Fas ligand (FasL) induces apoptosis in susceptible Fas-bearing cells and is critically involved in regulating T-cell immune responses. It is highly expressed in several human malignancies, and a role in the suppression of antitumor immune responses has been suggested. We evaluated FasL expression in leukemia and normal hematopoietic cells. By Western blotting, all acute leukemic cell lines (n = 9) and primary samples of acute leukemic marrow (n = 4) revealed high levels of FasL. In contrast, much weaker signals were observed in samples of normal marrow (n = 5), and either weak or intermediate expression was seen in chronic myeloid leukemia (CML) in chronic phase (n = 7). Additional leukemic samples were examined by immunohistochemistry. Staining for FasL was negative in 7 of 9 cases of chronic-phase CML, whereas all cases of CML in blast crisis (n = 5) and primary samples of acute leukemia (n = 6) stained strongly in 60 to 100% of nucleated cells. FasL+ leukemic cell lines did not trigger Fas-mediated apoptosis in either Jurkat cells or activated human T lymphocytes, possibly related to the intracellular location of the ligand. Western analysis of normal marrow subpopulations revealed that most FasL+ in marrow mononuclear cells was expressed by CD7+ lymphocytes. FasL was also strongly expressed in CD34+ hematopoietic progenitor cells from both normal and chronic-phase CML marrow, suggesting a correlation with primitive maturation stage. In summary, high levels of FasL expression were associated with aggressive biologic behavior in leukemia, including transformation of CML to blast crisis. This could potentially represent a response to loss of proapoptotic Fas signaling, which is known to occur in acute leukemic blasts. © 1999 International Society for Experimental Hematology. Published by Elsevier Science Inc.

**Keywords:** Fas ligand—Leukemia—Blast crisis—Apoptosis—Hematopoietic progenitor cells

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**Introduction**

Fas (APO-1/CD95) is a cell surface receptor of the nerve growth factor/tumor necrosis factor receptor superfamily that binds a ligand structurally related to tumor necrosis factor [1]. When Fas is cross-linked by Fas ligand (FasL) or anti-Fas antibody, apoptosis is triggered in susceptible cells [1–3]. Both Fas and FasL are expressed by activated T lymphocytes and appear to play critical roles in the negative regulation of immune responses through mediating activation-induced cell death [4–6]. Mice defective in either Fas (lpr strain) or FasL (gld strain) develop lymphoproliferative disease and autoimmunity [7,8]. A similar disease occurs in humans with dominant-negative Fas mutations [9]. FasL also functions as a key effector mechanism in cytotoxic lymphocytes by triggering apoptosis in Fas+ targets [10]. More recently, evidence has accumulated that FasL frequently is expressed by human malignancies [11–17]. In some reports, a correlation between FasL expression and advanced clinical stage was found [14,17]. FasL+ tumor cells also were observed to induce apoptosis in lymphoid cells that expressed functional Fas receptor [11–15,17]. These results suggested that malignant cells could potentially suppress antitumor immune responses by engaging Fas on activated tumor-infiltrating lymphocytes.

In the current study, the expression and function of FasL in leukemia was investigated. We speculated that differences in FasL expression might explain the differential sensitivity to graft-vs-leukemia (GVL) reactions of chronic myeloid leukemia (CML) and acute leukemia. After allogeneic bone marrow transplantation, a vigorous GVL response is mounted by donor T cells against chronic-phase CML, but this is more difficult to demonstrate for acute leukemia or transformed CML [18,19]. Likewise, relapsed acute leukemia responds much less well to donor lymphocyte infusions [20,21]. We hypothesized that if high levels of functional FasL were expressed by acute leukemic blasts, this could suppress antileukemic donor immune cells and therefore circumvent GVL effects. FasL expression also was examined in normal hematopoietic cells. Because the Fas receptor may participate in the regulation of normal he-
matopoiesis and its loss may predispose to leukemogenesis [22–24], the distribution of FasL in hematopoietic tissues is of potential significance.

