Leukotriene B₄ plays a pivotal role in CD40 dependent activation of chronic B lymphocytic leukemia cells

Short title: B-CLL and leukotrienes

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Abstract

Biosynthesis of leukotrienes occurs in human myeloid cells and B lymphocytes. However, the function of leukotrienes in B lymphocytes is unclear. Here we report that B-cell chronic lymphocytic leukemia (B-CLL) cells produce leukotriene (LT) B4 and that specific leukotriene biosynthesis inhibitors counteracted CD40-dependent activation of B-CLL cells. Studies on the expression of the high affinity receptor for LTB4 (BLT1) by flow cytometry analysis showed that the receptor was expressed, to a varying degree, in all investigated B-CLL clones. The drugs BWA4C (a specific 5-lipoxygenase inhibitor) and MK-886 (a specific 5-lipoxygenase activating protein inhibitor), at a concentration of 100 nM, markedly inhibited CD40-induced DNA synthesis (45% and 38%, respectively) and CD40-induced expression of CD23, CD54 and CD150. Addition of exogenous LTB4 (150 nM) almost completely reversed the effect of the inhibitors on DNA synthesis and antigen expression.

Taken together, the results of the present study suggest that leukotriene biosynthesis inhibitors may have a therapeutic role in B-CLL.
Introduction

Leukotrienes (LT) are biologically active metabolites of arachidonic acid.\textsuperscript{1} Once liberated by phospholipase A\textsubscript{2} (E.C.3.1.1.4), arachidonic acid can be converted to prostaglandins, thromboxanes, and leukotrienes. The key enzyme in leukotriene biosynthesis is 5-lipoxygenase (5-LO) (E.C.1.13.11.34), which in a two step reaction catalyzes the formation of LTA\textsubscript{4} from arachidonic acid.\textsuperscript{1} Leukotriene A\textsubscript{4} can be further metabolized into LTB\textsubscript{4}, a reaction catalyzed by LTA\textsubscript{4} hydrolase (E.C.3.3.2.6). Cellular leukotriene biosynthesis is dependent on 5-LO activating protein (FLAP), a membrane protein which binds arachidonic acid and facilitates the 5-LO reaction.\textsuperscript{1}

In contrast to prostaglandins, which are produced by almost all type of cells, formation of leukotrienes from arachidonic acid is restricted to a few cell types in the human body. Biosynthesis of leukotrienes occurs mainly in myeloid cells and B lymphocytes.\textsuperscript{1} The production of LTB\textsubscript{4} and the biological effects of this compound on myeloid cells are well characterized, and LTB\textsubscript{4} stimulates neutrophil trafficking and activation at very low concentrations.\textsuperscript{1} However, the biosynthesis and function of leukotrienes in B lymphocytes are much less characterized. In contrast to myeloid cells, intact B cells do not produce LTB\textsubscript{4} after challenge with calcium ionophore A23187 only.\textsuperscript{2,3} The mechanism of activation of leukotriene biosynthesis in intact B cells is unclear, but there is accumulating evidence that the cellular redox status is of importance for biosynthesis of leukotrienes.\textsuperscript{3-6} Furthermore, the p38 mitogen-activated protein kinase appears also to be involved in stress-induced leukotriene synthesis in B cells.\textsuperscript{7} There is no convincing report demonstrating that T lymphocytes contain 5-LO and can produce leukotrienes. However, T lymphocytes express FLAP but the function of this protein in T cells is not known.\textsuperscript{3}

