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#### H. 知的財産権の出願・登録状況

(予定を含む)

1. 特許取得  
無し
2. 実用新案登録  
無し
3. その他  
無し

研究成果の刊行に関する一覧表

書籍

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## Differential effects of BAFF on B cell precursor acute lymphoblastic leukemia and Burkitt lymphoma

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**Abstract** B cell-activating factor belonging to the tumor necrosis factor superfamily (BAFF) is a crucial factor for B cell development and is involved in the survival of malignant B cells, but its effect on B cell precursors (BCPs) remains unclear. We investigated BCP acute lymphoblastic leukemia (-ALL) cells for BAFF receptor (-R) expression and compared the effect of BAFF on BCP-ALL cells and Burkitt lymphoma (BL) cells. Expression of BAFF-R was detected in some cell lines and some clinical specimens of both BL and BCP-ALL. BAFF acted on both BL and BCP-ALL cells and promoted proliferation by both. BAFF also inhibited apoptosis by BL cells induced by cross-linking of cell surface molecules and anticancer drugs, but failed to inhibit apoptosis by BCP-ALL cells. BAFF induced prompt and obvious activation of the NF- $\kappa$ B signaling pathway in BL cells, but only weak and delayed activation of the pathway in BCP-ALL cells. The results of this study indicate that some BCP-ALL cells and some BL cells express BAFF-R, but that the effects of BAFF on BCP-ALL cells are different from its effects on mature B cell malignancies.

**Keywords** BAFF · B cell precursor ALL · Burkitt lymphoma · Apoptosis

### 1 Introduction

B cell-activating factor belonging to the tumor necrosis factor (TNF) superfamily (BAFF), also called B-lymphocyte stimulator (BlyS), is a cytokine that is produced by monocytes, dendritic cells, and some T cells [1–6]. Transgenic mice overexpressing BAFF exhibit hyperplasia of the mature B cell compartment [7–9], whereas mice deficient in BAFF have a reduced number of peripheral B cells and exhibit an almost complete loss of follicular and marginal zone B cells in their secondary lymphoid organs [9–11]. This suggests that BAFF is a survival and maturation factor for B cells and crucial for B cell development [1, 12]. To date, three distinct BAFF receptors have been identified, namely, BAFF receptor (BAFF-R, also called BR3), B cell maturation antigen (BCMA), and transmembrane activator and CAML-interactor (TACI). While BAFF binds to all three with similarly high affinity, only BAFF-R is thought to be responsible for the survival and differentiation of B cells [6, 13–20].

It has been well documented that some of the malignant as well as normal B cells express BAFF-R, and thus BAFF-mediated signaling is involved in the survival and proliferation of malignant B cells [1]. Indeed, BAFF is thought to be produced in excess in patients with various B cell malignancies, including B cell chronic lymphocytic leukemia, multiple myeloma, Hodgkin lymphoma, and various types of non-Hodgkin lymphomas (NHLs), such as follicular lymphoma, diffuse large cell lymphoma, mantle cell lymphoma, and marginal zone lymphoma, either by the tumor cells themselves or by cells in their microenvironment, or by both,

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and to behave as an autocrine and paracrine survival and proliferation factor in these B cell malignancies [21–27].

The capacity of B cells to bind BAFF has been found to be correlated with their maturation stage, and the effect of BAFF on B cells is dependent on their degree of maturation. However, the precise role of BAFF in B cell development is still a matter of controversy [1, 28], and the role of BAFF in the development of B cell precursors (BCPs), in particular, remains largely unknown. In addition, the effect of BAFF on the neoplastic counterpart of BCPs, namely BCP acute lymphoblastic leukemia (-ALL)/lymphoma, is also still unclear. Since BCP-ALL is the most common malignancy in childhood, whether BAFF has any effect on BCP-ALL cells should be investigated.

In this study, we investigated the effect of BAFF on BCP-ALL cells in comparison with its effect on the cells of Burkitt leukemia/lymphoma (BL), which is a subtype of NHL and another common B-lineage malignancy in children. In this study, we report the findings that some BCP-ALL cells as well as some BL cells expressed BAFF-R, and that BAFF promoted proliferation by both. Interestingly, although BAFF inhibited apoptosis by BL cells, it failed to inhibit apoptosis by BCP-ALL cells, suggesting that BAFF acts on B-lineage malignant cells differentially in a maturation stage-related fashion. The molecular basis of the differential effects of BAFF on BCP-ALL and BL cells is also analyzed and discussed.

## 2 Methods

### 2.1 Reagents

Recombinant human BAFF was obtained from R&D Systems, Inc. (Minneapolis, MN). Dexamethasone (DEX) and etoposide (VP-16) were obtained from Sigma-Aldrich Co. (St. Louis, MO). The monoclonal Abs (mAbs) used were phycoerythrin (PE)-conjugated anti-BAFF-R from Santa Cruz Biotechnology (Santa Cruz, CA), fluorescein isothiocyanate (FITC) anti-CD10, FITC anti-CD21, FITC anti-HLA-DR, PE anti-CD40, PE-Texas Red (ECD) anti-CD45, PE-cyanin 5.1 (PC5) anti-CD33, and PE-cyanine 7 (PC7) anti-CD19 from Beckman/Coulter, Inc. (Westbrook, MA). The mouse mAbs used for the immunochemical analysis were anti-I- $\kappa$ B, anti-NF- $\kappa$ B p65, and anti-Bcl-2 from Santa Cruz and anti- $\beta$ -actin (AC-15) from Sigma. The rabbit Abs used were anti-TRAF-3, anti-NF- $\kappa$ B1, anti-NF- $\kappa$ B2, anti-phospho-specific NF- $\kappa$ B p65 (Ser536), anti-phospho-specific NF- $\kappa$ B p105 (Ser933), and anti-phospho-specific I- $\kappa$ B- $\alpha$  (Ser32) from Cell Signaling Technology, Inc. (Danvers, MA). Purified anti- $\mu$  chain rabbit polyclonal Ab from Jackson ImmunoResearch Laboratories, Inc. (West Grove, PA) was used to cross-link B cell receptors (BCRs).

