Stromal Endothelial Cells Establish a Bidirectional Crosstalk with Chronic Lymphocytic Leukemia Cells through the TNF-Related Factors BAFF, APRIL, and CD40L

Montserrat Cols, Carolina M. Barra, Bing He, Irene Puga, Weifeng Xu, April Chiu, Wayne Tam, Daniel M. Knowles, Stacey R. Dillon, John P. Leonard, Richard R. Furman, Kang Chen and Andrea Cerutti

_J Immunol_ published online 16 May 2012
http://www.jimmunol.org/content/early/2012/05/16/jimmunol.1102066

Supplementary Material  http://www.jimmunol.org/content/suppl/2012/05/16/jimmunol.11020666.DC1

Subscription  Information about subscribing to _The Journal of Immunology_ is online at: http://jimmunol.org/subscription

Permissions  Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts  Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
Stromal Endothelial Cells Establish a Bidirectional Crosstalk with Chronic Lymphocytic Leukemia Cells through the TNF-Related Factors BAFF, APRIL, and CD40L

Montserrat Cols,* Carolina M. Barra,† Bing He,* Irene Puga,† Weifeng Xu,* April Chiu,§ Wayne Tam,§ Daniel M. Knowles,§ Stacey R. Dillon,§ John P. Leonard,‖ Richard R. Furman,‖ Kang Chen,* and Andrea Cerutti* †, ‡, * Received for publication July 19, 2011. Accepted for publication April 13, 2012.

Chronic lymphocytic leukemia (CLL) is an incurable disease of unknown etiology characterized by progressive accumulation of clonal B lymphocytes with mature morphology and phenotype (1). The clinical course of CLL is variable and correlates with the mutational status of Ig H chain variable (VH) genes and the percentage of leukemic cells expressing CD38 and ζ-chain–associated protein 70 (ZAP-70) (2–4). The presence of unmutated VH genes and expression of CD38 and ZAP-70 are biological variables associated with worse prognosis. B cell intrinsic factors such as abnormal expression of antiapoptotic NF-κB and Bcl-2 proteins and accumulation of heterogeneous genomic lesions regulate the survival, expansion, and clonal evolution of CLL cells (5, 6). B cell extrinsic factors such as antigen stimulation may also be important, as leukemic B cells from unrelated groups of CLL patients express restricted Ig VD1 genes encoding stereotyped Ag-binding VH regions (7–9).

Accessory signals from the microenvironment further contribute to the pathogenesis of CLL (10, 11). Indeed,stromal cells such as nurselike cells, mesenchymal cells, follicular dendritic cells (DCs), and macrophages recruit CLL cells by secreting CXCL12 and CXCL13 chemokines and enhance CLL cell survival by releasing BAFF or BLYS and a proliferation-inducing ligand (APRIL) (12–14). These TNF family members are usually expressed by myeloid and epithelial cells and engage BAFF-R (BR3), transmembrane activator and CAML interactor (TACI), and B cell maturation Ag (BCMA) on B cells (15–21). The ensuing recruitment of TNFR-associated factor adaptor proteins leads to the activation of NF-κB, a transcription factor that regulates the survival, proliferation, diversification, and differentiation of B cells (19, 20, 22). A similar pathway becomes activated after engagement of the CD40 receptor on B cells by CD40L, a TNF family member typically expressed by T cells but also by CLL cells (5, 23).

CD40L, BAFF, and APRIL likely represent an important component of CLL proliferation centers, which consist of pseudofollicles containing foci aggregates of activated and proliferating CLL cells. Proliferation centers also include T cells expressing CD40L and stromal macrophages and nurselike cells releasing BAFF and APRIL (13, 24). Remarkably, malignant B cells shape the cellular composition of proliferation centers to...
generate a CLL-supportive microenvironment. Indeed, CLL cells release chemokines such as CCL22 to attract CD40L-expressing T cells and produce angiogenic molecules such as vascular endothelial growth factor (VEGF) to stimulate the formation of microvessels (24, 25). In addition to favoring leukemic dissemination, microvessels deliver survival signals to CLL cells through unknown factors released by endothelial cells (26).

We found that the CLL stroma contained abundant microvascular endothelial cells (MVECs) that constitutively expressed the B cell-stimulating factors BAFF and APRIL. MVECs increased the release of soluble BAFF and APRIL after engagement of CD40 on MVECs by CD40L on CLL cells. Signals from CD40 induced furin and TNF-α converting enzyme (TACE or ADAM17), which cleaved inactive BAFF and APRIL precursors into active soluble proteins. By activating NF-κB through TACI, BAFF-R and BCMA, endothelial BAFF and APRIL delivered survival, activation, and Ig DNA-modifying signals to CLL cells. Endothelial BAFF and APRIL also increased the expression of CD40L on CLL cells, indicating that BAFF, APRIL, and CD40L, link malignant B cells with stromal MVECs through an integrated bidirectional signaling network. Interruption of this network by specific inhibitors may be useful for the treatment of CLL.

Materials and Methods

Patients and samples

Leukemic cells were isolated from the peripheral blood of 21 CLL patients (Table I). The diagnosis of CLL was made according to standard criteria (27). All blood samples were obtained by phlebotomy upon request of consent according to a protocol approved by the Institutional Review Board of Weill Medical College of Cornell University. The V(D)J gene mutational status and Zap70 expression of each CLL sample were determined as previously reported (2-4). Normal peripheral blood B cells were isolated from buffy coats provided by the New York Blood Center. Frozen tissues samples from lymph nodes, bone marrow, and spleen of 10 healthy patients and tissues samples from lymph nodes, bone marrow, and spleen of 10 healthy tissues samples from lymph nodes, bone marrow, and spleen of 10 healthy controls and 10 CLL patients were obtained from tissue repositories according to a protocol approved by the Institutional Review Board of Weill Medical College of Cornell University.

