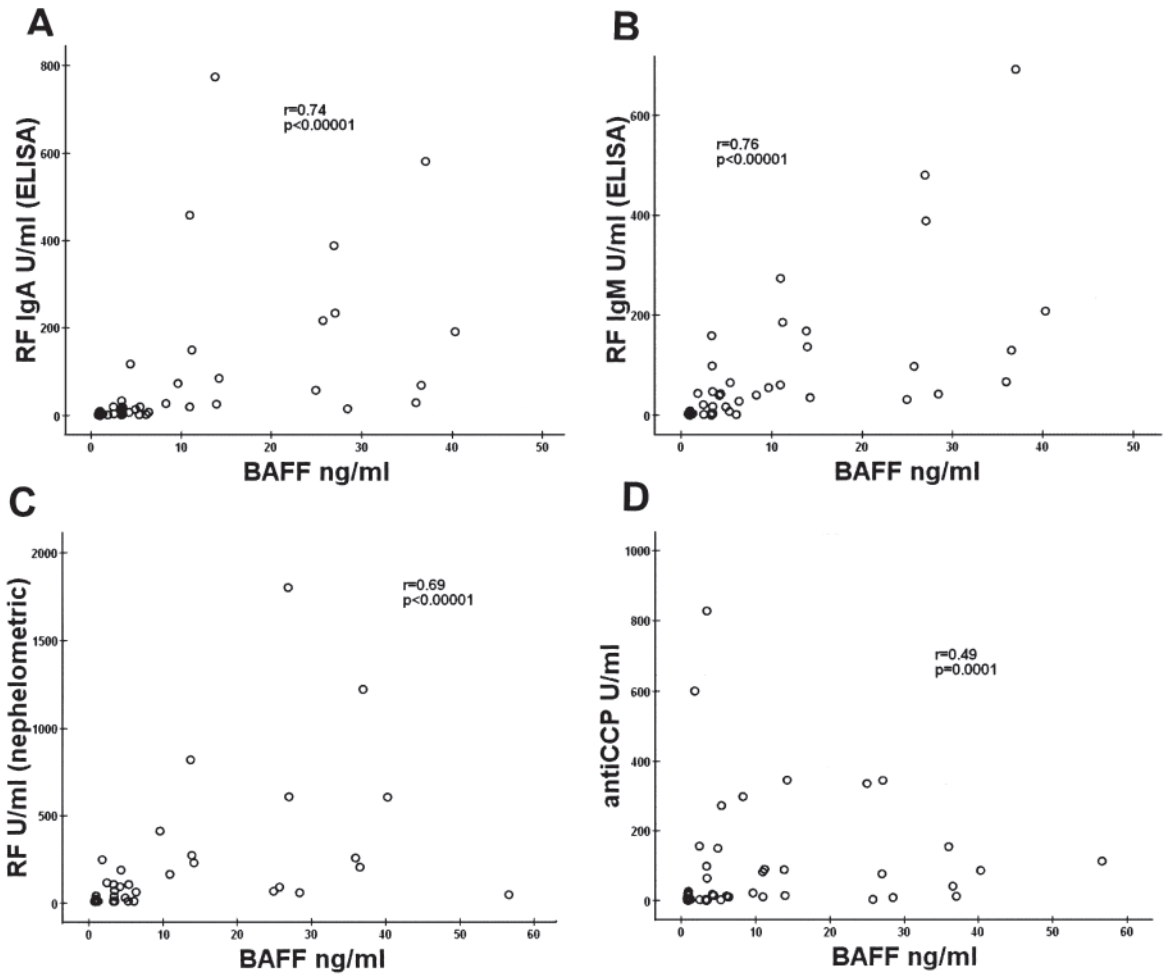

Figure 2. Correlations between levels of BAFF and (A) titers of IgA-RF by ELISA; (B) titers of RF-IgM ELISA; (C) levels of nephelometric RF; and (D) titers of anti-CCP antibodies in 48 patients with early RA. Each point indicates an individual blood sample. R values determined by Spearman correlation test.

