

BAFF levels are increased in lesional skin and sera in patients with cutaneous T-cell lymphoma

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Summary

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Conflicts of interest

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Background B-cell-activating factor belonging to the tumour necrosis factor family (BAFF) is known for its role in the survival and maturation of B cells. It has been recently suggested that BAFF also plays important roles in T-cell activation in T-cell mediated diseases such as psoriasis.

Objectives To investigate the role of BAFF in cutaneous T-cell lymphoma (CTCL).

Methods BAFF messenger RNA (mRNA) expression in skin samples (24 CTCL cases and seven healthy controls) and in skin-derived fibroblasts (five CTCL cases and five healthy controls) was examined by quantitative reverse transcription–polymerase chain reaction. We also performed immunohistochemical staining for BAFF and its receptors. Serum BAFF levels were measured in patients with CTCL ($n = 46$), atopic dermatitis ($n = 36$) or psoriasis ($n = 27$) and 27 healthy controls by enzyme-linked immunosorbent assay.

Results Lesional skin of CTCL contained higher levels of BAFF mRNA than normal skin and the expression levels correlated with disease activity. BAFF mRNA expression levels were elevated in fibroblasts from CTCL skin. Tumour cells in the lesional skin of CTCL expressed BAFF and its receptors, while fibroblasts expressed only BAFF. Serum BAFF levels of CTCL patients were significantly higher than those of healthy controls and correlated with types of skin lesions and clinical stages. They also significantly correlated with serum soluble interleukin-2 receptor and lactate dehydrogenase levels.

Conclusions BAFF expression in CTCL skin and serum BAFF levels are significantly increased and correlate with the severity of CTCL. These results suggest that BAFF may have important roles in the development of CTCL.

B-cell-activating factor belonging to the tumour necrosis factor family (BAFF) was identified by several research groups based on its homology to the tumour necrosis factor superfamily.^{1–4} This cytokine is expressed on the cell surface or cleaved and secreted. BAFF is produced by many different types of cells including myeloid cells (monocytes, macrophages, dendritic cells and neutrophils),^{5–10} T cells¹¹ and B cells.¹² Nasal fibroblasts¹³ and fibroblast-like synoviocytes¹⁴ are also reported to express BAFF.

BAFF binds to BAFF receptor (BAFF-R), transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI), and B-cell maturation antigen (BCMA).^{15,16} BAFF is known for its role in the survival and maturation of B cells.¹⁵ BAFF exhibits a strong costimulatory function for B-cell activation *in vitro*.^{1,2} Furthermore, excess BAFF rescues self-reactive B cells from anergy.¹⁷ These functions of BAFF may play a crucial role in autoimmune induction and development. In fact, the Baff-transgenic mouse model showed a

correlation between excess BAFF and autoimmunity.¹⁶ BAFF also plays important roles in T-cell activation through BAFF-R, which is expressed on activated/memory T cells.¹⁰ TACI is also expressed by activated T cells.¹⁸ Blockade of TACI suppresses activation of T cells *in vitro* and inhibits antigen-specific T-cell activation and priming *in vivo*.¹⁹ These results indicate that BAFF also regulates T-cell-mediated immune responses.

With regard to human diseases, BAFF was found to be expressed by tumour cells in B-cell chronic lymphocytic leukaemia (B-CLL),^{20,21} pre-B acute lymphoblastic leukaemia²² and multiple myeloma^{23–25} at greater levels than that observed in normal B cells. Serum BAFF levels are elevated in patients with B-cell lymphoma or leukaemia.²⁶ BAFF may contribute to tumour cell survival as an autocrine factor in these diseases.²¹ In addition, increased serum levels of BAFF have been reported in patients with various autoimmune conditions, such as systemic lupus erythematosus, rheumatoid arthritis, systemic sclerosis and autoimmune pancreatitis.^{27–31}

Chronic activation or survival of self-reactive B cells mediated by BAFF can lead to systemic autoimmune diseases.³² A relationship between BAFF and graft versus host disease (GVHD) has also been suggested. Elevated plasma BAFF levels 6 months after allogeneic stem cell transplantation can predict the development of chronic GVHD.^{33,34} Moreover, T cells infiltrating labial salivary glands from patients with Sjögren syndrome expressed BAFF.³⁵ TACI⁺ T cells were detected in synovial tissue samples from patients with rheumatoid arthritis and synovitis.³⁶ These results suggest that BAFF plays important roles in T-cell-mediated diseases. Indeed, increased serum BAFF levels are reported in patients with alopecia areata³⁷ and those with psoriasis.³⁸ These results indicate that the roles of BAFF in human diseases may be more extensive than first thought.