Cells

Normal and CLL B cells were MACSorted from PBMCs by negative selection using a B cell isolation kit (Miltenyi Biotec, Bergish-Gladbach, Germany). Marginal zone CD19IgD+IgM- CD27+ B cells, follicular naive CD19+IgD+IgM+CD27- cells, and memory CD19+IgD-CD27+ B cells were sorted from splenic mononuclear cells, using a BD FACSAria III (BD Biosciences, San Diego, CA). Sorting was preceded by labeling of splenic mononuclear cells with mouse mAbs to CD31 and CD68. Live, placental, or allophycocyanin-conjugated mouse mAbs to human CD19 (BD Pharmingen, San Diego, CA), IgD (Southern Biotech, Birmingham, AL), and CD27 (BD Pharmingen). HUVECs and lymphatic microvascular endothelial cells (LMVECs) are commercially available (Lonza, Walkersville, MD). Splenic microvascular endothelial cells (SMVECs) were MACSorted from enzymatically digested splenic cell suspensions stained with mouse mAbs to CD31 and mannose receptor (BD Pharmingen).

Cultures and reagents

Endothelial cells were propagated in endothelial basal medium (EBM-2) supplemented with endothelial growth medium (EGM-2MV) SingleQuots (Lonza). Endothelial cells were seeded with EBM-2 plus EGM-2MV medium in 12-well plates at a concentration of 1.5 × 10⁶ cells/well for 48 h prior to coculture with CLL cells. The EBM-2 plus EGM-2MV medium was mixed with standard RPMI 1640 medium supplemented with 10% FCS (1:4 vol:vol) after seeding CLL cells at 1 × 10⁶ cells/ml. This mix is referred to as endothelial medium throughout the text and was used in all cultures involving CLL cells unless specified otherwise. Conditioned endothelial medium was obtained after culturing SMVECs for 4 d in endothelial medium. Some endothelial cell–CLL cell cocultures were performed in a 24-well transwell system (Corning, Lowell, MA). Recombinant BAFF, APRIL, and CD40L MegaLigand (Alexis Biochemicals, San Diego, CA) were used at 100 ng/ml. TACI-Ig and CD40-Ig decoy receptors and Fc5 or IgG1 controls (ZymoGenetics, Seattle, WA) were used at 5 μg/ml. Furin convertase inhibitor (peptidyl choro- methylketone) and TNF-α processing inhibitor-2 were used at 100 μM and 5 mM, respectively (Enzo Life Sciences, Plymouth Meeting, PA), whereas IKK inhibitor III (BMS-345541) was used at 1 μM (Calbiochem, San Diego, CA). After a 3-h incubation with IKK inhibitor III (an NF-κB inhibitor) or control DMSO vehicle, CLL cells were washed twice with endothelial medium and then cocultured with MVECs.

Flow cytometry

Cells were stained with FITC-, PE-, allophycocyanin-, or cyanine-3-conjugated mouse mAbs to human CD5, CD19, CD38 (BD Pharmingen); BAFF; BAFF, CD31 (eBiosciences, San Diego, CA); CD40, CD40L (Ancell, Bayport, MN); CD54, CD102, CD144 (BioLegend, San Diego, CA); CD105 (ImmunoTools, Friesoythe, Germany); IgG, IgA, or control isotype-matched reagents (Southern Biotech). Biotinylated Abs to TACI (ZymoGenetics) were stained with PE-conjugated streptavidin (BD Pharmingen). A goat Ab to BCMA (Santa Cruz Biotechnologies, Santa Cruz, CA) was stained with an appropriate PE-conjugated secondary Ab (BD Pharmingen). Dead cells were excluded from analysis using 7-amino-actinomycin D. Events were acquired using a FACScanlibar or LSRII (BD Biosciences) and were analyzed by FlowJo software (Tree Star, Ashland, OR).

Immunofluorescence and histology

Endothelial cell lines were grown on glass cover slides in EGM until reaching confluence. Tissues and cells were fixed and washed as described elsewhere (15) and then stained with the following unconjugated or conjugated primary Abs to various human Ags: goat polyclonal Ab (PoAb) to IgD (Southern Biotech); mouse mAb to Pax-5 (Santa Cruz); rabbit PoAb to Pax-5 (NeoMarkers, Fremont, CA); mouse mAb to Ki-67, Factor VIII, CD31, and elastase (Dako, Carlsbad, CA); rabbit PoAb to von Willebrand factor (vWF)(Dako); rabbit PoAb anti-APRIL (ProSci, Poway, CA); mouse mAb to CD3, CD11c, CD21, CD206 (BD Pharmingen); goat PoAb to activation-induced cytokine deaminase (AID) (Santa Cruz Biotechnology); rabbit PoAb to furin (Santa Cruz Biotechnologies); rabbit PoAb to CD63 (Novus Biologicals, Littleton, CO); mouse mAb to CD68 (Abcam, Cambridge, MA); mouse mAb to CD40L (Ancell); and rabbit PoAb to TACE (Sigma-Aldrich, St. Louis, MO). Primary Abs were labeled with an appropriate Alexa 488-, 546-, or 647-conjugated or cyanine-3-conjugated secondary Ab (Jackson Immunotechnologies, West Grove, PA). Cell nuclei were visualized with DAPI (Boehringer Mannheim, Indianapolis, IN). Coverslips were applied with SlowFade reagent (Molecular Probes, OR). Images were acquired with a Zeiss Axioplan microscope (Atto Instruments, Rockville, MD).

Cell viability and proliferation assays

Cell viability was evaluated through a trypan blue exclusion test. To measure apoptosis, cells were double stained with FITC-conjugated Annexin V and propidium iodide (Calbiochem) and analyzed by flow cytometry. Cell proliferation and/or survival were monitored using a yellow tetracloaurum (MTT) cell proliferation assay (Trevigen, Gaithersburg, MD). In this assay, dehydrogenases expressed by metabolically active cells generate NADH and NADPH that convert MTT into formazan. Briefly, 100-μl aliquots of culture supernatant were transferred into a 96-well plate and incubated overnight with 20 μl MTT reagent at 37°C and 5% CO₂, followed by a 2-h incubation with 100 μl detergent reagent. Absorbance readings were performed at 570 nm in a microplate reader. Cell proliferation was further evaluated through a trypan blue exclusion test. The proliferation index was calculated according to the following formula: mean of counts per minute of triplicates with stimulus/mean of counts per minute of triplicates without stimulus. CFSE was used to trace cell division as instructed by the manufacturer (Invitrogen, Grand Island, NY).