Mouse anti-CD24 [29], anti-CD10, and anti-CD20 mAbs (Beckman) were used to cross-link each molecule. Secondary Abs, including enzyme-conjugated Abs, were purchased from Jackson and Dako (Denmark A/S).

### 2.2 Cells and cultures

The human BL-derived cell lines Daudi, Ramos, P32/ISH (Japanese Cancer Research Resource Bank, JCRB, Tokyo, Japan), EB-3, NAMALWA (Institution of Fermentation, Osaka, Japan), and BALM-18 (Dr. Y. Matsuo, Fujisaki Cell Center, Hayashibara Biochemical Labs, Inc.) and the human hairy cell leukemia cell line MLMA (JCRB) were used. The human BCP-ALL cell lines Reh (American Type Culture Collection), RS4;11, P30/OHK (JCRB), NALM-17, NALM-27 (Hayashibara), and NALM-6 (Tohoku University Cell Bank, Sendai, Japan) were also used. Cells were cultured at 37°C in RPMI 1640 medium (Sigma) supplemented with 10% FCS (Sigma) under a humidified 5% CO<sub>2</sub> atmosphere. Cells were cultured in the presence or absence of BAFF, and viable cell counts were made after staining with Trypan blue at the times indicated in the figures. In parallel, cells were plated on a 96-well culture plate (Corning, Inc., Corning, NY) and cell proliferation was also assessed by water-soluble tetrazolium salt (WST) assays (Cell Counting Kit-8, DojinDo, Kumamoto, Japan), a cell proliferation assay that colorimetrically determine dehydrogenase activity of the cells. Based on our preliminary experiments, we chose a concentration of 200 ng/ml as a sufficient dose of BAFF to achieve a maximum effect on cell proliferation.

Clinical specimens from pediatric patients, consisting of 20 patients with BCP-ALL, 10 with BL, and 2 with reactive lymphadenitis, were selected from the files of specimens collected in our laboratory between 1985 and 2001. The specimens are now kept under anonymous conditions, and all of the experiments included in this study adhered to the tenets of the Declaration of Helsinki and were performed with the approval of the local ethics committee.

The human bone marrow (BM) CD34+ cells used were purchased from Cambrex Bio Science Walkersville, Inc. (Walkersville, MD). A cloned murine BM stromal cell line, MS-5, was kindly provided by Dr. K. J. Mori (Niigata University, Niigata, Japan). BCP cells were induced from BM CD34+ cells by co-culture with MS-5 cells as described previously [30].

### 2.3 Immunofluorescence analysis and detection of apoptosis

To detect BAFF-R, cells were stained with fluorescein-labeled mAbs and analyzed by flow cytometry (FCM, FC500, Beckman) as described previously [31]. The frequency of apoptosis was quantified by MEBCYTO-

Apoptosis kit (Medical & Biological Laboratories, Co., Ltd., MBL, Nagoya, Japan) as described previously [29]. The caspase activation was quantified by APOPCYTOTM Intracellular Caspases Activity Detection Kit (MBL) and then analyzed according to the manufacturer's protocol.

#### 2.4 Reverse-transcriptase (RT)-PCR

Total RNA was extracted from cultured cells with an RNeasy plus Mini Kit (QIAGEN, Valencia, CA), and cDNA was generated with a FirstStrand cDNA Synthesis Kit (Pharmacia Biotech, Uppsala, Sweden). A set of primers (5'-caaattcagctcttctc-3', and 5'-tttgaaggcacaggaacag-3') was used to amplify the 193-bp fragment of BAFF-R transcripts by PCR as described previously [32], and the products were separated on a 2.0% agarose gel. The transcripts of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were amplified as an internal control [32].

#### 2.5 Statistical analysis

The statistical analysis was performed by means of a nonparametric Mann-Whitney test, and correlations were determined by using nonparametric statistics. A *p* value less than 0.05 was considered to be statistically significant.

#### 2.6 Immunoblotting

Immunoblotting was performed as described previously [33]. Briefly, a 50 µg sample of each cell lysate was electrophoretically separated on an SDS-poly acrylamide gel and transferred to a nitrocellulose membrane. The membrane was incubated with the appropriate combination of primary and secondary Abs, washed, and examined with an enhanced chemiluminescence reagent system.

### 3 Results

#### 3.1 Expression of BAFF-R in BL and BCP-ALL cells

First, we investigated whether the BL and BCP-ALL cell lines expressed BAFF-R. As shown in Fig. 1a, flow cytometric analysis revealed that most of the BL cell lines expressed BAFF-R. Of the 6 BL cell lines tested, 4, BALM-18, BALM-24, P32/ISH, and Ramos, exhibited a relatively high level of expression, but EB-3 cells did not express BAFF-R at all and the expression of BAFF-R by Daudi cells was very limited. On the other hand, testing the BCP-ALL cell lines for BAFF-R expression by FCM showed that most of the lines used in this study were positive, but expression by RS4;11 cells was very limited (Fig. 1b). As shown in Fig. 1c, hairy leukemia-derived MLMA cells exhibited high level

expression of BAFF-R. We confirmed BAFF-R expression by these cell lines at the mRNA level by RT-PCR (Fig. 1c). Similar examination for expression of the other types of BAFF receptors by FCM showed no significant expression of BCMA or TACI protein by any of the BL or BCP-ALL cell lines (data not shown).