Cutaneous T-cell lymphoma (CTCL) is a group of lymphoid neoplasms with diverse pathology and clinical course. The most common types are mycosis fungoides (MF) and Sézary syndrome (SS). Although the pathogenesis of CTCL is still unknown, some molecules, such as cutaneous lymphocyte antigen, CCR4 and CCR10, are reported to play roles in attracting T cells to the skin.^{39–41} Cytokine profiles in lesional skin and in peripheral blood from patients with MF change during clinical courses.^{42,43} In the early stage of MF, a T-helper (Th)1-dominant environment is observed, but it shifts to a Th2-dominant environment with disease progression.^{44,45} Other cytokines, such as interleukin (IL)-5, IL-10, IL-17, IL-22, and eotaxins are also reported to be associated with CTCL.^{45–48} In this study, we tried to investigate the roles of BAFF in patients with CTCL.

Materials and methods

Serum and tissue samples

Serum samples were obtained from 46 patients with CTCL [41 MF cases and five SS cases; age 60.3 ± 13.0 years (mean $\pm$ SD); 27 males and 19 females], 36 patients with atopic dermatitis (AD, 34.8 ± 10.9 years; 20 males and 16 females), 27 patients with psoriasis vulgaris (57.4 ± 14.5 years; 19 males and eight females) and 27 healthy controls (38.3 ± 15.5 years, 12 males and 15 females). All patients with CTCL were given diagnoses according to the current World Health Organization classification.⁴⁹ Patients with CTCL were subgrouped into patch/plaque (26 cases), tumour (12 cases) and erythroderma (eight cases) according to their types of skin lesions defined by the International Society of Cutaneous Lymphoma (ISCL) and the European Organization of Research and Treatment of Cancer (EORTC).⁵⁰ They were also subgrouped into stage I (25 cases), stage II (nine cases), stage III (five cases) and stage IV (seven cases) according to the staging system proposed by the ISCL/EORTC.⁵⁰ All patients with AD fulfilled the criteria of Hanifin and Rajka.⁵¹ The 27 healthy controls had no history of allergy, CTCL, AD, psoriasis or any skin diseases. All serum samples were stored at -20°C until use.

Messenger RNA (mRNA) was obtained from biopsy samples of lesional skin of patients with CTCL [$n = 24$; patch ($n = 7$), plaque ($n = 5$), tumour ($n = 5$) and erythroderma ($n = 7$); stage I ($n = 12$), stage II ($n = 5$), stage III ($n = 3$) and stage IV ($n = 4$)] and from normal skin ($n = 7$) using an Illustra QuickPrep Micro mRNA Purification Kit (GE Healthcare, Buckinghamshire, U.K.). We extracted mRNA from frozen samples stored at -80°C . All samples were collected during daily clinical practice. Serum samples were collected from almost all patients with lymphoma in our facilities; other samples were obtained from selected cases that were available. Clinical and laboratory data used in this study are summarized in Table 1. The medical ethical committee of the University of Tokyo approved all described studies and the study was conducted according to the Declaration of Helsinki principles. Informed consent was obtained to use blood and skin samples.

Enzyme-linked immunosorbent assay

Enzyme-linked immunosorbent assay kits were used for measuring serum BAFF levels (R&D systems, Minneapolis, MN, U.S.A.), according to the manufacturer's protocols. The measured values from individual patients were plotted by dots. The detection limit of the assay was 3.38 pg mL^{-1} .

Immunohistochemistry

We performed immunohistochemical staining for BAFF, BAFF-R and TACI with lesional skin of patients with CTCL [$n = 15$, patch ($n = 2$), plaque ($n = 4$), tumour ($n = 4$) and erythroderma ($n = 5$); stage I: ($n = 6$), stage II ($n = 3$), stage III ($n = 1$) and stage IV ($n = 5$)] and normal skin ($n = 4$). Briefly, 5- μm thick tissue sections from formaldehyde-fixed and paraffin-embedded samples were dewaxed and rehydrated. These sections were then stained with rat antihuman BAFF monoclonal antibody (ALX-804-131; Alexis Biochemicals, San Diego, CA, U.S.A.), goat antihuman BAFF-R polyclonal antibodies (AF1162; R&D Systems), rabbit antihuman TACI polyclonal antibodies (IMG-249; Imgenex, San Diego, CA, U.S.A.) or control antibodies followed by ABC staining (Vector Lab, Burlingame, CA, U.S.A.). Diaminobenzidine was used for visualizing the staining, and counterstaining with Mayer's haematoxylin was performed, according to the manufacturer's instructions. The degrees of positive staining for BAFF were evaluated as +, ++ or +++.