ELISA

Human IgG, IgA, IgM, BAFF, and APRIL were measured by ELISA as described elsewhere (29).

RT-PCR, quantitative RT-PCR, and Southern blot analysis

RNA was extracted with the RNeasy Mini Kit (Qiagen, Valencia, CA), and cDNA was synthesized as described (29). Germline 1¼-Cy1, 1¼-Cy2, and 1¼-Cy3 transcripts, as well as 1¼-Cy3, 1¼-Cy4, and 1¼-Cy4 switch circle transcripts, were RT-PCR amplified and hybridized with appropriate radiolabeled probes by Southern blot analysis as described (15). TACI forward: 5′-CTGGCACAACTCGGGGAATCT-3′, reverse: 5′-GCCACCCGTAGTCTGACCACTCCTC-3′, BAFF forward: 5′-ACCCCGGGACTG- AAAATCT-3′, and BAFF-R reverse: 5′-CAGCCTATTTTCTGTTTCTG- GA-3′, BCMA forward: 5′-GCCCTGACATCCTGTCACAATCGAT-3′.
and BCMA reverse: 5′-GAACTCGATTCGTTCTTTCTACTG-3′; CD40 forward: 5′-CACATTGCCACCCAGCACAA-3′, and CD40 reverse: 5′-GGCTTCTTCACAGGTCGAG-3′; AID forward: 5′-AGGGAGGTCGAGTGCTCA-3′; and AID reverse: 5′-TGTTAGGCCGATCTTCAGTTCAG-3′; TACE forward: 5′-GGGACGGAGGGCACTCA-3′; and TACE reverse: 5′-TGGAGGCCCCAGCTTCA-3′;furin forward: 5′-GGCCCTGGCTGTTCAATAC-3′; and furin reverse: 5′-TCGTCCAGCTGCTTTCTAT-3′; and β-actin forward: 5′-GCGTTCATACCTGGTCACTTG-3′, and β-actin reverse: 5′-GAAATGGCATTCTTCCTTACTG-3′ were used to quantify specific mRNAs by RT-PCR, as previously reported (29).

ImmunobLOTS

Equal amounts of total protein lysate, cytoplasmic extract, or nuclear extract were fractioned onto a 10% NaDodSO4-polyacrylamide gel and transferred onto nylon membranes (Bio-Rad, Hercules, CA). After blocking, membranes were probed with primary Abs to APRIL (ProSci), BAFF (Millipore, Bedford, MA), TACE, furin, or β-actin (Santa Cruz Biotechnologies), as reported (29). Then, membranes were washed and incubated with an appropriate secondary Ab (Santa Cruz Biotechnologies). Proteins were detected with an ECL detection system (Amersham, Little Chalfont, United Kingdom).

RNA interference

The following small interfering RNA (siRNA) duplexes were used after validation with three distinct siRNAs for each target gene of interest: 5′-GGAAATCAAATTCAGCTCTTTCACTG-3′ siRNA to BAFF-R, 5′-CAGCGGAGGGCACTCA-3′ siRNA to TACE (Ambion, Austin, TX); and 5′-ACATTTAAGGACGAGATTTA-3′ siRNA to BCMA (Qiagen); and 5′-AGGGACCCUCAAAACAGATT-3′ siRNA to CD40 (Qiagen). The specificity of each siRNA was validated using a scrambled siRNA as control. Transfection of specific or control (scrambled) siRNA in CLL cells was performed as recommended by the manufacturer (Ambion). Briefly, 5 μM siRNA was incubated with 100 μl nucleofection mix (Amaxa, Walkersville, MD) and 100 μl CLL cell suspension (3 × 10⁶ cells) for 2 min before transfection, using the Amaxa nucleofector protocol U-15. After 24 h, cells were seeded in 1 ml complete medium. Transfection efficiency was monitored after 48 h by measuring CLL cells positive for a control GFP-expressing plasmid through flow cytometry. Specific suppression of TACI, BCMA, and BAFF-R gene expression was validated through quantitative RT-PCR (QRT-PCR). These measurements demonstrated highly specific targeting of TACI, BCMA, or BAFF-R expression by distinct siRNAs. Transfection of CD40 or control siRNA in endothelial cells was performed using a SuperFect protocol (Qiagen). Briefly, endothelial cells were cultured on a monolayer with complete EGM until optimal confluence was reached. Then, 5 nM siRNA duplexes and 7.5 μl HiPerFect Transfection Reagent were diluted in 100 μl endothelial cell culture medium without serum. The mixture was incubated at room temperature to allow formation of transfection complexes and further added to the cells dropwise. Transfection efficiency was monitored by measuring the expression of CD40 through QRT-PCR after 48 h.

Statistical analysis

One-way ANOVA followed by a one-tailed unpaired Student t test was used to determine statistical significance.

Results

MVECs promote CLL cell survival

To elucidate the composition of the CLL stroma, we immunostained lymph node, spleen, and bone marrow tissues from CLL patients for molecules typically expressed by stromal cells. Immunohisto-fluorescence showed that all CLL tissues included microvessels expressing CD31 (PECAM-1) and containing factor VIII and vWF coagulation proteins (Fig. 1A, 1B). MVECs from these microvessels established an intimate contact with malignant B cells expressing IgD (a molecule associated with mature B cells and CLL cells) and generally were more abundant than macrophages, follicular DCs, DCs, and neutrophils expressing CD68 (Fcy receptor), CD21 (complement receptor 2), CD11c (integrin α X), and elastase (a granule protease), respectively.

