Interaction of Fibroblast Growth Factor (FGF) With Megakaryocytopoiesis and Demonstration of FGF Receptor Expression in Megakaryocytes and Megakaryocytic-Like Cells

By Andreas Bikfalvi, Zong C. Han, and Guy Fuhrmann

We have investigated the interaction of fibroblast growth factor (FGF) with megakaryocytopoiesis. Acidic FGF (aFGF) stimulated the proliferation of murine megakaryocytes and human erythroleukemia (HEL) cells in a concentration-dependent manner. The concentrations of aFGF required to elicit half-maximum and maximum effects were similar for HEL and megakaryocytic colony formation. The effect of aFGF was comparable to that of basic FGF (bFGF) in both cell types. The effect of both FGFs was found to be synergistic because it presents phenotypic properties of megakaryocytes. The expression of megakaryocytic and platelet glycoproteins (GPs) has been shown using this cell line. We evidenced here that both FGF act on megakaryocytopoiesis.

MATERIALS AND METHODS

Reagents

Recombinant human aFGF (1-140) has been produced by recombinant DNA technology (gift of Dr D. Mayaux, Rhone Poulenc, Paris). It has been purified to homogeneity by heparin-Sepharose chromatography and by reversed-phase high performance liquid chromatography (HPLC). It gives rise to a single band on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels. Its activity has been described in Mascarel et al. Recombinant human bFGF was kindly donated by Carlo Erba (Milan, Italy). Recombinant interleukin-3 (IL-3), granulocyte-macrophage colony-stimulating factor (GM-CSF), mouse and human IL-6, and rat antinmous anti–IL-6 and anti–IL-3 monoclonal antibodies (MoAbs) were purchased from Genezyme Corp (Boston, MA). All plasticware for cell culture was from Costar (Cambridge, MA). All cell culture reagents and media were from Gibco (Grand Island, NY) and Eurobio (Paris, France). Electrophoretic reagents, including glycine, bishydroxymethylamino-l-sulfate, SDS, acrylamide, ammonium persulfate, bisacrylamide, 2-mercaptoethanol, Teemed, and Coomassie blue R 250, were from BioRad (Richmond, CA). 125Iodine (125I) was purchased from Amersham (Les Ulis, France); all other materials and reagents were research grade.

Cells

The human megakaryocytic-like cell lines HEL and Dami were cultured in α-medium (Eurobio) with 10% fetal bovine serum (FBS), glutamine (0.15 mg/mL), and penicillin (15 U/mL)-streptomycin (15 μg/mL). Murine BM was taken from the femurs of Balb/c mice (IFBA, Paris, France), suspended in α-medium, and centrifuged, and processed for the experiments as indicated.

Human megakaryocytes were isolated by immunomagnetic beads using a MoAb directed against GPIIb/IIIa (p2; Immunotech, Marseille, France) as described. Briefly, BM cells from hematopoietically normal subjects were resuspended in α-medium and centrifuged on a lymphocyte-separating medium density gradient (Eurobio). The mononuclear cells were resuspended in 1 mL α-medium (107 cells/mL) with 20 μL (1 mg/mL) MoAb against human platelet GPIIb/IIa (p2; Immunotech), mixed, and incubated at room temperature for 60 minutes. Five microliters of dynal magnetic beads coated with antimouse IgG (Dynal, Oslo, Norway) was subsequently added and incubated for another 30 minutes. The beads and their attached cells were collected with a dynal magnet bead separator and washed eight times with α-medium. After the final wash, some attached cells were centrifuged onto

From INSERM U 118, Paris; and the Institut des Vaisseaux et du Sang, Paris, France.


A.B. was a recipient of “Fondation pour la Recherche Médicale.”