Materials and methods

Cell lines
The MOLT-13, RS4, CEM, and Jurkat cell lines were kindly provided by Dr Chris Pennell, and the BLIN-1 and NALM-16 lines by Dr Tucker LeBien (both at the University of Minnesota Cancer Center). K562 and KG-1 cells were purchased from the American Type Culture Collection (ATCC, Manassas, VA). The HL-60 line was a gift from Dr Yoji Shimizu of the University of Minnesota Center for Immunology. All cell lines were grown in RPMI medium 1640 supplemented with 10% fetal bovine serum, and 1% glutamine, penicillin, and streptomycin. Cells were incubated at 37°C in a humidified atmosphere containing 5% CO₂. In preparation for the studies, between 1.0 × 10⁶ and 4.2 × 10⁶ cells were removed from each culture, washed twice in phosphate buffered saline (PBS), and stored as cell pellets at −80°C.

Bone marrow samples
Bone marrow aspirates were obtained from normal volunteer donors and patients with CML, acute lymphoblastic leukemia (ALL), or acute myeloid leukemia (AML) undergoing marrow examination for clinical indications. All subjects gave written informed consent as approved by the Committee on the Use of Human Subjects in Research at the University of Minnesota. Marrow was aspirated into a heparinized syringe and either processed immediately or stored at 4°C or stored at −80°C.

Because the anti-FasL antibody used for immunohistochemical analysis did not reliably stain decalcified bone marrow biopsy sections, marrow clots containing leukemia tissue fixed in formalin or 4% formalin-fixed lymph nodes involved with transformed CML were studied.

Isolation of marrow subpopulations
Bone marrow MNC from normal donors or patients with CML in chronic phase were isolated as described earlier. For separation of cells on the basis of CD7 expression, total MNC were incubated at 4°C for 30 minutes with fluorescein isothiocyanate (FITC)-conjugated anti-CD7 monoclonal antibody (mAb) (Becton-Dickinson, San Jose, CA) prior to sorting into CD7⁺ and CD7⁻ fractions on a Becton-Dickinson FACStar-Plus flow cytometer. A total of 1.5 × 10⁶ cells from each fraction was collected, washed twice with PBS, and stored as a cell pellet at −80°C. To obtain CD34⁺ cells, marrow MNC were purified using the MACS CD34 Progenitor Cell Isolation kit (Miltenyi Biotec, Auburn, CA), according to the manufacturer’s instructions. In some cases, the column-purified cells were further sorted by flow cytometry after staining with FITC-conjugated anti-CD34 mAb (Becton-Dickinson). CD34⁺ samples (approximately 1 × 10⁶ cells) were washed twice with PBS and stored as cell pellets at −80°C.

Western blotting
Cell pellets were removed from the −80°C freezer and immediately placed in lysis buffer (50 mM Tris HCl pH 7.5, 250 mM NaCl, 5 mM EDTA pH 8, 0.1% NP-40, 50 mM NaF, and 1 mM PMSF). The resulting lysates were cleared by centrifugation and protein concentrations were determined using the Bio-Rad protein assay (Bio-Rad, Hercules, CA). An equal amount of protein from each sample was resolved by SDS polyacrylamide gel electrophoresis under reducing conditions using a standard method [25]. Transfer to polyvinylidene difluoride (PVDF) membranes was performed by electroblotting in a Mini Trans-Blot Cell transfer apparatus (Bio-Rad), under conditions recommended by the manufacturer. After incubating overnight at 4°C in blocking buffer (5% skim milk powder and 0.1% Tween-20 in PBS), blots were incubated for 1 hour at room temperature (RT) with anti-human FasL mAb (clone 33; Transduction Laboratories, Lexington, KY) diluted 1:1000 in blocking buffer. They were then washed and incubated for 1 hour at RT with peroxidase-conjugated goat anti-mouse immunoglobulin antibody (Jackson Laboratories, West Grove, PA) diluted 1:10,000 in blocking buffer. After further washing, bound antibody was detected with ECL Western blotting detection reagents (Amersham, Little Chalfont, England). To confirm equal loading of the gels with protein, blots were stripped in 0.1 M glycine, pH 2.9 at RT for 20 minutes and reprobed with anti-β-actin mAb (clone AC-15; Sigma). Methods were identical to those used with the anti-FasL antibody, except that the primary and secondary antibody incubations were performed at dilutions of 1:2,000 and 1:5,000, respectively.