In CLL spleens, MVECs expressed CD206 (mannose receptor, a molecule highly expressed by sinusoidal vessels) and were in close proximity with neoplastic B cells. Similar to MVECs from normal lymphoid tissues, MVECs from CLL tissues expressed CD54 (ICAM-1), CD102 (ICAM-2), CD105 (endoglin), and CD144 (VE-cadherin) (Fig. 1C). Flow cytometry showed that model MVECs from healthy individuals expressed a phenotype similar to that of MVECs from CLL patients (Fig. 1B, Supplemental Fig. 1). Indeed, UVECs, SMVECs, and LMVECs expressed CD31, CD54, CD102, CD105, and CD144 as MVECs from CLL patients did (Fig. 1D). As in SMVECs from CLL patients, MVECs further expressed CD206, whereas UVECs and LMVECs did not (Fig. 1D).

Given their phenotypic similarity to MVECs from CLL tissues, SMVECs, LMVECs, and UVECs were used in a simplified coculture model to establish the role of MVECs in the survival of malignant B cells. In the presence of SMVECs, ~90% of CLL cells survived after 7 d of culture, as established by a canonical viability assay (Fig. 1E). The prosurvival effect of SMVECs was partly dependent on cell–to-cell contact, because conditioned medium from SMVEC cultures induced the survival of only 40% of CLL cells after 7 d. Of note, SMVECs and conditioned medium induced stronger survival signals than did endothelial medium or standard medium alone, which indeed induced only 30% and 5% CLL cell survival, respectively. The partial prosurvival activity of endothelial medium alone was likely mediated by VEGF, a known CLL cell survival cytokine (30, 31). Compared with endothelial medium alone, MVECs increased CLL survival and/or proliferation, as measured in an MTT assay in which metabolically active cells convert MTT into formazan (Fig. 1F).

In agreement with these findings, proliferation centers from CLL tissues included factor VIII-positive MVECs that were in close contact with actively cycling IgD-positive malignant B cells expressing the proliferation-associated nuclear molecule Ki-67 (Fig. 2A). The possible proliferation-inducing activity of MVECs was suggested by means of CFSE dilution assays, which documented the division of a fraction (from 5% to 15% of total B cells) of the leukemic clone upon exposure of CLL cells to UVECs or SMVECs but not endothelial medium alone (not shown). Thymidine incorporation assays demonstrated induction of DNA replication in CLL cells exposed to SMVECs but not endothelial medium alone (Fig. 2B). Unlike CLL cells, nonmalignant B cells expressing IgM and IgD did not augment DNA replication in response to SMVECs, suggesting that MVECs require interaction with a CLL-specific factor (or factors) to induce the proliferation and/or survival of leukemic B cells. These data show that MVECs are a major component of the CLL stroma and indicate that MVECs deliver survival and possibly proliferation signals to CLL B cells.

MVECs trigger CLL cell activation and differentiation

In addition to active proliferation, a fraction of the CLL clone shows ongoing Ig DNA remodeling via class switch DNA recombination (CSR) (32, 33). This process is highly dependent on AID, a DNA-editing enzyme expressed by actively proliferating CLL cells in response to poorly understood signals generated by the activated microenvironment (32–37). To further elucidate the stimulating activity of MVECs on the leukemic clone, we determined whether MVECs induce AID expression and CSR in CLL cells (32). RT-PCR followed by Southern hybridization analysis showed that, compared with CLL cells exposed to endothelial medium alone, CLL cells incubated with SMVECs induced or enhanced the expression of molecular hallmarks of ongoing CSR from IgM to IgG1/IgG2, IgG3, or IgA (15), including switch circle transcripts, respectively (Fig. 2C). SMVECs also upregulated the expression of germline transcripts, an early event in IgM...
to IgG1, IgG2, and IgG3 CSR, respectively (Fig. 2C). In the majority of cases, CLL cells constitutively expressed germline Igα-Cα transcripts, and MVECs did not further augment this expression (not shown).

SMVECs also upregulated the expression of transcripts for AID in CLL cells (Fig. 2D). This in vitro finding correlated with tissue data showing MVEC–proximal CLL cells that expressed AID (Fig. 2E), a hallmark of ongoing B cell activation and Ig gene diversification (32). ELISA and flow cytometric studies showed that SMVECs also induced secretion of class-switched IgG and IgA as well as unswitched IgM proteins (Fig. 2F), expression of surface IgG and IgA proteins (Fig. 2G), and upregulation of surface CD38 (Fig. 2H), a microenvironment-inducible activation molecule that activates neoplastic B cells by binding to CD31 on MVECs (38–41). UVECs induced similar activation and differentiation signals to CLL cells. Similar to endothelial medium alone, a CLL cell survival factor such as the cytokine IL-4 did not induce CLL cell proliferation, AID expression, or Ig secretion, despite enhancing the viability of CLL cells (data not shown). These findings demonstrate that MVECs provide powerful stimulating signals to CLL cells, including AID-inducing and CSR-activating signals.

**MVECs release BAFF and APRIL**

Myeloid and stromal cells enhance the survival of peripheral B cells and under specific conditions elicit AID expression and CSR by releasing BAFF and APRIL (20). These immune mediators are also involved in the pathogenesis of CLL (13, 42–46), which prompted us to determine the involvement of BAFF and APRIL in the activation of CLL cells by MVECs. ELISAs demonstrated constitutive BAFF and APRIL release by MVECs, including SMVECs, LMVECs, and UVECs (Fig. 3A). Contact-dependent exposure of MVECs to CLL cells further increased endothelial secretion of BAFF and APRIL, whereas contact-independent exposure of MVECs to conditioned medium from CLL cell cultures did not increment steady state BAFF and APRIL secretion (Fig. 3A).

