CONCISE REPORT

Increase of B cell-activating factor of the TNF family (BAFF) after rituximab treatment: insights into a new regulating system of BAFF production

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Background: The cytokine B cell-activating factor of the TNF family (BAFF) is involved in the pathogenesis of autoimmune diseases.

Objective: To access changes in serum protein and mRNA levels of BAFF after rituximab treatment.

Methods: Serum and peripheral blood mononuclear cells (PBMCs) were isolated from five patients (two with lupus, two with Sjögren's syndrome, one with rheumatoid arthritis) before and 12 weeks (range 7–17) after a first course of rituximab infusion. Monocytes and B cells were selected from healthy controls and cocultured for 72 h. BAFF protein and mRNA levels were assessed by ELISA and real-time PCR, respectively.

Results: After rituximab treatment, median serum BAFF protein level and BAFF to actin mRNA ratio in PBMCs significantly increased. In monocytes cocultured with autologous B cells, BAFF protein level decreased, whereas the mRNA level was stable. In one closely monitored patient, the mRNA ratio of BAFF to actin in PBMCs increased later than the BAFF serum level.

Conclusions: Two distinct mechanisms are probably involved in the increase in BAFF level after B cell depletion: (1) the decrease in its receptors leading to a release of BAFF; (2) a delayed regulation of BAFF mRNA transcription. This could favour the re-emergence of autoreactive B cells.

The cytokine B cell-activating factor of the TNF family (BAFF), also called B lymphocyte stimulator (BLyS), is mainly expressed by monocytes and plays a key role in B cell activation and survival through three receptors: BCMA, TACI, and BAFFR or BR3.[1–3] BAFF is highly involved in the pathogenesis of autoimmune diseases.[4] Thus, transgenic BAFF mice develop systemic autoimmune disease mimicking systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), primary Sjögren's syndrome (pSS), and have a delayed increase in B cell lymphoma incidence.[5–8]

The serum level of BAFF has been found to be increased in patients with autoimmune diseases like SLE, RA or pSS,[8–11] with a correlation with the level of autoantibodies in some cases.[12] These data underline the potential key role of B cells in systemic autoimmune diseases and are reinforced by the possible effectiveness of rituximab, a monoclonal anti-CD20 antibody, demonstrated in RA[13] and probably in SLE and pSS.

We therefore investigated changes in BAFF level at the protein and mRNA levels in patients with severe autoimmune disease treated with rituximab. Our results demonstrate that the BAFF level increased after rituximab treatment by two distinct mechanisms.

PATIENTS AND METHODS

Patients

We studied five patients with severe and refractory autoimmune diseases treated with rituximab. Two patients had pSS according to AECG criteria, including one with MALT lymphoma. One patient had RA and two had SLE according to ACR criteria. Serum and PBMCs were isolated from all patients before and 12 weeks after (range 7–17) the first rituximab infusion. Patients gave their informed consent to participate in the study, which was approved by the local ethics committee. Patient characteristics are shown in table 1.

Cell cultures

Monocytes and B cells from the peripheral blood of four healthy donors were selected by use of anti-CD14 and anti-CD19 microbeads, respectively, and magnetic activated cell-sorting columns (Miltenyi Biotec, Auburn, California, USA). B cell and monocyte purity was always more than 90%. Monocytes were cultured in duplicate to a final concentration of 5×10^5 cells/ml alone, or with autologous B cells at a ratio of 1:1 or 1:2. After 72 h culture, supernatants and cells were stored at −20°C, cells were later stored in RNA (Qiagen, Courtaboeuf, France).

ELISA

BAFF level in serum or cell supernatants was assessed by ELISA (R&D systems, Minneapolis, Minnesota, USA). The presence of rheumatoid factor in serum does not interfere with this assay.

Real-time quantitative PCR

Total RNA was isolated from PBMCs and from cultured monocytes. BAFF and β-actin cDNA levels were determined by quantitative RT–PCR as described previously.[14] Primers were designed to be specific to full-length BAFF, excluding any amplification of ΔBAFF.

