FLT3 Receptor Expression on the Surface of Normal and Malignant Human Hematopoietic Cells

By Anne M. Turner, Nancy L. Lin, Surasit Issarachai, Stewart D. Lyman, and Virginia C. Broudy

FLT3 ligand is a hematopoietic growth factor that plays a key role in growth of primitive hematopoietic cells. FLT3 receptor mRNA is found in early hematopoietic progenitors and in human myeloid leukemia blasts. Much less is known about the surface expression of FLT3 receptor on human hematopoietic cells. Using human 125I-FLT3 ligand, we have identified and characterized surface FLT3 receptors on normal and malignant human hematopoietic cells and cell lines. Our results showed that surface display of FLT3 receptor was greatest in fresh myeloid leukemia blast cells and myeloid leukemia cell lines. Erythroleukemic and megakaryocytic leukemia cell lines (n = 5) bound little to no 125I-FLT3 ligand. Scatchard analysis of 125I-FLT3 ligand binding data shows that three myeloid leukemia cell lines, ML-1, AML-193, and HL-60, as well as normal human marrow mononuclear cells, exhibit high affinity FLT3 receptors. Crosslinking of 125I-FLT3 ligand to FLT3 receptors on the surface of ML-1 myeloid leukemia cells indicates that the FLT3 receptor forms homodimers in the presence of FLT3 ligand. The rates of FLT3 ligand internalization and degradation were determined by binding 125I-FLT3 ligand to ML-1 cells and acid stripping to distinguish surface bound from internalized ligand. Internalized 125I-FLT3 ligand was detected within 5 minutes after binding to ML-1 cells. In addition, we evaluated the effect of FLT3 ligand on megakaryocytic colony growth and nuclear endoreduplication, alone or in the presence of thrombopoietin. FLT3 ligand did not promote colony forming unit megakaryocyte (CFU-Meg) colony growth or megakaryocyte nuclear maturation, nor did FLT3 ligand augment the effects of thrombopoietin on these measures of megakaryopoiesis. These data indicate that the FLT3 receptor shares several characteristics with the c-kit receptor including dimerization and rapid internalization. However, the more restricted cellular distribution of the FLT3 receptor may target the effects of FLT3 ligand to primitive hematopoietic cells and to myeloid and lymphoid progenitor cells, in contrast to the pleiotropic effects of the c-kit receptor ligand, stem cell factor.

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THE FLT3 RECEPTOR is a recently identified member of the subclass III family of tyrosine kinase receptors and is similar in structure to the c-kit, PDGF, and c-fms receptors.1,2 Studies of hematopoietic colony growth in the presence of the FLT3 ligand, and of the effect of FLT3 receptor antisense oligonucleotides on hematopoiesis in long-term bone marrow cultures, indicate that FLT3 ligand may be involved in growth of primitive hematopoietic cells, as is the ligand for the c-kit receptor, stem cell factor.3,12 Additionally, mice engineered to lack expression of the FLT3 receptor exhibit defects in pluripotent hematopoietic stem cells and in lymphopoiesis.13 Unlike the c-kit receptor, which is found in large numbers on the surface of cells of both normal and malignant tissues, studies of the cellular distribution of FLT3 receptor mRNA suggest that the FLT3 receptor is more restricted in expression.2,14-19 Although FLT3 receptor mRNA has been detected in a variety of normal and malignant tissues, little is known about the binding characteristics and distribution of the FLT3 receptor protein on human cells. We sought to identify and characterize FLT3 receptor display on normal and malignant human hematopoietic cells and cell lines, using 125I-FLT3 ligand. We identified several myeloid leukemia cell lines that express high affinity FLT3 receptors on the cell surface. We used the ML-1 myeloid leukemia cell line and affinity crosslinking techniques to directly demonstrate that the FLT3 receptor undergoes ligand-induced dimerization. In addition, we found that FLT3 ligand is rapidly internalized and degraded after binding to its receptor.

MATERIALS AND METHODS

Cytokines. Purified recombinant human FLT3 ligand, expressed in yeast, was provided by Immunix Corporation (Seattle, WA). Recombinant human granulocyte macrophage colony stimulating factor (GM-CSF) was provided by Dr Ken Kaushansky (University of Washington, Seattle, WA).