Fluorescence microscopy and confocal microscopy confirmed the constitutive expression of BAFF and APRIL proteins by MVECs, including SMVECs from CLL tissues, and further showed that these MVECs stored BAFF and APRIL proteins in intracellular endosomal and granular structures that also contained the tetraspan protein CD63 and the coagulation proteins factor VIII and vWF (Fig. 3B, 3C). Flow cytometry and QRT-PCR assays demonstrated that MVECs did not express membrane-bound BAFF.
FIGURE 2. MVECs promote division, activation, and differentiation of CLL cells. (A) Immunofluorescence analysis of CLL lymph node (LN) tissue samples (one of five cases) stained for Ki-67 (green or red), factor VIII (red), and IgD (blue or green). DAPI (blue) counterstains nuclei. Original magnification ×10 (left panel) or ×63 (right panels). (B) [3H]Thymidine deoxyribose incorporation of B cells from CLL patients or healthy individuals (five cases in each group) cultured for 4 d with endothelial medium alone (control) or SMVECs. (C) Southern blot analysis of germline IgH-Cμ, IgH-Cγ2, and IgH-Cγ3 transcripts, as well as IgH-Igμ, IgH-Cμ, and IgH-Cγ3 switch circle transcripts RT-PCR amplified from CLL cells (one of five cases with little or no constitutive CSR) cultured for 4 d with endothelial medium alone (control), UVECs, or SMVECs. β-actin is a loading control. (D) QRT-PCR analysis of AID mRNA from CLL cells (five cases) cultured for 4 d as in (C). (E) CLL LN tissue (one of five cases) stained for AID (green), factor VIII (red), and Pax5 (blue). Arrowheads point to an intercellular bridge that connects factor VIII-positive MVECs with an AID-positive CLL cell, AID being a hallmark of ongoing B cell activation. Original magnification ×63. (F) ELISA of IgM, IgG, and IgA secreted by CLL cells (five cases) cultured for 7 d, as in (C). (G) Flow cytometric analysis of IgG and IgA on CLL cells (one of five cases) cultured with endothelial medium alone (red open profile) or UVECs (blue open profile) for 7 d. Gray solid profile, isotype-matched control. (H) Flow cytometric analysis of CD19 and CD38 on CLL cells (one of five cases) cultured for 7 d, as in (C). Data are from one of five experiments yielding similar results (A, C, E, G, H) or summarize three different experiments (B, D, F). Error bars, SEM; *p < 0.05 (ANOVA followed by one-tailed unpaired Student t test). RE, Relative expression as compared with freshly isolated CLL cells.

(Fig. 3D) and yet contained more BAFF and APRIL transcripts than did myeloid cell types with well-defined B cell-licensing functions (20), including DCs and macrophages (Supplemental Fig. 2). Compared with CLL cells exposed to endothelial medium alone, CLL cells exposed to endothelial medium supplemented with BAFF or APRIL showed increased proliferation and/or survival, AID transcription, as well as surface IgA expression and IgA secretion (Fig. 3E–I). BAFF and APRIL also increased the secretion of IgM and IgG (Fig. 3I). These data indicate that MVECs promote CLL cell survival and diversification through both contact-dependent and contact-independent mechanisms, including BAFF and APRIL.

**MVECs stimulate CLL cells through BAFF and APRIL**

Published data show that BAFF and APRIL deliver survival signals to normal and malignant B cells through BAFF-R, plasma cell survival signals through BCMA, and CSR signals through TACI (19, 20, 44, 45, 47–49). TACI can also deliver B cell survival and proliferation signals (20, 50). As shown by flow cytometry, BAFF-R was detected on malignant B cells from all 21 CLL cases analyzed, whereas TACI and BCMA were found on malignant B cells from 12 and 9 CLL cases, respectively (Fig. 4A, Table I). BAFF-R expression was generally more elevated than TACI and BCMA expression (Fig. 4A). QRT-PCR analysis demonstrated that TACI, BCMA, and BAFF-R transcripts increased after incubation of CLL cells with CD40L (Fig. 4B), a molecule aberrantly expressed by CLL cells and a powerful inducer of BAFF and APRIL, release by myeloid cells (5, 15, 44).

A soluble TACI-Fc5 decoy receptor, which prevents binding of BAFF and APRIL to B cells, decreased CLL cell survival, AID expression, as well as IgM and IgA secretion as induced by SMVECs or UVECs (Fig. 4C–E). In these cocultures, an Fc5 molecule was used as control (Fig. 4C–E). CLL cells transfected with siRNAs specifically suppressing the expression of either BAFF-R or TACI or BCMA receptor showed not only decreased survival but also attenuated expression of AID as induced by SMVECs (Fig. 4F–H). These data indicate that MVECs elicit survival and activation signals in CLL cells through a mechanism that involves engagement of multiple BAFF and APRIL receptors, including BAFF-R, as well as TACI and BCMA receptors.

**MVECs express CD40 and enhance CD40L expression on CLL cells**

Stromal cells enhance BAFF and APRIL release in response to various immune signals, including CD40L (15), an NF-κB-inducible T cell molecule aberrantly expressed by CLL cells (5, 23, 51). Given the ability of CLL cells to upregulate endothelial release of BAFF and APRIL in a contact-dependent manner, we wondered whether this upregulation involved engagement of CD40 on MVECs by CD40L on CLL cells. Fluorescence microscopy showed that factor VIII-positive MVECs from CLL or nonmalignant lymphoid tissues expressed CD40 (Fig. 5A). In
addition, CD40L was detected in CLL cells expressing the B cell-specific membrane protein IgD and nuclear protein Pax5, but lacking the T cell-specific membrane protein CD3 (Fig. 5B). Some of these CD40L-expressing CLL cells also contained the proliferation-associated nuclear protein Ki-67 and seemingly occupied CLL proliferation centers (Fig. 5B). In nonmalignant lymphoid tissues, only some germinal center B cells expressed CD40L (Fig. 5C). These CD40L-expressing B cells had a germinal center phenotype, as they expressed AID but lacked IgD (Fig. 5C).