Address reprint requests to A. Bikfalvi, MD, PhD, Departement of Cell Biology, New York University Medical Center, 550 First Ave, New York, NY 10016.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1992 by The American Society of Hematology.
slides by cytospin for immunofluorescence staining; others were resuspended in RNA extraction buffer. Human platelets were isolated according to the technique of Patscheke and Worner. The published method of Gaudernack et al was used to isolate mouse hematopoietic cells. Briefly, mouse macrophages, granulocytes, T cells, and B cells were, respectively, separated from the BM of Balb/c mice by immunomagnetic beads using the rat MoAbs to mouse macrophages (MI-70.15), to mouse granulocytes (RA6-8e), to mouse Ly-2 (CD86), and to mouse B cells (RA3-6B2) (Caltag Laboratories, San Francisco, CA). After isolation, immunofluorescence was performed with a sample of each cell type using relevant antibodies. Immunofluorescence showed a 100% positivity of the cells, demonstrating the absence of contaminating cells of other lineages.

**Proliferation Assay With Plasma Clot Cultures**

**Megakaryocytes.** Megakaryocytic progenitor cells were assayed using a plasma clot system. Briefly, 2 × 10³ marrow cells were cultured in five-plexes in 1 mL α-medium containing 1% bovine serum albumin (BSA; Sigma, St Louis, MO) 10% bovine plasma deionized with AG 501-X8 mixed bed resin (BioRad), and 1 × 10⁻⁴ mol/L 2-mercaptoethanol with or without various concentrations of growth factors or antibodies that were diluted in α-medium in a total volume of 50 µL. After 7 days of incubation, the clots were dried in situ with filter papers, fixed with methanol, and stained with hematoxylin. A leukemic colony was defined as containing 10 to 20 cells.

**HEL cells.** Colony formation assay of HEL cells was performed using a plasma clot system according to Piao et al. and Han et al. Briefly, 1 × 10³ cells/well were cultured in 24-well tissue culture plates in 0.25 mL of α-medium containing 10% FBS, 10% bovine-citrate plasma, 0.34 mg/mL CaCl₂, 1 × 10⁻⁵ mol/L phorbol myristate acetate (PMA) with or without FGF. FGF was diluted with α-medium at various concentrations. A total volume of 25 µL of FGF dilutions was added to each well, giving a final FGF concentration of 0 to 20 ng/mL. Cultures were incubated at 37°C in a humidified atmosphere and 5% CO₂. After 4 days of incubation, the clots were dried in situ with filter papers, fixed with methanol, and stained with hematoxylin. A leukemic colony was defined as containing 20 cells, whereas a cluster was defined by containing 10 to 20 cells. With or without 2 µg of cold ligand in a Eppendorf tube under gentle agitation (final volume, 500 µL). After 2 hours of incubation at 4°C, the cells were washed three times with phosphate-buffered saline (PBS) (pH 7.4) and bound ¹²⁵I-aFGF was cross-linked at 4°C with 0.3 mmol/L disuccinimidyl suberate (DSS; Pierce, Rockford, IL) in 1 mL PBS (7.4) for 20 minutes. Subsequently, 20 µL of 2 mol/L Tris HCl (pH 8) was added and the cells were again washed with PBS (pH 7.4).

The cells were extracted with 100 µL extraction buffer (Tris, pH 7.5, 1 mmol/L EDTA, 0.1 phenylmethylsulfonyl fluoride, 200 mmol/L NaCl, 1% Triton X-100). Insoluble material was removed by centrifugation at 15,000 g for 10 minutes and two times concentrated SDS sample buffer was added. Electrophoresis was performed on a 7.5% polyacrylamide gel under reduced conditions. The gels were fixed, stained with Coomassie blue, dried, and subjected to autoradiography. Development was performed for 2 to 6 weeks of exposure.