Next, we examined BL and BCP-ALL cells in the clinical material for BAFF-R expression. As shown in Fig. 2a, flow cytometric analysis revealed that the lymphoma cells in some of the BL cases expressed BAFF-R. Of the 10 cases tested, 3 were clearly positive for BAFF-R. We also confirmed that the leukemic cells in some of the BCP-ALL cases expressed BAFF-R, and 7 of the 20 cases were positive for BAFF-R (Fig. 2b). These findings indicate that some BL and BCP-ALL cells express BAFF-R proteins on their surface.

We also investigated whether non-leukemic B cells express BAFF-R. FCM of BCP cells induced from bone marrow CD34+ cells in vitro by co-culture with MS-5 cells revealed that some BCP cells expressed BAFF-R (Fig. 2c), indicating that the expression of BAFF-R is a characteristic of particular BCP cells. Similarly, some non-leukemic mature B cells in peripheral lymph nodes also expressed BAFF-R (Fig. 2d).

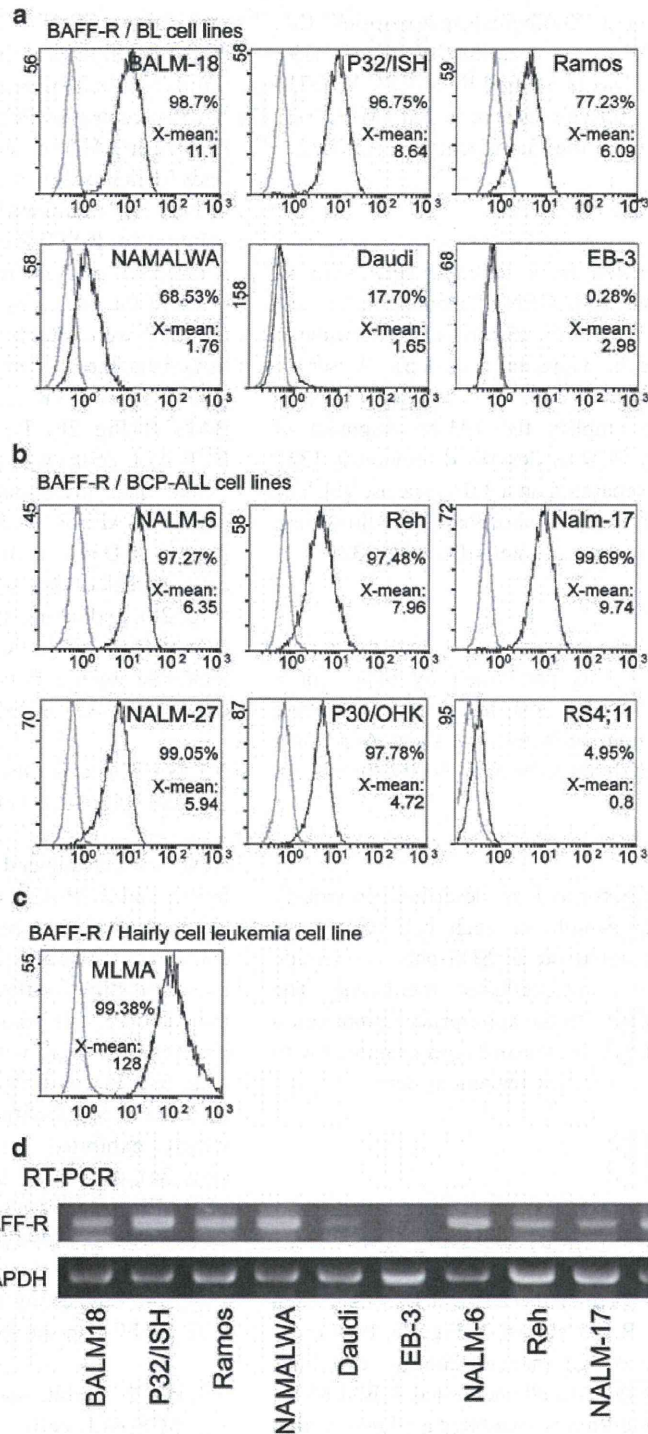
#### 3.2 BAFF accelerates proliferation by both BL and BCP-ALL cells

Next, we investigated whether BAFF affects proliferation by BL and BCP-ALL cells. The Trypan blue dye exclusion assay showed that proliferation of BAFF-R-positive BL cell lines (BALM-18, P32/ISH, Ramos, and NAMALWA) increased slightly after BAFF was added to the culture, and the increase was statistically significant (Fig. 3a). A cell proliferation assay with WST revealed a similar tendency (Fig. 3a). In contrast, BAFF failed to induce a significant increase in cell proliferation by Daudi cells and EB-3 cells, which exhibited no significant BAFF-R expression (Fig. 3a), indicating that the proliferation-inducing effect of BAFF is specific to BAFF-R-positive cells. We investigated whether BAFF affects proliferation by BCP-ALL cell lines in a similar manner and observed a similar effect (Fig. 3b), suggesting that BAFF promotes the growth of both BAFF-R-positive BL cells and BCP-ALL cells.

