Signaling Pathways Implicated in Hematopoietic Progenitor Cell Proliferation and Differentiation

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The objective of this study was to investigate the signal transduction pathways associated with the clonal development of myeloid and erythroid progenitor cells. The contribution of particular signaling molecules of protein tyrosine kinases (PTKs), mitogen-activated protein (MAP) kinase, and PI-3 kinase signaling to the growth of murine bone marrow colony forming unit–granulocyte-macrophage (CFU-GM) and erythroid (burst forming unit-erythroid [BFU-E] and colony forming unit-erythroid [CFU-E]) progenitors was examined in studies performed in the presence or absence of specific signal transduction inhibitors. The results clearly pointed to different signal transducing intermediates that are involved in cell proliferation and differentiation depending on the cell lineage, as well as on the progenitors' maturity. Lineage-specific differences were obtained when chemical inhibitors specific for receptor- or nonreceptor-PTKs, as well as for the main groups of distinctly regulated MAPK cascades, were used because all of these compounds suppressed the growth of erythroid progenitors, with no major effects on myeloid progenitors. At the same time, differential involvement of MEK/extracellular signal-regulated kinase (ERK) MAPK transduction pathway was observed in the proliferation and/or differentiation of early, BFU-E, and late, CFU-E, erythroid progenitor cells. The results also demonstrated that phosphatydylinositol (PI)-3 kinase and nuclear factor kappaB (NF-κB) transcriptional factor were required for maintenance of both myeloid and erythroid progenitor cell function. Overall, the data obtained indicated that committed hematopoietic progenitors express a certain level of constitutive signaling activity that participates in the regulation of normal steady-state hematopoiesis and point to the importance of evaluating the impact of signal transduction inhibitors on normal bone marrow when used as potential therapeutic agents.
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Introduction

Hematopoiesis is a dynamic process resulting in continuous production of various mature blood cells from a small population of pluripotent stem and progenitor cells through diverse proliferative and differentiative events. Different biological functions of hematopoietic cells are regulated coordinately by multiple growth factors and cytokines, as well as by interactions with other cells and extracellular matrix. Although the effects of hematopoietic regulators are well described, the exact intracellular signaling events leading to various cellular responses are not fully understood.

Studies conducted with a variety of hematopoietic growth factors and cytokines have provided evidence that binding of ligands to their receptors is able to activate multiple signal transduction intermediates, especially the Janus kinase (Jak)/signal transducers and activators of transcription (Stat), the mitogen-activated protein (MAP) kinase and the phosphatydylinositol (PI)-3 kinase pathways. Early insight into the cellular signaling led to the identification of a family of nonreceptor protein tyrosine kinases (PTKs) called Jak and their target proteins Stats, which bind to specific DNA motifs and modulate the expression of target genes mediating diverse biological processes, including cell growth, differentiation, migration, and apoptosis (1, 2). Beside Jaks, many other receptor and nonreceptor PTKs were shown to be key mediators of physiological cellular responses such as proliferation, differentiation, motility, and survival (3, 4). The members of three major groups of distinctly regulated MAP kinase cascades, the p38 family, the extracellular signal-regulated kinase (ERK) family and c-Jun amino-terminal kinase (JNK) family, also participate and mediate effects in the generation of different cellular responses, including gene

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transcription, induction of cell death or maintenance of cell survival, regulation of cell-cycle progression, cell growth and cell differentiation (5, 6). PI-3 kinase signaling pathway involves membrane phospholipids, which activate downstream targets, such as Akt serine-threonine kinase and influence many different cellular processes, including cell survival, cell-cycle progression, proliferation, and cytoskeletal reorganization (7). As an important component of cell survival machinery, PI-3K-Akt pathway activates several transcription factors, including nuclear factor kappaB (NF-κB), known to induce a number of genes involved in cell survival, activation, and regulation of immune response (8).