The actions of LTB\textsubscript{4} on leukocytes are mainly mediated by BLT1, a high-affinity G-coupled LTB\textsubscript{4} receptor expressed on neutrophils and monocytes.\textsuperscript{8-10} BLT1 is also expressed on activated T lymphocytes, both CD8\textsuperscript{+} cells and CD4\textsuperscript{+} cells\textsuperscript{11-13} and weakly on peripheral human non-activated B lymphocytes.\textsuperscript{14} A second LTB\textsubscript{4} receptor, BLT2, with lower ligand affinity and wider tissue distribution has also been characterized.\textsuperscript{15,16}
Leukotriene B₄ is an immunomodulator and this compound activates both B cells, T cells and NK cells.¹⁷ Several reports indicate that LTB₄ enhances activation, proliferation and antibody production in tonsillar B lymphocytes¹⁸-²⁰ and stimulates various T-cell functions.²¹,²² Leukotriene B₄ is a very potent chemotactic compound for activated T lymphocytes and BLT1-receptor deficient mice have an impaired trafficking of activated CD8⁺ cells and CD4⁺ cells.¹¹-¹³

The B-cell surface protein CD40 belongs to the tumor necrosis factor/nerve growth factor receptor family, and plays an important role in T-cell dependent B-cell activation. Ligation of this receptor with antibodies or with CD40-ligand (CD40L) generates an intracellular signal that induces a variety of stimulatory events in both normal and malignant B lymphocytes. Stimulation of B cells with CD40L and IL-4 leads to homotypic adhesion, proliferation, and differentiation into Ig producing cells.²³-²⁶ The expression of CD23 is a marker of activation of B cells, and CD54 (ICAM-1) is an important adhesive molecule expressed to various extent on many B-cell chronic lymphocytic leukemia (B-CLL) clones. CD150 is an antigen involved in the bidirectional stimulation of T- and B cells and is upregulated on activated B cells.²⁷-²⁹

Microarray studies have demonstrated an abundant expression of 5-LO in certain B-cell malignancies.³⁰-³³ In fact, 5-LO was one of the most abundantly expressed genes of 1,024 selected “lymphocyte” genes in B-CLL samples compared with a mixture of normal human tissues used as the reference sample.³³ Certain types of diffuse large B-cell lymphoma also have a comparatively high expression of 5-LO.³⁰

In light of these studies, it was of interest to explore the biosynthesis of leukotrienes in B-CLL cells and the effect of specific leukotriene biosynthesis inhibitors on the activation of B-CLL cells.
Materials and Methods

Reagents and cell lines: The calcium ionophore A23187 was purchased from Calbiochem-Behring (La Jolla, CA, U.S.A.). HPLC solvents were obtained from Rathburn chemicals (Walkerburn, U.K.) and the synthetic standards of LTB4 and prostaglandin (PG) B1 were from Biomol (Plymouth, PA, U.S.A.). BWA4C was a kind gift from Lawrie G Garland (Wellcome Research Laboratories, U.K.) and MK-886 from Jilly F. Evans (Merck Research Laboratories, Rahway, NJ, U.S.A.). Azodicarboxylic acid bis(dimethylamide) (diamide) was purchased from Sigma (Stockholm, S). Mouse fibroblastic L cells transfected with the human CD40L (CD40L-L) were used for activation and untransfected L cells (L) as control.34

Patients: Cell samples were studied from six patients with B-CLL. There were 3 women and 3 men with a median age of 66 years. The median time since diagnosis was 73 months and two patients were chemotherapy naive. The remaining four patients had received one to six different treatments.

Isolation of cells: B cells were isolated from patients suffering of B-CLL (Table 1) or B-prolymphocytic leukemia (B-PLL) who had not received chemotherapy during the previous six weeks. Peripheral blood samples were obtained after informed consent and with local ethics committee approval. Blood samples were Ficoll-Isopaque purified and washed twice in phosphate buffered saline (PBS) and either frozen in PBS with 50% human AB serum and 10% dimethylsulfoxide or analyzed fresh. Frozen cell samples were thawed and washed in ice cold fetal calf serum (FCS) and subsequently suspended in PBS before analysis. Cells from two patients were used twice, both freshly isolated cells and after freezing. However, similar results were obtained (data not shown). The purity of the isolated cells was estimated by flow cytometric analysis with FACS Calibur (Becton Dickinson, Mountain View, CA, U.S.A.). Morphological analysis was performed after staining with May-Grunewald/Giemsa solution. The purity of B-CLL and B-PLL cells was >98%.
Incubation of intact B-CLL cells: Freshly isolated cells (10^7) were suspended in one ml PBS and incubated for two minutes with/without diamide (100 µM), prior to stimulation with arachidonic acid (40 µM) and/or calcium ionophore A23187 (1 µM). The cells were stimulated for five minutes at 37°C and the incubations were terminated with 1 ml methanol.