**Message RNA (mRNA) Analysis**

**Northern blot analysis.** RNA was isolated by the guanidinium/isoctocyanate technique of Chirgwin et al. Ten micrograms of total RNA of BM cells or HEL cells was subjected to electrophoresis through a 1% agarose gel in 2% formaldehyde, transferred onto nitrocellulose filters (Hybond N; Amersham, Arlington Heights, IL), and prehybridized for 1 hour at 42°C in 45% (vol/vol) formamide, 6x SSC, 0.1% (wt/vol) SDS, 2% Denhardt’s solution, 500 µg denatured salmon sperm DNA/mL, and 2 mmol/L EDTA. Hybridization was started by addition of a random-primer-³²P (specific activity, 10⁶ cpm/µg) probe. For both FGF receptors (type 1 [FGF-R1] or type 2 [FGF-R2]), two cDNA probes were tested, corresponding either to the total extracellular region (1,200-bp length) or to the 5′-coding region extending up to the middle of the first Ig-like domain (350-bp length) (mouse FGF-R1 probes were kindly donated by Dr N. Fasel [Institut de Biochemie, University of Lausanne, Switzerland]; human FGF-R2 probes were kindly given by Dr R. Breathnach [Faculte des Sciences, University of Nantes, France]). Hybridization was continued at 42°C for 24 hours.

Filters were washed twice in high stringency at 60°C in 0.1 SSC, 0.1% SDS. Exposure was performed for 2 to 4 days at −70°C according to the probe used.

**Polymerase chain reaction (PCR) analysis.** mRNA was extracted from total BM, HEL cells, Dami cells, megakaryocytes, macrophages, granulocytes, T cells, and B cells. One microgram of these RNAs was reverse transcribed according to Newman et al using random hexamers. Complementary DNA first-strand reaction was performed at 37°C. After 60 minutes, the reaction was stopped by boiling for 10 minutes and chilling to 4°C. PCR amplification was performed at 90°C. One-fifth of the preparation was diluted in PCR buffer according to the manufacturer (Perkin Elmer, Nor-
Oligonucleotides were constructed by the Pasteur Institut (Paris, France). The antisense oligomer ASA was a 21-mer degenerate oligonucleotide, located in the common acidic region of FGF-R1 and FGF-R2 (GAATTCGTCGTCATCATC; Tm = 54°C).

The sense oligomer SR1 was an 18-mer oligonucleotide, located in the 5'-coding region of FGF-R1 (GAATTCGTCGTCATCATC; Tm = 51°C). The sense oligomer SR2 was a 20-mer oligonucleotide, located in the 5'-coding region of FGF-R2 (GAATTCGTCGTCATCATC; Tm = 57°C). The expected lengths of the PCR products were 377 and 380 bp for ASA-SR1 and ASA-SR2 pairs, respectively.

Amplification was performed for 30 cycles, with denaturation for 2 minutes at 94°C, primer annealing for 2.5 minutes at 55°C or 50°C (according to the species tested), and primer extension for 6 minutes at 72°C. Final extension was conducted for 15 minutes at 72°C. Analysis of PCR products was performed as follows. One-tenth of each PCR reaction was subjected to 1% agarose gel electrophoresis, transferred onto nylon (Hybond N, Amersham) hybridized at high stringency with the probes described above, and exposed to autoradiography (exposure time, 2 to 12 hours).

To further characterize the PCR products, amplified DNA bands of the expected size were recovered from agarose gel, purified by the gene clean procedure (Bio 101, La Jolla, CA), and subjected to limited restriction map analysis. The experiments were performed with different starting RNAs with or without reverse transcription to insure the specificity of the PCR reaction and the absence of DNA contamination.

**RESULTS**

**FGFs Stimulate the Proliferation of Megakaryocytes and Megakaryocytic-Like Cells**

Murine BM cells and HEL cells cultured on plasma clots were incubated with increasing concentrations of aFGF and bFGF. Colony formation was stimulated for both cell types in a concentration-dependent manner (Fig 1A and 1B). As shown for megakaryocytes, maximum HEL colony formation was noted between 10 and 20 ng/mL of aFGF. Half-maximum stimulation for HEL cell colony formation was similar to the half-maximum stimulation of megakaryocytic colony formation (2 ng/mL). The same plateau was reached in both cell types. When expressed in percentages, the stimulatory effect was found to be lower for HEL cells than for megakaryocytes. In HEL cells, there was an...
increase of ~160% to 200% in colony formation in the presence of maximal stimulatory concentrations of aFGF. In megakaryocytes, the percentage was much higher (~600%). bFGF stimulated megakaryocytic and HEL colony formation in a way similar to aFGF (half-maximum and maximum stimulation of 2.5 to 4 and 10 to 20 ng/mL, respectively).