Accumulating evidence indicates that all the hematopoietic growth factors and cytokines seem able to activate all the major signal transduction pathways simultaneously. Thus, the Jak/Stat pathway is implicated in interleukin (IL)-3, IL-6, erythropoietin (Epo), and granulocyte-macrophage–colony stimulating factor (GM-CSF) signaling (1), the MAP kinase pathway in Epo, GM-CSF, granulocyte-colony stimulating factor (G-CSF), and IL-3 signaling (6), and the PI-3 kinase pathway in Epo, IL-6, GM-CSF, G-CSF, and macrophage-colony stimulating factor (M-CSF) signaling (9). However, the pleotropic nature of both cytokines and the signal transduction pathways they activate has prompted questions about the signal specificities that lead to the unique biological events of the particular cytokine, as well as the specificities that determine lineage-specific blood cell differentiation.

The objective of this work was to analyze whether similar or different signaling pathways are involved in the proliferation and differentiation of murine myeloid and erythroid progenitor cells. With the help of specific inhibitors of known signal transduction pathways, we have examined the contribution of particular signaling molecules of PTKs signaling, MAP kinase, and PI-3 kinase pathways in the growth of murine bone marrow colony-forming unit–granulocyte-macrophage (CFU-GM) and erythroid (burst forming unit-erythroid [BFU-E] and colony forming unit-erythroid [CFU-E]) progenitor cells. In addition, we investigated whether NF-κB activation is required for hematopoietic progenitor cell colony formation. Since CFU-GM and BFU-E progenitors were simultaneously assayed, in the presence of the same cytokine combination, differences observed in their growth while using specific inhibitors, pointed to different signal transduction intermediates regulating myeloid and erythroid progenitor cell proliferation. On the other hand, differences in the inhibitory activity on the growth of early, BFU-E, and late erythroid progenitors, CFU-E, indicate signaling molecules differentially involved in the erythroid colony growth dependent on progenitors' maturity.

Materials and Methods

Animals. Adult male inbred CBA mice, 6 to 8 weeks old, weighing 20 to 22 g, were purchased from the Breeding Facilities of the Institute for Medical Research, Military Medical Academy, Belgrade, and used as donors of bone marrow cells. The animals were housed under conventional conditions, with food and water provided *ad libitum*. The experimental protocols were approved by the Institute for Medical Research Institutional Animal Care and Use Committee.

Chemicals and Reagents. Signal transduction inhibitors were obtained from Tocris Cookson Ltd (Bristol, UK). As PTKs reagents genistein (specific inhibitor of protein tyrosine kinases including epidermal growth factor receptor [EGFR] kinase), AG490 (Jak2/Jak3 inhibitor, also EGFR-kinase inhibitor), and PP2 (selective inhibitor of Src-family tyrosine kinases) were used. MAPK reagents SB203580, SP600125, and PD98059 were used as selective inhibitors of p38, JNK, and MEK1/2-ERK1/2, respectively. To inhibit PI-3 kinase a highly selective inhibitor-Ly 294002, a potent, selective and irreversible inhibitor, Wortmanin, and nonselective inhibitor, Quercetin, were used. The cell-permeable antioxidant pyrrolidine dithiocarbamate (PDTC), used as potent inhibitor of NF-κB activation, was also obtained from Tocris Cookson. MTT (3-(4,5-dimethyl-thiazol-2-yl)-2,5 diphenyl-tetrazolium bromide) was purchased from ICN Biomedicals (Aurora, Ohio). Dulbecco's modified Eagle's medium (DMEM), fetal calf serum (FCS), and analytical grade chemicals were purchased from Sigma (St. Louis, MO). Semisolid methylcellulose media (MethoCult M3434 and MethoCult M3334) were obtained from Stem Cell Technologies (Vancouver, BC, Canada).

Cells and Cell Culture. Mice were sacrificed and their femurs were removed for processing and subsequent cell preparation. Bone marrow cells were flushed out of the femurs with DMEM and pooled cell suspensions were prepared in DMEM supplemented with 10% FCS.