Cell lines and human hematopoietic cells. The human myeloid leukemia cell line ML-120 was provided by Dr Donald Small (Johns Hopkins University, Baltimore, MD). The AML-193, THP-1, CEM, HL-60, KG-1, K562, U937, and Daudi cell lines were obtained from American Type Culture Collection (Rockville, MD). The human erythroleukemia cell lines HEL, OCIM1, KU812F were obtained from Dr Thalia Papayannopoulos (University of Washington, Seattle, WA).21 The ML-1 and Daudi cell lines were grown in RPMI 1640 (MA Bioproducts, Walkersville, MD) supplemented with 10% fetal calf serum (FCS) (Hyclone Laboratories Inc, Logan, UT) and 1% penicillin, streptomycin, and fungizone (PSF). All other cell lines were grown in Iscove’s modified Dulbecco’s media (IMDM; GIBCO, Grand Island, NY) supplemented with 10% FCS and 1% PSF. The factor-dependent cell line M07E22 was grown in IMDM supplemented with 10% FCS and 3 ng/mL recombinant human GM-CSF.

Bone marrow was aspirated from the posterior iliac crest of consenting normal adults. This protocol was approved by the University of Washington Human Subjects Committee. Marrow mononuclear cells were obtained by density centrifugation through Lymphocyte Separation Medium (Organon Teknika Co, Durham, NC). The low
density cells were washed twice, resuspended in IMDM with 10% FCS, and incubated overnight at 37°C in tissue culture flasks. Nonadherent marrow mononuclear cells were collected and used for experiments or passed for CD34⁺ selection. Purification of CD34⁺ cells was achieved by direct immune adherence using the 12.8 anti-CD34 monoclonal antibody (a gift from Dr Irv Bernstein, Seattle, WA) as previously described. For one experiment, CD34⁺ cells were isolated from G-CSF mobilized peripheral blood mononuclear cells using the Ceprate column (CellPro, Bothell, WA). Cytopreserved marrow samples from patients with newly diagnosed acute myelogenous leukemia were obtained from the Children's Cancer Group, CCG AML (Seattle, WA).

Screening of cell lines and human marrow cells for surface display of FLT3 receptors. Human FLT3 ligand was iodinated using the chloramine T method. Self-displacement analysis was used to test the radiologic specific activity. Cell lines were examined for FLT3 receptor display by incubating the cells (10⁶) with 200 pmol/L ¹²⁵I-FLT3 ligand with or without a 100-fold excess of unlabeled FLT3 ligand in binding buffer consisting of RPMI 1640 supplemented with 1% bovine serum albumin (BSA) (Sigma Chemical Co, St Louis, MO), 50 mmol/L HEPES (pH 7.4), 0.1% sodium azide, and 10 μg/mL cytochalasin B (Sigma) for 1 hour at 37°C shaking water bath. After the incubation, the cells were sedimented through phthalate oil (dibutyl phthalate [Sigma]:dinonyl phthalate 3.5:2.0 [Fluka, Buchs, Switzerland]) to separate cell bound from free ¹²⁵I-FLT3 ligand. Both cell associated and free ¹²⁵I were quantitated using a Packard Cobra II gamma counter (Packard Instrument Co, Downers Grove, IL). All measurements were performed in duplicate.

Binding affinity and number of FLT3 receptors on cell lines and human hematopoietic cells. The binding affinity and number of FLT3 receptors on the surface of the ML-1, HL-60, and AML-193 cell lines or on human marrow mononuclear cells were determined by Scatchard analysis of ¹²⁵I-FLT3 ligand equilibrium binding experiments. The cells (1-3 × 10⁶) were incubated with varying amounts of ¹²⁵I-FLT3 ligand (30 pmol/L to 4 nmol/L) with or without a 100-fold excess of unlabeled FLT3 ligand in binding buffer for 4 hours in a 15°C shaking water bath. After the incubation, the cells were separated from the binding buffer containing free ¹²⁵I-FLT3 ligand by sedimentation through phthalate oil as described above. Measurements of ¹²⁵I were performed in duplicate, and the data were analyzed using the ligand program.

Biotinylation of FLT3 ligand. Purified FLT3 ligand (50 μg) was incubated with biotin-X-NH₂ (1 μg) (Calbiochem-Novabiochem Corp, La Jolla, CA) in 0.1 mol/L NaHCO₃ buffer (pH 8.4) for 30 minutes at room temperature. Unbound biotin-X-NH₂ was removed by dialysis.