#### 3.3 BAFF inhibits apoptosis by BL cells, but not by BCP-ALL cells

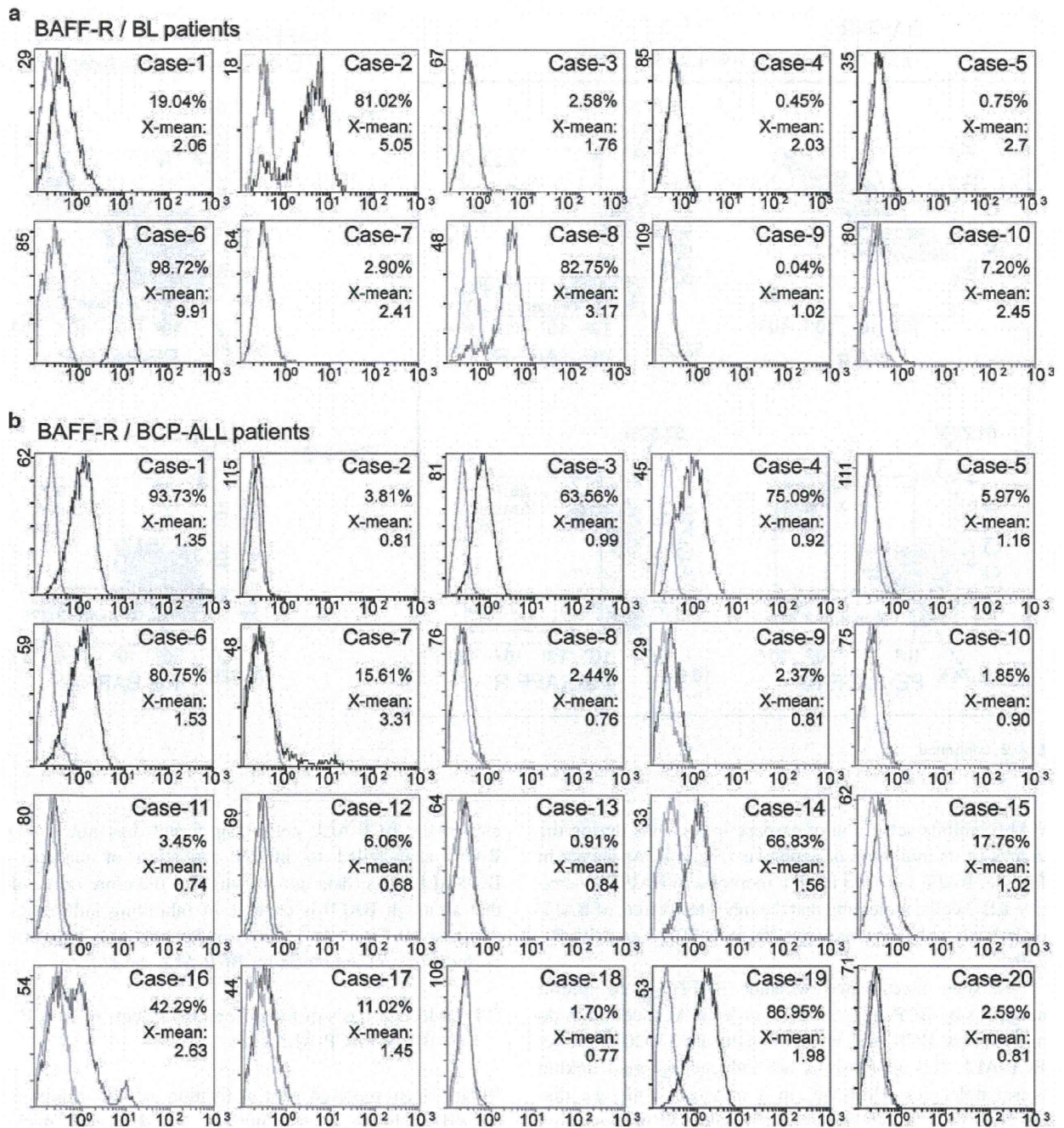
It has been well documented that stimulation of cultured BL cells via particular surface molecules, including BCR and CD20, induces apoptosis [34–36]. It has also been reported that BAFF is responsible for B cell survival and prevents apoptosis by cultured B cells [37, 38]. We therefore investigated whether BAFF is able to inhibit BL cell apoptosis that

**Fig. 1** Expression of BAFF receptor (BAFF-R) in Burkitt lymphoma (BL) cell lines and B cell precursor acute lymphoblastic leukemia (BCP-ALL) cell lines. **a** Six BL cell lines were stained with PE-labeled anti-BAFF-R antibody and analyzed by flow cytometry. The histograms obtained (*dark lines*) are shown superimposed on those of the negative control (cells stained with isotype-matched control mouse Ig, *light lines*). Positivity (%) and mean fluorescence (X-mean) values are shown. X-axis, fluorescence intensity; Y-axis, relative cell number. **b** Six BCP-ALL cell lines were examined for BAFF-R expression as described in **a**. **c** Hairy cell leukemia-derived cell line MLMA was tested as a positive control and presented as in **a**. **d** The expression of BAFF-R mRNA in both groups of cell lines and MLMA cells was investigated by the reverse-transcriptase polymerase chain reaction (RT-PCR)



is mediated by cross-linking of CD20 and BCR. As shown in Fig. 4a, assessment by annexin-V binding showed that the incidence of P32/ISH cell and BALM-18 cell apoptosis induced by both BCR- and CD20-mediated stimuli was reduced to a statistically significant extent by the addition of

BAFF to the culture. We also investigated the inhibitory effect of BAFF on apoptosis by BL cells mediated by DEX and observed a similar inhibitory effect, but this effect on the apoptosis mediated by VP-16 was very limited (Fig. 4a). Although a higher dose of BAFF tended to inhibit induction



**Fig. 2** Analysis of BAFF receptor (BAFF-R) expression in clinical material and non-leukemic samples. The histograms for BAFF-R in ten Burkitt lymphoma (BL) cases (a) and twenty B cell precursor acute lymphoblastic leukemia (BCP-ALL) cases (b) are shown as in Fig. 1a. c BCP cells induced from bone marrow CD34+ cells in vitro were examined for expression of BAFF-R and other leukocyte

antigens by flow cytometry, and the cytograms are shown. d Mononuclear cells obtained from lymph nodes of two non-leukemic reactive lymphadenitis patients were examined for the expression of BAFF-R as in c

of apoptosis more effectively (Fig. 4b), the inhibitory effect of BAFF on induction of apoptosis was always partial (data not shown). As shown in Fig. 4c, when BAFF was added to

the culture prior to the induction of apoptosis, its inhibitory effect on induction of apoptosis was greater than when added at the start of apoptosis induction. We also confirmed that