BAFF. As an approach to the issue of whether an increased synthesis of BAFF is instrumental to autoantibody production, ERA patients were stratified into 2 groups according to level of BAFF (Table 1). Seventeen patients (Group A) had levels ≥ 8.6 ng/ml, and 31 patients (Group B) < 8.6 ng/ml. Interestingly, all 17 ERA patients of Group A had RF (100.0%), and all but one had anti-CCP antibody (94.1%), compared to 9 (29.0%) and 16 (51.6%), respectively, in Group B ($p < 0.0001$ both comparisons). Group A patients also displayed higher levels of nephelometric RF [median 256.0 U/ml (5th-95th 45–1800.0) vs median 10.0 U/ml (5th-95th 10.0–221.0); $p < 0.0001$], IgA-RF [median 116.9 U/ml (5th-95th 14.7–773.8) vs median 6.7 U/ml (5th-95th 0.8–71.2); $p < 0.0001$], IgM-RF [median 132.4 U/ml (5th-95th 30.7–691.0) vs median 5.8 U/ml (5th-95th 0.1–122.2); $p < 0.0001$], and anti-CCP antibody [median 81.6 U/ml (5th-95th 2.4–345.4) vs median 5.7 U/ml (5th-95th 0.5–691.0); $p = 0.009$] than Group B.

Similarly, in LSRA, controls with levels of BAFF ≥ 8.6 ng/ml had higher titers of autoantibody (Table 2). There were more RF and anti-CCP antibodies in patients with elevated levels of BAFF than in the remainder: 84% RF-positive in the presence of BAFF+ compared with 50% in its absence (OR 5.2, 95% CI 1.4–20.0), and 88% anti-CCP-positive compared with 45.8% (OR 8.7, 95% CI 2.0–36.9).

In patients with ERA the level of BAFF was also related to the number of swollen joints, considering either the 28-joint count (12.3 ± 6.1 vs 8.7 ± 6.1; $p = 0.04$) or the 44-joint count (17.2 ± 10.6 vs 11.5 ± 8.3 U/ml; $p = 0.04$). No correlation was found with DAS values. Finally, considering all ERA patients, BAFF correlated with the 28-swollen joint count ($r = 0.37$; $p = 0.01$) as well as the 44-swollen joint count ($r = 0.40$; $p = 0.005$) (Figure 4). No correlation was found between swollen joint count and BAFF levels in the LSRA cohort.

Serial assessment of ERA patients. In 21 ERA patients, BAFF was measured before and after 6.7 ± 2.8 months of MTX treatment. BAFF levels diminished from 6.1 ng/ml (5th-95th 1.0–39.9) to 2.8 ng/ml (5th-95th 0.6–35.0) ($p =$

