A Functional Receptor for B-Cell–Activating Factor Is Expressed on Human Acute Lymphoblastic Leukemias

Reshmi Parameswaran1, Markus Müschen2,3, Yong-mi Kim2,4, John Groffen1,2,3,4, and Nora Heisterkamp1,2,3,4

Abstract

B-lineage acute lymphoblastic leukemia (ALL) arises by transformation of a progenitor (pre-B) cell. Cure rates in adults remain low and treatment is complicated by support provided by the microenvironment to the leukemic cells, indicating an urgent need to better understand the factors that promote their survival. B-cell–activating factor (BAFF) and its receptor BAFF-R are important for survival and growth of mature normal and malignant B cells but are not expressed on pre-B cells. Unexpectedly, all cells in the primary Philadelphia chromosome (Ph)–positive and Ph-negative ALL samples tested were positive for high BAFF-R cell surface expression. BAFF-R was fully competent to bind BAFF, and stimulation of the receptor activated both the classic and the noncanonical NF-κB pathways. Recombinant BAFF supported survival of the ALL cells in the absence of stroma, and it significantly attenuated the rate of apoptosis caused by exposure to nitotinib, a drug used therapeutically to treat Ph-positive ALLs. Surprisingly, BAFF mRNA and protein were also expressed in the same cells but BAFF was not shed into the medium. Our report is the first showing universal expression of BAFF-R by pre-B ALL cells and opens the possibility of blocking its function as an adjuvant therapeutic strategy. Cancer Res; 70(11) June 1, 2010; 4346–56. ©2010 AACR.

Introduction

Acute lymphoblastic leukemia (ALL) is the most common malignancy affecting children and is a major cause of mortality from hematopoietic malignancies in adults (1). The t(9;22)(q34;q11) translocation, leading to the generation of the Philadelphia chromosome (Ph), is the most common cytogenetic abnormality in adult ALL and is associated with a poor prognosis (2). The translocation moves ABL on chromosome 9 to BCR on chromosome 22 and results in the formation of a BCR-ABL fusion gene (3). The high incidence of such unfavorable genetic alterations accounts for the low overall cure rate in adult patients with ALL, and the identification of new and more effective therapies remains an urgent goal. To this end, a thorough understanding of the survival signals and microenvironment contributing to the establishment of the leukemic clone and its resistance to therapy is required.

ALL develops by transformation of normal B-cell progenitors in the bone marrow. Pre-B ALL cells depend on bone marrow stroma for in vitro proliferation and survival (4, 5). Bone marrow stromal cells support leukemic cell growth by the production of soluble factors such as CXCL12, interleukin-7, and Wnt proteins, as well as via cell-cell adhesion mediated by molecules such as VLA-4/VCAM1 (6–8). Previously, we and others have shown that stroma exerts a protective effect that contributes to the poor response of leukemic cells to chemotherapeutic drugs (9–11), and the examination of the molecular nature of the protection provided by stroma remains the focus of significant interest. Several studies have shown that malignant B cells from patients with chronic lymphocytic leukemia (CLL), non–Hodgkin lymphoma B, and multiple myeloma express abnormal levels of B-cell–activating factor (BAFF), which protects these cells from spontaneous or drug-induced apoptosis (12–14). BAFF belongs to the tumor necrosis factor (TNF) ligand family and either is displayed on the cell surface or is released in a soluble form after cleavage from the plasma membrane by a furin-like protease (15, 16). BAFF is crucial for the survival, maturation, and differentiation of normal B cells (17, 18).

The three known receptors for BAFF on B cells include BCMA (B-cell maturation antigen), TACI (transmembrane activator, calcium modulator, and cyclophilin ligand interactor), and BAFF-R/BR3 (BAFF receptor/BLyS receptor 3). Both...
BAFF and the related cytokine APRIL bind to TACI and BCMA, whereas only BAFF binds to BAFF-R. The BAFF-R/BR3 pathway in particular is crucial to the differentiation of late primary B cells and survival of mature B cells (19, 20). Interestingly, neoplastic B-lineage lymphocytes express not only BAFF but also the receptors for BAFF, which, when ligated, can promote their cell survival in vitro (21).