Immunohistochemistry
Paraflin sections of fixed bone marrow clots or lymph node were cut at thicknesses of 4 µm and mounted on glass slides. After removal of paraffin in xylene, slides were rehydrated with graded alcohol solutions. Mercury was removed from samples fixed in B5 after the 80% alcohol step by sequential treatment with alcoholic iodine and 5% sodium thiosulfate. Heat-induced epitope retrieval in a pressure cooker was performed, using published methods [26]. After blocking for 15 minutes with 15 µg/mL of horse serum, sections were incubated at room temperature for 1 hour with a 1:400 dilution of either anti-human FasL mAb (clone 33; Transduction Laboratories) or nonimmune mouse serum (Dako, Carpenteria, CA). Detection of bound antibody was performed using an avidin-biotin complex immunoperoxidase technique (mouse Elite ABC kit; Vector Laboratories, Burlingame, CA) followed by treatment with 3,3′-diaminobenzidine. Finally, a hematoxylin counterstain was applied and slides were examined microscopically. A total of 500 to 1,000 cells were counted. Cells showing granular cytoplasmic staining were scored positive for FasL and their number was expressed as a percentage of the total nucleated cells counted. Cases were classified as negative if fewer than 1% of nucleated cells were positive.

Detection of soluble FasL
Fifteen-milliliter tubes containing 1 × 10⁶ KG-1 or HL-60 cells in 1 mL of medium were incubated for 20 hours at 37°C. Aliquots of culture medium were removed and briefly centrifuged at maximum speed in a microcentrifuge. Supernatants were immediately frozen at −80°C. Detection of soluble FasL was performed using the sFas Ligand ELISA kit (MBL, Nagoya, Japan) according to the manufacturer’s instructions. Standards consisted of serial dilutions.
of purified soluble FasL (MBL). Culture supernatants were thawed and diluted 1:1 with the provided assay buffer immediately prior to analysis. Soluble FasL concentrations were determined by measuring the optical density of samples at 450 nm in a Thermomax microplate reader (Molecular Devices, Menlo Park, CA).

Activated primary T cells
MNC were isolated from normal peripheral blood by density gradient centrifugation and grown in X-Vivo 15 medium (Bio-Whittaker, Walkersville, MD) at 37°C in a 5% CO₂ atmosphere. Cultures were supplemented with 10 ng/mL of OKT3 (Ortho Biotech, Raritan, NJ) and 1,000 U/mL of interleukin 2 (Chiron, Emeryville, CA) to induce differentiation to an activated T-cell phenotype. After 13 days of culture, aliquots of 2 × 10⁵ cells were washed in PBS containing 0.3% bovine serum albumin and incubated either with FITC-labeled anti-Fas mAb (Pharmingen, San Diego, CA) or FITC-labeled IgG, isotype control antibody (Becton-Dickinson). Fas receptor expression was evaluated using a FACS Calibur flow cytometer and Cellquest software (Becton-Dickinson).