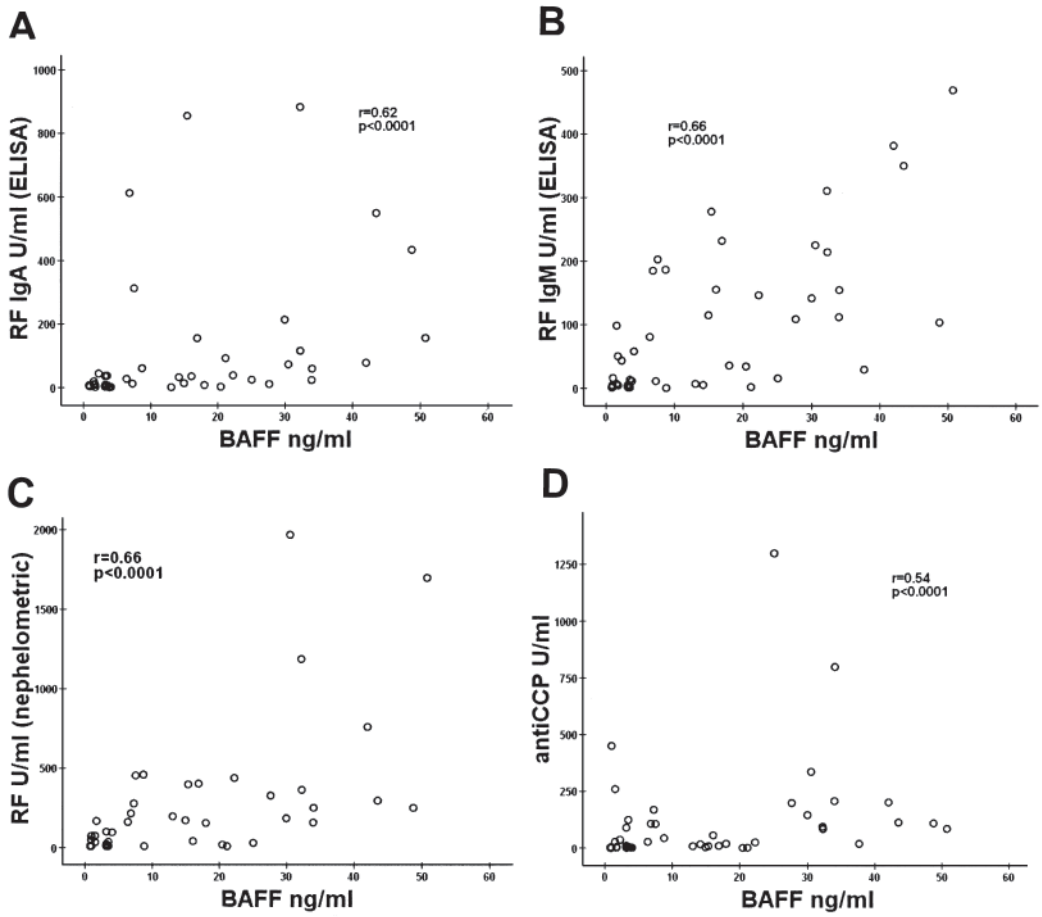

Figure 3. Correlations between levels of BAFF and (A) titers of IgA-RF by ELISA; (B) titers of RF-IgM ELISA; (C) levels of nephelometric RF; and (D) titers of anti-CCP antibodies in 49 patients with longstanding RA. Each point indicates an individual blood sample. R values determined by Spearman correlation test.

0.003), and the DAS28 from 6.4 ± 1.3 to 4.0 ± 1.9 ($p < 0.0001$; Table 3). There was also a reduction in the titer of RF-IgA, from 19.8 U/ml (5th-95th 1.6–563.5) to 9.5 U/ml (5th-95th 0.7–279.1) ($p = 0.03$), of RF-IgM from 43.4 U/ml (5th-95th 0.1–660.7) to 9.8 U/ml (5th-95th 0.3–476.2) ($p = 0.008$), and of anti-CCP from 81.6 U/ml (5th-95th 0.7–804.7) to 21.3 U/ml (5th-95th 0.3–234.8) ($p = 0.002$).

Over this followup period, the levels of BAFF diminished in 17 patients, were stable in one, and increased in the remaining 3 (Figure 5). After 6.7 ± 2.8 months, 17 patients (81%) showed a positive clinical response, with an improvement of DAS28 ≥ 1.2, and 14 patients (67%) had a decrease of DAS28 up to 3.2.

At baseline, the levels of BAFF were ≥ 8.6 ng/ml in 9 patients (42.8%) and < 8.6 ng/ml in 12 (57.2%). After a 6.7 ± 2.8-month treatment, there remained only 2 (9.5%) patients with BAFF levels ≥ 8.6 ng/ml (OR 7.13, 95% CI 1.11–58.04). Seven of the 9 patients with BAFF ≥ 8.6 ng/ml at start had BAFF < 8.6 ng/ml after treatment; one had a reduction but with BAFF level still > 8.6 ng/ml, and one had an increase. In other words, the levels of BAFF diminished in all the patients but one, and DAS28 was reduced by 1.2 in all of these 8 patients. The sole patient with increased BAFF developed cutaneous vasculitis and rheumatoid nodules, and did not have a clinical response.