Flow cytometry confirmed the expression of CD40 on MVECs as well as the expression of CD40L on malignant B cells from some CLL cases, but not on naive, marginal zone, and memory B cells from healthy donors (Fig. 5A–D, Table I). CD40L expression on malignant B cells ranged from high to low among CLL cases and did not seem to preferentially target unmutated versus mutated cases or ZAP-70–positive versus ZAP-70–negative cases (Table I). QRT-PCR identified CD40L transcripts in malignant B cells from both CLL cases with high CD40L protein expression and low CD40L protein expression (Fig. 5D), indicating that transcription of the CD40L gene is not always associated with surface CD40L protein expression. The QRT-PCR assay was highly specific because, unlike CLL cells, nonmalignant naive, marginal zone, and memory B cells expressed little or no CD40L transcripts. The expression of CD40L transcripts in CLL cells further increased upon exposure to SMVECs, but this increase did not occur after a short preincubation of CLL cells with a specific inhibitor of the NF-κB pathway (Fig. 5D). We next wondered whether CD40L on CLL cells exerted a positive feedback effect on CD40 expression by MVECs. Exposure of MVECs to recombinant CD40L induced more expression of CD40 transcripts in MVECs (Fig. 5E). Simi-
larly, exposure of MVECs to CD40L-positive CLL cells upregulated the expression of CD40 on MVECs, but this upregulation did not occur in the presence of a soluble CD40-Ig decoy receptor that inhibits engagement of CD40 by CD40L (Fig. 5F). These data show that endothelial NF-κB–inducing factors upregulate CD40L expression on CLL cells and show that leukemic CD40L estab-

Table I. Genotypic and phenotypic features of CLL cases

<table>
<thead>
<tr>
<th>ID</th>
<th>Ig V_{H}</th>
<th>Zap-70</th>
<th>CD38</th>
<th>CD40L</th>
<th>TACI</th>
<th>BCMA</th>
<th>BAFF-R</th>
</tr>
</thead>
<tbody>
<tr>
<td>011</td>
<td>UM, V_{H}4–31</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>—</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>014</td>
<td>UM, V_{H}1–31</td>
<td>+/-</td>
<td>—</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>+</td>
</tr>
<tr>
<td>021</td>
<td>MU, V_{H}3–48</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
<td>+/-</td>
<td>—</td>
<td>+</td>
</tr>
<tr>
<td>025</td>
<td>MU, V_{H}2–5</td>
<td>+/-</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>031</td>
<td>MU, V_{H}3–74</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>045</td>
<td>UM, V_{H}1–69</td>
<td>+</td>
<td>—</td>
<td>+</td>
<td>+</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>048</td>
<td>MU, V_{H}4–4</td>
<td>+</td>
<td>+/-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>051</td>
<td>UM, V_{H}2–5</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>+</td>
</tr>
<tr>
<td>062</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>067</td>
<td>N/A</td>
<td>+/−</td>
<td>—</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>078</td>
<td>MU, V_{H}3–30</td>
<td>+/-</td>
<td>—</td>
<td>+</td>
<td>—</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>085</td>
<td>MU, V_{H}2–5</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>090</td>
<td>UM, V_{H}3–7</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>091</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>—</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>095</td>
<td>UM, V_{H}3–11</td>
<td>—</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>098</td>
<td>UM, V_{H}1–69</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>107</td>
<td>MU, V_{H}6–1</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>112</td>
<td>N/A</td>
<td>+/−</td>
<td>—</td>
<td>—</td>
<td>N/A</td>
<td>—</td>
<td>+</td>
</tr>
<tr>
<td>123</td>
<td>N/A</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>126</td>
<td>MU, V_{H}4–34</td>
<td>+/−</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>128</td>
<td>MU, V_{H}1–18</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

MU, Mutated (>-2% mutations compared with putative germline VDJ gene); N/A, not available; UM, unmutated (≤2% mutations compared with putative germline VDJ gene); +/-, intermediate expression (<15% but >5% of the cells).
lishes positive feedback by enhancing CD40 expression on MVECs.

**MVECs release BAFF and APRIL in response to CD40L**

Considering that CD40L is a powerful inducer of BAFF and APRIL (15, 52), we wondered whether engagement of CD40 on MVECs by CD40L on CLL cells elicits endothelial release of BAFF and APRIL. QRT-PCR and ELISA assays demonstrated that SMVECs, UVECs, and LMVECs upregulated the expression of BAFF and APRIL transcripts as well as the release of soluble BAFF and APRIL proteins as early as 3 h after incubation with CD40L or CLL cells expressing CD40L (red open profile) on CLL cells (three cases). Gray solid profile, isotype-matched control. Numbers indicate specific CLL cases. (C) Left panels, Normal tonsillar (TO; five cases) tissue stained for IgD (green), AID (red), and CD40L (blue). Original magnification ×10 (upper panel) or ×20 (lower panel). Right panels, CD40L (red open profile) on naive, marginal zone, or memory B cells from a healthy spleen (three cases). Gray solid profile, isotype-matched control. (D) QRT-PCR of CD40L mRNA in naive, marginal zone, or memory B cells from healthy spleens (three cases), freshly isolated CLL cells (six cases, each analyzed in triplicates), or CLL cells exposed to SMVECs (two cases, each analyzed in triplicates) for 4 d. CLL cells were preincubated with control vehicle (DMSO) or an NF-κB inhibitor (IKK inhibitor III) for 3 h, washed, and then cocultured with SMVECs. Results are normalized to freshly isolated naive B cells. Numbers indicate CLL cases. (E) QRT-PCR of CD40 mRNA in UVECs, SMVECs, or LMVECs incubated with endothelial medium alone (control) or CD40L. (F) Flow cytometric analysis of CD40 on SMVECs incubated with control Ig or CD40L in the presence of CD40Lhigh CLL cells (blue open profile; one of three cases) or absence of CD40Lhigh CLL cells (red open profile) for 48 h. Gray solid profile, isotype-matched control. Data are from one of six experiments yielding similar results (A–C, E, F) or summarize three different experiments (D). Error bars, SEM.

