# Interferon-$\alpha$ Restores Normal Adhesion of Chronic Myelogenous Leukemia Hematopoietic Progenitors to Bone Marrow Stroma by Correcting Impaired $\beta$1 Integrin Receptor Function

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## Abstract

**Treatment of chronic myelogenous leukemia (CML) with interferon-$\alpha$ frequently results in normalization of peripheral blood counts and, in up to 20% of patients, reestablishment of normal hematopoiesis. We hypothesize that interferon-$\alpha$ may restore normal adhesive interactions between CML progenitors and the bone marrow microenvironment and restore normal growth regulatory effects resulting from these progenitor–stroma interactions. We demonstrate that treatment with interferon-$\alpha$ induces a significant, dose-dependent increase in the adhesion of primitive long-term culture initiating cells and committed colony-forming cells (CFC) from CML bone marrow to normal stroma. Adhesion of CFC seen after interferon-$\alpha$ treatment could be inhibited by blocking antibodies directed at the $\alpha$4, $\alpha$5, and $\beta$1 integrins and vascular cell adhesion molecule, but not CD44 or intracellular adhesion molecule, suggesting that interferon-$\alpha$ induces normalization of progenitor-stroma interactions in CML. Because FACS® analysis showed that the level of $\alpha$4, $\alpha$5, and $\beta$1 integrin expression after interferon-$\alpha$ treatment is unchanged, this suggests that interferon-$\alpha$ may restore normal $\beta$1 integrin function. Normalization of interactions between CML progenitors and the bone marrow microenvironment may then result in the restoration of normal regulation of CML progenitor proliferation, and explain, at least in part, the therapeutic efficacy of interferon-$\alpha$ in CML. (*J. Clin. Invest.* 1994. 94:384–391.) Key words: chronic myelogenous leukemia • hematopoiesis • interferon • integrins • extracellular matrix**

## Introduction

Chronic myelogenous leukemia (CML),[1] a malignancy arising from the hematopoietic stem cell, is characterized by a vast expansion of hematopoietic cells belonging to the malignant clone resulting from unregulated proliferation of hematopoietic progenitors (1). In addition, greatly increased numbers of circulating progenitors are seen as a result of their premature release into the circulation (2). Malignant CML cells are characterized cytogenetically by a translocation between chromosomes 9 and 22 (t9:22), the Philadelphia chromosome (3), and at the molecular level by the bcr/abl fusion oncogene (4).

Unregulated proliferation and abnormal circulation of CML malignant progenitors can, at least in part, be explained by abnormal adhesive interactions between the bone marrow microenvironment and CML progenitors. Close contact between normal progenitors and stroma is thought to be important not only for localization of hematopoiesis in the bone marrow but also for regulation of progenitor cell growth. Normal primitive hematopoietic progenitors present in close contact with bone marrow stroma are usually quiescent (5). This may be the result of increased availability of growth inhibitory cytokines such as transforming growth factor-$\beta$ (TGF-$\beta$) and macrophage inflammatory protein-1$\alpha$ (MIP-1$\alpha$) in proximity with the bone marrow stroma (6–9). Recent studies from our laboratory suggest also that direct contact per se between normal progenitors and stroma may inhibit progenitor proliferation (10, 11). Normal progenitors adhere to stroma through a variety of cell surface adhesion receptors including the $\alpha$4$\beta$1 integrin receptors which can bind to VCAM and the CS-1 region in the COOH-terminal heparin binding domain of fibronectin and $\alpha$5$\beta$1 integrin receptors which recognize the RGD containing cell binding domain of fibronectin (12–16). Unlike normal progenitors, CML progenitors fail to adhere to normal stromal layers (17, 18) and to fibronectin or its proteolytic fragments (18). However, $\alpha$4$\beta$1 and $\alpha$5$\beta$1 integrin receptors are present at normal levels on CML progenitors, which suggests that the function of these receptors may be impaired in CML (18). Since $\alpha$4$\beta$1 and $\alpha$5$\beta$1 receptors may transmit proliferation inhibitory signals from the microenvironment to normal hematopoietic progenitors, decreased adhesion of CML progenitors to the microenvironment through these receptors may underly the observation that CML progenitors in the stromal adherent layer of long-term bone marrow cultures (LTBMC) are continuously proliferating.

Interferon-$\alpha$ has been used successfully in the treatment of CML. Treatment with interferon-$\alpha$ induces hematologic remissions in up to 75% of patients treated. Moreover, interferon-$\alpha$ treatment can result in a gradual, selective suppression of the malignant clone with restoration of normal nonclonal hematopoiesis (19). The mechanism(s) by which interferon-$\alpha$ restores normal hematopoiesis in CML is not clear. It has been demonstrated that interferon pretreatment of normal bone marrow stroma followed by culture of CML progenitors in the continuing presence of interferon results in enhanced adhesion of progenitors to stroma (20). It is, however, not clear if this effect is the result of alterations in the progenitors, the stroma or both.

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1. *Abbreviations used in this paper:* BM, bone marrow; BMMNC, bone marrow mononuclear cells; CFC, colony forming cells; CM, conditioned medium; CML, chronic myelogenous leukemia; LTBMC, long-term bone marrow culture; LTC-IC, long-term culture-initiating cells; MIP, macrophage inflammatory protein.

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In this regard, interferon-$\alpha$ can restore the deficient expression of LFA-3 on CML progenitor cells suggesting that it may similarly enhance expression of other critical adhesion receptors that may be deficient on CML progenitors (21).