We have subsequently investigated whether FGF was able to stimulate megakaryocytic and HEL colony formation under serum-free conditions. Figure 2 shows that both FGFs were capable of inducing colony stimulation. Thus, serum components may not be required for the action of FGF on cells of the megakaryocytic lineage.

To ensure that the effect on cell proliferation of HEL cells was accompanied by differences in the cloning efficiency, the number of colonies (more than 20 cells) versus the number of clusters (less than 20 cells) was counted (Figs 1B and 2B). These experiments showed that only the number of colonies increased significantly after FGF stimulation, in contrast to the number of clusters. The effect of FGF is therefore mediated through a real increase in the number of colonies.

The Effect of Both FGFs Is Synergistic With IL-3 and Abrogated by Anti-IL-6 Antibodies

Experiments were performed to evaluate the effect of aFGF and bFGF in combination with IL-3, IL-6, and GM-CSF at optimal stimulatory concentrations (100 U/mL for IL-3, 5 ng/mL for GM-CSF, 20 ng/mL for IL-6) on megakaryocytopenesis using the plasma clot system. The combination of both FGFs with IL-3 causes a significant synergistic action, whereas the combination with IL-6 or GM-CSF did not cause a significant change (Fig 3).

To investigate whether the effect of FGF is mediated through an endogenous stimulation of either IL-3 or IL-6, blocking experiments using neutralizing antibodies against IL-3 or IL-6 were performed. These experiments show that the activity of murine IL-3 was effectively neutralized by antimonoclonal IL-3 antibodies, but had no effect on the activity of FGFs or mouse IL-6 (data not shown). In contrast, anti-IL-6 antibodies were able to neutralize the activity of FGFs as well as mouse IL-6, but had no effect on the activity of human IL-6 or mouse IL-3 (Table 1).

Binding of $^{125}$I-aFGF to Megakaryocytic-Like Cells and Megakaryocytes

HEL cells were incubated with $^{125}$I-aFGF for 2 hours and the bound ligand was subsequently cross-linked using DSS as cross-linking agent (Fig 4, lanes 1 and 2). A band of ~120 Kd was detected by autoradiography. The appearance of this band was completely inhibited by a 200-fold excess of cold ligand. We have made attempts to obtain quantitative data of $^{125}$I-aFGF binding to HEL cells in terms of dissociation constants and number of binding sites. However, Scatchard analysis could not be performed because of a very high nonspecific binding under our assay conditions.

Single-cell autoradiography of megakaryocytes in BM and plasma clot cultures showed binding sites for $^{125}$I-aFGF (Fig 5). Incubation of the cells with unreduced $^{125}$I-aFGF showed a strong signal on megakaryocytes. However, the

<table>
<thead>
<tr>
<th>Factor Added</th>
<th>No. of Megakaryocyte Colonies/2 x 10^5 Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without Anti-IL-6</td>
</tr>
<tr>
<td>None</td>
<td>5 ± 1</td>
</tr>
<tr>
<td>Human IL-6</td>
<td>29 ± 4</td>
</tr>
<tr>
<td>Mouse IL-6</td>
<td>31 ± 3</td>
</tr>
<tr>
<td>Mouse IL-3</td>
<td>92 ± 7</td>
</tr>
<tr>
<td>aFGF</td>
<td>28 ± 3</td>
</tr>
<tr>
<td>bFGF</td>
<td>30 ± 2</td>
</tr>
</tbody>
</table>

BM cells (2 x 10^6 cells/mL) were cultured for 7 days with recombinant human and mouse IL-6 (20 ng/mL), IL-3 (100 U/mL), or FGFs (20 ng/mL). Monoclonal anti-IL-6 antibodies were added at 0.5 or 1 µg/mL. Data represent the mean ± SE of determinations from two separate experiments.