BAFF-R expression was reported to be restricted to more mature B cells, starting at the T1 transitional B-cell stage (22). Thus, BAFF-R is not present on murine pro-B or pre-B cells (20). In agreement with the proposed lack of significance of this receptor/cytokine for early cell development, mice lacking BAFF-R or BAFF have normal pro- and pre-B-cell compartments (18, 19). Because Ph-positive ALL cells typically represent transformed pre-B cells that do not produce surface IgM, the BAFF-R/BAFF pathway would not be anticipated to play a role in their survival or growth. However, the expression of both BAFF and its receptor BAFF-R in leukemias and lymphomas involving more mature B-lineage cells prompted us to examine a possible role of BAFF and its receptors in ALL. We here present evidence that pre-B ALL is characterized by the abnormal expression of a functional BAFF/BAFF-R pathway.

Materials and Methods

Human ALL cell culture

ALL cells used in this study are listed in Supplementary Table S1. US7 and US7R were from one patient before and after the patient developed drug resistance. US6 has a t(7;14) translocation. TXL-2 and TXL-3 were from patients at diagnosis. Primary viable frozen human ALL cells were engrafted through tail vein injections into female NOD.Cg-PrkdcscidIl2rgtm1Wjll/SzJ mice (The Jackson Laboratory). Leukemia cells harvested from spleens were plated on irradiated OP9 feeder layers and grown in αMEM (Life Technologies), 20% fetal bovine serum (FBS), 1% 1-glutamine, and 1% penicillin/streptomycin. Murine OP9 (CRL-2749), U937, and 293 were purchased from the American Type Culture Collection. Viability of ALL cells collected from the medium was determined by examining and manually counting total and trypan blue–excluding lymphoblasts, under a microscope.

Expression studies

RNA was isolated using Trizol from ALL cells harvested without stroma. First-strand synthesis used oligo(dT) as primer. Primers for reverse transcription-PCR (RT-PCR) of BAFF-R, TACI, BCMA, BAFF, and β2-microglobulin have been described (13). We used ELISA to detect soluble human BAFF (Apotech Corp.) in ALL culture supernatant concentrated using Amicon filters (Millipore).

We used a Becton Dickinson FACScan instrument for fluorescence-activated cell sorting (FACS) analysis. Isotype-matched antibody was used to verify staining specificity. ALL cells were Fc blocked by treatment with 1 μg of human IgG/10^5 cells for 15 minutes at room temperature before staining. An Annexin apoptosis detection kit (BD Pharmingen) was used for detecting apoptotic cells. For detection of NF-κB (p65 and p52), a nuclear extraction kit (Imgenex) was used to separate nuclear and cytoplasmic fractions. For Western blot BAFF, BAFF-R, and Pim-2 detection, 20 μg of cell lysates prepared in radioimmunoprecipitation assay buffer containing 1 mmol/L phenylmethanesulfonyl fluoride, 0.28 trypsin inhibitor units/mL aprotinin, 50 μg/mL leupeptin, 1 mmol/L benzamidine, and 0.7 μg/mL pepstatin were used.

Antibodies used in this study are described in detail in Supplementary Table S2.

Isolation of mononuclear cells from ALL and normal control bone marrow

Viably frozen bone marrow from a normal donor and a patient with ALL was obtained from the National Disease Research Interchange (Philadelphia, PA). Samples were thawed, RBC lysis was performed, and the cells were pelleted. Cells were suspended in PBS−/−, and mononuclear cells were isolated by centrifugation on a Ficoll gradient (according to Miltenyi Biotec). Peripheral blood mononuclear cells from U8.7R had been viably frozen directly from the patient. Mononuclear cells were incubated with CD19, IgM, CD10, and BAFF-R antibodies. Cells were gated for the CD19+, IgM+, CD10− fraction and then examined for BAFF-R expression.