To further elucidate the mechanisms whereby interferon-$\alpha$ restores normal hematopoiesis in CML, we studied the effect of interferon-$\alpha$ treatment of CML progenitors on their adhesion to normal BM stroma. Treatment of CML progenitors with interferon-$\alpha$ resulted in enhanced progenitor adhesion to stroma in a dose-dependent fashion. We demonstrate that adhesion of interferon-$\alpha$–treated cells to normal stroma involves the $\alpha 4\beta 1$ and $\alpha 5\beta 1$ integrin receptors and that this is the result of modulation of receptor function rather than an increase in their quantitative expression.

## Methods

### *Bone marrow samples*

14 patients with CML and 9 normal healthy volunteers were evaluated after informed consent was obtained using guidelines approved by the Committee on the Use of Human Subjects at the University of Minnesota. All patients were in the chronic phase of CML and had received treatment with hydroxyurea only. For each patient, treatment was discontinued at least 4 d before study. Heparinized bone marrow samples were obtained by aspiration from the posterior iliac crest. Bone marrow mononuclear cells (BMMNC) were isolated by Ficoll-Hypaque (Sigma Diagnostics, St. Louis, MO) density gradient separation (specific gravity 1.077) for 30 min at 37°C and 400 *g*.

### *Selection of purified progenitor populations*

Selection of purified progenitor populations was performed using methods previously described (22, 23). CML or normal BMMNC were injected into an elutriator system (Beckman Instruments, Palo Alto, CA) with rotor speed maintained at 1,950 rpm and temperature at 10°C (24). After loading, 200 ml of effluent was collected at a flow rate of 12 ml/min for CML bone marrow and 14 ml/min for normal marrow. Fraction 12 or 14 cells were depleted of T lymphocytes and NK cells by sheep erythrocyte rosetting (25) and depleted further of committed B lymphocytic, myelomonocytic and erythroid cells using immunomagnetic beads (Advanced Magnetics Inc., Cambridge, MA) and monoclonal antibodies directed at CD19, CD11b (Becton Dickinson, Mountain View, CA), MY-8 (Coulter Cytometry, Hialeah, FL), and glycophorin-A (Amac, Inc., Westbrook, ME). The resultant lineage negative population (lin−) was labeled with anti-CD34-PE and anti-HLA-DR-FITC antibodies and sorted on a FACStar$^{PLUS®}$ laser flow cytometry system equipped with a CONSORT 32 computer system. Cells were selected for low vertical and horizontal light scatter properties and for expression of CD34 and HLA-DR antigens based on isotype control stains. In normal individuals primitive progenitors capable of initiating LTBMC (long-term culture initiating cells, LTC-IC) are concentrated in the Lin-34+DR− (DR−) fraction and more differentiated progenitors capable of forming colonies in short-term methylcellulose progenitor cultures (colony forming cells, CFCs) in the Lin−34+DR+ fraction (DR+) (22). In CML patients, however, CFC and LTC-IC derived from the malignant clone are both present in the DR+ fraction. The CML DR− fraction on the other hand contains cells that give rise to colonies of nonclonal origin during LTBMC (23).

### *Bone marrow stromal layers*

Bone marrow stromal layers were established in T-75 or T-150 flasks by plating normal bone marrow in LTBMC medium (IMDM; Gibco Laboratories, Grand Island, NY with 12.5% fetal calf serum [Hyclone Laboratories Inc., Logan, UT], 12.5% horse serum [Terry Fox Laboratories, Vancouver, BC, Canada], 2 mM L-glutamine, penicillin [1,000 U/ml] and streptomycin [100 U/ml; Gibco] and $10^{-6}$ M hydrocortisone [A-Hydrocort; Abbott Laboratories, North Chicago, IL]). Confluent stromal layers were formed after 4–5 wk of culture and were irradiated at 1,250 cGy using a Cesium irradiator to eliminate hematopoietic cells. Adherent cells collected after digestion with 0.1% collagenase (Boehringer Mannheim Corp., Indianapolis, IN) were subcultured at a concentration of 350,000 stromal cells/well in 24-well plates (Costar Corp., Cambridge, MA) (22).