Incubation of sonicated B-CLL cells: Freshly isolated cells (10^7) were suspended in one ml calcium free PBS including EDTA (2 mM) and sonicated 3 x 5 s. The cells were incubated for two minutes in the presence of ATP (1 mM) prior to addition of calcium chloride (2 mM) and arachidonic acid (40 µM). The reaction was terminated with 1 ml methanol after five minutes of incubation at 37°C.

HPLC analysis of leukotrienes: After addition of 0.5 ml PBS and the internal standard PGB₁ (100 pmol) to the samples, the cells were centrifuged (800 x g, 5 min). The supernatant was subsequently subjected to solid phase extraction on Sep-Pak Vac C₁₈ columns (100 mg, Waters). The methanol eluate was analyzed on Waters Alliance 2695 reverse phase HPLC and detected with Waters PDA 996. Methanol:water:trifluoroacetic acid (70:30:0.007, v/v) was used as mobile phase at a flow rate of 1.2 ml/min. Qualitative analysis was performed by comparison of retention times of synthetic standards and online analysis of UV-spectra of eluted compounds. Quantitative determinations were performed by computerized integration of the area of eluted peaks.

Flow cytometry analysis of BLT1: Fresh blood samples from healthy donors and fresh samples from patients were Ficoll-Isopaque separated and washed in PBS. For analysis of whole blood leukocytes (including granulocytes) from healthy donors, cells were washed in PBS and lysed with FACS lysing solution (Becton Dickinson). Frozen patient samples (B-CLL and B-PLL) were thawed (as described above) and washed in PBS. After resuspending cells in 100 µl PBS, antibodies were added according to manufacturer’s instructions and incubated at room temperature for 10 minutes. The cells were washed in 2 ml PBS and fixed in 1% paraformaldehyde, before analysis with FACS Calibur (Becton Dickinson) using the CellQuest software (Becton Dickinson). In this study all the
antibodies used for flow cytometry were directly conjugated with either fluorescein isothiocyanate (FITC), phycoerythrin (Pe) or peridinin chlorophyll protein (PerCP). The BLT1 antibody 7B1 FITC was raised in-house. IgG1-FITC, IgG1-Pe, IgG1 PerCP, CD4-Pe, CD5-Pe, CD8-Percp, CD14-FITC, CD14-Pe, CD19-FITC, CD19-Pe, CD20-Percp, CD22-Pe, CD33-FITC, CD33-Pe and IgG2a-FITC were purchased from Becton Dickinson (Mountain View, CA).

**Measurement of DNA synthesis:** Purified B-CLL cells were treated with MK-886 (1 nM- 1 µM) or BWA4C (1 nM- 100 nM) and/or LTB₄ (150 nM) in RPMI 1640 medium for 30 minutes. The B-CLL cells (4 × 10⁵) were thereafter seeded in 200 µl culture medium in precoated 96-well plates with irradiated (15,000 Rad) CD40L-L cells or control L cells. The culture medium contained RPMI 1640 medium, supplemented with 10% FCS, 2 mM L-glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin. The cells were incubated at 37°C in an atmosphere of 5% CO₂ for 96 hrs. ³H-thymidine (1 µCi) was present in the wells for the final eight hours of the incubation period. The cells were harvested onto glass fiber filter and radioactivity was measured in a liquid scintillation counter. Each sample was represented by triplicates.