![Figure 5](http://www.jimmunol.org/)

**FIGURE 5.** MVECs express CD40 and up-regulate CD40L on CLL cells. (A) Upper panels, Immunofluorescence analysis of CLL lymph node (LN; five cases) or normal tonsillar (TO; five cases) tissues stained for CD40 (green), factor VIII (red), and IgD (blue). Bottom panels, Flow cytometric analysis of CD40 (red open profile) on UVECs, SMVECs, and LMVECs. Gray solid profile, isotype-matched control. Original magnification ×10 (upper panels) and ×40 (bottom panels). (B) Upper panels, CLL bone marrow (BM; three cases) stained for IgD or Pax5 (green), CD40L (red or blue), CD3 (yellow), and Ki-67 (blue). Inset in large left panel shows merged IgD and CD40L. Original magnification ×10 (left panels) or ×63 (right panels). Bottom panels, CD40L (red open profile) on CLL cells (three cases). Gray solid profile, isotype-matched control. Numbers indicate specific CLL cases. (C) Left panels, Normal tonsillar (TO; five cases) tissue stained for IgD (green), AID (red), and CD40L (blue). Original magnification ×10 (upper panel) or ×20 (lower panel). Right panels, CD40L (red open profile) on naive, marginal zone, or memory B cells from a healthy spleen (three cases). Gray solid profile, isotype-matched control. (D) QRT-PCR of CD40L mRNA in naive, marginal zone, or memory B cells from healthy spleens (three cases), freshly isolated CLL cells (six cases, each analyzed in triplicates), or CLL cells exposed to SMVECs (two cases, each analyzed in triplicates) for 4 d. CLL cells were preincubated with control vehicle (DMSO) or an NF-κB inhibitor (IKK inhibitor III) for 3 h, washed, and then cocultured with SMVECs. Results are normalized to freshly isolated naive B cells. Numbers indicate CLL cases. (E) QRT-PCR of CD40 mRNA in UVECs, SMVECs, or LMVECs incubated with endothelial medium alone (control) or CD40L. (F) Flow cytometric analysis of CD40 on SMVECs incubated with control Ig or CD40L in the presence of CD40Lhigh CLL cells (blue open profile; one of three cases) or absence of CD40Lhigh CLL cells (red open profile) for 48 h. Gray solid profile, isotype-matched control. Data are from one of six experiments yielding similar results (A–C, E, F) or summarize three different experiments (D). Error bars, SEM.
TACE and furin proteins, which were predominantly localized in perinuclear compartments together with APRIL and (not shown) BAFF (Fig. 6F, Supplemental Fig. 3). Furthermore, QRT-PCRs and immunoblotting showed rapid upregulation of the expression of furin and TACE transcripts as well as furin and TACE proteins in SMVECs or UVECs exposed to CD40L (Fig. 6G, 6H).
The importance of CD40L in the generation of active BAFF and APRIL by MVECs was further documented by experiments showing that the prosurvival activity of SMVECs or UVECs on CLL cells decreased when SMVECs or UVECs were either made deficient of CD40 through RNA interference (Fig. 6I, Supplemental Fig. 4A) or exposed to a CD40L inhibitor such as CD40-Ig (Fig. 6J). Accordingly, interruption of CD40 signaling in MVECs attenuated the release of BAFF and APRIL elicited by CD40L-expressing CLL cells (Supplemental Fig. 4B). These data indicate that MVECs enhance CLL cell survival by releasing more BAFF and APRIL in response to leukemic CD40L, possibly as a result of an increased intracellular processing of BAFF and APRIL proproteins through furin and TACE cleavases.

MVECs upregulate CD40L on CLL cells via BAFF and APRIL

Given that MVECs upregulate CD40L expression on CLL cells via NF-κB, we hypothesized that this upregulation could involve MVEC production of NF-κB–inducing factors such as BAFF and APRIL. Consistent with this possibility, flow cytometry showed that blockade of BAFF and APRIL by TACI-Ig inhibited not only the upregulation of class-switched IgG and IgA isotypes but also the upregulation of CD40L on CLL cells exposed to SMVECs (Fig. 7A). This inhibition was specific because blockade of BAFF and APRIL by TACI-Ig did not decrease TACI, BCMA, and BAFF-R expression on CLL cells exposed to SMVECs. In agreement with these findings, CLL cells upregulated CD40L expression on CLL cells exposed to BAFF or APRIL (Fig. 7B). This inhibition was reversed by preincubating CLL cells with a specific NF-κB inhibitor (data not shown). The functionality of CD40L on CLL cells was further evaluated by verifying its ability to deliver autocrine survival signals independently of MVECs. Consistent with this possibility, a CD40-Ig decoy receptor attenuated the viability of CLL cells cultured in the absence of MVECs (Fig. 7C). These data indicate that leukemic CD40L requires endothelial BAFF and APRIL signals for its upregulation by MVECs and promotes CLL cell expansion and differentiation not only by inducing paracrine release of BAFF and APRIL from MVECs but also by stimulating CLL cells in an autocrine manner (Fig. 7D).

Discussion

We found that stromal MVECs delivered survival, activation, Ig gene remodeling, and differentiation signals to CLL cells by releasing BAFF and APRIL. Engagement of CD40 on MVECs by CD40L aberrantly expressed on CLL cells enhanced BAFF and APRIL release through induction of furin and TACE cleavases, which elicted processing of BAFF and APRIL, proproteins into soluble molecules. In addition to stimulating CLL cells through TACI, BCMA, and BAFF-R, endothelial BAFF and APRIL augmented leukemic expression of CD40L, suggesting that MVECs and malignant B cells establish a bidirectional crosstalk via a complex signaling network involving multiple TNF family members.

CLL is characterized by a dynamic balance between malignant B cells circulating in the blood and malignant B cells located in permissive niches of lymphoid organs (57, 58). Circulating CLL cells show resistance to apoptosis, but little or no proliferation, whereas a significant fraction of tissue-based CLL cells show active proliferation, suggesting that the tissue microenvironment provides a distinct set of signals to the leukemic clone (10, 11). In this regard, the CLL stroma has been proposed to provide Ig-driven antigenic signals by facilitating the interaction of stereotypic leukemic Igs with self-antigens exposed on apoptotic cells (59). The CLL stroma also contains nurselike cells, macrophages, and follicular DCs that provide accessory survival signals, in-

**FIGURE 7.** MVECs upregulate CD40L expression on CLL cells through BAFF and APRIL. (A) Flow cytometric analysis of CD40L, IgG, IgA, BAFF-R, TACI, and BCMA on CLL cells (one of six cases) incubated with endothelial medium alone (red open profile; also shown in center and right panels), SMVECs plus control Fc5 (blue open profile), or SMVECs plus TACI-Ig (blue open profile) for 7 d. Gray solid profile, isotype-matched control. (B) Flow cytometric analysis of CD40L on CLL cells (one of six cases) incubated with endothelial medium alone (red open profile; also shown in center and right panels) or endothelial medium supplemented with BAFF or APRIL (blue open profile) for 7 d. Gray solid profile, isotype-matched control. (C) Viable trypan blue-excluding CLL cells (one of six cases) cultured with standard medium in the presence of control Ig or CD40-Ig for 0, 24, or 48 h. Data in (A)–(C) are from one of six experiments yielding similar results. (D) Model summarizing crosstalk between MVECs and CLL cells via CD40L, BAFF, and APRIL.
cluding BAFF and APRIL (13, 14, 60, 61). Moreover, the CLL stroma contains T cells expressing CD40L (24), a B cell-stimulating molecule linked to BAFF and APRIL (19, 20).