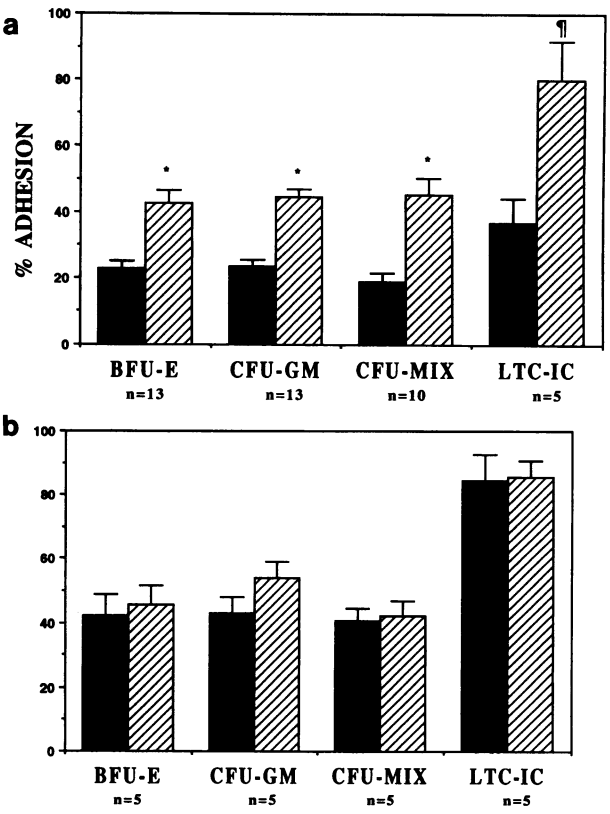


*Figure 1.* Increased adhesion of CML but not normal progenitors to normal bone marrow stroma after interferon-$\alpha$ treatment. CML DR+ cells (*a*) and normal DR− and DR+ cells (*b*) were preincubated with interferon-$\alpha$ (10,000 U/ml) for 48 h. Cells were washed, panned, or plated on normal stromal layers for 2 h, and then replated in either methylcellulose progenitor culture or LTBMC to assay for CFCs and LTC-ICs, respectively. Percent adhesion of progenitors was calculated by dividing the number of progenitors in cultures initiated with panned cells by the number of progenitors in cultures initiated with plated cells. Results are expressed as mean±SEM. Black bars represent adhesion in the absence of interferon, while cross-hatched bars represent adhesion after interferon exposure. Significance levels: comparison of adhesion of progenitors with and without interferon-$\alpha$ treatment; $*P < 0.0001$, $\P P < 0.05$. The number of BFU-E, CFU-GM, CFU-MIX, and LTC-IC in 5,000 CML DR+ cells was 210.6±25.7, 97.8±12.3, 15.3±1.8 and 236.4±53.7, respectively. The number of BFU-E, CFU-GM and CFU-MIX in 5,000 normal DR+ cells was 119.7±20.6, 128.9±15.8 and 15.9±2.6, respectively. The number of LTC-IC in 5,000 normal DR− cells was 105.9±15.9.

### *Cell adhesion assays*

Normal DR− and DR+ and CML DR+ progenitor cells were incubated in bone marrow stroma conditioned medium (CM) containing interferon-$\alpha_{2b}$ (Schering Corp., Kenilworth, NJ) at 37°C in a humidified atmosphere with 5% $CO_2$. The interferon concentrations used ranged from 100 to 10,000 U/ml. Cells were exposed to interferon-$\alpha$ for time periods ranging from 20 min to 48 h. A control population of cells was incubated under identical conditions but without interferon-$\alpha$. After incubation, cells were washed three times to remove excess unbound interferon,

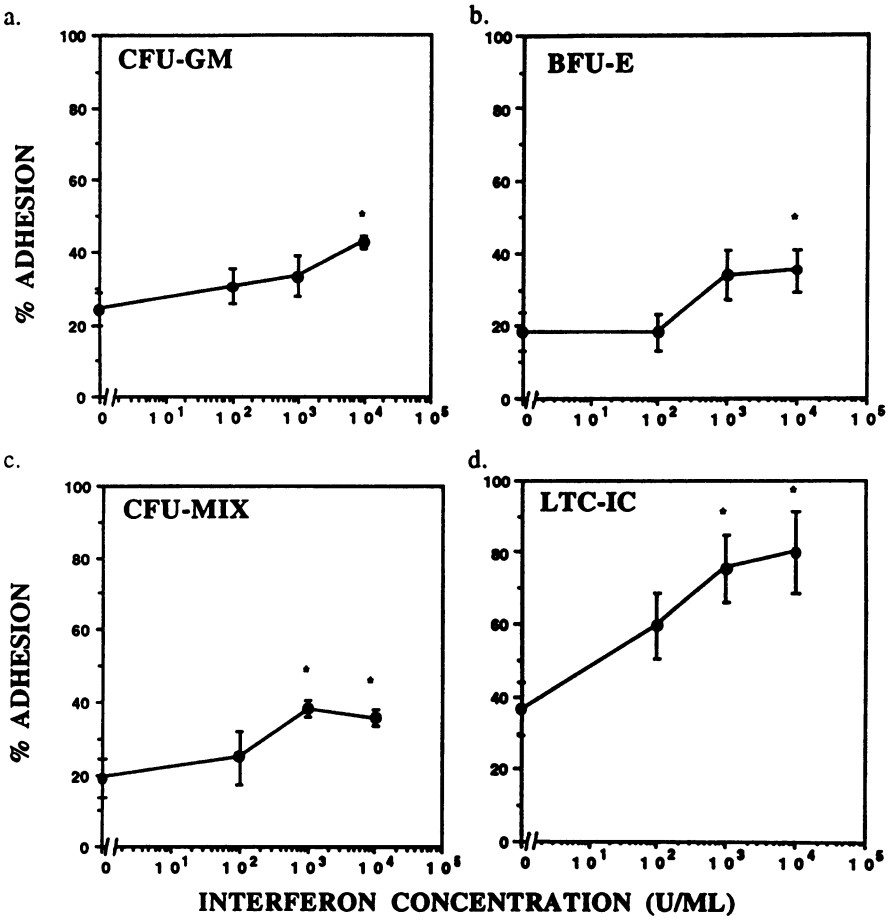


*Figure 2.* Interferon-$\alpha$ enhances adhesion of CML progenitors to normal bone marrow stroma in a dose-dependent manner. CML DR+ cells ($n$ = 5) were preincubated with increasing concentrations of interferon-$\alpha$ for 48 h. Cells were washed, panned and plated on normal stromal layers for 2 h, and then replated in either methylcellulose progenitor culture or LTBMC. Percent adhesion of ($a$) CFU-GM, ($b$) BFU-E, ($c$) CFU-MIX, and ($d$) LTC-IC was calculated by dividing the number of progenitors in cultures initiated with panned cells by the number of progenitors in cultures initiated with plated cells. Results are expressed as mean±SEM. Comparison of adhesion of progenitors with and without interferon-$\alpha$ treatment; $*P < 0.05$.

resuspended at 5,000 cells/ml in LTBMC media and coincubated with normal bone marrow stromal layers in 24-well plates for 2 h at 37°C. Nonadherent cells were removed by three vigorous washes using warm IMDM (panning). Tightly adherent progenitor cells were harvested using trypsin and plated in short-term methylcellulose assay to evaluate the percent adherent CFCs and in LTBMC culture to evaluate the percent adherent LTC-ICs (22). For normal progenitors, DR+ cells were used for CFC assays and DR− cells for the LTC-IC assays. For CML patients, DR+ cells were used in both assays. The percent adherent progenitors was calculated as: [the number of progenitors adherent to stroma (panned cells) divided by the total input of progenitor cells (plated cells)] × 100. Experiments were carried out simultaneously with normal and CML progenitors.