Coculture experiments
Four leukemia cell lines that expressed FasL at high levels (KG-1, HL-60, K562, and BLIN-1; see Fig. 1) were assessed for their ability to trigger apoptosis in Fas⁺ Jurkat cells. Cocultures containing 1 × 10⁶ Jurkat cells and 1 × 10⁵ FasL⁺ leukemic cells in 1 mL of medium were incubated in 15 mL round-bottom tubes for 20 hours at 37°C. Controls were Jurkat cells alone, Jurkat cells supplemented with 0.25 μg/mL of agonistic anti-human Fas mAb (clone CH-11; Immunotech, Westbrook, ME), and each leukemic cell line alone. Additional cultures were supplemented with 10 μg/mL of soluble Fas-Fc fusion protein (R&D Systems, Minneapolis, MN) to inhibit Fas signaling. Jurkat cell apoptosis was evaluated by dual staining with phycocerythrin (PE)-conjugated anti-CD3 mAb (Becton-Dickinson) and FITC-conjugated annexin V (Pharmingen), followed by flow cytometry. Staining was performed at 4°C for 30 minutes in annexin V binding buffer (10 mM HEPES, pH 7.4, 140 mM NaCl, 2.5 mM CaCl₂). Cells were washed once in the same buffer and then analyzed on the FACS Calibur flow cytometer. Ten thousand events were acquired from cultures containing only Jurkat cells and 10⁵ events from cocultures. Using results from KG-1, HL-60, K562, and BLIN-1 cells incubated alone, a CD3⁺ gate containing more than 99% Jurkat cells was established for the cocultures. The percentage of cells positive for annexin V in this gate was used to calculate Jurkat cell apoptosis. Results were expressed as the mean ± SEM of triplicate cultures. To assess the effect of leukemic FasL on primary lymphocytes, additional experiments were performed in duplicate using T cells activated with OKT3 and interleukin 2 for 13 days. Coculture conditions and flow cytometric analysis were as described for assays involving Jurkat cells.

Results

Western analysis of FasL expression
When Western blots of acute leukemic cell lines were probed with anti-human FasL mAb, a single strong band that migrated as expected for full-length FasL (approximately 37 kDa) was observed in all samples (Fig. 1). These lines were derived from patients with T-cell ALL (MOLT-13, CEM, and Jurkat), pro-B-cell ALL (NALM-16), pre-B-cell ALL (BLIN-1), AML (KG-1 and HL-60), undifferentiated leukemia (RS4), and CML in erythroleukemic blast crisis (K562). To examine expression in primary hematopoietic cells, MNC were isolated from bone marrow and similarly analyzed by Western blotting (Fig. 2). Strong FasL expression was evident in all primary acute leukemia samples (3 AML, 1 ALL), with signals of comparable intensity to those seen in the Jurkat-cell positive controls. In contrast, much weaker signals were observed in five normal marrow samples, and weak or intermediate expression was seen in seven samples of chronic-phase CML marrow. Comparable β-actin signals in all lanes indicated that differences in FasL signals were not due to uneven loading of the gels.

Morphologic examination of aspirates drawn at the same time as samples for Western analysis revealed blast counts greater than 80% in all the acute leukemias studied (data not shown). Consequently, it is likely that the FasL observed in these cases was derived from leukemic blast cells. All CML samples were obtained from marrow with chronic-phase morphology. However, one sample showing intermediate FasL expression (CML1) was isolated from a patient with...
cytogenetic evidence of transformation to accelerated phase (new isochromosome 17q abnormality).

Immunohistochemical detection of FasL in leukemia
To examine FasL expression in a larger number of primary leukemia samples and obtain information about the morphology and distribution of positive cells, immunohistochemistry was performed on fixed tissue from patients with CML, ALL, and AML. Bone marrow from 33 patients and lymph nodes involved with blast-phase CML from two patients were studied. Photomicrographs of representative sections of CML in chronic phase, CML in lymphoid and myeloid blast crisis, ALL, and AML are shown in Figure 3. Results from all samples studied are summarized in Table 1. All cases of acute leukemia and CML in blast crisis revealed strong FasL expression in the majority (60–100%) of nucleated cells. In contrast, 7 of 9 samples of chronic-phase CML were negative for FasL (<1% positive cells) and the two positive cases showed fewer FasL+ cells (16-32%) than in blast-phase disease. In the three cases of accelerated-phase CML examined, FasL expression was present in 6 to 40% of nucleated cells. FasL+ cells revealed myeloid morphology in CML in chronic or accelerated phase (data not shown), and blastic morphology in blast-phase CML and acute leukemia (Fig. 3).