Flow cytometry. Flow cytometric analysis of biotinylated FLT3 ligand binding to CD34⁺ normal human hematopoietic cells and to ML-1 cells was performed as follows. CD34⁺ cells or ML-1 cells (10⁶) were incubated with 1 nmol/L biotinylated FLT3 ligand in PBS containing 2% heat-inactivated FCS for 2 hours at 4°C. The cells were washed and resuspended with a 1:100 dilution of phycoerythrin-conjugated streptavidin (Immuno Research Laboratories, West Grove, PA) for 30 minutes at 4°C, washed, and analyzed in a Coulter Epics Elite flow cytometer. Parallel experiments were performed with CD34⁺ cells or ML-1 cells incubated with biotinylated FLT3 ligand plus a 100-fold excess of unlabeled FLT3 ligand.

Affinity crosslinking. To determine the molecular weight of the FLT3 receptor on the surface of ML-1 cells, ¹²⁵I-FLT3 ligand was crosslinked to its receptor using techniques previously described. The cells (10⁶) were incubated with 1 nmol/L ¹²⁵I-FLT3 ligand with or without a 100-fold excess of unlabeled FLT3 ligand for 1 hour at 37°C in a shaking water bath. After binding, crosslinking was performed by adding 1 mmol/L bisulfosuccinimidyl suberate (BS3; Pierce, Rockford, IL) and incubating for 30 minutes at 22°C. The reaction was quenched with 20 mmol/L Tris HCl (pH 7.0). The cells were washed and then lysed in buffer consisting of 1% Triton X-100, 20 mmol/L Tris HCl (pH 8.0), 10 mmol/L EDTA, 1% glycerol, 1 mmol/L PMSF, 10 μg/mL leupeptin, 0.5 mg/mL aprotinin, and 10 μmol/L pepstatin for 1 hour at 4°C. The protein inhibitors were obtained from Boehringer Mannheim (Indianapolis, IN). The cell lysates were spun at 8,000g for 10 minutes to remove cellular debris, washed in sample buffer (final concentration 60 mmol/L Tris HCl [pH 6.8] 2% sodium dodecyl sulfate [SDS], 0.004% bromophenol blue, 10% glycerol), and subjected to SDS polyacrylamide gel electrophoresis (SDS-PAGE). High molecular weight standards (39,200-340,000 kD; Boehringer Mannheim) were electrophoresed in parallel.

 Autoradiography. The distribution of FLT3 receptors on normal human marrow mononuclear cells, CD34⁺ marrow cells and marrow cells obtained from AML patients was determined by autoradiography. The marrow cells (1 × 10⁶) were resuspended in binding buffer and incubated with ¹²⁵I-FLT3 ligand (1 nmol/L) with or without a 100-fold excess of unlabeled FLT3 ligand for 1 hour at 37°C. After the incubation, the cells were layered on Percoll (density 1.030 g/mL) and centrifuged for 2 minutes at 400g to separate the cells from the free ¹²⁵I-FLT3 ligand. The cells were resuspended in phosphate-buffered saline containing 0.1% BSA. Cytopreparations were made using a Shandon Southern cytospin. The cells were fixed with methanol and dipped in radiographic emulsion as previously described then exposed for 2 weeks before being developed with Kodak developer and fixed with Kodak fixer (Eastman Kodak Co, Rochester, NY). The slides were stained with Wright-Giemsa and cells with increased numbers of grains above background were identified. Autoradiography was performed on leukemia cell lines known to be positive for FLT3 receptor (ML-1) and negative for FLT3 receptors (M07e) in parallel as controls.

FLT3 ligand internalization. To determine the rate of FLT3 ligand internalization and degradation, we used the acid stripping method. Briefly, ML-1 cells (2-4 × 10⁶) were incubated for 4 hours at 4°C with 1 nmol/L ¹²⁵I-FLT3 ligand to saturate FLT3 surface receptors with labeled ligand. For these experiments, the binding buffer lacked sodium azide and cytochalasin B. The radioabeled cells were separated from free ¹²⁵I-FLT3 ligand by centrifugation through Percoll (density 1.030 g/mL). An aliquot of cells (3-10 × 10⁶) was removed at time 0 and after a 5, 10, 15, 20, 30, and 45 minute incubation at 37°C in a shaking water bath. Each aliquot of cells was resuspended in 0.6 mL sodium barbital:acetate buffer (pH 2.6) and incubated for 6 minutes, then spun at 400g to separate the stripped cells from the supernatant. The supernatant was removed and an ice cold solution of 10% trichloroacetic acid (TCA) was added to precipitate the proteins. The solution was spun at 8,000g for 10 minutes, and the supernatant was removed. ¹²⁵I was quantitated in the cell-associated fraction, in the TCA precipitate, and in the TCA soluble fraction, representing the relative amount of internalized ¹²⁵I-FLT3 ligand, cell surface intact ¹²⁵I-FLT3 ligand, and degraded free ¹²⁵I-FLT3 ligand, respectively. From these results, we estimated the rate of internalization and degradation of the labeled FLT3 ligand.