MTT Assay. Cell viability and reagent cytotoxicity were measured by a colorimetric assay using MTT originally developed by Mosmann (10), based on the ability of cells to convert soluble MTT into an insoluble formazan. In brief, freshly isolated bone marrow cells (5×10^5cells/100 μl/well) were plated in triplicate in 96-well microtitre flat-bottomed plates in the absence or presence of increasing concentrations of a particular inhibitor and were cultured at 37°C in a humidified atmosphere with 5% CO_2 in air for 1 or 24 hrs. Ten microliters of 5 mg/ml of MTT were then added to each well and incubated at 37°C in a humidified atmosphere for additional 3 hrs. The solution was then removed and formazan salts dissolved with sodium dodecyl sulfate (SDS)-HCl (10% SDS-0.1 *N* HCl) during overnight incubation. The optical densities were measured at 540 nm in a microplate reader. Cell viability was defined relative to corresponding control value from the same time point (i.e., relative cell viability = absorbance of treated sample/absorbance of control sample). The experiments were repeated at least two times.

Hematopoietic Progenitor Cell Assays. The

number of CFU-GM–, BFU-E–, and CFU-E–derived colonies was determined using semisolid media purchased from Stem Cell Technologies. To investigate the role of signal transduction pathways in the growth of normal murine hematopoietic cell progenitors, we performed experiments in which bone marrow cells were cultured in methylcellulose in the presence or absence of specific signal transduction inhibitors. For CFU-GM and BFU-E progenitor assays, the same methylcellulose medium supplemented with 50 ng/ml recombinant mouse stem cell factor (rmSCF), 10 ng/ml rmIL-3, 10 ng/ml recombinant human IL-6 (rhIL-6), and 3 units/ml rhEpo (MethoCult GF M3434, Stem Cell Technologies) was used and expected to discriminate between cell lineage specifications. Bone marrow cells (1×10^5) were plated in duplicate in 35-mm tissue culture plates (Falcon, BD Biosciences, Franklin Lakes, NJ) in 1ml methylcellulose medium in the absence or presence of increasing concentrations of inhibitors. Colony formation was assessed 7 days later, after incubation at 37°C in a humidified atmosphere with 5% CO_2 in air. CFU-E progenitors were assayed in methylcellulose medium containing only 3 units/ml erythropoietin, without other cytokines (MethoCult M3334, Stem Cell Technologies), thus enabling to discriminate the erythroid colony growth dependent on progenitors' stage of differentiation. Bone marrow cells (2×10^5/ml) were also plated in duplicate in the absence or presence of various concentrations of inhibitors and incubated for 2 days at 37°C in a humidified atmosphere with 5% CO_2 in air. The experiments were conducted at least three times.

Statistical Analysis. Statistical analysis was performed by Student's *t* test, using the Origin PC program (OriginLab Corp., Northampton, MA) with the actual numbers of each investigated parameter, and *P* values less than 0.05 were considered to be significant. Data are presented as the percentage of the value for the corresponding control (100%).

Results

Viability of the Cells. Initially, we carried out a series of viability/cytotoxicity tests to determine the workable concentrations of the kinase inhibitors used. Bone marrow cells were treated with increasing concentrations of each inhibitor, ranging from 0.01–500 μ*M*, for up to 24 hrs, and then viability was measured by MTT assay (data not shown). For further experiments on signal transduction inhibition in hematopoietic progenitor cell assays, each compound was applied at concentrations that did not cause appreciable loss of bone marrow cells viability after continuous exposure for 24 hrs.