Statistical analysis

Results are shown as means (SD). Statistical comparison involved the Wilcoxon signed ranks test using Analyse-it for Microsoft Excel (Leeds, England, UK). $p \leqslant 0.05$ was considered significant.

RESULTS

BAFF serum and mRNA level evolution under rituximab treatment

Twelve weeks after the first rituximab infusion, B cells were undetectable in all evaluated patients (4/5). The median (SEM)

Abbreviations: BLyS, B lymphocyte stimulator; MALT, mucosa-associated lymphoid tissue; PBMC, peripheral blood mononuclear cells; pSS, primary Sjögren's syndrome; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus.

Table 1 Patient characteristics

Number	Age	Sex	Rituximab infusion regimen	Time between t1 and t2 (weeks)	Diagnosis
1	25	F	2×1 g (W0, 2)	9	SLE
2	59	F	4×375 mg/m^2 (W0, 1, 2, 3)	11	pSS+MALT
3	57	F	2×1 g (W0, 2)	7	pSS
4	49	F	2×1 g (W0, 2)	15	RA
5	31	M	4×375 mg/m^2 (W0, 1, 2, 3)	17	SLE

MALT, mucosa-associated lymphoid tissue; pSS, primary Sjögren's syndrome; RA, rheumatoid arthritis; SLE, systemic erythematosus; t1 = before rituximab infusion; t2 = 7–17 weeks after rituximab infusion (mean 12 weeks).

serum protein level of BAFF increased threefold, from 1.3 (0.5) to 3.6 (1) ng/ml (p = 0.008) (fig 1A).

Changes in BAFF mRNA level paralleled changes in BAFF protein level: the median (SEM) mRNA ratio of BAFF to actin in PBMCs increased from 12.8 (4.4) to 30.9 (10.4) (p = 0.05) (fig 1B). Monocyte count did not vary significantly for each patient (median (SEM) increase in total count: from 400 (63.2) to 480 (68.2)/mm^3.

Level of BAFF protein and mRNA in monocytes in the presence or absence of autologous B cells

We tested the hypothesis of a negative regulation of B cells on BAFF secretion by monocytes by coculturing purified monocytes and B cells from four healthy subjects. Median (SEM) BAFF protein level in supernatants as assessed by ELISA significantly decreased when monocytes were cultured in the presence of autologous B cells at ratios of both 1:2 and 1:1 (20.7 (4.9) pg/ml, p = 0.03, and 27.8 (2.8) pg/ml, p = 0.03, respectively) as compared with culture without B cells (36.3 (10) pg/ml) (fig 1C). Conversely, the mean mRNA ratio of BAFF to actin did not change significantly (p = 0.3) (fig 1D).

BAFF protein and mRNA levels closely monitored in one patient

The sequential data obtained for mRNA and protein levels at weeks 0, 1, 2, 3 and 11 in patient 2 treated with four weekly infusions of rituximab provide some clues to understanding the phenomenon. In this patient, an increase in BAFF protein level occurred early, at week 3, whereas the BAFF mRNA level was still low at week 3 and increased between week 3 and 11 (no sample was obtained between week 3 and week 11) (fig 2).

DISCUSSION

In five patients (one with RA, two with SS and two with SLE) with severe and refractory autoimmune disease treated with