Megakaryocytic colony formation. B6D2F1 mice (8-12 weeks old) were obtained from the Jackson Laboratories (Bar Harbor, ME). The mice were killed by rapid cervical dislocation. Murine marrow cells were obtained by flushing the femurs of the mice with RPMI 1640 supplemented with 2% FCS. The murine marrow cells were plated at a final concentration of 2 × 10⁵ cells/mL in semi-solid media consisting of 0.275% agar in IMDM (Difco, Detroit, MI) supplemented with 10% horse serum (HyClone) and 1% PSF. The growth factors used included human FLT3 ligand (100 ng/mL), murine IL-3 (100 U/mL), murine SCF (50 ng/mL), and murine Tpo (1,000 U/mL) alone or in combination. Prior reports indicate that human FLT3 ligand is active on murine cells. The plates were
incubated at 37°C in a humidified atmosphere supplemented with 5% CO₂ and megakaryocytic colonies were counted on day 5, using previously defined criteria.³³

Megakaryocyte DNA content. To determine if FLT3 ligand enhanced megakaryocyte nuclear endoreduplication, we cultured normal murine bone marrow cells (10⁶/mL) in serum-free media consisting of IMDM supplemented with 1% Nutridoma-SP (Boehringer Mannheim) in the presence of hematopoietic growth factors (FLT3 ligand, Tpo) alone or in combination. After 5 days of culture, the cells were prepared for flow cytometric analysis of megakaryocyte ploidy as previously described.²² Briefly, the cells were labeled with 15 µg/mL of 4A5 rat monoclonal antibody or with control rat IgG antibody (Southern Biochemical Associates, Inc, Birmingham, AL) for 30 minutes on ice. The 4A5 antibody recognizes an antigen present on murine platelets and megakaryocytes,²² and was generously provided by Dr Sam Burstein (University of Oklahoma, Oklahoma City, OK). The cells were washed and incubated with FITC-conjugated goat anti-rat IgG (30 µg/mL, Jackson ImmunoResearch Labs, Inc, West Grove, PA), then washed and resuspended in a hypotonic citrate solution (0.1% sodium citrate, 50 µg/mL propidium iodide, 30 µg/mL DNAse-free RNAse [Boehringer Mannheim] and 0.1% Triton X-100) for 30 minutes at 22°C. The DNA content of the 4A5 positive cells was analyzed in a Coulter Epics Elite flow cytometer.³³

RESULTS

Screening for FLT3 receptors. Human bone marrow cells from normal adults or from patients with acute myelogenous leukemia were tested for the presence of FLT3 receptor display by ability to bind °²⁵I-FLT3 ligand. In addition, a number of human hematopoietic cell lines were tested for the presence of cell surface FLT3 receptors (Table 1). Each of the four independent samples of normal human bone marrow cells, and the bone marrow cells from four patients presenting with acute myelogenous leukemia, were positive for FLT3 receptors (Table 1). Of the five bone marrow samples from patients with megakaryoblastic leukemia studied, one sample bound °²⁵I-FLT3 ligand (Table 1), and four samples did not (data not shown). The majority of myeloid leukemia cell lines tested were capable of binding °²⁵I-FLT3 ligand. However, the two cell lines with predominantly megakaryocytic features (MO7e and KU812F) did not display detectable FLT3 receptors on the cell surface (Table 1). Two of the three cell lines with erythroid features (OCI1M1 and HEL) displayed detectable but low level °²⁵I-FLT3 ligand binding.