*P < .05 compared with control cultures without anti-IL-6 (Student’s t-test).
binding under this condition was not significantly displaced by a 200-fold excess of cold ligand (not shown). A 2 mol/L NaCl and a heparin wash displaced some, but not all, of the cell-surface bound $^{125}$I-aFGF (Fig 5B and D).

To inhibit aggregate formation between labeled and unlabeled aFGF, aFGF was incubated before binding with 1% 2-mercaptoethanol. It has been shown that disulfide bonds are not required for the activity of aFGF. $^{125}$I-aFGF reduced with 2-mercaptoethanol did not bind as well, but still bound significantly to megakaryocytes (Fig 5C and E).

This binding could be displaced more efficiently by cold aFGF. Binding of $^{125}$I-aFGF was also detected on megakaryocytes in early plasma clot cultures. Besides megakaryocytes, other cell types were found to bind aFGF, including cells from the granulocytic lineage and, probably, erythroblasts (not shown).

To ensure that specific cell surface receptors, through which FGF would possibly stimulate megakaryocytopoiesis directly or indirectly, are present in the BM, $^{125}$I-aFGF was cross-linked to total murine BM cells (Fig 4, lanes 3 and 4). A similar radiolabeled band of $\sim 120$ Kd, as observed in HEL cells, was detected. The appearance of this band could be blocked by an excess of $\sim 200$-fold cold ligand. However, cross-linking experiments using human platelets were unable to show a similar radiolabeled band (data not shown).

Murine BM Cells, Megakaryocytes, and Megakaryocytic-Like Cells Express mRNA for FGF Receptors

mRNA was extracted from total BM, HEL cells, Dami cells, megakaryocytes, platelets, macrophages, granulocytes, T cells, and B cells to analyze whether FGF receptor type 1 and type 2 mRNAs were expressed in these cells. The mRNAs were analyzed either directly by Northern blotting using specific probes of 350 bp and 1,200 bp for FGF receptor type 1 and type 2, respectively, or reverse transcribed and amplified by PCR before blotting using specific FGF receptor oligonucleotides. As seen in Fig 6, Northern blotting showed that mRNA from murine BM cells and HEL cells hybridized with both specific FGF receptor probes, giving a signal of 4.4 kb. Thus, FGF receptor type 1 and type 2 are coexpressed in both total BM cells and HEL cells. The relative amounts of the transcripts of both receptors was found to be the same in the two cell types.

To reinforce these results and also to investigate whether platelets that represent the final "cellular" structure of megakaryocytes contain vestigial amounts of FGF receptor mRNA, PCR was performed. This method has been re-
reported to be capable of amplifying specific DNA sequences greater than $10^9$ times.21 For this analysis, mRNA of BM cells, HEL cells, Dami cells, megakaryocytes, and platelets were subjected after retrotranscription to in vitro amplification. The amplified products were analyzed by specific blot hybridization for the presence of FGF receptor type 1 and type 2 mRNA (Fig 7). The intensity of the signal essentially depends on the species studied. The degenerate primers of both receptors preferentially recognize mouse RNA. Controls were run to ensure the specificity of the cDNAs amplified by PCR. First, different retrotranscripts amplified with oligonucleotides pairs specific for FGF receptor type 1 (or FGF receptor type 2) were hybridized with the specific $^{32}$P-labeled FGF receptor type 2 (or FGF receptor type 1) probe. No hybridization signal was detected. Second, the different directly amplified mRNA samples (non-reverse transcribed) showed an absence of amplified FGF receptor type 1 and type 2 cDNAs. Third, the specificity of the PCR products has also been tested by restriction mapping, which was the same for FGF receptor type 1 or type 2 cDNA (data not shown).