Effect of Protein Tyrosine Kinase Inhibitors on Bone Marrow Cell Colony Formation. Three different inhibitors were used as PTKs reagents: Genistein, phytoestrogen, which inhibits receptor tyrosine kinases, especially EGFR-kinase; AG490, tyrphostin, which selectively inhibits Jak2 and Jak3 protein tyrosine kinases; and PP2, selective inhibitor of Src family tyrosine kinases. Despite different specificities, all three tested PTKs inhibitors significantly inhibited only the growth of erythroid progenitors, with no major effects on the formation of CFU-GM–derived colonies. At the tested concentrations, 0.1–25 μ*M*, Genistein inhibited the growth of BFU-E–derived colonies by 15% to 33% and CFU-E–derived colonies by 36% to 58% (Fig. 1A). As shown in Figure 1B, AG490 also suppressed only the growth of erythroid progenitors, expressing more profound effect on CFU-E formation. Namely, AG490 inhibited the formation of CFU-E by almost 50% over a range of 0.01–10 μ*M*, while the suppression of BFU-E was marked only at higher concentrations (10 and 25 μ*M*). The selective inhibitor of Src family tyrosine kinases, PP2, also inhibited the colony formation for both BFU-E and CFU-E progenitors, but expressed more prominent effects on BFU-E. At the concentrations used, 0.1 to 10 μ*M*, PP2 suppressed the CFU-E colony formation by 5% to 40% and BFU-E colony formation by 15% to 83% (Fig. 1C).

Effect of MAP Kinase Inhibitors on Bone Marrow Cell Colony Formation. To provide evidence for the involvement of MAP kinases in the hematopoietic progenitor cells'growth, murine bone marrow cells were treated with specific pharmacological inhibitors: SB203580 for the p38 MAP kinase pathway, SP600125 as selective JNK inhibitor, and PD98059 for MEK1/2-ERK1/2 signaling. Treatment of bone marrow cells with SB203580 had no significant effect on CFU-GM colony formation at concentrations ranging from 0.01 up to 25 μ*M*. The inhibition of BFU-E occurred in a dose-dependent manner with maximal inhibition obtained at the concentration of 25 μ*M*, while the profound inhibitory effect of SB203580 on the CFU-E colony formation was evident at concentrations as low as 0.01 μ*M* (Fig. 2A). The presence of SP600125 inhibitor in semisolid media caused approximately 40% and 70% reduction in BFU-E and CFU-E growth, respectively. This effect was noticed with concentrations ranging from 0.01 up to 10 μ*M*, while in higher concentration of 25 μ*M*, SP600125 suppressed the formation of all tested progenitors, including CFU-GM (Fig. 2B). In contrast, the inhibitor of MEK1/2-ERK1/2 signaling, PD98059, at the tested concentrations from 0.1 to 25 μ*M*, had no significant effect on the growth of both CFU-GM and CFU-E progenitor cells, while the reduction in the number of BFU-E that developed ranged from 18% to 38% compared with the control cultures without PD98059 (Fig. 2C).

Effect of PI-3 Kinase Inhibitors on Bone Marrow Cell Colony Formation. To gain insight into the role of PI-3 kinase signaling in murine hematopoietic progenitors' colony growth, the bone marrow cells were assayed with or without increasing concentrations of three PI-3 kinase inhibitors: selective inhibitor-Ly294002, irreversible inhibitor-Wortmanin, and nonselective inhibitor-Quercetin. All three inhibitors exhibit almost the same activity on the myeloid and erythroid progenitors tested. As can be seen in