Statistical analysis

Results are shown as mean ± SD of at least three samples each. For statistical comparison between groups, the Student’s t test was used, with a P value <0.05 considered significant.

Results

Human ALL cells express the BAFF receptor BR3

Primary cells from different B-lineage ALL patients were passaged in NOD.Cg-PrkdcscidIl2rgtm1Wjll/SzJ mice using previously published protocols (23). To culture these cells ex vivo, we used OP9 stromal cells because we found that murine embryonic fibroblasts (MEF), which we previously used to support the ex vivo growth of murine ALL cells, failed to support the long-term growth of these human ALL cells.

In initial experiments, we examined the human ALL cells for mRNA expression using semiquantitative RT-PCR for BAFF-R, TACI, and BCMA. Ramos and Jurkat cells were used as positive and negative controls. To our surprise, as shown in Fig. 1A, all of the four human ALL cells tested were positive for BAFF-R and TACI. However, they showed very weak or no expression of BCMA. To examine whether the detected mRNA resulted in production of substantial amounts of cell surface receptors, flow cytometric analysis was done on 12 different ALL samples. Surprisingly, all showed high expression of BAFF-R (Fig. 1B; Supplementary Table S1). Remarkably, within the individual samples, >90% of the cells were positive. Of the different ALL samples tested for the expression of TACI and BCMA, all were weakly positive for TACI (Fig. 1B; Supplementary Table S1) but negative for

www.aacrjournals.org Cancer Res; 70(11) June 1, 2010 4347
BCMA (Fig. 1B; Supplementary Table S1). Although 25% to 40% of the cells expressed TACI, the intensity of expression was much lower compared with the BAFF-R expression on these cells (Fig. 1B). Both Ph-positive and Ph-negative ALL samples expressed BAFF-R (Supplementary Table S1).

**Pre-B ALL cells isolated from fresh bone marrow and peripheral blood of ALL patients express BAFF-R**

The samples that tested positive for BAFF-R had all been passaged in mice and then plated on stroma. We therefore considered the possibility that passage in mice had induced BAFF-R expression in the ALL cells. To address this question, we obtained uncultured fresh bone marrow and peripheral blood and isolated mononuclear cells from these ALL samples. We also obtained bone marrow from a healthy donor. We gated for CD19+CD10+IgM− cells to identify pre-B cells and tested these for the expression of BAFF-R, TACI, and BCMA using flow cytometry. Consistent with earlier reports, normal pre-B cells were negative for expression of BAFF-R, BCMA, and TACI (Fig. 2A). About 60% of the population of mononuclear cells in the ALL patient’s bone marrow consisted of CD10+, CD19+, IgM− cells (data not shown). More than 50% of gated pre-B ALL cells from the bone marrow of this patient (Fig. 2B) and from the peripheral blood of a different patient (Fig. 2C) were positive for BAFF-R and uniformly negative for BCMA and TACI expression (Fig. 2B and C).

To confirm the expression of BAFF-R by noncultured human ALL cells, we used Western blot analysis on lysates made from independent peripheral blood lymphocytes of two Ph-positive and three Ph-negative ALLs. As shown in

![Figure 1](https://cancerres.aacrjournals.org/content/70/11/4348/F1.large.jpg)

**Figure 1.** Human ALL cells cultured in vitro express BAFF receptors. A, semiquantitative RT-PCR on RNA from human ALL cells cultured on OP9 stroma using the primers indicated to the right. β2-Microglobulin serves as control. Ramos and Jurkat are positive and negative controls, respectively. B, FACS analysis on the indicated ALL cells (P-2, BLQ-1, BLQ-11, and TXL-2) cultured on OP9 stroma using anti–BAFF-R, anti-BCMA, and anti-TACI antibodies. Ramos and Jurkat are positive and negative controls, respectively. Histograms show overlay of isotype control (black line) and receptor antibodies (gray line).
Fig. 2D, all five samples tested were positive for BAFF-R and expression levels were comparable with those of the positive control Ramos cells.