### *Adhesion inhibition assays*

CML DR+ cells were incubated for 48 h with or without 10,000 U/ml interferon-$\alpha$ in stroma conditioned medium in a humidified atmosphere with 5% $CO_2$ at 37°C. Cells were washed three times and resuspended in LTBMC media at 5,000 cells/well. Interferon treated CML DR+ cells or untreated CML and normal DR+ cells were incubated with the indicated antibodies for 30 min before the adhesion assays. Alternatively, stroma was incubated with antibodies to VCAM or ICAM for 30 min before the adhesion assays. Progenitors were then plated in contact with stroma in the presence of the indicated antibodies for 2 h after which nonadherent cells were removed and adherent cells plated in methylcellulose progenitor culture as described above. Percent adhesion of interferon-treated CML progenitors in the presence of these antibodies was calculated as [adhesion in the presence of antibody divided by adhesion in the absence of antibody] × 100%.

### *Progenitor culture*

*Long-term bone marrow culture.* Long-term bone marrow cultures were established by plating or panning 5,000 CML DR+ cells or normal DR− cells in direct contact with normal stromal layers subcultured in 24-well plates. Cultures were maintained for 5 wk in a humidified atmosphere, at 37°C and 5% $CO_2$. Weekly media changes were carried out by removing half the cell free supernatant medium and replacing it with fresh LTBMC medium. To determine the number of CFC present in long-term cultures, cells were harvested by digesting the stromal layers with trypsin and replated in methylcellulose progenitor culture (22).

*Short-term methylcellulose progenitor culture.* DR+ cells recovered from panning assays or the progeny of cells in LTBMC were plated in methylcellulose (final concentration 1.12%) (Fisher Scientific, Fair Lawn, NJ) with IMDM supplemented with 30% FCS, antibiotics, 5 × $10^{-5}$ M 2-mercaptoethanol (Sigma Chemical Co.), 3 IU recombinant erythropoietin (Epoetin; Amgen Biologicals, Thousand Oaks, CA) and 4 ng/ml recombinant interleukin 3 (a kind gift from Dr. Wong, Genetics Institute, Boston, MA). Cultures were incubated in a humidified atmosphere at 37°C and 5% $CO_2$ for 14–18 d. The cultures were then assessed for the presence of CFU-GM, BFU-E, and CFU-MIX colonies as previously described (25).

### *FACS analysis of cell surface adhesion receptors on CML progenitors*

Lineage negative CML cells were incubated with or without interferon-$\alpha$ in stroma-conditioned medium for 48 h, washed, and labeled with antibodies to adhesion receptors in conjunction with antibodies to CD34