Characterization of FLT3 receptors. The binding affinity and number of FLT3 receptors per cell were determined on human bone marrow nonadherent cells and on three of the cell lines that were found to be strongly positive for FLT3 receptor display in the screening experiments (ML-1, AML-193, and HL-60). All three cell lines displayed a single class of high affinity FLT3 receptors (Fig 1, Table 2). The binding affinities ranged from 200 to 500 pmol/L. The ML-1 cell line, with approximately 4,500 receptors per cell, displayed the greatest number of FLT3 receptors on the cell surface (Fig 1). In two separate experiments, nonadherent normal human marrow mononuclear cells were found to exhibit high affinity FLT3 receptors with a Kₚ of approximately 100 pmol/L and an average of 40 receptors per cell.

Affinity crosslinking. The molecular weight of the FLT3 receptor complex was determined by binding to °²⁵I-FLT3 ligand followed by crosslinking with BS₃, and analysis by SDS-PAGE (Fig 2). Two bands were identified on the film, a faint band at 180 kD (that is not readily apparent on the photograph) consistent with the FLT3 ligand-receptor monomer, and a dark band at 360 kD consistent with the dimerized form of the FLT3 receptor (Fig 2, lane A). After accounting for the 30 kD molecular weight of FLT3 ligand, the molecular weight of the receptor is estimated to be 150 kD, and that of the receptor dimer approximately 300 kD. These radiolabeled complexes were not seen when binding was performed in the presence of 100-fold excess of unlabeled FLT3 ligand (Fig 2, lane B).

Autoradiography. Nonadherent marrow mononuclear cells are a heterogeneous population of hematopoietic cells. To better characterize the cells that display FLT3 receptors autoradiography was used to identify °²⁵I-FLT3 ligand binding to individual cells. Numerous grains were found on ML-1 cells and on AML marrow cells (Fig 3) in comparison with cells incubated in parallel with excess unlabeled FLT3 ligand. The density of grains per cell was higher on the ML-1 cells than on the fresh AML blasts. Autoradiographs performed on nonadherent normal human marrow mononuclear cells did not show a specific population that was labeled with °²⁵I-FLT3 ligand. Because of previous reports that FLT3 receptor mRNA is found within the CD34⁺ fraction of nor-
Fig 1. Binding of $^{125}$I-FLT3 ligand to ML-1 cells. (A) ML-1 cells (2 x 10^5) were incubated with $^{125}$I-FLT3 ligand (30 pmol/L to 4 nmol/L) for 4 hours at 15°C. (B) Scatchard analysis of $^{125}$I-FLT3 ligand binding to ML-1 cells. The data were analyzed using the ligand program and are presented as the average of duplicate determinants. Four independent experiments gave similar results.

Flow cytometry. Flow cytometry was used to compare the FLT3 receptor positive population of CD34+ marrow cells with that of the ML-1 cell line. There was a readily detectable shift in fluorescence in CD34+ normal human hematopoietic cells incubated with biotinylated FLT3 ligand in comparison with cells incubated with biotinylated FLT3 ligand plus a 100-fold excess of nonbiotinylated FLT3 ligand (Fig 4A). Consistent with the binding experiments with $^{125}$I-FLT3 ligand, there was a substantially greater shift in fluorescence in ML-1 cells studied in the same experiment (Fig 4B).

Receptor internalization. $^{125}$I-FLT3 ligand was permitted to bind to cell surface FLT3 receptor on ML-1 cells at 4°C in the absence of metabolic inhibitors, then the temperature was raised to 37°C, and acid stripping was used to estimate the proportions of cell surface and internalized $^{125}$I-FLT3 ligand. As shown in Fig 5, receptor-bound $^{125}$I-FLT3 ligand is internalized by 5 minutes at 37°C, and internalization is maximal after 15 minutes at 37°C. Degraded $^{125}$I-FLT3 ligand is detectable after 20 to 30 minutes at 37°C. Similar results were obtained in three separate experiments.

Table 2. Binding of $^{125}$I-FLT3 Ligand to Human Myeloid Leukemia Cell Lines

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<tr>
<th>Cell Line</th>
<th>Binding Affinity</th>
<th>No. Receptors/Cell</th>
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<tbody>
<tr>
<td>ML-1</td>
<td>200 ± 31 pmol/L</td>
<td>4,500 ± 380</td>
</tr>
<tr>
<td>AML-193</td>
<td>500 pmol/L</td>
<td>1,800</td>
</tr>
<tr>
<td>HL-60</td>
<td>220 ± 20 pmol/L</td>
<td>575 ± 300</td>
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The results for the ML-1 and HL-60 cell line represent the mean ± SEM of values obtained from three or four independent experiments. The results from the AML-193 cell line represents the average of two experiments. The binding data were analyzed using the ligand program.