**BAFF receptor expressed by ALL cells shows high affinity toward its ligand BAFF**

Early B cells can differentiate into more mature stages when cocultured with feeder stromal layers (24, 25). Acute myeloid leukemia cells (26) and CLL cells (27) were reported to differentiate in vitro, and hence, we considered the possibility that the presence of stromal feeder layers had changed the immunophenotype of the cells. However, all the human ALL cells tested retained their phenotype (CD19^+CD10^+IgM^-) after in vitro culture (TXL-2; Fig. 3A; similar data for the other samples; data not shown). We also examined the expression of BAFF-R on different ALL cells growing in the absence of stroma for 5 days using flow cytometry. As shown in Fig. 3B, cells remained positive for BAFF-R also in the absence of stroma.

We next asked whether the BAFF-R expressed by these cells is functional. We used a specific monoclonal antibody (clone 11c1) in this experiment to detect cell surface BAFF-R on the ALL cells after incubating overnight with 100 ng/mL recombinant BAFF. Due to spatial competition, the 11c1 anti–BAFF-R antibody is unable to bind
to B cells in the presence of high amounts of added recombinant BAFF (28). As shown in Fig. 3C, >90% of the ALL cells that had previously been incubated with recombinant BAFF were negative for detection of BAFF-R with this antibody; thus, the BAFF-R expressed by ALL cells was occupied with BAFF. This indicates that the BAFF-R expressed by ALL cells shows a high affinity toward its ligand BAFF.

**Expression of BAFF and its signaling in human ALL cells**

We considered the possibility that the pre-B ALL cells could also express the ligand for BAFF-R, BAFF. We first evaluated the cells for BAFF mRNA by RT-PCR. As shown in Fig. 4A, all four of the ALL cells tested were positive for BAFF mRNA expression. BAFF is a member of the broader family of TNF-like cytokines, existing as a membrane-bound and as a secreted form. As shown in Fig. 4B, flow cytometry analysis showed that the membrane-associated form of BAFF was present on the surface of BLQ-1, US.7, and TXL-2. The ALL cells were also evaluated for the presence of secreted human BAFF by ELISA using conditioned medium collected from ALL cell culture. However, we did not detect expression of this form of human BAFF (data not shown). We also examined the expression of BAFF protein by Western blot. As shown in Fig. 4C, BAFF was clearly present in the ALL cells.

BCMA and TACI signaling activates NF-κB through the canonical pathway, whereas signaling through BAFF-R was reported to activate both the canonical and the alternative pathways (29–31). We therefore examined intracellular signaling pathways induced by BAFF in the ALL cells. In B cells, the BAFF-mediated activation of the alternative NF-κB pathway involves processing of p100 to p52, with subsequent translocation of p52 to the nucleus (31, 32). When ALL cells incubated with BAFF for 20 hours were separated into a cytoplasmic and a nuclear fraction, there was a substantial (>8-fold) increase of processed p52 in the nuclear fraction and a concomitant decrease of p100 in the cytoplasm compared with non-BAFF-treated controls (Fig. 4D, left).

Although most studies suggest that BAFF activates the classic NF-κB pathway through TACI and BCMA (29), Enzler and colleagues (33) reported that BAFF-R also contributes to activation of the classic pathway. We therefore also examined nuclear levels of p65, which migrates to the nucleus on activation of the classic NF-κB pathway. Compared with control ALL cells without BAFF treatment, nuclear levels of p65 were increased ∼2-fold after 30 minutes in the presence of BAFF (Fig. 4D, right).