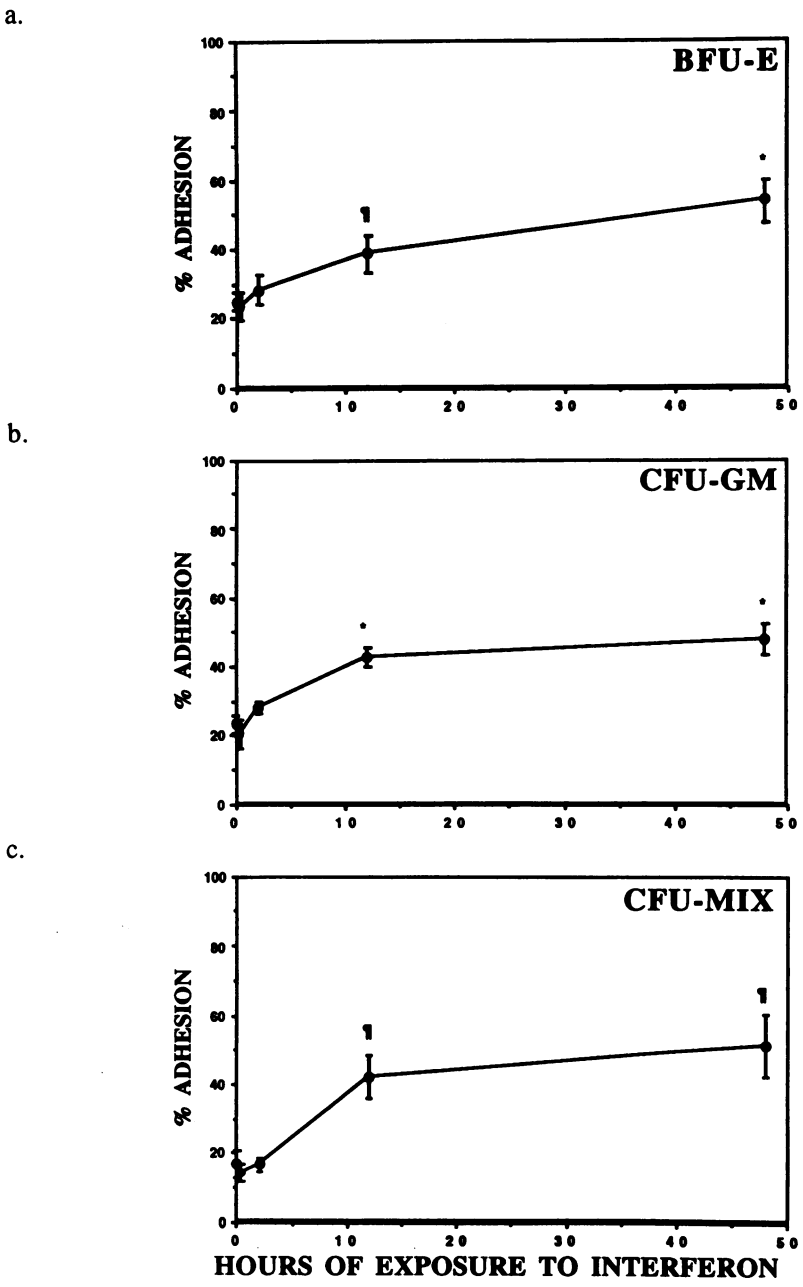


*Figure 3.* Effect of duration of preincubation with interferon-$\alpha$ on the adhesion of CML progenitors to normal stroma. CML DR+ cells ($n$ = 4) were preincubated with interferon-$\alpha$ (10,000 U/ml) for time periods ranging from 20 min to 48 h. Cells were washed, panned and plated on normal stromal layers for 2 h, and then replated in methylcellulose progenitor culture. Percent adhesion of ($a$) CFU-GM, ($b$) BFU-E and ($c$) CFU-MIX was calculated by dividing the number of progenitors in cultures initiated with panned cells by the number of progenitors in cultures initiated with plated cells. Results are expressed as Mean±SEM. Significance levels: comparison of adhesion of progenitors with and without interferon-$\alpha$ treatment; $*P < 0.005$, $\P P < 0.05$.

as follows: 100,000 lineage negative cells were sequentially incubated with primary antibodies to the $\alpha$4, $\alpha$5, and $\beta$1 integrins, FITC conjugated goat anti-mouse antibody (Tago Inc., Burlingame, CA), mouse IgG (Sigma, Chemical Co.) to block unoccupied binding sites on the goat anti-mouse antibody and PE-conjugated anti-CD34 antibody (Becton Dickinson). Controls were labeled sequentially with control mouse IgG, goat anti-mouse FITC, mouse IgG, and then either $IgG_1$-PE or anti-CD34-PE. Cells with low horizontal and vertical light scatter properties were selected and the expression of adhesion receptors on cells coexpressing CD34 within this population measured using FACS®.

### *Antibodies*

Antibodies against $\alpha$-4 (P4C2), $\alpha$-5 (P3D10), $\beta$-1 (P5D2), ICAM (P4F11) and VCAM (P8B1) were developed by Dr. Elizabeth Wayner. Anti-CD44 Hermes 3 was a kind gift from Dr. Eugene Butcher, Palo Alto, CA and antibody 50B4 was a kind gift from Dr. Dominique Letarte, Toronto, Canada. Mouse IgG was obtained from Sigma Chemical Co.

### *Statistical analysis*

Results of experimental points obtained from multiple experiments were reported as mean±SEM. Significance levels were determined by two-sided $t$ test analysis.

## Results

*Interferon-$\alpha$ treatment of CML progenitors restores their adhesion to stroma.* The effect of interferon-$\alpha$ on the adhesion of CML and normal progenitors to normal stroma was assessed by incubating CML progenitors for 48 h in the presence or absence of interferon-$\alpha$ before performing adhesion assays. As we have previously demonstrated, adhesion of malignant LTC-IC present in the CML DR+ population to normal bone marrow stroma is significantly decreased (18). Similarly, CFC present

in the CML DR+ population adhere significantly less to normal stroma compared with normal CFC. Interferon-$\alpha$ treatment resulted in a significant increase in the adhesion of CML LTC-IC to normal bone marrow stroma ($P < 0.05$, $n = 5$) (Fig. 1 *a*). Similarly, significantly more committed CML progenitors [BFU-E ($P < 0.001$, $n = 13$), CFU-GM ($P = 0.001$, $n = 13$) and CFU-MIX ($P < 0.001$, $n = 10$)] (Fig. 1 *a*) adhered to normal stroma after interferon-$\alpha$ treatment. In contrast, adhesion of normal bone marrow derived LTC-IC, BFU-E, CFU-GM and CFU-MIX was not significantly increased after interferon-$\alpha$ treatment (Fig. 1 *b*). Although growth of CML progenitors could be suppressed following pretreatment with interferon-$\alpha$ [progenitor growth after interferon-$\alpha$ treatment compared with growth of controls not exposed to interferon: BFU-E, 77.9±7.3% ($n = 13$); CFU-GM, 94.8±7.3% ($n = 13$); CFU-MIX, 92.1±4.3% ($n = 10$) and LTC-IC, 89.2±8.5% ($n = 5$)], this did not affect the results of adhesion assays, since the percent adherent progenitor cells was calculated by dividing the number of colonies generated in cultures initiated with panned cells by the number of colonies in cultures initiated with plated cells under identical conditions.

The increased adhesion of interferon-$\alpha$ treated CML progenitors to bone marrow stroma was dose-dependent (Fig. 2). Adhesion to stroma of both CML LTC-IC and CFC did not differ significantly from that of normal progenitors once CML progenitors were pretreated with $1 \times 10^3$ to $1 \times 10^4$ U/ml interferon-$\alpha$. At least 12 h of incubation with interferon-$\alpha$ was required before significantly increased adhesion of CML progenitors to bone marrow stroma was observed (Fig. 3) although a further increase in adhesion could be seen after 48 h of incubation.

*Receptors involved in the adhesion of interferon-$\alpha$–treated CML progenitors to stroma.* The role of $\alpha4\beta1$, $\alpha5\beta1$ and VCAM, known to be involved in the adhesion of normal committed progenitors to BM stroma, in the adhesion of interferon-$\alpha$–treated CML progenitors was evaluated by studying the ability of antibodies directed against these receptors to block adhesion. CML DR+ cells, incubated with or without interferon-$\alpha$ (10,000 U/ml) for 48 h, were washed and allowed to adhere to normal BM stromal layers for 2 h in the presence of antibodies against $\alpha4$, $\alpha5$, $\beta1$, CD44, VCAM, and ICAM-1.