In addition, 9 of the 12 patients with BAFF levels < 8.6 ng/ml had a further reduction, one had no changes and only 2 had an increase. Interestingly, the 21 ERA patients exhibited a significant correlation between the modification of BAFF and either the modification of RF-IgM ($r = 0.55$; $p = 0.02$) or the number of swollen joints ($r = 0.53$; $p = 0.01$) (Figure 6). Hence a decrease in BAFF levels was associated with an improvement in clinical manifestations in patients with ERA.

DISCUSSION

We describe the largest cohort of patients with early RA examined for their serum levels of BAFF prior to any drug treatment. These patients displayed higher levels of BAFF compared with the HC and the DC[17,18]. The levels were similar in early RA and control RA, suggesting that BAFF is involved from the outset of the disease. This was not a surprise, as we know that autoantibodies (anti-CCP and

Table 2. Demographic, biological, and clinical characteristics of all RA patients and those with longstanding RA (LSRA) and patients with elevated and normal levels of BAFF.

	RA Patients, n = 97	Subgroup A RA pts (n = 42) BAFF ≥ 8.6 ng/ml	Subgroup B RA pts (n = 55) BAFF < 8.6 ng/ml	p*	Subgroup A 25 LSRA pts (51%) BAFF ≥ 8.6 ng/ml	Subgroup B 24 LSRA pts (49.0%) BAFF < 8.6 ng/ml	p**
Age, yrs (mean ± SD)	58.2 ± 12.9	59.0 ± 11.2	57.5 ± 14.2	NS	60.7 ± 10.3	55.6 ± 13.9	NS
F/M	72/25	28/14	44/11	NS	16/9	18/6	NS
Disease duration, mo (mean ± SD)	52.3 ± 86.3	76.9 ± 114.6	33.4 ± 49.4	0.016	124.8 ± 128.6	70.3 ± 56.4	0.002
RF positivity, %	60.8	90.5	38.2	< 0.0001	84.0	50.0	0.02
RF (nephelometric assay), median U/ml (5th-95th)	75 (10–1208.8)	254 (10.0–1808.4)	22.0 (10–263.5)	< 0.0001	252.0 (10.0–1915.2)	37.5 (10.0–438.3)	0.004
IgA-RF (ELISA), median U/ml (5th-95th)	19.5 (1.6–593.7)	73.7 (3.6–856.4)	7.3 (1.2–186.0)	< 0.0001	61.2 (2.3–878.9)	7.9 (1.2–567.9)	0.001
IgM-RF (ELISA), median U/ml (5th-95th)	35.3 (0.5–383.9)	136.1 (2.5–479.4)	6.9 (0.1–166.5)	< 0.0001	142.2 (0.9–443.7)	9.0 (1.0–200.1)	0.001
Anti-CCP-positive, %	67.0	90.5	49.1	< 0.0001	88.0	45.8	0.002
Anti-CCP, median U/ml (5th-95th)	19.7 (0.6–472.5)	81.6 (1.7–754.5)	3.5 (0.4–480.0)	< 0.0001	70.7 (1.3–1175.0)	3.3 (0.3–402.5)	0.015
BAFF levels, median ng/ml (5th-95th)	6.1 (0.8–42.1)	25.4 (8.9–50.4)	3.2 (0.8–7.3)	< 0.0001	25.0 (8.7–50.1)	2.7 (0.8–7.5)	< 0.0001
ESR, mm/h (mean ± SD)	34.3 ± 27.5	34.6 ± 27.6	33.9 ± 27.7	NS	28.9 ± 22.6	29.4 ± 28.8	NS
CRP, mg/dl (mean ± SD)	23.7 ± 30.8	21.3 ± 21.8	25.6 ± 36.2	NS	18.2 ± 16.4	25.1 ± 42.0	NS
SJ28 (mean ± SD)	9.9 ± 5.8	11.3 ± 6.3	8.8 ± 5.1	0.05	10.6 ± 6.5	8.9 ± 5.5	NS
TJ28 (mean ± SD)	14.2 ± 6.3	14.6 ± 6.1	13.9 ± 6.4	NS	14.2 ± 5.5	13.8 ± 6.7	NS
SJ44 (mean ± SD)	13.1 ± 8.9	14.9 ± 9.7	11.7 ± 7.9	NS	13.4 ± 8.2	12.0 ± 7.6	NS
TJ44 (mean ± SD)	19.0 ± 8.8	19.3 ± 8.6	18.8 ± 9.3	NS	18.8 ± 7.5	19.3 ± 10.5	NS
DAS28 (mean ± SD)	5.9 ± 1.3	6.0 ± 1.4	5.8 ± 1.3	NS	5.3 ± 1.1	5.7 ± 1.2	NS
HAQ (mean ± SD)	1.5 ± 0.7	1.5 ± 0.8	1.4 ± 0.7	NS	1.5 ± 0.8	1.3 ± 0.7	NS