**Flow cytometry analysis of CD23, CD54 and CD150 expression:** Purified B-CLL cells were treated with MK-886 (1 nM- 1 µM) or BWA4C (1 nM- 100 nM) and/or LTB₄ (150 nM) in RPMI 1640 medium for 30 minutes. The B-CLL cells (6 × 10⁶) were thereafter seeded in 3 ml culture medium in precoated 12-well plates with irradiated (15,000 Rad) CD40L-L cells or control L cells. The culture medium contained RPMI 1640 medium, supplemented with 10% FCS, 2 mM L-glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin. The cells were incubated at 37°C in an atmosphere of 5% CO₂ for 96 hrs. B-CLL cells were collected (without the plastic attached L cells) and used for FACS detection. Surface marker expression was detected by indirect immunofluorescence. The cells (10⁶/sample) were washed in cold PBS containing 1% FCS and 0.1% sodium azide and then exposed to the relevant antibodies. The cells were washed and incubated with the RPE conjugated secondary antibody. All incubations were done at 4°C. Samples were run on a FACScan flow cytometer (Becton Dickinson). Ten thousand events were
collected per sample and the CellQuest software was used both for acquisition and analysis. Only the viable cells were considered for analysis based on their light scatter (FSC/SSC) characteristics. The following antibodies were used: MAb MHM-6 (anti-CD23, from Dr. M. Rowe, University of Wales, Cardiff, Wales, U.K.), MAb LB-2 (anti-CD54, from E.A. Clark, University of Washington, Seattle, WA), MAb IPO-3 (anti-SLAM, kind gift from S. Sidorenko, Acad. of Science of Ukraine, Kiev, Ukraine) and RPE conjugated rabbit anti-mouse Ig F(ab')2 (Dako, Copenhagen, Denmark) were used as secondary antibody.

Results

Biosynthesis of leukotrienes in B-CLL cells: The capacity of B-CLL cells to produce leukotrienes was investigated. The cells were challenged with either calcium ionophore A23187, arachidonic acid or calcium ionophore A23187 plus arachidonic acid. No cell clones produced detectable amounts of leukotrienes after challenge with either calcium ionophore A23187 or arachidonic acid alone (Figure 1). Activation of the cells with calcium ionophore A23187 and arachidonic acid led to the formation of LTB4 (mean 2.6 ± 0.8 pmol/10⁶ cells). The B-CLL cells did not produce LTC4 (data not shown).

Preincubation of intact cells with the thiol-reactive agent diamide, prior to addition of calcium ionophore and arachidonic acid, led to a markedly increased production of LTB4 (mean 33.5 ± 1.2 pmol/10⁶ cells) in comparison to untreated intact cells. Similar amounts of LTB4 (mean 34.8 ± 1.7 pmol/10⁶ cells) were produced in sonicated cells, incubated with arachidonic acid. There was no obvious correlation between the capacity to produce leukotrienes and the clinical stage of the disease (data not shown). Taken together, the results demonstrated that all investigated B-CLL clones had the capacity to produce LTB4 and that all B-CLL clones contained substantial amounts of 5-LO which could be activated under certain conditions.
Figure 1. **Biosynthesis of LTB₄ by B-CLL cells.** Intact B-CLL cells (10 x 10⁶) were incubated for five minutes at 37°C with either calcium ionophore A23187 (1 µM), arachidonic acid (AA, 40 µM) or A23187 (1 µM) plus AA (40 µM). The cells were preincubated for two minutes at 37°C, in the presence or absence of diamide (100 µM), prior to addition of indicated compound(s). Sonicated cells were preincubated with ATP (1 mM) for two minutes at 37°C and then incubated with calcium chloride (2 mM) and AA (40 µM) for 5 minutes. The results show the mean ± SD from six B-CLL patients.