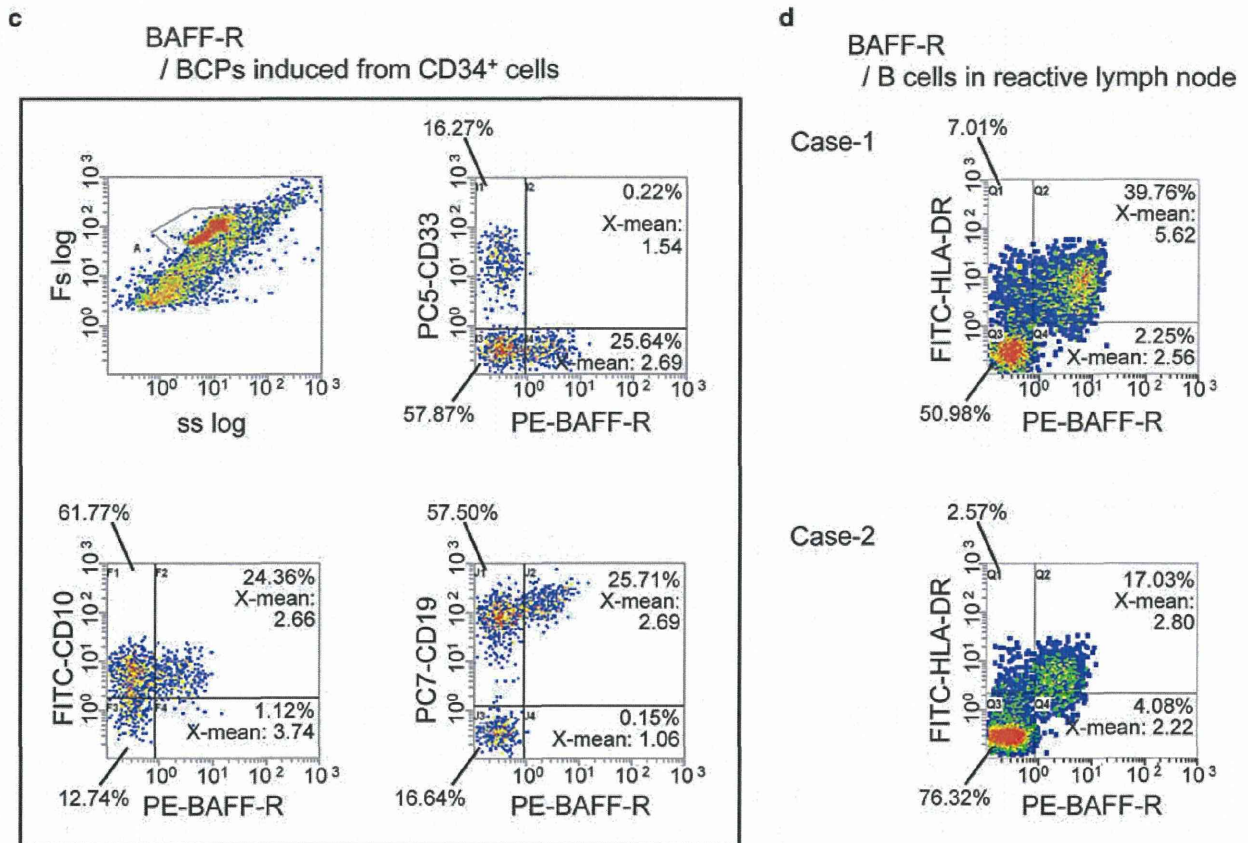


Fig. 2 continued

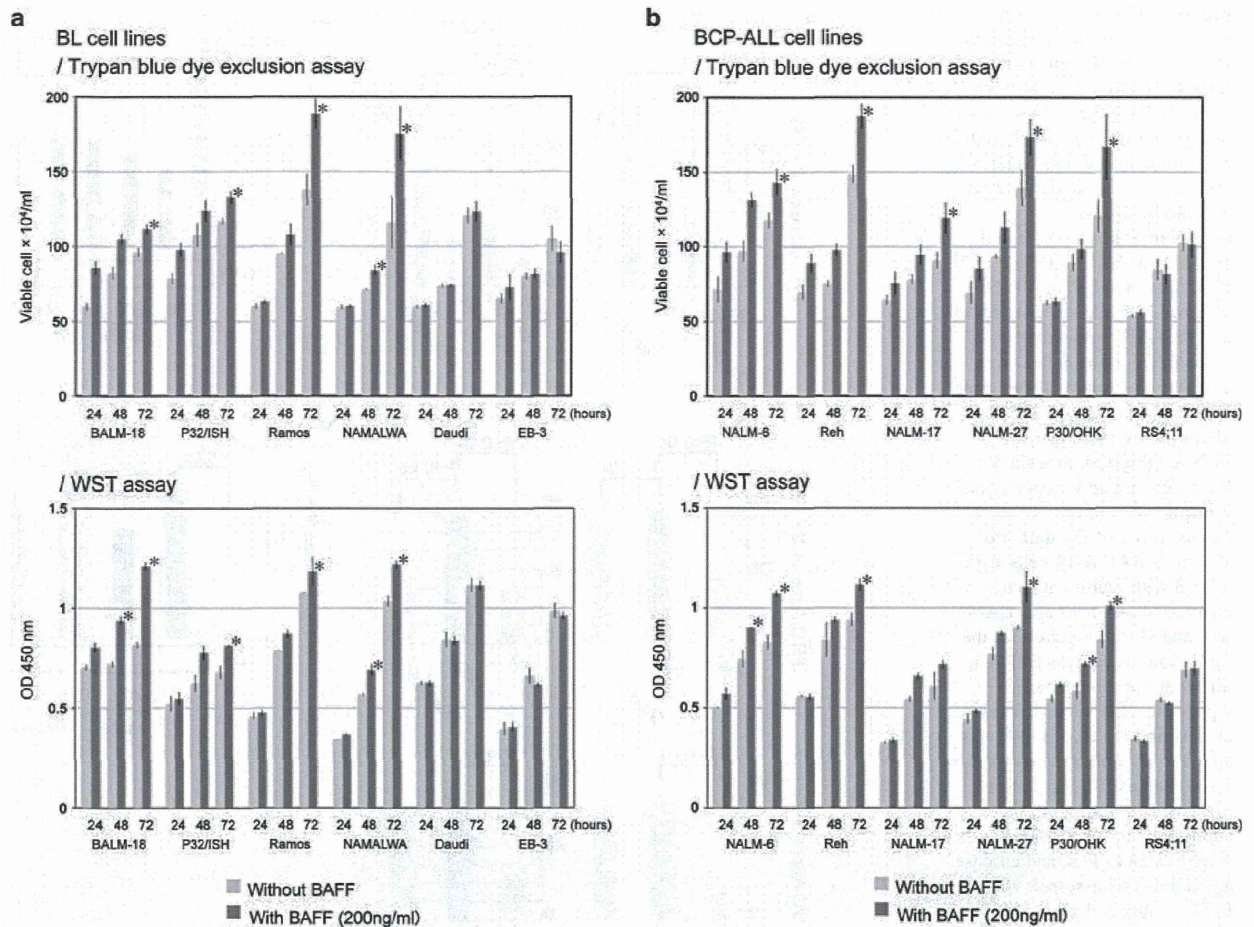
BAFF inhibits activation of caspase in BL cells during the course of its inhibition of apoptosis (Fig. 4d). As shown in Fig. 4e, BAFF failed to inhibit apoptosis in BAFF-R-negative EB-3 cells, indicating that the inhibitory effect of BAFF on induction of apoptosis is specific for BAFF-R-positive BL cells.