Our findings indicate that MVECs constitute a key component of the CLL stroma by establishing an intimate crosstalk with neoplastic B cells through BAFF, APRIL, and CD40L. CLL tissues contained MVECs that were proximal to malignant B cells expressing molecular hallmarks of ongoing proliferation and Ig gene remodeling, including Ki-67 and CD19, respectively. Consistent with these data, MVECs stimulated leukemic cell survival, activation, and differentiation, including CD38 expression, AID expression, CSR, and Ab production, through both contact-dependent and contact-independent mechanisms, including soluble BAFF and APRIL, as well as membrane-bound CD40L molecules. That MVECs may play a pivotal role in the pathogenesis of CLL is in agreement with evidence showing that malignant B cells from aggressive CLL cases express CD49d, a receptor for VCAM-1 (or CD106) expressed by endothelial cells (62). It is also in agreement with data showing interaction of leukemic CD38 with the endothelial adhesion molecule CD31 (39) and with additional studies demonstrating that CLL cells produce angiogenic factors such as VEGF and generate survival signals via VEGF receptors (25, 30).

In mice, BAFF from radiation-resistant stromal cells is mandatory for the survival of peripheral B cells (63), but the nature of BAFF-producing stromal cells remains unclear. We found that MVECs contained more BAFF transcripts than did DCs and macrophages and released large amounts of BAFF and APRIL, both constitutively and after activation. Tonic release of BAFF and APRIL may involve CD63-expressing endosomes widely dispersed in the cytoplasm of MVECs, whereas inducible release of BAFF and APRIL may involve perinuclear Weibel–Palade bodies containing vWF and factor VIII. These endothelial storage granules release their content in response to various signals, including CD40L (64). In agreement with this, engagement of CD40 on MVECs by CD40L aberrantly expressed on CLL cells augmented endothelial processing of pro-BAFF and pro-APRIL proteins into soluble molecules through a mechanism involving furin and TACE cleavages, which were constitutively stored in Weibel–Palade bodies. Of note, CD40L rapidly increased furin and TACE transcription and expression by MVECs. This process may be important for MVECs to rapidly undergo post-degranulation replenishment of furin and TACE proteins. The relative contribution of furin and TACE to BAFF and APRIL cleavage remains uncertain, but one possibility is that these proteases form alternative proteolytic pathways to maximize BAFF and APRIL release by MVECs (54, 55). Consistent with this interpretation, the furin cleavage site in the stalk region of pro-BAFF and pro-APRIL proteins was found to be adjacent to a putative TACE cleavage site.

In addition to augmenting CLL cell survival and diversification, MVECs increased CD40L expression on CLL cells. Indeed, unlike nonmalignant B cells, leukemic cells from several CLL cases aberrantly expressed surface CD40L and contained CD40L transcripts. MVECs upregulated the expression of CD40L through a BAFF- and APRIL-dependent mechanism requiring NF-κB, a transcription factor that plays a crucial pathogenic role in CLL (5, 45, 46). Indeed, CLL cells exposed to MVECs downregulated CD40L expression upon preincubation with an NF-κB inhibitor or in the presence of TACI-Ig, a decoy receptor that prevents binding of BAFF and APRIL to B cells. Conversely, soluble BAFF and APRIL upregulated CD40L expression on CLL cells.

In agreement with previously published findings (5), leukemic CD40L delivered autocrine CD40-dependent survival signals to CLL cells. Moreover, leukemic CD40L increased CD40 expression in MVECs, as well as TACI, BCMA, and BAFF-R expression in neoplastic B cells, suggesting that CD40, TACI, BCMA, and BAFF-R form a CLL-enhancing signaling network that bidirectionally links the microvascular stroma with the leukemic clone. Consistent with this possibility, published data show that CD40L and BAFF cooperatively enhance B cell survival (44, 65). Remarkably, TACI and BCMA contributed as much as BAFF-R to the survival and activation of CLL cells exposed to MVECs. Although the important function of BAFF-R and TACI in nonmalignant and malignant B cells is well established (19, 20, 43–45, 47–49, 66–71), less is known about the function of BCMA. This receptor delivers survival signals to nonmalignant plasma cells (72, 73), but growing evidence indicates its additional involvement in B cell and plasma cell tumors (44, 45, 47, 49, 74).

Blockade of endothelial BAFF and APRIL by TACI-Ig or blockade of leukemic CD40L by CD40-Ig reduced the survival, activation, and diversification of CLL cells cocultured with MVECs. The inhibitory effect of TACI-Ig on leukemic AID expression as induced by MVECs may have clinical relevance, because Ig and non-Ig gene lesions induced by dysregulated AID expression may play a role in the survival and clonal evolution of CLL cells (6, 33, 75). In conclusion, our findings show that stromal MVECs establish a bidirectional interplay with CLL cells through multiple functionally related members of the TNF family, including BAFF, APRIL, and CD40L. Blocking these molecules with specific inhibitors may be beneficial for the treatment of CLL.

Acknowledgments

We thank Lauren Tyrell (Weill Medical College of Cornell University, New York, NY) for administrative and technical assistance.

Disclosures

S.R.D. is employed by ZymoGenetics (a Bristol-Myers Squibb company) and helped with the early development of TACI-Ig. The other authors have no financial conflicts of interest.

References


document the dynamic cellular kinetics of chronic lymphocytic leukemia B cells. 