Values are percentage or mean ± SD or median (5th-95th percentiles), per normal or non-normal distribution. RF: rheumatoid factor, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, anti-CCP: anti-cyclic citrullinated antibodies, SJ28: swollen joint count 28 joints, TJ28: tender joint count 28 joints, SJ44: swollen joint count 44 joints, TJ44: tender joint count 44 joints, DAS28: Disease Activity Score for 28 joints, HAQ: Health Assessment Questionnaire. LSRA: > 1 year disease duration. Cutoff level for BAFF was set at 2 SD above the mean of 50 normal samples (BAFF ≥ 8.6 ng/ml). * Patients with BAFF ≥ 8.6 ng/ml (subgroup A) vs patients with BAFF < 8.6 ng/ml (subgroup B), considering the whole RA patient cohort; Mann-Whitney nonparametric test; ** patients with BAFF ≥ 8.6 ng/ml (subgroup A) vs patients with BAFF < 8.6 ng/ml (subgroup B) in LSRA group, Mann-Whitney nonparametric test.

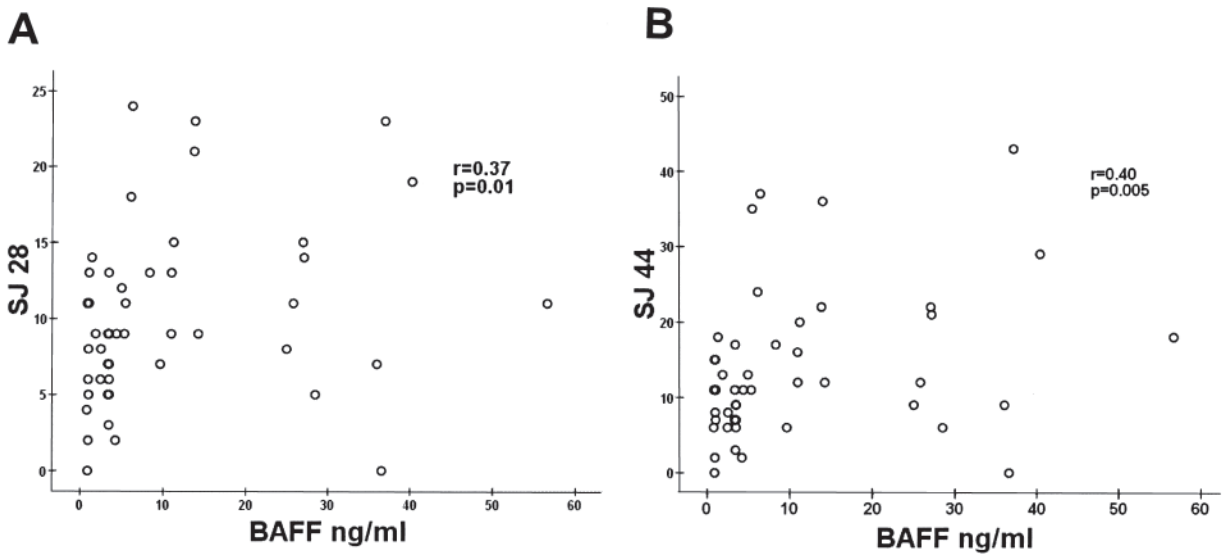

Figure 4. Correlation between BAFF levels and 28-swollen joint count (A) and 44-joint count (B) in 48 early RA patients. Each point indicates an individual blood sample. R values determined by Spearman correlation test.