Thus, these results clearly show the presence of FGF receptors type 1 and type 2 in the different megakaryocytic lineages. In addition, platelets also contain vestigial amounts of FGF receptor type 1 and type 2 mRNA. Furthermore, other hematopoietic lineages, including macrophages, granulocytes, T cells, and B cells, express FGF receptor type 1 and type 2 mRNA. The presence of transcripts of these FGF receptor types is therefore not restricted to the megakaryocytic lineage.

**DISCUSSION**

We have evaluated the effect of FGFs on in vitro megakaryocytogenesis. We have shown that FGFs significantly stimulate the proliferation of megakaryocytes and megakaryocytic-like cells. In addition, our results show a synergistic effect with IL-3 and support the contention that the effect of aFGF and bFGF is mediated by IL-6. Finally, we show that these cells exhibit binding sites for $^{125}$I-aFGF and express mRNA for FGF receptor type 1 (fg) and type 2 (bek). These results provide, therefore, the first complete description of FGF receptors in hematopoietic cells.

FGF induces proliferation of HEL cells and megakaryocytic colony formation at similar half-maximum and maximum concentrations. These concentrations are higher than the concentrations required for other cell types, such as endothelial cells.1,2 One could only speculate about the reason for these differences. Perhaps nonadherent cells such as hematopoietic cells require the presence of a higher amount of FGF because matrix interactions in anchorage-dependent growing cells have been shown to be important for the bioactivity of FGF.3,23,24 Alternatively, differences in the FGF signal transduction pathways might exist.

We also show that FGF stimulates growth under serum-free conditions, and that it significantly increases the number of colonies, but not the number of clusters. These results demonstrate that serum components are not required for the action of FGF on cells of the megakaryocyte lineage and that this effect is a real effect on cell proliferation by significantly increasing the number of colonies.

In support of the contention that FGF may stimulate megakaryocytogenesis by interacting with specific cell surface receptors, cross-linking experiments using HEL cells were performed. These experiments showed the presence of specific cell surface receptors for FGF. The molecular weight of this receptor complex is ~ 120 Kd, which is similar to the molecular weight of known FGF receptors in different cells.25-29 Thus, FGF might interact with this receptor to stimulate the proliferation of HEL cells. We have further studied whether FGF receptor mRNA could be detected in HEL cells. Partanen et al30 have recently cloned a new FGF receptor (FGF receptor type 4) by using a HEL cell library. This receptor seems to be specific for aFGF. Our data provide evidence that FGF receptors type
1 and type 2 are also present in HEL cells, thus increasing the FGF receptor repertoire and the possibility of response to FGF. We have also observed that HEL cell growth is stimulated by bFGF. Because FGF receptor type 1 and type 2 can bind both aFGF and bFGF, these data suggest strongly that the stimulation of HEL cell proliferation by FGF is, at least in part, mediated through these receptors.

Because it has not been possible to obtain a sufficient amount of purified megakaryocytes, similar cross-linking experiments using 125I-FGF could not be performed on this cell type. Instead, we have performed single-cell autoradiography with megakaryocytes from BM smears and BM cultures to study whether they are able to bind iodinated FGF. Indeed, single-cell autoradiography on megakaryocytes showed binding sites that were barely displaced by cold aFGF, but more efficiently by a heparin and a 2 mol/L NaCl wash, suggesting that proteoheparan sulfate-FGF binding sites are present on the surface of these cells. In attempt to reduce the nonspecific binding in the single-cell binding assay, we have reduced aFGF with 2-mercaptoethanol before incubation with the cells. It has been shown that disulfide bonds are not required for the activity of recombinant aFGF and that reduction of aFGF may prevent intermolecular aggregate formation (Y. Courtois, personal communication, April 1991). Indeed, under reduced conditions, the displacement of the 125I-aFGF binding by cold ligand was more efficient, suggesting that aggregate formation was present on the cell surface. However, these data provide no proof that these binding sites are real FGF receptors.