We then investigated whether BAFF would inhibit apoptosis by BCP-ALL cells. Since BCP-ALL cell lines do not express BCR and most of them are CD20-negative, BCP-ALL cell apoptosis is not induced by cross-linking these molecules. However, in a previous study we discovered that cross-linking of other molecules expressed on BCP-ALL cells, including CD24, can induce apoptosis by BCP-ALL cells [39]. When we investigated the effect of BAFF on CD24- and CD10-mediated apoptosis by NALM-6 cells, however, no inhibitory effect of BAFF was observed on apoptosis mediated by either of them (Fig. 5). We also discovered that BAFF was unable to inhibit NALM-6 cell apoptosis mediated by DEX or VP-16 (Fig. 5). We tested other BCP-ALL cell lines, including Reh and NALM-17, in a similar manner, but no inhibitory effect of BAFF on induction of apoptosis was observed in

any of the BCP-ALL cells (Fig. 5 and data not shown). BAFF also failed to inhibit activation of caspase in BCP-ALL cells (data not shown). We therefore concluded that although BAFF is capable of inhibiting induction of apoptosis by BL cells, it does not have an inhibitory effect on induction of apoptosis by BCP-ALL cells.

#### 3.4 Molecular basis of the differential effects of BAFF on BL and BCP-ALL cells

It has been reported that activation of the alternative NF- $\kappa$ B pathway (processing of NF- $\kappa$ B2 and nuclear translocation of p52/RelB heterodimers) is a major outcome of BAFF-R-stimulation, but that BAFF-R also weakly activates the classical NF- $\kappa$ B pathway (processing of NF- $\kappa$ B1 and nuclear translocation of p50/RelA) [1, 20]. When expression of signaling molecules related to the NF- $\kappa$ B pathways, including NIK, I- $\kappa$ B, NF- $\kappa$ B1, NF- $\kappa$ B2, and p65 NF- $\kappa$ B (RelA), was investigated by immunoblot analysis, no clear differences were found between the BL cell lines (Ramos, BALM-18, P32/ISH) and BCP-ALL cell lines (NALM-6, Reh, LC4-1) (data not shown). In contrast,



**Fig. 3** Effect of BAFF on proliferation by Burkitt lymphoma (BL) cells and B cell precursor acute lymphoblastic leukemia (BCP-ALL) cells. The effect of BAFF (200 ng/ml) on proliferation by BL cell lines (a) and BCP-ALL cell lines (b), each at a starting cell

concentration of  $5 \times 10^5$ /ml, was investigated by Trypan blue dye exclusion assay and WST assays. Each experiment was performed in triplicate, and the means  $\pm$  SD of the data are shown. \*Statistically significant ( $p < 0.05$ )

protein expression of TRAF-3 was detected in all three BL cell lines and in NALM-6 cells, but not in other BCP-ALL cell lines (data not shown).