**BLT1 expression:** Peripheral blood leukocytes from healthy donors were analyzed with FACS for the expression of BLT1. Gates for granulocytes, lymphocytes and monocytes were set on the basis of forward and side scatter. Virtually all cells gated as granulocytes (CD33 positive) expressed BLT1 (Figure 2A). Cells in the monocyte gate (CD14 positive) showed the same pattern of BLT1 expression (data not shown). In the lymphocyte gate, no expression of BLT1 was observed on peripheral non-activated CD4⁺- or CD8⁺-positive T lymphocytes (Figure 2B and 2C). These results are in agreement with the observation that naive non-activated mouse T lymphocytes do not express BLT1.¹³ In contrast, 30-50% of the peripheral B lymphocytes (CD19⁺, CD20⁺,
CD22⁺) stained positively for BLT1 (Figure 2D). The BLT1 expression on peripheral B lymphocytes was weaker than on granulocytes and monocytes. B cells from six patients with B-CLL and two with B-PLL were analyzed with FACS for BLT1 expression. BLT1 expression varied from about 15% to 85% in the investigated B-CLL clones (average 42%) (Figure 2E). In the B-PLL group, the average expression of BLT1 was 74% in the two investigated clones. (Figure 2F).

Figure 2. Expression of BLT1 on human leukocytes. The expression of BLT1 was analysed in various leukocytes by FACS. A) PMNL, B) peripheral CD8⁺ T cells, C) peripheral CD4⁺ T cells, D) normal peripheral B cells, E) B-CLL cells and F) B-PLL cells. Large panel shows expression of BLT1 and the cell specific antigen. Small panel shows results with negative control antibodies. The figure depicts one typical experiment out of six except for B-PLL (two experiments).
Effects of leukotriene synthesis inhibitors on DNA synthesis in B-CLL cells: In order to elucidate if leukotrienes are of importance for proliferation of B-CLL, the cells were cultivated in the presence of leukotriene biosynthesis inhibitors. B-CLL cells were cultivated together with CD40L-L cells or control L cells for 96 hrs in the absence or presence of MK-886 (a specific FLAP inhibitor) or BWA4C (a specific 5-LO inhibitor). CD40-CD40L interactions activated B-CLL cells and resulted in an increased DNA synthesis, measured as ^3^H-thymidine incorporation during the final eight hours of four days cultures (Figure 3). MK-886, at a concentration of 100 nM, markedly inhibited DNA synthesis induced by CD40L stimulation (Figure 3A). Due to the relatively high binding of MK-886 to serum proteins, the effect of 1 µM MK-886 on DNA synthesis was also investigated in three experiments. This concentration of the inhibitor only caused a slightly more pronounced inhibition of DNA synthesis. The inhibitory action of 1 µM and 100 nM MK-886 on thymidine incorporation was 46 and 38 %, respectively. Leukotriene B₄ (final concentration 150 nM) alone did not amplify CD40-induced thymidine incorporation. However, exogenously added LTB₄ almost completely reversed the inhibitory effect of MK-886 on thymidine incorporation. The specific 5-LO inhibitor BWA4C was an even more potent inhibitor than MK-886 to block DNA synthesis (Figure 3B). Significant inhibitory effect of BWA4C on thymidine incorporation was observed at 10 nM. In line with the results obtained with MK-866, exogenous LTB₄ (150 nM) almost completely reversed the inhibitory action of 100 nM BWA4C on thymidine incorporation (Figure 3B). The cell survival after four days cultivation was about 80 % in all B-CLL cultures stimulated with CD40L-L, both in the absence and presence of inhibitor or LTB₄ (data not shown). Taken together, these data demonstrate that specific inhibitors of leukotriene synthesis cause a pronounced inhibition of DNA synthesis which could be reversed by addition of exogenous LTB₄.
Figure 3. **Effects of leukotriene biosynthesis inhibitors on CD40L-induced thymidine incorporation in B-CLL cells.** *B-CLL* cells (*4 x 10^5*) were cultivated together with either

A) **Effects of leukotriene biosynthesis inhibitors on CD40L-induced thymidine incorporation in B-CLL cells.**