Cell culture

Dermal fibroblasts were obtained from lesional skin of patients with CTCL [$n = 5$, plaque ($n = 1$), tumour ($n = 2$) and erythroderma ($n = 2$); stage I ($n = 1$), stage II ($n = 2$), stage III ($n = 1$) and stage IV ($n = 1$)] and normal skin adjacent to benign skin tumours ($n = 5$). Fibroblasts were separated from skin by the skin explant culture protocol described in a previous paper.⁵² They were cultured at 37°C , 5% CO_2 in Minimum Essential Medium Eagle (MEM) (Sigma, St Louis, MO,

Table 1 Summary of clinical data

Age	Sex	Stage	Clinical stage	Serum levels			IHC	Real-time RT-PCR
				BAFF (pg mL ⁻¹)	sIL-2R (U mL ⁻¹)	LDH (IU L ⁻¹)	BAFF	BAFF
83	F	I	Patch	1254.8	180	190	ND	0.0011
82	F	I	Patch	1027.5	280	208	ND	0.0276
54	M	I	Patch	ND	409	156	++	0.0116
49	M	I	Patch	ND	270	180	+	0.0039
63	M	I	Plaque	848.0	449	193	+	0.0038
52	F	I	Plaque	916.2	180	172	ND	0.0160
63	M	I	Plaque	1243.8	664	241	++	0.0037
65	M	I	Plaque	459.0	558	199	+	ND
56	F	I	Plaque	1053.9	211	191	ND	0.0021
59	M	I	Plaque	638.0	1478	168	+	0.0025
43	M	II	Tumour	1689.8	4035	453	ND	0.0145
47	M	II	Tumour	865.7	581	242	ND	0.0048
63	F	II	Tumour	1448.8	689	356	++	ND
69	M	II	Tumour	1235.1	239	233	ND	ND
57	M	II	Tumour	1116.5	359	301	++	0.0085
67	F	II	Tumour	2246.1	780	324	ND	0.0032
70	M	II	Tumour	ND	962	209	+	0.0016
41	M	III	Erythroderma	957.8	300	191	ND	0.0130
31	F	III	Erythroderma	876.7	189	190	ND	0.0227
64	M	III	Erythroderma	2037.0	2276	277	+++	0.0166
43	M	IV	Erythroderma	1941.2	1535	343	ND	0.0164
38	F	IV	Erythroderma	1511.2	1519	384	+++	0.0161
58	F	IV	Erythroderma	2548.3	3475	951	+++	0.0160
42	F	IV	Erythroderma	1351.4	698	372	+++	ND
65	F	IV	Erythroderma	1352.6	479	264	+++	0.0328
75	F	IV	Tumour	ND	7392	429	+++	ND

BAFF, B-cell-activating factor belonging to the tumour necrosis factor family; IL, interleukin; LDH, lactate dehydrogenase; IHC, immunohistochemistry; ND, not done; RT-PCR, reverse transcriptase–polymerase chain reaction; sIL-2R, soluble IL-2 receptor. +, ++, +++, degrees of positive staining for BAFF.

U.S.A.) containing 10% fetal bovine serum, penicillin G sodium, streptomycin sulphate and amphotericin B. Third- to fourth-passage cells were used in all experiments. Total RNA was prepared by using TRIZOL[®] Reagent (Invitrogen, Carlsbad, CA, U.S.A.).

Quantitative reverse transcriptase–polymerase chain reaction assay

cDNA was synthesized using TaqMan[®] Reverse Transcription Reagents (Applied Biosystems, Foster City, CA, U.S.A.). Quantitative reverse transcription-PCR (RT-PCR) was performed using the following primers: BAFF forward, 5'-AAG ACC TAC GCC ATG GGA CAT CAT C-3' and reverse, 5'-TCT TGG TAT TGC AAG TTG GAG TTC A-3'; BAFF-R forward, 5'-AGG ACG CCC CAG AGC C-3' and reverse, 5'-AGT GTC TGT GCT TCT GCA GG-3'; TACI forward, 5'-AGT GAA CCT TCC ACC AGA GC-3' and reverse, 5'-CTC TTC TTG AGG AAG CAG GC-3'; glyceraldehyde-3-phosphate dehydrogenase (GAPDH) forward, 5'-ACC CAC TCC TCC ACC TTT GA-3' and reverse, 5'-CAT ACC AGG AAA TGA GCT TGA CAA-3'. mRNA expression levels of BAFF, BAFF-R, and TACI were quantified relative to GAPDH expression levels.

Intracellular cytokine staining

Peripheral blood mononuclear cells were obtained by density centrifugation over Ficoll-Paque (GE Healthcare, Piscataway, NJ, U.S.A.). T-cell receptor V β expression was determined by staining with fluorescein–isothiocyanate-conjugated V β specific monoclonal antibody (Beckman Coulter, Brea, CA, U.S.A.). Intracellular cytokine staining was carried out using fixation/permeabilization concentrate, fixation/permeabilization diluent, and 10 $\times$ permeabilization buffer (all from eBioscience, San Diego, CA, U.S.A.) according to the manufacturer's protocol. Phycoerythrin (PE)-conjugated anti-human BAFF monoclonal antibody (137314; R&D systems) was used to detect intracellular expression of BAFF. Samples were analysed with an FACScan flow cytometer (Becton Dickinson, Franklin Lakes, NJ, U.S.A.).