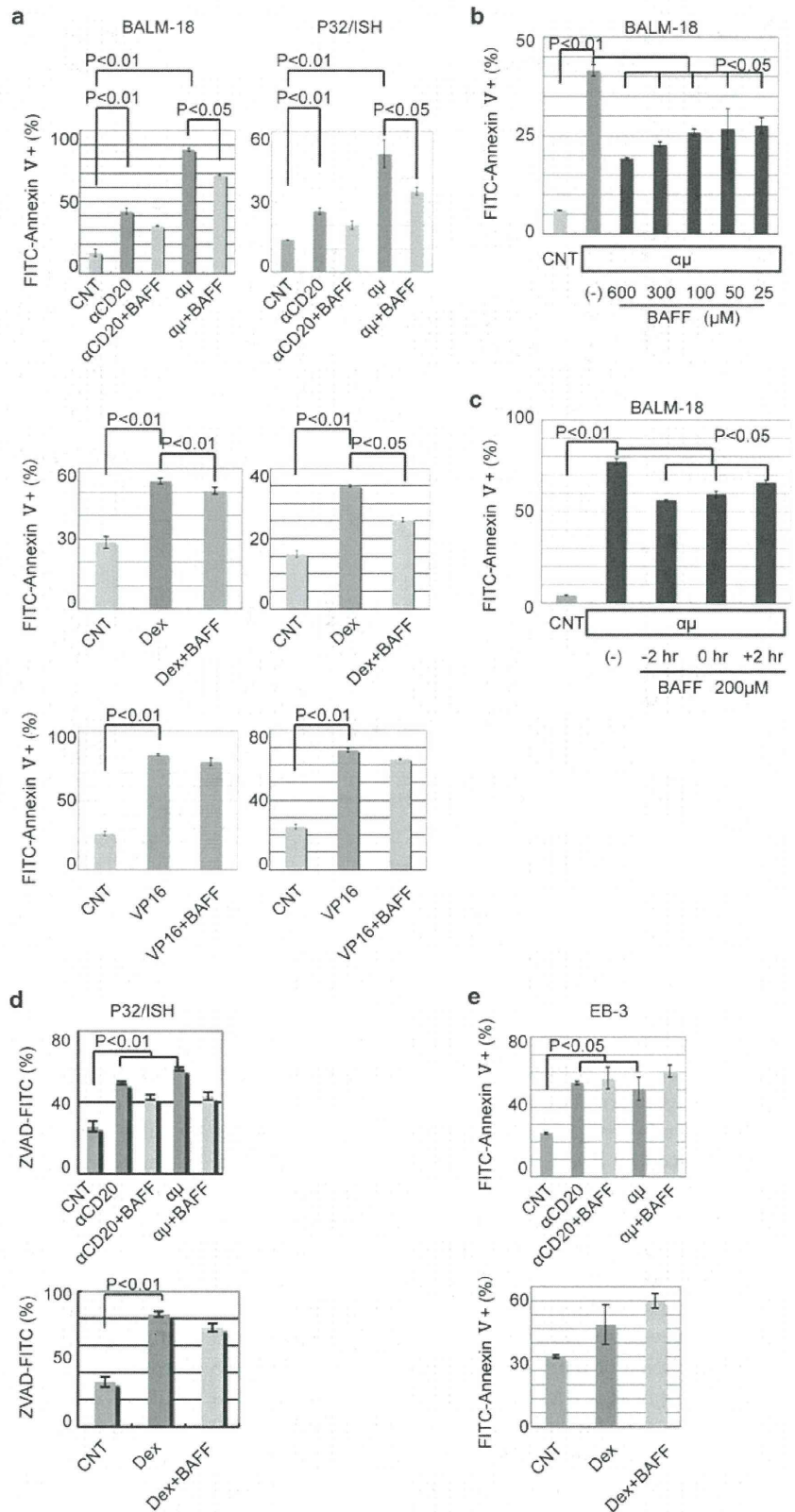
Next, we investigated the activation of signaling molecules induced by BAFF stimulation. As shown in Fig. 6a, investigation of the phosphorylation state of I- $\kappa$ B- $\alpha$ , p65 NF- $\kappa$ B, and p105 NF- $\kappa$ B, the regulatory molecules of the NF- $\kappa$ B1 signaling cascade revealed a temporary increase in their phosphorylation state following BAFF stimulation in P32/ISH cells (peaking in 5–10 min), but not in NALM-6 cells. However, as shown in Fig. 6a, a slight increase in phosphorylation of p105 NF- $\kappa$ B was detected in NALM-6 cells at 60 min after BAFF stimulation. We also observed a clear increase in the active forms of both NF- $\kappa$ B1 (p50) and NF- $\kappa$ B2 (p52) in P32/ISH cells after stimulation with BAFF (Fig. 6b). In contrast, the increase in active forms of NF- $\kappa$ Bs mediated by BAFF in NALM-6 cells was smaller than that in P32/ISH cells (Fig. 6b). Immunoblot analysis

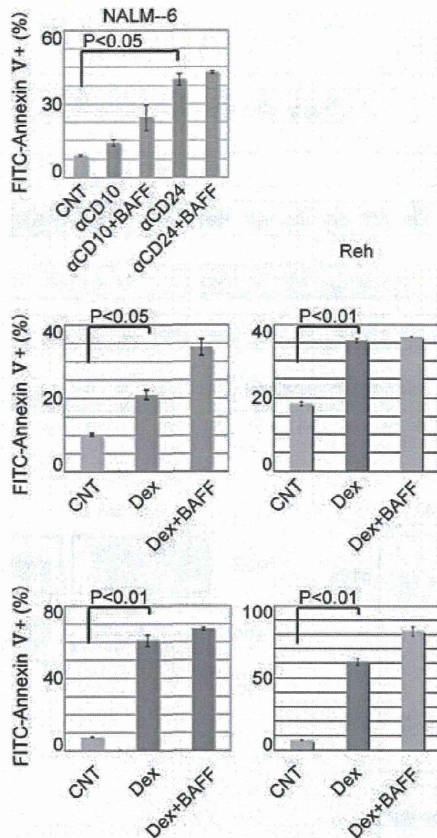
revealed a more marked increase in the active form of NF- $\kappa$ B1 than in the active form of NF- $\kappa$ B2 (Fig. 6b).

We also investigated the activation of signaling molecules induced by BAFF stimulation in hairy leukemia-derived MLMA cells in the same manner as a positive control for mature B cell malignancy. As shown in Fig. 6, BAFF induced prompt and sustained phosphorylation of I- $\kappa$ B- $\alpha$ , p65 NF- $\kappa$ B, and p105 NF- $\kappa$ B (Fig. 6a), whereas the active form of NF- $\kappa$ B1 (p50) was already present prior to stimulation with BAFF, and whether its level increased after the addition of BAFF was unclear (Fig. 6b). In contrast, BAFF significantly increased the level of active form of NF- $\kappa$ B2 (p52) in MLMA cells (Fig. 6b).