B) **Effects of leukotriene biosynthesis inhibitors on CD40L-induced thymidine incorporation in B-CLL cells.**
irradiated L cells alone (L) or irradiated CD40L-L cells plus indicated compound(s) for 96 hrs. When inhibitors and/or LTB₄ were used, B-CLL cells were pretreated with the indicated compound(s) for 30 minutes in a serum-free medium. ³H-thymidine (1 µCi) was present for the final eight hours of the incubation period. A) MK-886 (1 µM- 1 nM) or B) BWA4C (100 nM- 1 nM) with or without LTB₄ (150 nM). Control in the figure represents B-CLL cells cultured together with irradiated CD40L-L cells alone. Activation of B-CLL cells with CD40L-L treatment led to between 3580 and 15369 cpm (³H-thymidine) incorporation in the different experiments (control). This was set as 100 % in each experiment. Each sample was represented by triplicates. The results show the mean ± SD from eight separate experiments (B-CLL cells from two patients were analyzed two times). The highest concentration of MK-886 (1 µM) was only used in three experiments. Student’s t-test was used to calculate statistics i.e. control vs control plus indicated compound(s) (** P<0.01, *** P<0.001).

Effects of leukotriene biosynthesis inhibitors and LTB₄ on CD23, CD54 and CD150 expression in B-CLL cells: FACS analysis demonstrated that CD40-CD40L interactions caused an increased expression of CD23, CD54 and CD150 (Figure 4). MK-886 and BWA4C, at a concentration of 100 nM, markedly counteracted this CD40-induced expression of CD23, CD54 and CD150. Leukotriene B₄ alone did not cause any significant effect on the expression of the investigated antigens. However, addition of exogenous LTB₄ (150 nM) almost completely reversed the inhibitory effect of the inhibitors on antigen expression (Figure 4). These results show that LTB₄ is involved in the expression of these antigens which are associated with activation of B-CLL cells.
Figure 4. Effects of leukotriene biosynthesis inhibitors on the expression of CD23, CD54 and CD150 in CD40L activated B-CLL. Purified B-CLL cells were cultivated together with either L cells or CD40L-L cells in absence or presence of MK-886 (100 nM), BWA4C (100 nM), and/or LTB₄ (150 nM) for 96 hrs. When inhibitors and/or LTB₄ were used, B-CLL cells were pretreated with the compound(s) for 30 minutes in a serum-free medium prior to cultivation together with L cells or CD40L-L cells. B-CLL cells were collected and analysed by FACS with antibodies against CD23, CD54 or CD150. The figure depicts one typical experiment out of six. In order to more clearly demonstrate
the different degree of expression of indicated antigen in the various samples, the inserted dotted line represents the expression of the indicated antigen in B-CLL cells stimulated with CD40L-L only.

Discussion

The enzyme 5-LO is abundantly expressed in B-CLL cells\textsuperscript{4,31-33} and the cells have capacity to produce LTB\textsubscript{4} (Figure 1). The biosynthesis of LTB\textsubscript{4} by B cells seems not to occur in low differentiated malignant B lymphocytes since the most immature B cell phenotypes do not have the capacity to produce leukotrienes.\textsuperscript{38} The cellular events which activate the endogenous formation of LTB\textsubscript{4} is not yet known. However, although the B-CLL clones produced comparatively low amounts of LTB\textsubscript{4} after activation with calcium ionophore A23187 and arachidonic acid (in comparison to calcium ionophore-activated granulocytes), sonicated and thiol-activated B-CLL cells produced markedly more LTB\textsubscript{4} (Figure 1), which is in agreement with earlier reports.\textsuperscript{3,4} Thus, B-CLL cells have under certain conditions the capacity to produce and release LTB\textsubscript{4} in similar amounts as myeloid cells. Furthermore, it is possible that the 5-LO pathway in B-CLL may generate LTB\textsubscript{4} both for export and as a messenger in an intrinsic signal transduction system.