**Effect of BAFF on growth of ALL cells**

Primary ALL cells do not initially grow in culture without stromal support and are prone to apoptosis in vitro due to lack of proper survival signals. As mentioned above, we found that MEFs did not support ALL growth in long-term culture. However, OP9 bone marrow stromal cells were able to provide the required support to sustain the in vitro growth of ALL cells in long-term culture (Fig. 5A). Interestingly, we found that OP9 produced significantly higher amounts of BAFF than MEFs (Fig. 5B). This suggested that the human ALL cells require BAFF for long-term growth. To test this, we cultured ALL cells in the absence of stroma and using 5% FBS, with or without recombinant BAFF. Viability of the
Figure 4. BAFF expression and BAFF-induced NF-κB signaling in ALL cells. A, BAFF expression using semiquantitative RT-PCR. U937, positive control; 293, negative control. B, FACS analysis for cell surface expression of BAFF. Black, isotype control; gray, BAFF antibody. C, Western blot analysis on the indicated cells. U937 and 293 are positive and negative controls, respectively. D, left, Western blot using antibodies for p52 on cytoplasmic and nuclear extracts of BLQ-1 ALL cells incubated for 15 or 20 h with or without (con) 200 ng/mL of recombinant BAFF. Tubulin and lamin are cytoplasmic and nuclear fraction controls, respectively. Densitometric analysis of the nuclear p52 is shown. Ratio of nuclear p52 to lamin of control is taken as 1. Right, nuclear extract of BLQ-1 ALL cells treated for 0.5 or 2 h with recombinant BAFF (200 ng/mL) blotted with an antibody against p65. Control, sample not treated with BAFF at 2-h time point. Densitometric analysis of the nuclear p65 is shown. Ratio of nuclear p65 to lamin of control is taken as 1.
ALL cells dropped under these stringent conditions, but
the presence of BAFF significantly delayed the decrease in
viability starting at day 7 of incubation (Fig. 5C, left). There
are no small-molecule inhibitors available to block BAFF-R
signaling. However, antibodies have been used to attempt
to block BAFF-R function. We therefore incubated the ALL
cells with a neutralizing BAFF-R antibody (5 μg/mL) for 9
days. Although there was only a partial inhibition of BAFF-
induced NF-κB signaling in ALL cells by this antibody (data
not shown), there was a measurable reduction in cell viability
after 5 days of exposure to the neutralizing antibody as shown
in Fig. 5C (right).

An autocrine loop could be present on the ALL cells
because they express both BAFF and BAFF-R. To investi-
gate this possibility, we incubated a Ph-negative and a Ph-
positive ALL with a human BAFF decoy receptor-Fc fusion
protein, BCMA-Fc, which can neutralize the effect of human
BAFF (19). Interestingly, as shown in Fig. 5D, at day 5 in the
absence of stroma, there was a significant reduction in
cell viability in the presence of BCMA-Fc in comparison
with control human IgG-Fc–treated wells. Addition of human
recombinant BAFF reduced this inhibitory effect, indicating
a role of autocrine BAFF signaling in the survival of the
ALL cells.

Recombinant BAFF reduces drug-induced ALL cell
apoptosis
It has been reported that autocrine and paracrine BAFF sig-
naling promotes the accumulation of non–Hodgkin lymphoma
B cells by attenuating apoptosis (14). BAFF also protected pri-
mary myeloma cells cultured with the patient’s own bone mar-
row stroma from dexamethasone-induced apoptosis (13).
Therefore, we investigated whether BAFF could protect ALL cells from apoptosis induced by a chemotherapeutic drug. Nilotinib is a selective and potent Bcr/Abl tyrosine kinase inhibitor that is currently used to treat Ph-positive leukemias (34). To exclude the possibility that nilotinib might affect the ability of OP9 stroma to support ALL cell growth, we cultured a Ph-negative ALL (US.7) in the presence of nilotinib. Because nilotinib is a Bcr/Abl tyrosine kinase inhibitor, it has no effect on Ph-negative cells (34). As shown in Fig. 6A (right), neither viability nor proliferation of US.7 cells growing over OP9 stroma was affected by the presence of nilotinib, which indicates that the capacity of OP9 stroma to support ALL growth is not affected by nilotinib. We then cultured Ph-positive TXL-2 ALL cells in the presence of nilotinib with or without recombinant BAFF.