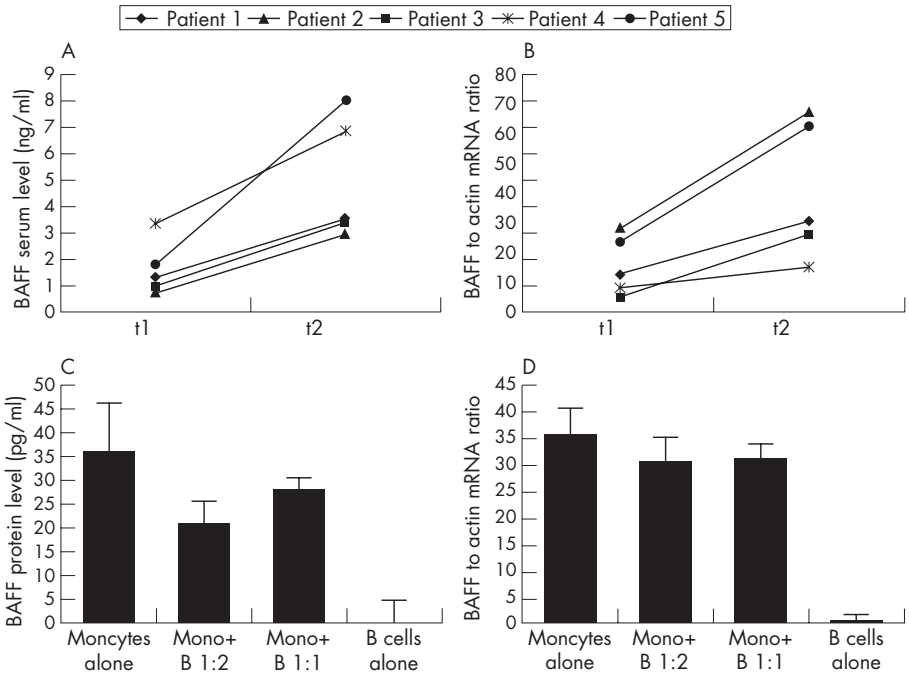

Figure 1 In vivo and in vitro assessment of B cell-activating factor (BAFF) protein and mRNA level evolution after B cell depletion. (A) Serum BAFF protein level (ng/ml) before (t1) and 7–17 weeks after (t2) rituximab infusion. Each line relates to one patient. The median (SEM) serum BAFF level increased from 1.3 (0.5) to 3.6 (1.0) ng/ml (p = 0.008). (B) mRNA ratio of BAFF to actin in peripheral blood mononuclear cells before (t1) and 7-–17 weeks after rituximab infusion (t2). Each line relates to one patient. The median (SEM) mRNA ratio of BAFF to actin increased from 12.8 (4.4) to 30.9 (10.4) (p = 0.05). (C) Median (SEM) BAFF protein level in supernatants after 72 h of culture of monocytes from four healthy subjects in the absence or presence of autologous B cells at a ratio of 1:2 or 1:1. A significant decrease in BAFF level was observed when monocytes were cultured with B cells, whatever the ratio. Results are the mean of six experiments. (D) Median (SEM) mRNA ratio of BAFF to actin after 72 h culture of monocytes from four healthy subjects in the presence or absence of autologous B cells at a ratio of 1:2 or 1:1. No significant difference was observed in BAFF mRNA in monocytes cultured alone or in the presence of B cells. *p = 0.03

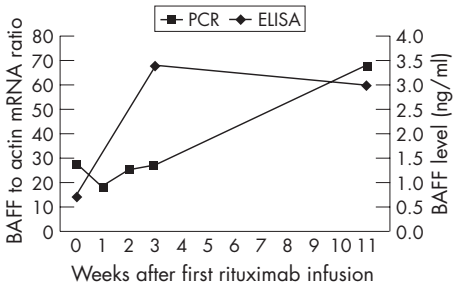

Figure 2 Kinetics of B cell-activating factor (BAFF) increase at both the protein and mRNA levels after rituximab infusion in one closely monitored patient. Serum BAFF protein and mRNA levels were assessed by ELISA in patient two at weeks 0 (before rituximab infusion), 3 and 11. The mRNA ratio of BAFF to actin was assessed in peripheral blood mononuclear cells from the same patient at weeks 0, 1, 2, 3 and 11. BAFF serum level increased as soon as week 3, whereas the mRNA ratio of BAFF to actin increased only between weeks 3 and 11 (no sample was obtained between weeks 3 and 11).

rituximab, we found a significant increase in serum protein level of BAFF 12 weeks after treatment, which extends recent previous results in 15 patients with RA.[15] Thus, the effect of rituximab on the serum protein level of BAFF seems to be the same in different autoimmune diseases.