We previously reported finding that BAFF induces Bcl-2 expression in MLMA cells [40]. When we investigated the effect of BAFF on Bcl-2 expression in P32/ISH and NALM-6 cells by immunoblot analysis, we found that BAFF induced a small amount of Bcl-2 protein expression

**Fig. 4** Effect of BAFF on apoptosis by Burkitt lymphoma (BL) cells. **a** BALM-18 and P32/ISH were treated with either rabbit anti- $\mu$  heavy-chain ( $\alpha\mu$ ) antibody (Ab), a combination of anti-CD20 Ab ( $\alpha$ CD20) and secondary rabbit anti-mouse Ig Ab, dexamethasone (DEX, 2 and 100  $\mu$ M for BALM-18 and P32/ISH, respectively), or VP-16 (0.5 and 12.5  $\mu$ M for BALM-18 and P32/ISH, respectively) for 24 h. BAFF (200 ng/ml) was added to the culture at the same time, and apoptotic cells were identified by binding with FITC-conjugated annexin V. Each experiment was performed in triplicate, and the means  $\pm$  SD of the data are shown. **b** BALM-18 cells were treated with rabbit anti- $\mu$  heavy-chain antibody ( $\alpha\mu$ ) and BAFF was added to the culture at the same time as  $\alpha\mu$  was added at different concentrations. Apoptotic cells were examined as described in **a**. **c** BAFF was added to the culture either at the same time as (0), 2 h before (-2), or 2 h after (+2) the addition of  $\alpha\mu$ . **d** P32/ISH cells were treated as in **a**, and caspase activation was assessed with FITC-conjugated z-VAD-fmk. The results are shown in the form of a graph (left). **e** BAFF-R-negative EB-3 cells were examined as a negative control as in **a**





**Fig. 5** BAFF does not inhibit apoptosis by B cell precursor acute lymphoblastic leukemia (BCP-ALL) cells. BCP-ALL cell lines NALM-6 cells and Reh cells were treated with DEX (20 and 200  $\mu$ M for NALM-6 and Reh, respectively) or VP-16 (50 and 12.5  $\mu$ M for NALM-6 and Reh, respectively) for 24 h, and the effect of BAFF on induction of apoptosis was assessed in the same manner as in Fig. 4. The effect of BAFF on induction of NALM-6 cell apoptosis induced by either anti-CD10 Ab ( $\alpha$ CD10) or anti-CD24 Ab ( $\alpha$ CD24) in the presence of secondary rabbit anti-mouse Ig Ab was also tested. Since CD24 and CD10 failed to induce apoptosis in Reh cells, the assay was not performed with Reh cells

in P32/ISH cells, whereas Bcl-2 was already expressed in NALM-6 cells, and that BAFF did not affect its expression (Fig. 6c).

### 3.5 Effects of BAFF on CD40 expression in BL and BCP-ALL cells

We also previously reported discovering that expression of CD40, a TNF-receptor family molecule critical for B cell survival, was up-regulated in hairy leukemia-derived MLMA cells after BAFF stimulation [40]. As shown in Fig. 7, flow cytometric analysis revealed that BAFF also induced up-regulation of CD40 in P32/ISH cells, but no clear change in CD40 expression was observed in NALM-6 cells.

## 4 Discussion

As stated above, expression of BAFF-R has been reported in some of B cell malignancies [21, 26, 27], whereas BAFF-R expression in BL and BCP-ALL/lymphoma has not been well characterized. The results of the present study clearly showed expression of BAFF-R by tumor cells of BL and BCP-ALL, both of which are major B-lineage hematologic malignancies in children, but that BAFF-R expression was limited in some cases for each of these diseases.

The results of this study have also shown that BAFF acts on both BL and BCP-ALL cells and promotes their proliferation. Furthermore, BAFF was found to inhibit the BL cell apoptosis mediated by the cross-linking of cell surface molecules, including BCR and CD20, and induced by anticancer drugs, including DEX. Interestingly, however, BAFF failed to inhibit apoptosis by BCP-ALL cells, indicating that BAFF has a differential effect on B-lineage malignancies that have originated at different stages of B cell development. As mentioned above, BAFF has been reported to act as a survival and proliferation factor in B cell malignancies and a significant correlation between clinical outcome and BAFF-R expression in tumor cells or serum BAFF levels has been demonstrated in a certain subtype of NHLs [21, 27, 41]. Studies to clarify the relations between BAFF-R expression and the clinical features of BL and BCP-ALL are now being prepared.

Expression of BAFF-R has been reported to increase with the stage of B cell maturation. Rodig et al. [26] reported that up-regulation of BAFF-R occurs during the transition from BCPs to mature B cells and that BAFF-R is present in most B cells circulating in peripheral blood. They also reported that BAFF-R expression appears to be highest on the naive B cells in the mantle zones, whereas BAFF-R is only detectable in a subset of B cells in the germinal centers (GCs) [26]. Since BL cells are thought to be a malignant counterpart of centroblasts present in germinal centers, our finding that some BL cells express BAFF-R seems to be consistent with the report by Rodig et al. In contrast, we found that BAFF-R is expressed in some BCP cells, indicating that BAFF may also affect the proliferation and development of some BCP cells. All of the above data indicate that distinct subsets, namely, a BAFF-R-positive subset and a BAFF-R-negative subset, are present in both BCP and GC B cells. Further analysis of the effect of BAFF on BCP and GC B cells should provide useful information with regard to the biological differences between BAFF-R-positive and BAFF-R-negative cases of both BL and BCP-ALL.

As reported in this study, although both BL and BCP-ALL cells express BAFF-R, BAFF was found to have an anti-apoptotic effect on BL cells, but not on BCP-ALL