Fig 2. Dimerization of the FLT3 receptor in the presence of $^{125}$I-FLT3 ligand. ML-1 cells were incubated with $^{125}$I-FLT3 ligand in the absence (lane A) or the presence (lane B) of a 100-fold excess of unlabeled FLT3 ligand, and affinity crosslinking was performed.
Effect of FLT3 ligand on megakaryopoiesis. The effects of FLT3 ligand on CFU-Meg colony growth were examined alone and in the presence of other growth factors including Tpo, SCF, and IL-3. Human FLT3 ligand alone or in the presence of Tpo, SCF, or IL-3 displayed no effect on CFU-Meg colony numbers at the concentrations tested (Table 3). In contrast, human FLT3 ligand was able to stimulate CFU-GM colony growth, demonstrating its activity on murine cells (Table 3).

To evaluate the effect of FLT3 ligand on megakaryocyte endoreduplication, we examined the ploidy of megakaryocytes generated in suspension cultures in the presence or absence of FLT3 ligand. No significant change in megakaryocyte ploidy was seen by flow cytometric analysis of DNA content of megakaryocytes produced in the presence of FLT3 ligand in comparison with those produced with no added growth factor (data not shown). Additionally, the combination of FLT3 ligand and Tpo did not enhance megakaryocyte endoreduplication above that seen with Tpo alone (data not shown).

DISCUSSION

The FLT3 receptor and its ligand have been shown to play a role in hematopoiesis and may play an important role in the survival and proliferation of primitive hematopoietic cells. Although FLT3 receptor mRNA has been detected in various normal and malignant hematopoietic cells and cell lines, relatively little is known about the FLT3 ligand binding characteristics of the receptor protein expressed on the surface of these cells.

FLT3 receptors have been identified on a subpopulation of murine marrow cells using autoradiography, and CD34+ human bone marrow cells and some human hematopoietic cell lines display FLT3 receptors detectable by flow cytometry. We characterized FLT3 receptors on normal and leukemic human marrow cells and leukemia cell lines in terms of binding affinity, number of receptors per cell, receptor dimerization, and ligand internalization. Our results indicate that normal human marrow cells exhibit high affinity FLT3 receptors (Kd approximately 100 pmol/L). Because nonadherent marrow mononuclear cells consist of a heterogeneous population of primitive and mature cells, we attempted to use autoradiography to identify the marrow cells that display FLT3 receptors. This method has been used to demonstrate the presence of c-kit receptors on blasts from normal human marrow and on hemoglobinized progeny of BFU-E. Rasko et al detected binding of FLT3 ligand to a portion of blasts from murine marrow, as well as to more purified sub-
Fig 4. FLT3 receptor expression on (A) CD34+ cells obtained from G-CSF mobilized peripheral blood mononuclear cells from a normal donor and (B) ML-1 cells. The cells were incubated with biotinylated FLT3 ligand (——), or with biotinylated FLT3 ligand plus 100-fold excess unlabeled FLT3 ligand (—), and then with streptavidin-PE, and analyzed by flow cytometry. An experiment with CD34+ normal human marrow mononuclear cells gave similar results.

sets of primitive murine hematopoietic cells and to pro-B lymphocytes. Although our results demonstrate the presence of FLT3 receptors on normal hematopoietic cells by competitive binding with 125I-FLT3 ligand and by flow cytometric analysis of biotinylated FLT3 ligand binding, we were unable to identify by our autoradiography techniques the subpopulation of normal hematopoietic cells that binds 125I-FLT3 ligand. This indicates that the number of FLT3 receptors on human marrow cells is below the level readily detectable by autoradiography, and suggests that FLT3 receptor density on normal human blasts may be less than that of the c-kit receptor.

The cell lines that were positive for FLT3 ligand binding expressed relatively low numbers of receptors on the cell surface. The highest number of receptors, 4,500 receptors per cell, was found on the myeloid leukemia cell line ML-1. In general, hematopoietic cell lines with myeloid features displayed 125I-FLT3 ligand binding, consistent with prior studies demonstrating the presence of FLT3 receptor mRNA predominantly in myeloid-monocytic cell lines and in pre-B cell lines. Human erythroleukemia cell lines known to display large numbers of high affinity c-kit receptors (up to 80,000 per cell) such as OCIM1 and HEL expressed few detectable FLT3 receptors on the cell surface, consistent with studies of the cellular distribution of FLT3 receptor mRNA.