Assay of leukemic FasL function
To test whether FasL in leukemic cells was functional, cocultures were performed with the Jurkat lymphoid line, which is highly susceptible to apoptosis mediated by Fas signaling [4]. Apoptosis of Jurkat cells was assessed by flow cytometry after staining with PE-labeled anti-CD3 mAb and FITC-labeled annexin V. Because most Jurkat cells expressed CD3 and all other cells used were negative (data not shown), Jurkat cell apoptosis was measured as the percentage of cells binding annexin V in the CD3+ gate (Fig. 4A). This was 6.8% ± 1.0% in Jurkat cells incubated alone and increased to 50.1% ± 2.4% following exposure to CH-11 agonistic anti-Fas mAb (Fig. 4B). As expected, soluble Fas-Fc chimera completely reversed the effects of CH-11 antibody. Despite expressing high levels of FasL (Fig. 1), KG-1 and HL-60 leukemic cells did not induce Fas-mediated apoptosis in Jurkat cells (Fig. 4B). Incubation with HL-60 cells resulted in 6.3% ± 0.4% apoptotic Jurkat cells, which was similar to the control cultures. KG-1 cells caused a small increase in Jurkat cell apoptosis (to 12.8% ± 0.8%), but the lack of inhibition by soluble Fas-Fc indicated a Fas-independent mechanism. Two other FasL+ leukemic cell lines also failed to show evidence of Fas-mediated killing. Cocultures with K562 and BLIN-1 cells resulted in 7.5% ± 0.7% and 5.3% ± 0.2% apoptotic Jurkat cells, respectively.

The effect of leukemic FasL on primary T cells activated with OKT3 and interleukin-2 was studied. After 13 days in culture, immunophenotyping revealed that 95% of cells were CD3+ and 92% expressed Fas receptor. A functional Fas signaling pathway in the T cells was confirmed by the observation of greater than 85% apoptosis following exposure to CH-11 mAb. Nonetheless, apoptosis was not increased in cocultures with FasL+ leukemic cell lines (KG-1, HL-60, K562, or BLIN-1) compared to T cells incubated alone (data not shown), again indicating a failure of leukemic FasL to kill Fas+ targets.

Soluble FasL in culture supernatants
Matrix metalloproteinases cleave the extracellular domain of FasL and release it into solution, where it may act to

Figure 2. FasL expression in primary MNC from normal and leukemic marrow. After isolation by density gradient centrifugation, cells were lysed and 10 μg of protein from each sample was analyzed by Western blotting. Blots were probed with anti-human FasL mAb, then stripped and reprobed with anti-β-actin monoclonal antibody to confirm equal loading of the gels. The size (in kDa) and position of protein standards are indicated at left. Three separate blots are shown. Samples were as follows: N1 to N5, normal; CML1 to CML7, chronic myeloid leukemia in morphologic chronic phase; ALL1, acute lymphoblastic leukemia; AML1 to AML3, acute myeloid leukemia. Ten micrograms of protein from lysed Jurkat cells were included on each blot as a positive control.
Figure 3. Immunohistochemical analysis of FasL expression in leukemia. Sections of bone marrow clots (rows A, D, and E) or lymph nodes (rows B and C) containing leukemic tissue were incubated with anti-human FasL monoclonal and stained using an indirect immunoperoxidase technique. (A) chronic myeloid leukemia (CML) in chronic phase; (B) CML in lymphoid blast crisis; (C) CML in myeloid blast crisis; (D) acute lymphoblastic leukemia; (E) acute myeloid leukemia. (Left panels: original magnification × 500; right panels: original magnification × 1250).
modulate the effects of surface-bound FasL [27,28]. Using an enzyme-linked immunosorbent assay, we examined conditioned medium from the FasL-expressing leukemic lines KG-1 and HL-60 for the presence of soluble FasL. In both cases, the soluble FasL level was below the limits of detection of the test (<0.1 ng/mL), indicating that biologic activity was unlikely under the conditions studied [29].