Table 3. Clinical and biological characteristics of 21 early RA patients with followup evaluation. Clinical and biological evaluations were performed at Time 0 before the beginning of therapy and after 6.7 ± 2.8 months of therapy with methotrexate 10–20 mg/week with or without 5 mg/day prednisone.

	Time 0	Followup	p*
BAFF level, median ng/ml (5th-95th)	6.1 (1.0–39.9)	2.8 (0.6–35.0)	0.003
BAFF ≥ 8.6 ng/ml, %	42.8	9.5	0.03
RF-positive, %	66.6	52.4	NS
RF (nephelometric method), median U/ml (5th-95th)	105.0 (10.0–1160.5)	46.0 (4.3–1125.5)	NS
IgA-RF (ELISA), median U/ml (5th-95th)	19.8 (1.6–563.5)	9.5 (0.7–279.1)	0.03
IgM-RF (ELISA), median U/ml (5th-95th)	43.4 (0.1–660.7)	9.8 (0.3–476.2)	0.008
Anti-CCP-positive, %	85.7	71.4	NS
Anti-CCP, median U/ml (5th-95th)	81.6 (0.7–804.7)	21.3 (0.3–234.8)	0.002
ESR, mm/h (mean ± SD)	47.0 ± 31.0	18.9 ± 13.7	< 0.0001
CRP, mg/l (mean ± SD)	36.9 ± 36.5	5.9 ± 12.7	< 0.0001
SJ28 (mean ± SD)	10.5 ± 6.3	4.1 ± 5.1	0.001
TJ28 (mean ± SD)	15.9 ± 7.2	6.6 ± 6.8	< 0.0001
SJ44 (mean ± SD)	15.6 ± 11.9	5.8 ± 8.0	0.002
TJ44 (mean ± SD)	20.9 ± 10.7	9.9 ± 10.6	0.001
DAS28 (mean ± SD)	6.4 ± 1.3	4.0 ± 1.9	< 0.0001
HAQ (mean ± SD)	1.7 ± 0.8	0.9 ± 0.8	0.001

Values are percentage or mean ± SD or median (5th-95th percentiles) per normal or non-normal distribution. SJ28: swollen joint count 28 joints, TJ28: tender joint count 28 joints, SJ44: swollen joint count 44 joints, TJ44: tender joint count 44 joints, DAS28: Disease Activity Score for 28 joints, HAQ: Health Assessment Questionnaire. * Clinical and biological evaluations at Time 0 vs evaluations at followup; Wilcoxon nonparametric test.