Statistical analysis

Statistical analysis between two groups was performed using the Mann–Whitney U-test. When multiple groups were compared, one-way ANOVA followed by Tukey–Kramer multiple comparisons test was performed. Correlation coefficients were

determined by using the Spearman's rank correlation test. *P*-values of < 0.05 were considered statistically significant.

Results

BAFF mRNA expression in lesional skin of cutaneous T-cell lymphoma

We first assessed BAFF mRNA expression in lesional skin of CTCL and in normal skin by quantitative RT-PCR. Lesional skin of CTCL contained higher levels of BAFF mRNA than normal skin (Fig. 1a; $P < 0.01$). BAFF mRNA expression levels in erythroderma were significantly higher than those in normal skin ($P < 0.005$), patch/plaque ($P < 0.01$) and tumour ($P < 0.05$; Fig. 1b). BAFF mRNA expression levels in lesional skin of stage IV patients were significantly higher than those in normal skin ($P < 0.01$), stage I patients ($P < 0.05$) and stage II patients ($P < 0.05$; Fig. 1c). BAFF mRNA expression levels in lesional skin of stage III patients were also elevated compared with normal skin ($P < 0.05$). We next examined BAFF mRNA expression levels in cultured fibroblasts obtained from CTCL lesional skin and normal skin because nasal fibroblasts are reported to express BAFF.¹³ As shown in Figure 1d, BAFF mRNA expression levels were higher in fibroblasts from lesional skin of CTCL than in those from normal skin ($P < 0.01$). Thus, expression of BAFF mRNA is upregulated in lesional skin of CTCL and BAFF mRNA expression levels increase with disease progression.

BAFF, BAFF-R and TACI expression in lesional skin of cutaneous T-cell lymphoma

We next immunolabelled lesional skin samples of CTCL and normal skin for BAFF, BAFF-R and TACI. In normal skin,

BAFF, BAFF-R or TACI were not expressed (Fig. 2e–h). In lesional skin of CTCL, atypical lymphocytes infiltrating into the epidermis and the dermis were positive for BAFF (Fig. 2a,b). In addition, dermal fibroblasts also expressed BAFF (Fig. 2b). BAFF-R and TACI, both of which were receptors for BAFF, were expressed on infiltrating atypical lymphocytes (Fig. 2c,d). Thus, tumour cells in CTCL express both BAFF and its receptors, BAFF-R and TACI, whereas dermal fibroblasts in CTCL express only BAFF.

Serum BAFF levels and their correlation with disease severity

Serum BAFF levels in patients with CTCL were 1101.6 ± 84.8 pg mL⁻¹, which were significantly higher than those of healthy controls (966.8 ± 143.6 pg mL⁻¹; $P < 0.05$) (Fig. 3a). Serum BAFF levels in patients with AD and those in patients with psoriasis were 715.3 ± 37.1 and 1238.6 ± 83.8 pg mL⁻¹, respectively. The latter was significantly higher than those of healthy controls ($P < 0.05$), which was consistent with a previous report.³⁸ We next examined serum BAFF levels in patients with different skin lesions and clinical stages. Serum BAFF levels in patients with patch/plaque, tumour and erythroderma were 1041.1 ± 48.9 , 1327.5 ± 129.6 and 1597.7 ± 215.7 pg mL⁻¹, respectively (Fig. 3b). Serum BAFF levels in patients with tumours were significantly higher than those in healthy controls ($P < 0.01$). Serum BAFF levels with erythroderma were extremely high, which were significantly higher than patients with patch/plaque ($P < 0.005$) as well as those with healthy controls ($P < 0.005$). Serum BAFF levels in patients at stages I, II, III and IV were 1044.6 ± 50.8 , 1149.8 ± 90.2 , 1345.8 ± 263.4 and 1798.9 ± 202.9 pg mL⁻¹, respectively (Fig. 3c). Serum BAFF levels in stage IV patients were significantly higher than those with healthy controls