**Figure 6.** Exogenous BAFF reduces effects of drug treatment on ALL cells. A, left, viable cell counts (cell number x 10^5/mL) of Ph-positive TXL-2 on OP9 stroma incubated with 1 μmol/L nilotinib and human recombinant BAFF (200 ng/mL) for 10 d. Wells without BAFF or nilotinib served as controls. *, P < 0.05, compared with nilotinib only–treated cells. Right, viable cell counts of Ph-negative US.7 cells treated with 1 μmol/L nilotinib. B, phase-contrast image of TXL-2 cell cultures at day 10. Scale bar, 100 μm. C, FACS analysis for Annexin V (top) and PI (bottom) staining of TXL-2 at day 6. D, Western blot for Pim-2 expression in TXL-2 after 6 d of incubation with nothing (control), nilotinib, or nilotinib + BAFF. Tubulin, loading control.
BAFF for 10 days. At day 7, as expected, the total viable cell number in wells treated with nilotinib had decreased (Fig. 6A, left). However, there were significantly more viable cells in the nilotinib + BAFF–treated wells, as is exemplified in Fig. 6B. To examine the rate of cell death and apoptosis induced by the drug, cells were collected at day 6 and stained with propidium iodide (PI) and Annexin V. A significant reduction in the number of PI-positive and Annexin V–positive cells was noticed in the presence of BAFF compared with the drug alone–treated wells (Fig. 6C), indicating that BAFF reduced the rate of apoptosis caused by the nilotinib treatment.

Although the effect of BAFF on in vitro B-cell survival is mediated through the classic and alternative NF-κB pathways, each pathway makes a distinct contribution to the expression of antiapoptotic genes. Activation of either the classic or alternative pathway enhances expression of the antiapoptotic proteins A1/Bi1 and Bcl-Xi, whereas induction of the prosurvival Pim-2 kinase exclusively depends on the alternative pathway (33). Hence, we analyzed Pim-2 expression in the ALL cells treated with nilotinib or nilotinib and BAFF. As shown in Fig. 6D, compared with nilotinib only–treated cells, the addition of BAFF clearly induced the expression of Pim-2, suggesting that the decreased apoptosis of nilotinib-treated ALL cells in the presence of BAFF might be mediated at least in part by upregulation of Pim-2.

Discussion

The cytokine BAFF is produced by a number of cell types, including monocytes, neutrophils, macrophages, dendritic cells, and some subsets of T cells (17). Receptors for BAFF, however, were initially thought to be restricted to more differentiated B-lineage cells. Therefore, the expression of BAFF receptors on transformed B-lineage lymphocytes in CLL was not entirely unexpected. In contrast, based on BAFF-null and BAFF-R–null mutants as well as other studies, it has been generally accepted that precursor B-lineage cells do not express this receptor. Our studies confirm that there is no expression of this receptor in normal bone marrow pre-B cells. Interestingly, Rodig and colleagues (35) also performed FACS on two pre-B ALL samples and reported that these were negative for expression of BAFF-R. Therefore, the prominent expression of the BAFF-R that we detected on both Ph-positive and Ph-negative ALL samples was unanticipated. In fact, all 12 samples that were tested by us, including original ALL bone marrow and blood samples, were BAFF-R positive, and BAFF-R was detected using both Western blots and FACS analysis. We conclude that this receptor is an excellent tumor-specific marker that distinguishes malignant pre-B cells from their normal counterparts.