The monoclonal antibodies used in this study for analysis of the BLT1 expression has previously been used to demonstrate BLT1 expression on granulocytes and differentiated HL-60 cells.\textsuperscript{35} Here we have demonstrated the expression of BLT1 in normal peripheral B lymphocytes, B-PLL cells and B-CLL cells (Figure 2). The degree of expression of BLT1 varied between different B-CLL clones but all investigated cell clones expressed BLT1 to some extent. BLT1 is very important for trafficking of T lymphocytes.\textsuperscript{11-13} The role of BLT1 on migration of B-CLL, and other types of B cells, remains to be elucidated. The presence of BLT1 on B-CLL cells suggests that LTB\textsubscript{4} might influence the function of B-CLL cells either in an autocrine and/or paracrine manner.

In order to understand the function of the 5-LO pathway in B-CLL cells, we investigated the effects of specific leukotriene biosynthesis inhibitors on the activation of...
these cells. For that purpose, the CD40-CD40L model system, which imitates T-cell dependent activation of B cells, was used. MK-886 and BWA4C are inhibitors of the 5-LO pathway which inhibit the synthesis of leukotrienes by completely different mechanism of action i.e. MK-886 is a specific FLAP inhibitor and BWA4C is a specific 5-LO inhibitor. At a concentration of 100 nM, both inhibitors markedly inhibited DNA synthesis in B-CLL cells (Figure 3). In addition, exogenous LTB₄ completely reversed the effects of the drugs, indicating that the effects of the inhibitors did not reflect an unspecific effect of the drugs (Figure 3). However, LTB₄ alone did not further stimulate CD40-induced DNA synthesis suggesting that endogenous LTB₄ caused maximal effects. Leukotriene B₄ has also been reported to stimulate proliferation of myeloid cells.³⁹,⁴⁰ MK-886, at a concentration of 100 nM, has an antiproliferative effect and induces apoptosis in HL-60 cells.⁴¹ Addition of exogenous LTB₄ could reverse the effect of the inhibitor on these cells. Furthermore, MK-866 has been found to be a potent inhibitor of DNA synthesis in a subset of acute myeloid leukemia cells.⁴²

CD54/intercellular adhesion molecule-1 (ICAM-1) is a single chain membrane glycoprotein, which is expressed on many types of cells such as leukocytes, endothelial and epithelial cells. Normal peripheral B lymphocytes barely express CD54, while B lymphocytes from CLL patients have an increased expression of CD54.²⁸ High expression is associated with poor prognostic features including increased tumor burden and sometimes a short lymphocyte doubling time.²⁷,²⁸ Soluble CD54 (sICAM-1) levels are high in patients with advanced clinical stage/high tumor burden.⁴³ Both the FLAP inhibitor and the 5-LO inhibitor counteracted the stimulatory action of CD40-CD40L interaction on the expression of CD54 (Figure 4). In these experiments, exogenous LTB₄ (150 nM) also reversed these effects of the inhibitors (Figure 4).

CD23 is a low-affinity receptor for IgE (FceRII) and is involved in the feedback regulation of IgE synthesis. CD23 has been proposed to be involved in cell viability and proliferation.⁴⁴ High serum levels of soluble CD23 (sCD23) was associated with high tumor burden and shorter time to progression in B-CLL.⁴⁵,⁴⁶ The expression of CD23 on B-CLL cells was inhibited by the leukotriene biosynthesis inhibitors and reversed by
LTB₄ (Figure 4). In agreement with these findings, LTB₄ has been reported to stimulate the expression of CD54 on endothelial cells and CD23 on B cells.¹⁸,¹⁹,⁴⁷

In summary, this study demonstrates that LTB₄ plays an important role in the activation of B-CLL cells. Inhibitors of leukotriene synthesis have so far only been used for treatment of asthma. The present report indicates that leukotriene biosynthesis inhibitors, LTA₄ hydrolase inhibitors or BLT1 antagonists, alone or in combination with conventional therapy, might also be useful in the treatment of B-CLL.
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