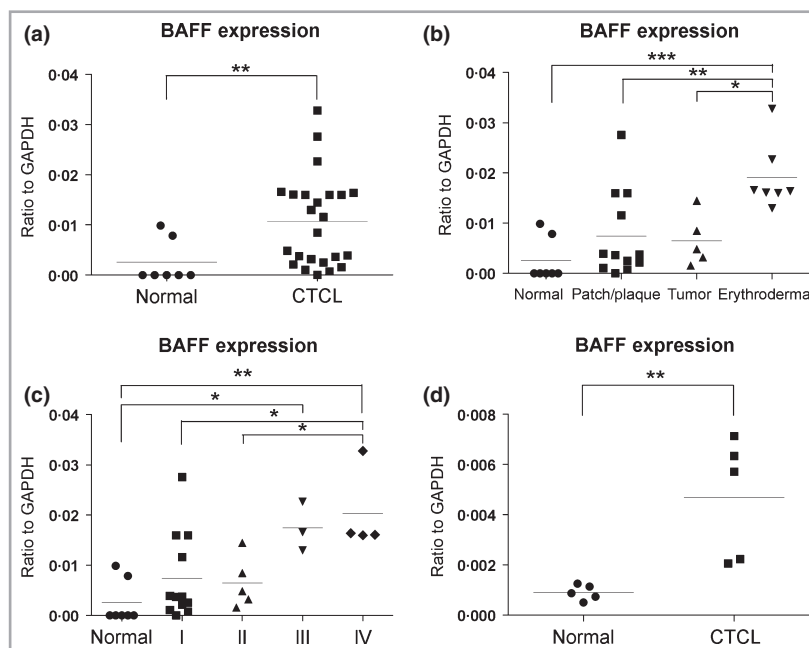
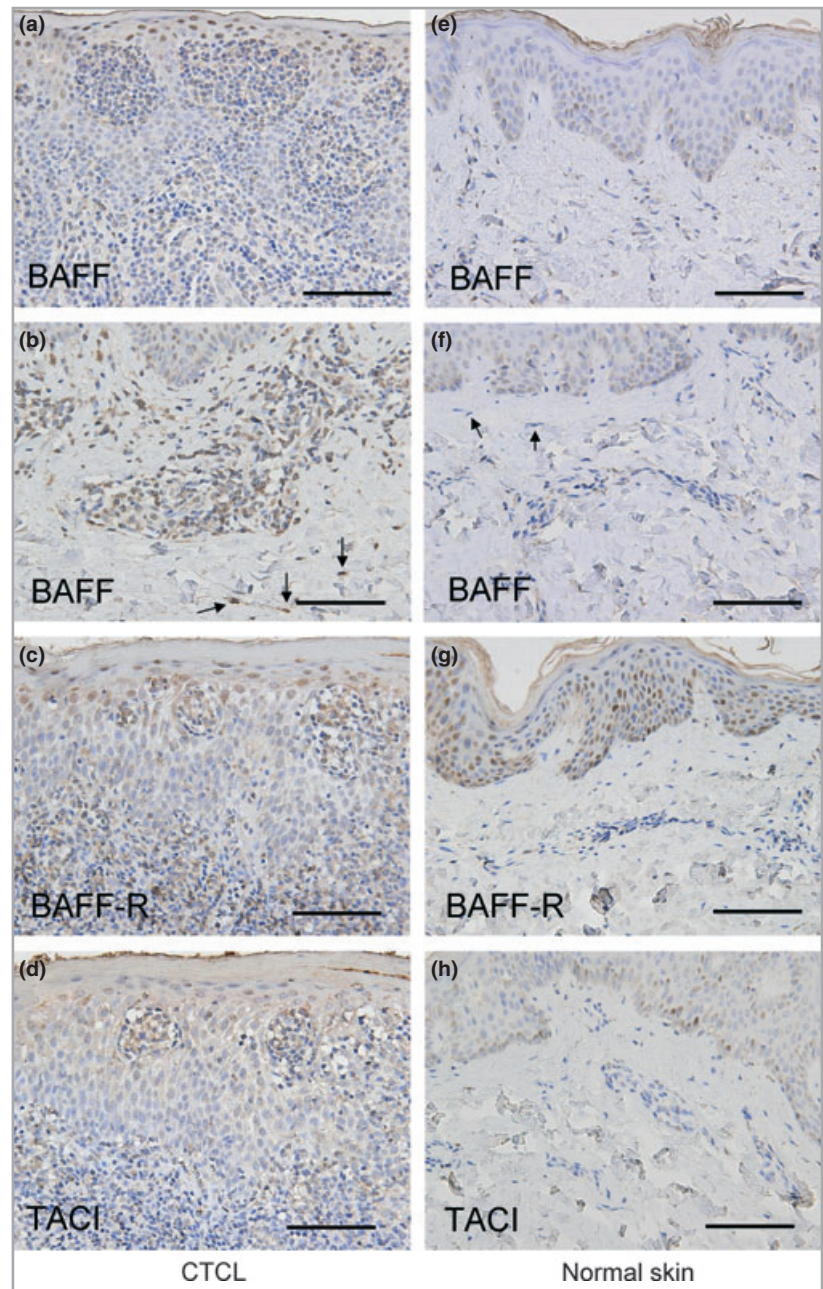


Fig 1. Messenger RNA (mRNA) expression levels of B-cell-activating factor belonging to the tumour necrosis factor family (BAFF) relative to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). (a–c) BAFF mRNA expression in lesional skin of cutaneous T-cell lymphoma (CTCL; $n = 24$) and in normal skin ($n = 7$) assessed by quantitative reverse transcription–polymerase chain reaction (RT-PCR); (d) BAFF mRNA expression in dermal fibroblasts from lesional skin of CTCL ($n = 5$) and from normal skin ($n = 5$). The measured values from individual patients are plotted by dots. Each bar indicates the mean of each group. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$.