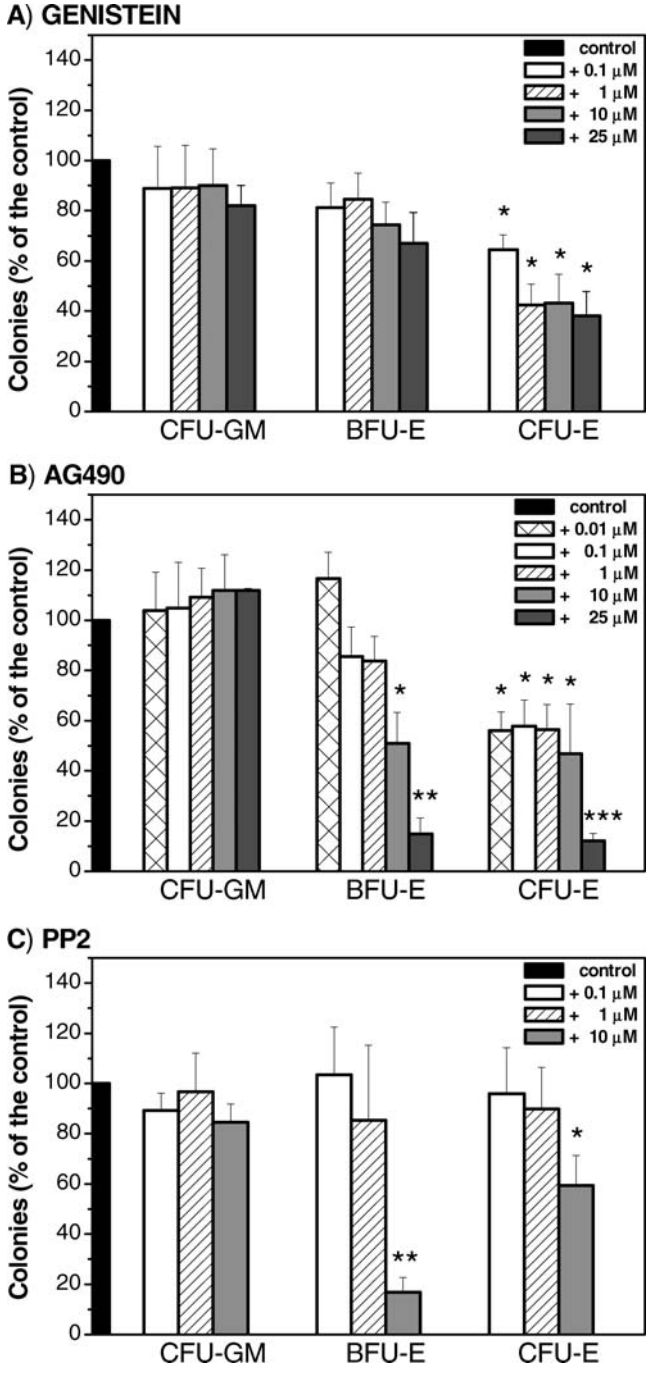

Figure 1. The effect of (A) protein tyrosine kinase inhibitor Genistein, (B) Jak2/Jak3 inhibitor AG490, and (C) Src kinases inhibitor PP2 on CFU-GM–, BFU-E–, and CFU-E– derived colonies grown by bone marrow cells obtained from normal mice. The chemical compounds were directly added to the corresponding MethoCult methylcellulose medium in the indicated final concentrations at the onset of the experiment. The data are shown as percentage of the value for the corresponding control, cultured in the absence of inhibitor (100%). There was an average of 1532.0 ± 62.8 CFU-GM, 228.6 ± 4.1 BFU-E, and 1497.1 ± 145.8 CFU-E per 10^6 bone marrow cells in the controls. The data points represent means ± SEM of three to four experiments each performed in duplicate. Significant difference from the control by *t* test: $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