We considered the possibility that BAFF-R expression would be downregulated when the ALL cells lost contact with stromal support, but BAFF-R expression was remarkably stable over time and remained even when stromal contact was removed for 5 days. The presence of BAFF-R on 50% to 99% of all the cells in the individual samples suggests that it has been selected for and has some important function. Because BAFF-R promotes survival of normal B cells, it could also provide a survival signal to the ALL cells. Indeed, a role for BAFF-R in the survival of neoplastic B cells, such as CLL, lymphoma, and myeloma, has been reported (13, 36–38). We provide evidence that the presence of BAFF-R on the pre-B ALL cells similarly increases their survival, although viability of ALL cells cultured with recombinant human BAFF alone was not as high as that of cells cultured with OP9 stroma. This was not unexpected because physical (cell-cell) contact is known to constitute one component of the stromal support to ALL and other leukemia cells (39). In addition, recombinant exogenously added BAFF may not be qualitatively equivalent to that processed by cells, such as that produced in significant amounts by the OP9 cells.

In B cells, BAFF activates the classic and alternative NF-κB pathways. Signals through BAFF-R mainly activate the alternative pathway, whereas the classic NF-κB pathway is stimulated through BAFF-R, TACI, and BCMA. Both mechanisms of NF-κB activation play important roles in B-cell development and survival (33, 40–42). Interestingly, constitutive NF-κB activation is a molecular signature that has been reported in several malignant B-cell types, including ALL cells (43, 44). We observed BAFF-induced degradation of p100 to p52 and translocation of p65 to the nucleus in ALL cells, suggesting that BAFF induces activation of both classic and alternative NF-κB pathways also in ALL cells.

In vivo and in vitro studies have shown that BAFF signal increases the expression of antiapoptotic molecules and promotes cell survival, cell cycle progression, and proliferation of B cells. Pim-2 is one of the antiapoptotic genes induced by BAFF signaling in B cells (33, 45). We also measured BAFF-induced Pim-2 expression in ALL cells that were exposed to a therapeutic drug, whereas the levels of Bcl-2 remained unchanged (data not shown). Because BAFF-induced long-term survival of B cells with upregulation of Pim-2 depends only on the alternative pathway (33), BAFF may provide protection against drug-induced apoptosis through activation of the alternative NF-κB pathway.

The simultaneous expression of BAFF-R as well as that of BAFF has been reported both in normal murine microglial cells and on malignant human lymphoma cells (14, 36, 46). Remarkably, we observed that the pre-B ALL cells also expressed BAFF mRNA and protein, which points to the possibility of an autocrine BAFF signaling loop in these cells. However, although the pre-B ALL cells expressed amounts of BAFF detectable by Western blots in total cell lysates, we did not detect soluble human BAFF in ALL culture-conditioned medium, with a detection level of <1 ng/mL. Interestingly, when we blocked human BAFF using a BCMA-Fc decoy receptor, the survival of the ALL cells was reduced, suggesting that the cell surface BAFF on the ALL cells provides an autocrine or paracrine BAFF survival signal.

Our data show that BAFF-R is a pre-B leukemia-specific cell surface antigen that could be used for diagnostic purposes. Moreover, inhibitors that block the BAFF/BAFF-R interaction could be valuable specific therapeutic agents in pre-ALL. This receptor-ligand combination is thought to play a role in autoimmune disorders such as rheumatoid arthritis (47), and different approaches to inhibit the interaction have been reported (48). Unfortunately, to date, a potent inhibitor has
not been identified. We suggest that specific targeting of pre-B ALL cells with antibody to BAFF-R conjugated to a toxin, for receptor-mediated delivery of the toxin to ALL cells, could be a promising approach, especially for pre-B ALL cells located in the protective bone marrow environment.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank Dr. Parameswaran Ramakrishnan for providing Ramos and Jurkat cells and Sonia Stoddart for help with growth of the human ALL cells. ALL samples for Western blots were a kind gift from Ralph Arlinghaus. Viably frozen bone marrow from a normal donor and a patient with ALL was obtained from the National Disease Research Interchange (Philadelphia, PA).

References

A Functional Receptor for B-Cell–Activating Factor Is Expressed on Human Acute Lymphoblastic Leukemias

Reshmi Parameswaran, Markus Müschen, Yong-mi Kim, et al.

Cancer Res 2010;70:4346-4356. Published OnlineFirst May 11, 2010.