Fig 2. Immunohistochemistry of B-cell-activating factor belonging to the tumour necrosis factor family (BAFF), BAFF receptor (BAFF-R) and transmembrane activator and cyclophilin ligand interactor (TACI) in lesional skin of CTCL ($n = 15$) and in normal skin ($n = 4$). Staining of lesional skin in patients with CTCL for BAFF (a, b), BAFF-R (c) and TACI (d). Lymphocytes infiltrating into the epidermis and the dermis positive for BAFF (a, b), BAFF-R (c) and TACI (d). Dermal fibroblasts positive for BAFF (b; arrows). Staining of normal skin for BAFF (e, f), BAFF-R (g) and TACI (h). Representative staining of tissue sections is shown. Original magnification $\times 400$. Scale bars represent 100 μm . CTCL, cutaneous T-cell lymphoma.



($P < 0.005$), stage I patients ($P < 0.005$) and stage II patients ($P < 0.005$).

We also compared serum BAFF levels with other clinical and laboratory data: age, sex and serum levels of soluble IL-2 receptor (sIL-2R) and lactate dehydrogenase (LDH). We found that serum BAFF levels significantly correlated with serum sIL-2R and LDH levels ($r = 0.47$, $P < 0.01$ and $r = 0.61$, $P < 0.01$, respectively; Fig. 3d,e), which have been reported to reflect disease activity of CTCL.^{53–55} Other factors, including the numbers of white blood cells and eosinophils, did not correlate with serum BAFF levels (data not shown). Moreover, there was a slight trend that cases with high serum BAFF levels showed high BAFF mRNA expression levels and enhanced

BAFF expression in lesional skin (Table 1). Thus, serum BAFF levels correlate with disease activity in CTCL.

Changes in serum BAFF levels with exacerbation or improvement of skin conditions

In five patients with CTCL (four cases with MF and one case with SS), we measured serum BAFF levels before and after disease progression. Despite aggressive therapy, such as topical steroids, UV phototherapy and chemotherapy, skin lesions continued to progress in these patients. The average serum BAFF levels increased from 1042.1 ± 140.6 to 2176.3 ± 631.6 pg mL^{-1} after progression of skin lesions (Fig. 4a). In