Expression of FasL in subsets of marrow cells
Western blots of normal marrow MNC revealed low-level FasL expression. To define cells that expressed FasL in normal marrow, Western analysis was performed on cell subpopulations isolated by flow cytometry. This revealed that CD7+ lymphocytes had significantly higher FasL levels than CD7- cells, suggesting that the majority of FasL in unsorted marrow MNC was expressed by lymphoid cells (Fig. 5). This is consistent with published data on the expression and function of FasL in activated T cells [30]. Strong FasL expression also was observed in CD34+ hematopoietic progenitor cells isolated from normal and chronic-phase CML marrow (Fig. 6). No differences in FasL levels were evident between progenitor cells derived from normal donors or patients with CML.

Discussion
We investigated FasL expression in leukemia to determine whether differential expression levels might contribute to differences in response to GVIL immune reactions. High levels of FasL were observed with either Western blotting or immunohistochemistry in all samples of acute leukemia

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Cases examined</th>
<th>FasL+ cases*</th>
<th>Percentage cells positive†</th>
</tr>
</thead>
<tbody>
<tr>
<td>CML, chronic phase</td>
<td>9</td>
<td>2</td>
<td>16–32</td>
</tr>
<tr>
<td>CML, accelerated phase</td>
<td>3</td>
<td>3</td>
<td>6–40</td>
</tr>
<tr>
<td>CML, lymphoid blast crisis</td>
<td>3</td>
<td>3</td>
<td>68–90</td>
</tr>
<tr>
<td>CML, myeloid blast crisis</td>
<td>3</td>
<td>3</td>
<td>60–95</td>
</tr>
<tr>
<td>ALL</td>
<td>6</td>
<td>6</td>
<td>72–99</td>
</tr>
<tr>
<td>AML</td>
<td>11</td>
<td>11</td>
<td>83–100</td>
</tr>
</tbody>
</table>

*Number of cases with ≥1% of nucleated cells staining positively for FasL.
†Percentage of nucleated cells staining positively in cases with ≥1% positive cells.

ALL = acute lymphoblastic leukemia; AML = acute myeloid leukemia.

Figure 4. Assessment of Jurkat cell apoptosis in cocultures with FasL+ leukemic cells. Cultures containing 1 x 10^5 Jurkat cells and 1 x 10^6 cells of a FasL+ leukemic line (KG-1 or HL-60) were incubated for 20 hours. Controls were 1 x 10^5 Jurkat cells incubated either alone or with 0.25 µg/mL of CH-11 agonistic anti-human Fas mAb. Apoptotic Jurkat cells were quantified by flow cytometry after staining with PE-conjugated anti-CD3 monoclonal antibody and FITC-conjugated annexin V. (A) Annexin V staining in the CD3+ gate in a representative coculture of KG-1 and Jurkat cells (middle panel), and in cultures of Jurkat cells alone (top panel) or with CH-11 antibody (bottom panel). The marker bar shown in each panel was used to define apoptotic Jurkat cells. (B) Summary of Jurkat cell apoptosis after incubation with KG-1 or HL-60 leukemic cells, or exposure to CH-11 antibody. Results are the means of triplicate cultures, and error bars indicate the standard error of the mean. Hatched bars show the effect of adding soluble Fas-Fc chimera to inhibit engagement of the Fas receptor.
and blast-phase CML. In contrast, chronic-phase CML marrow revealed weak or intermediate FasL expression by Western analysis and was negative by immunostaining in the majority of cases. Weak FasL signals also were detected in normal marrow MNC. These arose largely from CD7+ lymphocytes, as shown by cell sorting experiments. Variable contamination of marrow aspirates with peripheral blood lymphocytes may explain the differing FasL signals seen on Western analysis of chronic-phase CML samples. Alternatively, increasing FasL expression may indicate early stages of accelerating disease. The latter is supported by the observation that FasL+ cells in chronic- and accelerated-phase marrow examined by immunohistochemistry revealed myeloid morphology. Moreover, one of the chronic-phase samples showing relatively higher expression on Western analysis (CML1, Fig. 2) was derived from a patient with cytogenetic evidence of accelerated disease. Irrespective of how the variations in CML Western blot signals are interpreted, immunostaining showed a clear association between increasing FasL expression and advanced disease phase, with most chronic-phase samples negative, all accelerated-phase samples positive at a low or intermediate level, and all blast-phase samples strongly positive.