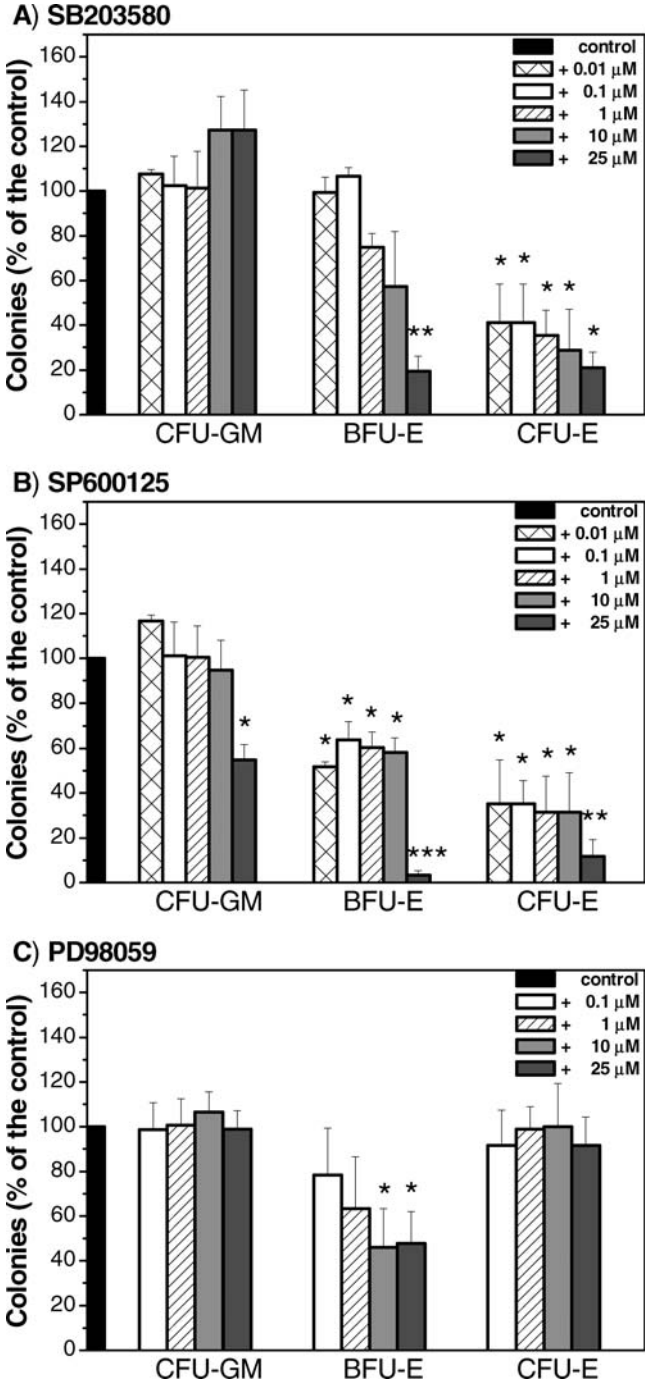

Figure 2. The effect of (A) p38 MAPK inhibitor SB203580, (B) JNK MAPK inhibitor SP600125, and (C) MEK1/2-ERK1/2 inhibitor PD98059 on CFU-GM–, BFU-E–, and CFU-E–derived colonies grown by bone marrow cells obtained from normal mice. The chemical compounds were directly added to the corresponding MethoCult methylcellulose medium in the indicated final concentrations at the onset of the experiment. The data are shown as percentage of the value for the corresponding control, cultured in the absence of inhibitor (100%). There was an average of 1587.4 ± 17.4 CFU-GM, 252.9 ± 21.5 BFU-E, and 1229.3 ± 183.8 CFU-E per 10^6 bone marrow cells in the controls. The data points represent means ± SEM of three to four experiments each performed in duplicate. Significant difference from the control by *t* test: $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Figure 3A, PI-3 kinase inhibition by Ly294002, resulted in dose-dependent suppression of both myeloid and erythroid progenitors. However, while the inhibition of mature erythroid progenitors, CFU-E, was evident at all concentrations used (0.1 to 25 μ*M*), the inhibition of both BFU-E and CFU-GM growth occurred only at higher concentrations (10 and 25 μ*M*). Similar results were obtained using Wortmanin (at concentrations ranging from 1 to 1000 n*M*) and Quercetin (at concentrations from 0.1 to 100 μ*M*) to inhibit PI-3 kinase (data not shown).