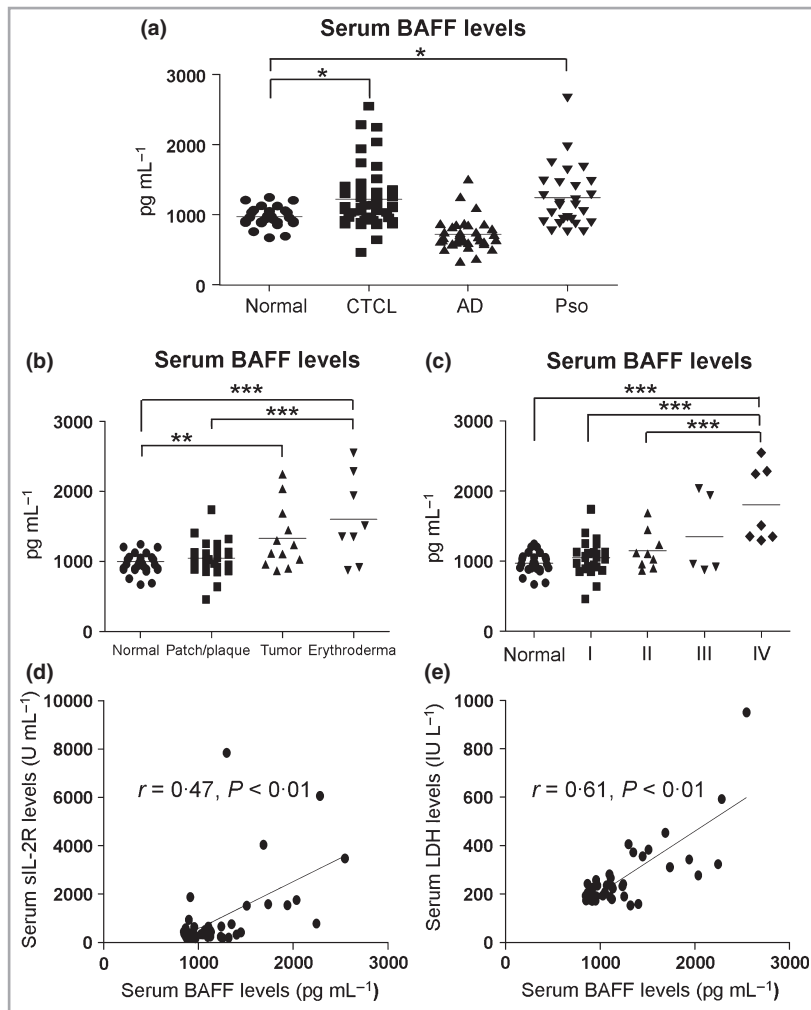


Fig 3. Serum BAFF levels and their correlations with other laboratory data. (a) Serum BAFF levels in patients with cutaneous T-cell lymphoma (CTCL; $n = 46$), atopic dermatitis (AD; $n = 36$), psoriasis ($n = 27$) and healthy controls ($n = 27$). The measured values from individual patients are plotted by dots. Each bar indicates the mean of each group. (b, c) Serum BAFF levels in patients with CTCL and healthy controls. The measured values from individual patients are plotted by dots. Each bar indicates the mean of each group. (d) Correlations between serum BAFF and soluble IL-2 receptor (sIL-2R) levels in patients with CTCL. (e) Correlations between serum BAFF and LDH levels in patients with CTCL. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$. BAFF, B-cell-activating factor belonging to the tumour necrosis factor family; CTCL, cutaneous T-cell lymphoma; LDH, lactate dehydrogenase.

addition, in four patients with CTCL (two cases with MF and two cases with SS), we measured serum BAFF levels before and after treatment. The treatment included topical and oral corticosteroids, UV phototherapy, electron beam and chemotherapy. All cases, at least temporarily, showed reduction of skin lesions. After treatment, the serum BAFF levels decreased from 1531.7 ± 283.8 to 843.0 ± 73.0 pg mL⁻¹, which correlated with the improvement in skin conditions (Fig. 4b). Thus, BAFF levels in sera collected at different time points from the same patients reflect disease activity during clinical courses.

Production of BAFF in peripheral blood mononuclear cells

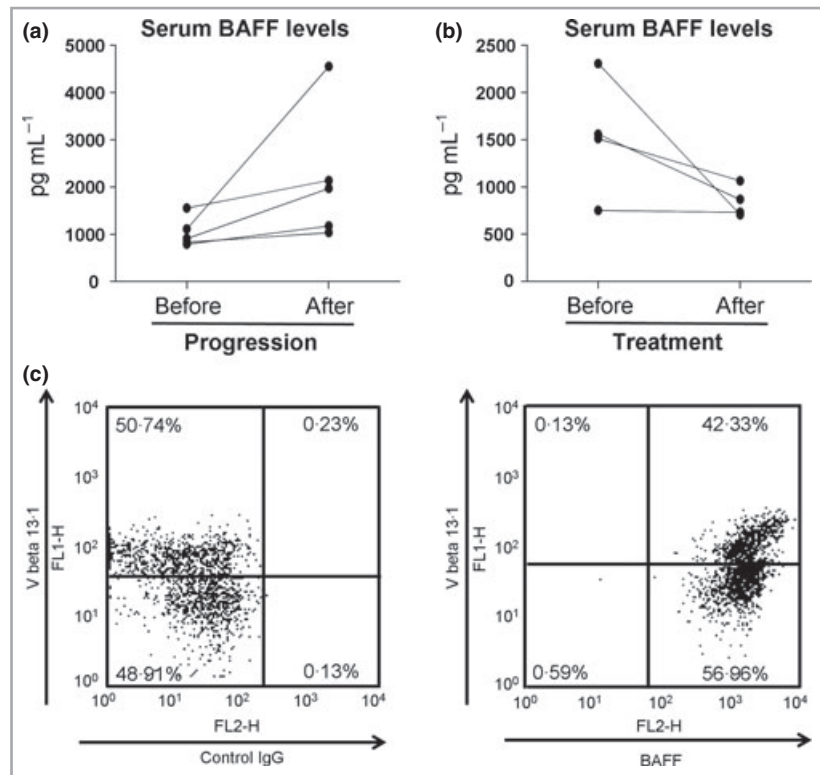
We next examined whether clonal malignant T cells or benign reactive cells produce BAFF, using peripheral blood of three patients with SS. Interestingly, almost all cells, both clonal malignant T cells and benign cells, produced BAFF (Fig. 4c). BAFF expression levels were not different among three patients (data not shown). Thus, both malignant and inflammatory T cells in peripheral blood express BAFF.