The low level adhesion to stroma of CML CFC, in the absence of interferon-$\alpha$, was not significantly inhibited by antibodies against $\alpha4$, $\alpha5$, $\beta1$, and VCAM [BFU-E: control adhesion 25.9±4.8%, with anti-$\alpha4$ 25.6±5.6%, anti-$\alpha5$ 25.8±4.8%, anti-$\beta1$ 20.8±5.0%, anti-VCAM 30.3±5.13%; CFU-GM: controls 22.3±3.3%, anti-$\alpha4$ 20.8±2.5%, anti-$\alpha5$ 21.3±3.2%, anti-$\beta1$ 26.1±3.0%, anti-VCAM 25.4±2.5%; ($n = 4$)]. This confirms previous observations that $\beta$-1 integrin receptor–mediated adhesion mechanisms are deficient in CML. However, as shown in Fig. 4 *a*, adhesion of CML BFU-E, treated with interferon-$\alpha$, to normal BM stroma was significantly inhibited by antibodies against the $\alpha$-4 ($P < 0.01$), $\alpha$-5 ($P = 0.005$), and $\beta$-1 ($P < 0.001$) integrin receptors as well as to VCAM ($P < 0.05$, $n = 4$). Antibodies against the CD44 receptor (50B4 and Hermes-3) and ICAM-1 did not have a significant effect on adhesion. Similar results were obtained for CML CFU-GM (Fig. 4 *b*). The effect of these antibodies on the adhesion of interferon-$\alpha$-treated CML progenitors was similar to their effect on the adhesion of normal progenitors to normal BM stroma. We have previously demonstrated that adhesion of normal BFU-E to BM stroma in the presence of anti-$\alpha4$ antibody is 65.8±5.6% of control ($P < 0.05$); anti-$\alpha5$, 24.3±12.6% ($P < 0.001$), and anti-$\beta1$, 28.0±10.4% ($P < 0.001$) ($n = 4$). Similarly, adhesion of normal CFU-GM in the presence of anti-$\alpha4$ is 65.8±6.9% of control ($P < 0.01$); anti-$\alpha5$, 42.3±12.1% ($P < 0.01$) and anti-$\beta1$, 45.3±13.1% ($P < 0.01$) ($n = 4$) (16). Adhesion of normal BFU-E and CFU-GM to BM stroma in the presence of anti-VCAM was 62.9±3.7% ($P < 0.001$) ($n = 7$) and 58.7±6.3% ($P < 0.001$) ($n = 7$) of control, respectively. These results indicate that interferon-$\alpha$ treatment of CML progenitors results in enhanced adhesion to stroma by restoring $\beta1$ integrin receptor–dependent mechanisms.

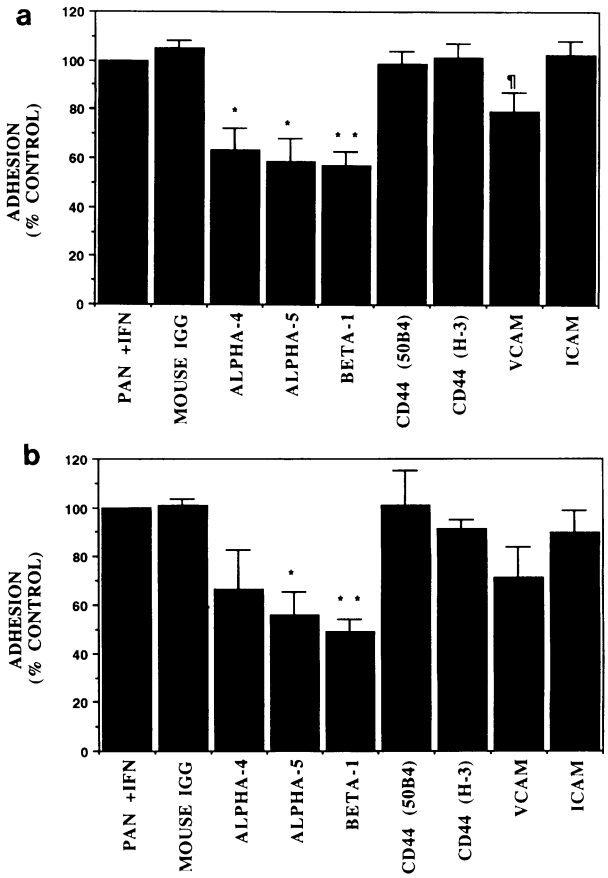


*Figure 4.* Increased adhesion of interferon-$\alpha$ treated CML progenitors to normal stroma involves $\alpha4\beta1$ and $\alpha5\beta1$ integrin receptors. CML DR+ cells ($n = 4$) were treated with interferon-$\alpha$ (10,000 U/ml) for 48 h, washed and subsequently plated and panned on normal stromal layers in the presence of blocking antibodies to the indicated receptors and control mouse IgG. Cells were replated in methylcellulose progenitor culture and the effect of these blocking antibodies on the adhesion of BFU-E (*a*) and CFU-GM (*b*) was assessed. Results are shown as the adhesion of progenitors in the presence of antibody expressed as the percentage of adhesion in controls without antibody. Significance levels; $*P < 0.01$, $**P < 0.001$, $\P P < 0.05$ ($n = 4$). The number (and percent) of adherent BFU-E and CFU-GM in 5,000 CML DR+ cells was 71.4±15.8 and 28.8±3.3, respectively.