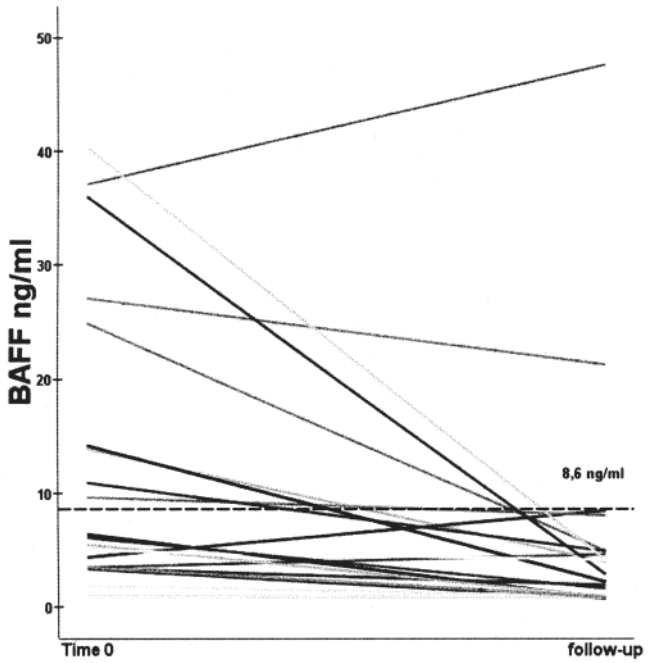

Figure 5. Longitudinal changes in serum BAFF levels in 21 early RA patients during the followup period (after 6.7 ± 2.8 mo). BAFF levels decreased in 17 patients, remained unchanged in 1, and increased in 3 patients. Each line indicates plasma levels of BAFF in each patient at Time 0 and after 6.7 ± 2.8 months. Broken line indicates cutoff value (mean + 2 SD of control samples).

RF) can antedate the appearance of clinical arthritis by several years.

Elevation of BAFF has repeatedly been reported in a proportion of patients with longstanding RA, and was related with their RF titers[16-20]. Our belief is that data from earlier studies might have overemphasized the relationship with autoantibodies; for example, in one report, the majority of the patients (37 of 42) had RF, thus confounding the possible association[17]. In our study, in which only 54.2% of ERA and 67.3% of LSRA were RF-positive, we confirm not only the strong correlation between BAFF levels and the titers of autoantibodies, but also a strong association with the simple positivity or negativity of the tests. These data reinforce the relationship between BAFF and the autoimmune response. Another potential consideration is that DMARD were not always taken into account in the calculations, raising the issue of their effects on the production of BAFF. In one study, patients taking DMARD had higher levels of BAFF in their SF than in their sera, and there was a correlation between the level in the SF and the number of local polymorphonuclear cells[19], but no correlations between clinical indices and BAFF were investigated. A significant correlation between DAS and BAFF levels was reported in a small group of RA patients[30].

Our data are clear in showing that BAFF levels are increased in patients with early RA, as in the RA controls, and suggest that B cells are part of an amplifying inflammatory process from the earliest phase of the disease. Another interesting finding is that high titers of IgA-RF and IgM-RF