To ensure that a molecule that would be able to be cross-linked to FGF was present in at least total BM, BM cells were incubated with 125I-aFGF, which was subsequently cross-linked to the cell surface. A similar complex as that found for HEL cells was detected. Thus, FGF is able to recognize a molecule in murine BM cells with a similar molecular mass as in HEL cells. To investigate whether cells of the murine megakaryocyte lineage present cell
surface proteins able to be cross-linked to FGF, human platelets were incubated with $^{125}$I-aFGF, assuming that platelets presents the “end stage” cells of megakaryocytes. However, we were unable to show a similar FGF receptor complex as in HEL cells and BM cells. Thus, we can not conclude definitively upon the presence of a real FGF receptor protein on megakaryocytes. Nevertheless, our data strongly support the contention that FGF receptor types are localized on cells of the megakaryocytic lineage, because megakaryocytic-like cells, such as HEL cells or Dami cells, as well as normal megakaryocytes and platelets, express FGF receptor type 1 and type 2 transcripts. Furthermore, we also show the presence of FGF receptor type 1 and type 2 mRNA in granulocytes, macrophages, and lymphocytes, suggesting an involvement of FGFs and their receptors in other hematopoietic lineages.

If one considers the potential mechanisms for the observed effect of FGF, the most proximate would include a direct effect on megakaryocytic progenitor cells or an indirect effect via the production of megakaryocytic growth factors by megakaryocytes itself or accessory marrow cells. The data we present favor an indirect mechanism. First, the extent of the FGFs and IL-3 synergistically stimulated cell proliferation was similar to that of IL-6 and IL-3. Second, anti-IL-6 antibodies, but not anti-IL-3 antibodies, were found to inhibit FGF-induced megakaryocytopeniosis. Thus, the effect of FGFs on murine megakaryocytopeniosis is most likely mediated by endogenously produced IL-6 in response to FGF.

In summary, these results indicate that (1) FGF is able to stimulate the proliferation of megakaryocytes and megakaryocytic-like cells; (2) specific FGF receptors are expressed in the BM and megakaryocytic cells; and (3) the effect of FGF is most likely mediated through an endogenous stimulation of IL-6. Taken together, these data suggest that FGF stimulates megakaryocytic colony formation by an autocrine or paracrine IL-6 loop through the presence of cell surface receptors on the surface of megakaryocytes and their precursors or on cells of the microenvironment. The observation that FGF interacts with megakaryocytopeniosis, including the description of FGF receptor types in megakaryocytic-like cells and BM, may lead to the investigation of the role of FGFs and their receptors in several diseases in which abnormal megakaryocytopeniosis is observed, in particular in essential thrombocytopenia, myelofibrosis, or thrombocytopenic disorders.

ACKNOWLEDGMENT
The authors thank Dr J.C. Jeanny and N. Fayemin for advice in the single cell binding assay, Dr Y. Courtois for helpful discussions, and Dr J. Caen for supporting this work. We also thank Dr D.B. Rifkin and Dr L. Wilson for their critical reading of the manuscript.

NOTE ADDED IN PROOF
During the review of this manuscript, Katoh et al. have also observed FGF receptor expression in megakaryocytic cell lineages. They also showed FGF receptor expression in platelets. However, they only concluded upon the presence of FGF receptor type 2 mRNA, detected after in vitro amplification by restriction enzyme experiments and ethidium bromide staining. The sensitive hybridization experiments with another cDNA probe (corresponding to the extracellular region and not to the tyrosine kinase domain) we related here also detected transcripts of FGF receptor type 1 in megakaryocytes, megakaryocytic-like cells, and platelets.

REFERENCES

17. Piao YF, Ichijo H, Miyagawa K, Oashi H, Takaku F, Miyazono K: Latent form of transforming growth factor-β1 acts as
a potent growth inhibitor on a human erythroleukemia cell line. Biochem Biophys Res Commun 167:27, 1990
Interaction of fibroblast growth factor (FGF) with megakaryocytopoiesis and demonstration of FGF receptor expression in megakaryocytes and megakaryocytic-like cells

A Bikfalvi, ZC Han and G Fuhrmann