The simplest mechanism proposed to explain this phenomenon is a mechanistic increase in serum BAFF level after rituximab-induced B-cell depletion because of the disappearance of most BAFF receptors mainly present on the surface of B cells, but other feedback mechanisms can be suggested.[15 16]

To determine the mechanisms involved in BAFF protein level increase after rituximab treatment, we carried out two sets of experiments.

Firstly, we used quantitative PCR to monitor BAFF mRNA level in PBMCs and ELISA to analyse BAFF levels in serum. Changes in BAFF mRNA level paralleled changes in BAFF protein level. We therefore concluded that there is a true transcriptional regulation of BAFF production induced by B-cell depletion.

Secondly, we cocultured purified monocytes and B cells to understand the effect of B cells on BAFF mRNA expression and BAFF protein secretion by monocytes. When monocytes were cultured in the presence of autologous B cells, BAFF protein level decreased whereas BAFF mRNA levels did not change. The sequential data obtained for mRNA and protein levels at weeks 0, 1, 2, 3 and 11 in one patient treated with four weekly infusions of rituximab provide some clues to understanding this discrepancy between the in vitro and ex vivo findings. In this patient, an increase in BAFF protein level occurred early, at week 3, whereas the BAFF mRNA level was still low at week 3 and increased only between weeks 3 and 11 (no sample was obtained between weeks 3 and week 11) (fig 2).

Thus, two successive mechanisms are suggested to explain the increase in BAFF level after rituximab treatment. First, a very early mechanistic increase in the serum protein level of BAFF may occur, demonstrated ex vivo and in vitro by the early increase in protein level with a stable level of mRNA. But this mechanistic increase due to the depletion of BAFF receptors is followed by a positive transcriptional regulation of BAFF, as demonstrated by the concomitant increase in serum BAFF protein and mRNA levels in all patients, 12 weeks after rituximab infusion.

How can this transcriptional regulation of BAFF be explained? We hypothesised that a negative signal might be delivered from B cells to monocytes, thus inhibiting BAFF production. Therefore, B cell depletion could abolish this negative signal, leading to an increased transcription of BAFF mRNA. Nevertheless, coculture experiments did not demonstrate a negative effect of B cells on BAFF mRNA transcription. The absence of a decrease in the BAFF or actin mRNA ratio in cocultures could even signify a positive regulation, since BAFF mRNA expressed by monocytes and not by B cells should have been diluted with the actin expressed by B cells. A possible explanation of this discrepancy is that cocultures were performed during only 3 days because B cells could not survive in vitro any longer, and that, as observed in one patient, regulation of BAFF mRNA transcription occurred late after B-cell depletion. Thus, we suggest that a negative regulation of B cells on BAFF mRNA transcription by monocytes could occur after a long-term persistent interaction between B cells and monocytes. Alternatively, the positive regulation of BAFF mRNA transcription after rituximab might not be related directly to B-cell depletion but to another indirect associated mechanism.

In conclusion, the increased serum BAFF level after rituximab infusion is probably the consequence of two distinct mechanisms: one mechanistically related to the large decrease in receptors after B-cell depletion, and the other to delayed regulation of the BAFF mRNA level. Therefore, the serum BAFF level must be interpreted with caution after rituximab treatment and probably does not reflect the level of activity of the autoimmune disease in these patients. However, this increase in BAFF level after rituximab could favour the re-emergence of autoreactive B cells leading to disease relapse. BAFF-antagonist treatment could therefore be considered to prolong the period of clinical remission after rituximab infusion in refractory autoimmune diseases.

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