The functional consequence of FasL overexpression in leukemic cells was investigated in cocultures with Jurkat cells and activated human T lymphocytes, both of which were highly susceptible to Fas-mediated killing. These assays revealed no evidence of lymphoid apoptosis induced by leukemic FasL. It is possible that costimulatory molecules required for Fas signaling were lacking in the cocultures, and an animal model of the GVL reaction may permit a more definitive assessment. Moreover, the location of FasL may be critical to its function; specifically, intracellular molecule is unlikely to participate in communication with other cells. It may be noteworthy that in leukemic marrow sections examined by immunohistochemistry, the majority of FasL was intracellular (Fig. 3). In addition, no soluble FasL was detected in culture supernatants of two FasL+ leukemic cell lines. Appropriate signals to externalize FasL on leukemic blasts may be absent from cell line cocultures, but might occur during in vivo interactions with lymphocytes. Nonetheless, based on the current results, a role for leukemic FasL in suppressing antileukemic immune responses remains speculative.

Our data indicate an association between high-level FasL expression and acute fully transformed leukemias. There are at least two possible explanations for this correlation. First, elevated FasL expression may simply be a feature of the blast cell phenotype; therefore, diseases with more blast cells would be expected to express higher levels of FasL. This is supported by our finding of strong FasL expression in purified CD34+ progenitor cells from normal and chronic-phase CML marrow. Many normal CD34+ cells show blastic morphology and, conversely, approximately half of acute leukemia cases are positive for the CD34 antigen [31,32]. The second possibility is that FasL overexpression may be related to loss of function of the Fas signaling pathway. This proapoptotic network appears critical for maintaining homeostasis in diverse cell populations through inducing programmed cell death in superfluous cells [30,33–35].

Figure 5. Western analysis of FasL expression in CD7+ and CD7− cells isolated by flow cytometry from normal marrow mononuclear cells (MNC). Samples of unsorted marrow MNC and Jurkat cells were included for comparison. Each sample consisted of 10 μg of cell lysate protein. Blots were probed with anti-human FasL mAb, stripped, and reprobed with anti-β-actin monoclonal antibody. Protein standards are indicated at left.

Figure 6. Western analysis of FasL expression in CD34+ progenitor cells isolated by flow cytometry from normal and chronic-phase chronic myeloid leukemia marrow. Samples of unsorted marrow mononuclear cells (MNC) and Jurkat cells were included for comparison. Methods were as described for Fig 5.
example, mice defective in either Fas receptor (lpr mutation) or FasL (gld mutation) develop lymphoid hyperplasia due to an inability to delete activated T cells. It is noteworthy that these mice also express high levels of FasL in their lymphoid tissues, presumably due to deficient FasL-Fas signaling [36]. Loss of Fas function has been described in acute leukemias [37,38] and may contribute to the prolonged survival and accumulation of leukemic cells. Such a role as a tumor suppressor was supported by a recent report on crossing lpr mice with mice overexpressing Bcl-2 in their myeloid cells. The offspring, deficient in two apoptotic pathways, developed a disease resembling AML, with an incidence of 15% [39]. If the uniformly high expression of FasL in leukemic blasts reported in the current study indicates loss-of-function mutations in the FasL-Fas pathway (as it does in lymphocytes in lpr and gld mice), then such mutations may be critical for the development of acute leukemia and transformation of chronic-phase CML to blast crisis.

FasL was expressed at relatively high levels in normal CD34+ hematopoietic progenitor cells. Because a proportion of progenitor cells express the Fas receptor [22], FasL may be involved in regulating the size of the normal progenitor pool by triggering physiologic apoptosis. Similarly elevated levels of FasL were observed in CD34+ cells from chronic-phase CML marrow. Interferon α is known to up-regulate Fas expression on CD34+ cells in this disease, resulting in increased sensitivity to Fas-mediated killing in vitro [40]. Thus, FasL expressed by the progenitor cells themselves may be an important effector of the clinical response to interferon in CML.

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