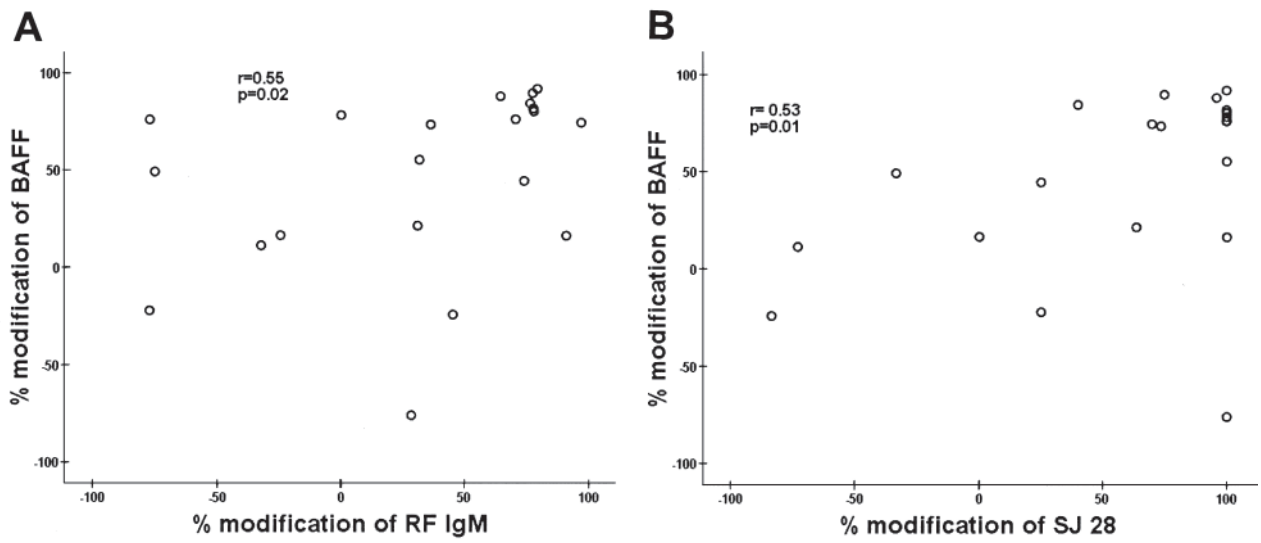

Figure 6. Correlations between modification of BAFF under MTX treatment and (A) titers of IgM-RF by ELISA; (B) number of swollen joints (SJ 28) in 21 patients with early RA. Each point indicates an individual blood sample. R values determined by Spearman correlation test.

are accompanied by elevated levels of BAFF, as reported in longstanding RA[17] and mixed cryoglobulinemia[31]. Nonetheless, our study is unique in that we demonstrated that the gradient of BAFF levels parallels RF and anti-CCP antibody titers. In light of this correlation, one may predict that BAFF is restricted to the most aggressive phenotype of RA. Anti-CCP antibody and IgA-RF are extremely precocious markers of the tolerance breakdown that triggers the disease.

We observed a correlation between the levels of BAFF and the numbers of swollen joints in patients with ERA, prior to any treatment influencing the severity of the synovitis. This suggests that BAFF production by myelomonocytic and FLS cells in synovial tissue can contribute to disease by allowing a survival niche for autoreactive B cells, and that the more abundant the synovial tissue, the more likely is the survival of B cells[9].

At the time of BAFF determination, the control RA patient cohort, with longstanding disease, presented a still-active synovitis and disease indices comparable to those of ERA patients at baseline. The lack of correlation between swollen joints in longstanding RA could certainly be due to modulation of the immunosuppressive treatment on BAFF production, as demonstrated longitudinally in our cohort of ERA. Under treatment with DMARD a relationship between BAFF levels and synovitis could become much more difficult to observe. That DMARD-treated control RA patients had similar levels of BAFF could also indicate that, once RA has become persistently active, the inflammation-amplifying loop seems to involve BAFF.

In addition, other factors can influence serum BAFF levels, such as immunosuppressive therapies, binding of BAFF to its cognate receptors on B cells[32], and the presence of heterotrimers of BAFF and APRIL not identified by the antibody used in the ELISA[10].

Because it has been reported that a therapy like anti-CD20 treatment can modify BAFF levels[30,33,34], we emphasize that levels of BAFF were for the first time determined in patients prior to any treatments — we confirmed the increased levels of BAFF, suggesting a pivotal role of this cytokine in the pathophysiology of the disease; but we also indirectly support its significant role by showing a decrease of BAFF levels after good clinical response was obtained with MTX.

In vivo B cell-depleting therapy has clearly shown that B cells are a credible target, and that B cell depletion results in substantial and persistent attenuation of the inflammatory process that leads to relevant clinical results[35,36]. Therapeutic approaches through a BAFF-receptor blockade are now at hand[37-39]. The increase of BAFF levels reported after rituximab treatment[30,33,34] suggests that B cell repopulation can be differently influenced by BAFF levels[31], and BAFF-antagonist treatment could therefore be considered to prolong the clinical remission after rituximab infusions. Whether targeting BAFF or BAFF receptors[40] will be more effective in early RA than in LSRA or in particular subgroups of RA, such as autoantibody-positive disease, will be major issues of future research.

We conclude that BAFF is elevated throughout the RA disease process, not only in longstanding but also in early RA, in the absence of any treatment. Irrespective of disease duration, levels correlate with all autoantibody titers and with the synovitis early in the course of the disease. This was established by our followup study showing an association between reduction in the level of BAFF and a decrease in disease activity. Treatment with anti-BAFF antibodies and receptor-Fc fusion decoy protein thus seems to be an attractive possiblity in ERA.

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