*Expression of cell surface adhesion receptors on CML progenitors after interferon-$\alpha$ treatment.* To determine if the increased adhesion through $\alpha4\beta1$ and $\alpha5\beta1$ adhesion receptors was the result of increased expression of these receptors, lineage negative cells from three CML patients were incubated with interferon (10,000 U/ml) for 48 h. Cells were washed and

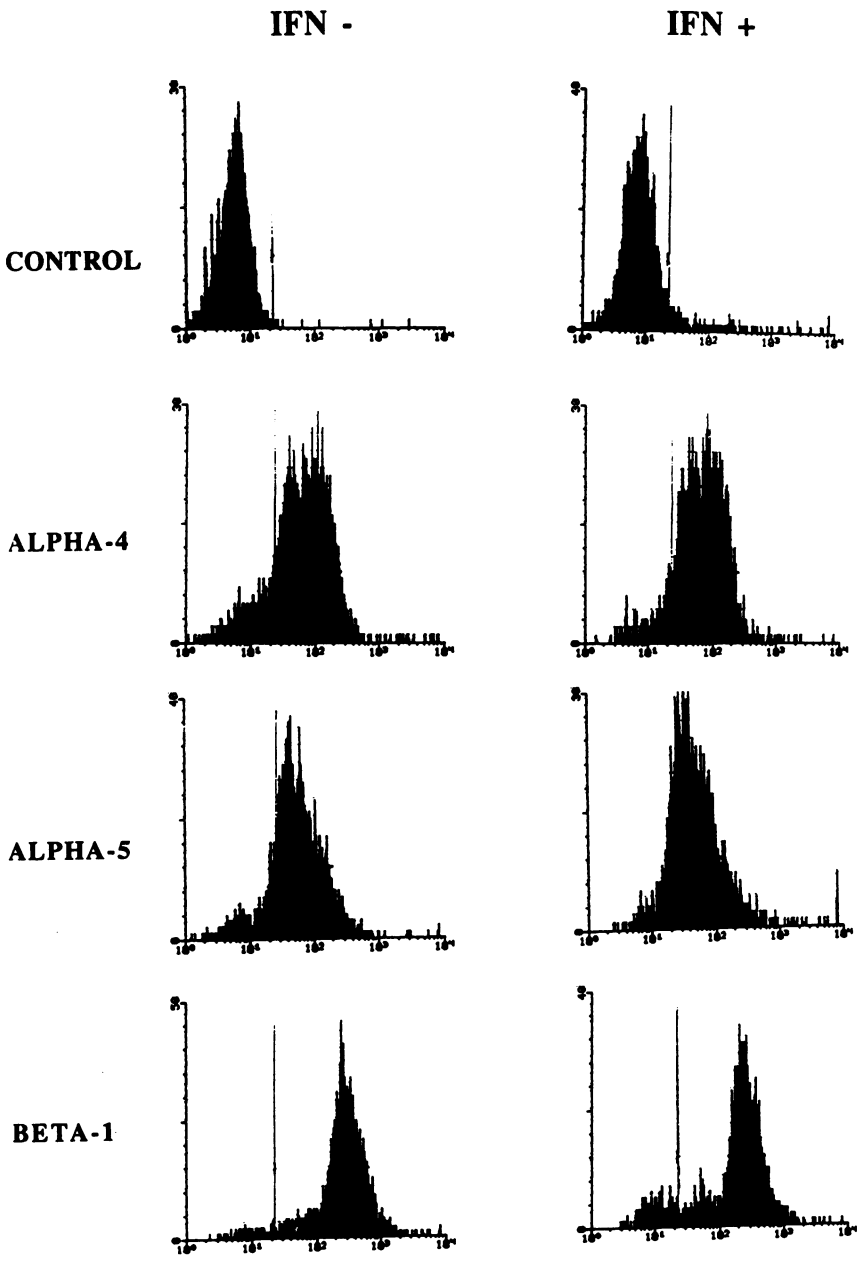


*Figure 5.* Interferon-$\alpha$ treatment does not change the level of expression of $\alpha$4, $\alpha$5 and $\beta$1 integrin receptors on CML progenitors. Lineage negative CML cells incubated with or without interferon-$\alpha$ (10,000 U/ml) for 48 h, were labeled with antibodies to the $\alpha$4, $\alpha$5, and $\beta$1 integrin receptors in conjunction with antibodies to CD34. The expression of adhesion receptors on cells coexpressing CD34 was measured using FACS®. Representative results from one patient are shown. Three patients were studied in this manner. No change in $\alpha$4, $\alpha$5, and $\beta$1 receptor expression was observed after interferon-$\alpha$ treatment.

labeled with antibodies against integrin receptors and CD34 and analyzed using FACS®. Cells with low vertical and horizontal light scatter properties and expressing high levels of CD34 antigen were evaluated for expression of integrin receptors by frequency histogram. Fig. 5 shows representative histograms obtained from one such patient. As we have previously demonstrated, CML CD34+ cells express similar levels of the $\alpha$4, $\alpha$5, and $\beta$1 integrins as normal CD34+ cells (18). No change in receptor expression was observed after interferon-$\alpha$ treatment, suggesting that interferon-$\alpha$ may restore $\beta$-1 integrin receptor function rather than up-regulating the level of receptor expression.