We found that, like other members of the subclass III tyrosine kinase receptors, the FLK3 receptor forms dimers in the presence of its ligand. Affinity crosslinking experiments demonstrated that the FLT3 receptors on ML-1 cells are of approximately 150 kD molecular weight. After the formation of receptor dimers, cytokine receptors are internalized and either degraded or recycled; the ligand is generally degraded. Our results show that FLT3 ligand is rapidly internalized.

Table 3. The Effect of FLT3 Ligand on CFU-Meg and CFU-GM Colony Growth

<table>
<thead>
<tr>
<th>Condition</th>
<th>No. CFU-Meg Colonies</th>
<th>No. CFU-GM Colonies</th>
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<tr>
<td>Tpo</td>
<td>44.3 ± 6.1</td>
<td>ND</td>
</tr>
<tr>
<td>FLT3 ligand</td>
<td>0</td>
<td>ND</td>
</tr>
<tr>
<td>Tpo + FLT3 ligand</td>
<td>41.3 ± 1.9</td>
<td>ND</td>
</tr>
<tr>
<td>IL-3</td>
<td>22.6 ± 3.6</td>
<td>185.3 ± 11.6</td>
</tr>
<tr>
<td>IL-3 + FLT3 ligand</td>
<td>20.0 ± 2.9</td>
<td>279.0 ± 13.7</td>
</tr>
<tr>
<td>SCF</td>
<td>3.7 ± 0.7</td>
<td>129.7 ± 8.4</td>
</tr>
<tr>
<td>SCF + FLT3 ligand</td>
<td>3.3 ± 1.0</td>
<td>195.7 ± 3.8</td>
</tr>
<tr>
<td>SCF + Tpo</td>
<td>57.3 ± 7.1</td>
<td>ND</td>
</tr>
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Murine marrow cells (2 x 10^5/mL) were cultured in semi-solid media in the presence of Tpo (1,000 U/mL), IL-3 (100 U/mL), or SCF (50 ng/mL) with or without FLT3 ligand (100 ng/mL). The data show the number of CFU-Meg or CFU-GM colonies per 2 x 10^6 cells plated (mean ± SEM of triplicate plates). Two additional experiments gave similar results. ND indicates not done.
internalized after binding to the FLT3 receptor. By 15 min-
utes, FLT3 ligand internalization was maximal and by 20 to
30 minutes, ligand degradation was detected. These results
parallel the rapid internalization of SCF after binding to the

FLT3 ligand alone does not support megakaryocytic col-
ony growth in vitro, but FLT3 ligand may augment the
effects of IL-3 or SCF on megakaryopoiesis. Because of
the finding of relatively high amounts of FLT3 receptor
mRNA in blasts from two patients with acute megakaryo-
blastic leukemia, and our demonstration of binding of FLT3
ligand to blast cells obtained from one patient with acute
megakaryoblastic leukemia, we reexamined the effects
of FLT3 ligand on megakaryopoiesis in conjunction with
the c-kit receptor, including high affinity ligand binding by
normal marrow cells, dimerization in the presence of ligand,
and rapid internalization of ligand. How-
Our results indicate that the FLT3 receptor shares many
characteristics with the c-kit receptor, including high affinity
ligand binding by normal marrow cells, dimerization in the
presence of ligand, and rapid internalization of ligand. How-
ever, FLT3 receptor cell surface expression differs from c-
kit receptor expression both in terms of cellular distribution
and receptor density. In contrast to the c-kit receptor, FLT3
receptor is not present on mast cells or megakaryocytes,
and was not readily detected on normal erythroblasts. Nor-
mal human marrow blasts, which have been shown to have
a high density of c-kit receptors, appear to display fewer
FLT3 receptors. Equilibrium binding experiments showed
that populations of normal human marrow mononuclear cells
display an average of 400 c-kit receptors per cell, and
approximately 40 FLK3 receptors per cell. In addition, our
results show that unlike SCF, the FLT3 ligand does not
augment megakaryocyte progenitor growth in the presence
of Tpo. These data provide further evidence supporting the
concept that the effects of FLT3 ligand are targeted to the
myeloid and lymphoid lineage. The more restricted cellular
distribution of the FLT3 receptor may have implications for
the clinical use of FLT3 ligand.

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