Discussion

In this study, we have revealed that CTCL skin expresses higher amounts of BAFF than normal skin. In addition, cultured fibroblasts isolated from CTCL skin express increased amounts of BAFF mRNA compared with those from normal skin. Tumour cells as well as dermal fibroblasts in lesional skin of CTCL express BAFF. They also express BAFF-R and TACI, receptors for BAFF. Furthermore, serum BAFF levels in patients with CTCL, especially at advanced stages, are significantly higher than those in healthy individuals. Serum BAFF levels correlate with serum sIL-2R and LDH levels, also showing significant correlation with disease activity. These results suggest that BAFF may have an important role in the development of CTCL in both autocrine and paracrine manners.

It is known that fibroblasts isolated from diseased skin maintain cellular characteristics even after isolation and in vitro culture.^{48,56} In this study, we showed that fibroblasts in CTCL skin expressed high levels of BAFF mRNA (Fig. 1d), suggesting that fibroblasts are one of the possible sources of BAFF. Thus, cytokines and chemokines expressed by fibroblasts, including BAFF, may function as a paracrine

Fig 4. Serum BAFF levels in patients with CTCL during clinical courses and BAFF production from peripheral blood mononuclear cells in patients with Sézary syndrome (SS). (a) Serum BAFF levels before and after progression of CTCL. (b) Serum BAFF levels before and after treatment of CTCL. (c) Representative flow cytometric histograms of intracellular BAFF of three patients with SS. Clonal malignant T cells express V β 13.1 in this case. BAFF, B-cell-activating factor belonging to the tumour necrosis factor family; CTCL, cutaneous T-cell lymphoma.



survival factor for tumour cells. To our knowledge, fibroblasts could be a previously unreported target of the treatment of CTCL.

In addition to dermal fibroblasts, tumour cells of CTCL expressed BAFF (Fig. 2a,b). There are emerging data suggesting that some cytokines contribute in an autocrine manner to cell survival of lymphoma and leukaemia. For example, IL-15,⁵⁷ IL-9⁵⁸ and CCL11⁵⁹ may play autocrine roles in the progression of SS, smoldering/chronic adult T-cell leukaemia, and anaplastic large cell lymphoma, respectively. BAFF is already known as a costimulator and activator for T cells.^{10,19} Therefore, excess BAFF may exacerbate CTCL via activation of malignant T cells. In fact, atypical lymphocytes in lesional skin of CTCL express BAFF-R and TACI, both of which are receptors for BAFF (Fig. 2c,d). BCMA was not expressed in tumour cells of CTCL (data not shown), which was consistent with previous reports that BCMA is specific to B cells.^{60–62}

Serum BAFF levels were found to be elevated in patients with B-CLL²¹ and non-Hodgkin lymphoma⁶³ compared with healthy donors. These findings suggest that the microenvironment of the malignancy may exacerbate the survival and persistence of malignant B cells. Until now, BAFF has not been reported to be associated with CTCL. In this study, we have shown that BAFF mRNA expression in lesional skin and serum BAFF levels in CTCL patients are elevated and are significantly associated with the severity of CTCL (Figs 1a–c, 3a–c). Th2-dominant environments have been reported in CTCL as well as AD.^{42–45} However, serum BAFF levels in AD patients were

not elevated, which was consistent with a previous report.⁶⁴ On the other hand, serum BAFF levels in patients with psoriasis are elevated,³⁸ suggesting that Th1–Th2 balance alone does not regulate BAFF expression. High BAFF expression in the lesional skin and sera may have an impact not only on malignant T cells but also on B cells. Although there were no or few, if any, B cells detected in lesional skin in this study, B-cell infiltration and follicular formation are sometimes reported in CTCL, especially at tumour stage. Cases with B-cell lymphoma secondary to CTCL have also been reported.⁶⁵ Our study suggests that BAFF expression in CTCL may account for these phenomena.

In summary, BAFF mRNA expression in lesional skin and serum BAFF levels in patients with CTCL are significantly increased and correlate with disease severity. These results suggest that BAFF may have an important role in the development of CTCL.

What's already known about this topic?

- B-cell-activating factor belonging to the tumour necrosis factor family (BAFF) is produced by many different types of cells, including B cells and T cells, and is best known for its role in the survival and maturation of B cells.
- Clinically, serum BAFF levels have been reported to be elevated in patients with B-cell lymphoma and autoimmune diseases.

What does this study add?

- BAFF mRNA is upregulated in lesional skin of cutaneous T-cell lymphoma (CTCL).
- Tumour cells and dermal fibroblasts in CTCL skin express BAFF.
- Tumour cells also express BAFF receptors.
- Furthermore, serum BAFF levels in patients with CTCL are significantly higher than those in healthy individuals.
- They correlate with serum levels of soluble interleukin-2 receptor and lactate dehydrogenase levels.
- The results suggest that BAFF may have important roles in the development of CTCL in both autocrine and paracrine manners.

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