## Discussion

Interferon-$\alpha$ treatment of CML can result in the selective suppression of malignant hematopoietic progenitors and resumption of normal hematopoiesis (19). The mechanism(s) whereby interferon achieves this effect are not clear. It has been demonstrated that CML progenitors, which fail to adhere to normal bone marrow stroma, can adhere to normal stromal layers after treatment of stroma with interferon-$\alpha$ (20). We demonstrate here that interferon-$\alpha$ can increase adhesion of CML progenitors to stroma through a direct effect on the progenitors involving upregulation of $\alpha4\beta1$ and $\alpha5\beta1$ integrin receptor function. Restoration of normal adhesive interactions between CML progenitors and stroma may then result in restoration of normal growth regulatory mechanisms, which in normal individuals are thought to be dependent on close progenitor–stroma interactions (5, 6, 10, 11).

Integrins are heterodimeric receptors consisting of $\alpha$ and $\beta$ chains which possess a large extracellular domain, a transmembrane domain and a short intracellular domain (26). The role of integrin receptors in hematopoiesis has recently gained interest.

$\alpha 4\beta 1$ and $\alpha 5\beta 1$ integrin receptors are present on normal progenitors and are involved in the adhesion of these progenitors to stroma (12–16). We have previously demonstrated that CML progenitors express similar levels of cell surface $\alpha 4\beta 1$ and $\alpha 5\beta 1$ integrin receptors as normal progenitors (18). However, CML progenitors are deficient in their ability to adhere to stroma or to purified fibronectin and fibronectin proteolytic fragments (18, 19) suggesting that these receptors are present in a functionally inactive state. A variety of studies have demonstrated that the mere presence of integrin receptors does not necessarily indicate that the receptor has functional significance (26). Integrins can be present in a nonfunctional low affinity state that can be switched to a high affinity state after stimulation of the cell with cytokines (9), through other cell surface receptors (27) or through its own ligands (28). The mechanisms underlying the decreased affinity of $\alpha 4\beta 1$ and $\alpha 5\beta 1$ integrins on CML progenitors are not clear.

One possibility is that the presence of the $p210^{bcr/abl}$ tyrosine kinase in CML progenitors results in phosphorylation and deactivation of $\beta 1$ integrins. Similar mechanisms have been suggested for other malignancies. Transformation of fibroblasts by the Rous sarcoma virus results in their attachment independent growth (29). It has been demonstrated that colocalization of $pp^{60src}$ with $\beta 1$ integrins at the cell membrane is associated with phosphorylation of the $\beta 1$ chain, which may result in decreased affinity of the $\beta 1$ integrin receptor (30). Similarly the abnormal $p210^{bcr/abl}$ phosphotyrosine kinase is also located in the cytoplasm (31, 32). Colocalization of $p210^{bcr/abl}$ and $\beta 1$ integrins may then lead to deactivation of the $\beta 1$ integrins through abnormal phosphorylation. In addition, impaired $\beta 1$ integrin function may result from abnormal phosphorylation of cytoskeletal or other proteins closely associated with the integrin receptor as has recently been demonstrated for bcr/abl transfected hematopoietic cell lines (33). Recent studies have demonstrated that interferon-$\alpha$ may prevent transcription of the bcr/abl mRNA, and hence expression of the $p210^{bcr/abl}$ tyrosine kinase (34). Removal of the $p210^{bcr/abl}$ neoprotein and its possible effect on integrin affinity may then explain the restored adhesion of progenitors following interferon-$\alpha$ treatment. The time required for interferon-$\alpha$ to cause enhanced adhesion of CML progenitors to stroma is consistent with an effect mediated by alteration in mRNA and/or protein synthesis. It is also possible that interferon-$\alpha$ may act on CML progenitors to upregulate certain other adhesion receptors, which we have not evaluated, such as members of the selectin family (35). Stimulation of CML progenitors through these receptors could subsequently result in enhanced affinity of $\beta 1$ integrin receptors (27).

Adhesion through integrins serves not only to localize cells within specific microenvironments, but may also have profound effects on cell growth and differentiation. Engagement of integrins results in cytoskeletal rearrangement (36), tyrosine phosphorylation of certain cytoplasmic proteins (37), and, depending on the cell type, in either increased proliferation (38), with associated increased expression of AP-1 (fos/jun) (39), or reduced proliferation and induction of differentiation (40). The importance of signal transduction through integrin receptors on hematopoietic progenitors has been less well studied. Normal primitive progenitors adherent to bone marrow stroma are usually quiescent (5). Recent studies from our laboratory suggest that inhibition of proliferation of normal progenitors in contact with stroma may result from the adhesive interaction itself with stroma and extracellular matrix, specifically fibronectin (10, 11). It is therefore possible that impaired integrin receptor function on CML progenitors (18) may lead to reduced growth inhibition, thereby providing an explanation for the observation that CML progenitors present within the adherent layer of stromal cultures are continuously proliferating (1). Modulation of $\alpha 4\beta 1$ and $\alpha 5\beta 1$ function on CML progenitors by interferon-$\alpha$ may then restore normal growth inhibitory signal transduction pathways, leading to normal regulation of proliferation. In addition, inhibition of proliferation of normal progenitors as a result of adhesion to stroma may result from colocalization of progenitors with negative growth regulatory factors, such as transforming growth factor-$\beta$ or macrophage inflammatory protein-$1\alpha$, which are bound and concentrated by the extracellular matrix (6–9). Such interactions would be expected to be impaired in CML because of impaired adhesion to stroma. Restored adhesion induced by interferon-$\alpha$ may then allow normal regulation of CML progenitors by these negative growth regulatory factors.

In conclusion, the present study demonstrates that interferon-$\alpha$ restores normal adhesive interactions between CML progenitors and the bone marrow microenvironment through upregulation of $\beta 1$ integrin function. Further studies will be required to characterize the functional defect of abnormal $\beta 1$ integrin receptors in CML and the mechanisms by which interferon-$\alpha$ modulates receptor function. This knowledge may help in the development of improved treatment strategies for this disease.

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