Effect of NFκB Inhibitor on Bone Marrow Cell Colony Formation. To determine if NF-κB was necessary for colony formation of normal murine hematopoietic cell progenitor cells, pyrrolidine dithiocarbamate (PDTC) was added to the media in the methyl cellulose colony-forming assays in a broad range of concentrations from 0.01 to 500 μ*M*. Blocking the activation of NF-κB resulted in almost complete suppression of the formation of all tested progenitors at PDTC concentration as low as 0.1 μ*M* (Fig. 3B).

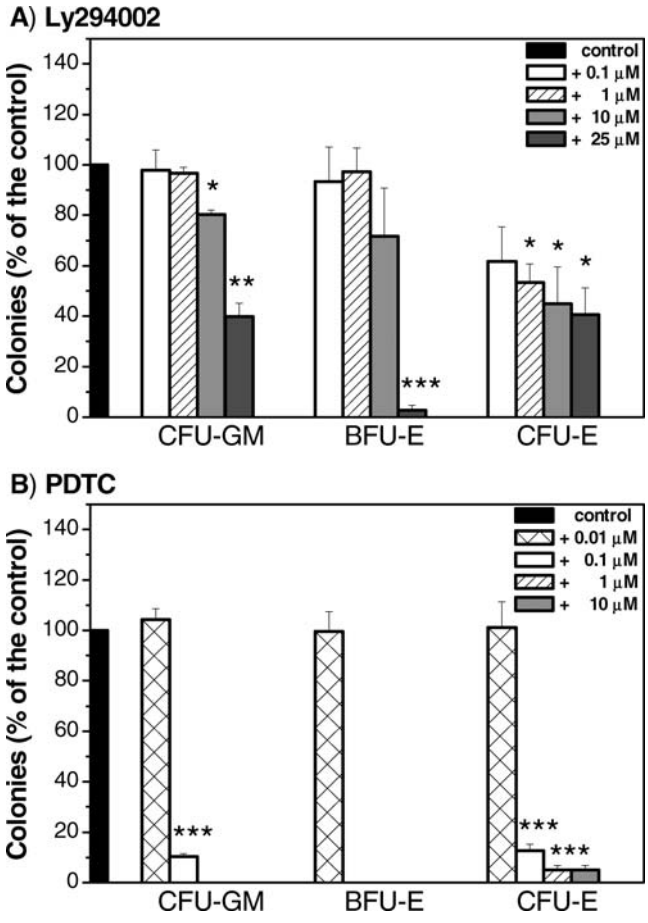

Figure 3. The effect of (A) PI-3K inhibitor Ly294002 and (B) NF-κB inhibitor PDTC on CFU-GM–, BFU-E–, and CFU-E–derived colonies grown by bone marrow cells obtained from normal mice. The chemical compounds were directly added to the corresponding MethoCult methylcellulose medium in the indicated final concentrations at the onset of the experiment. The data are shown as percentage of the value for the corresponding control, cultured in the absence of inhibitor (100%). There was an average of 1508.4 ± 72.0 CFU-GM, 258.0 ± 7.0 BFU-E, and 1541.4 ± 111.2 CFU-E per 10^6 bone marrow cells in the controls. The data points represent means ± SEM of three experiments each performed in duplicate. Significant difference from the control by *t* test: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

Discussion

The purpose of this study was to analyze the signal transduction intermediates associated with the clonal development of myeloid and erythroid progenitor cells. All the cytokines used in the applied colony cultures (i.e., SCF, IL-3, IL-6, and Epo) are known to activate multiple signal transduction pathways and most of them are of the same core signaling cascades (11–16). However, using an inhibitor-based strategy, different signal transducing molecules that allow selective lineage expression were demonstrated when inhibitors specific for PTKs and MAPKs were used, while erythroid progenitors' stage-specific differences were observed when the inhibitor specific for MEK/ERK MAPK transduction was applied. A potential criticism of the present study is the reliance on chemical inhibitors rather than molecular biological approaches to interfere with specific signaling pathways. But, it should be emphasized that cytokine-related signal transduction events have not been extensively studied in hematopoietic progenitor cells, mainly because of the difficulty in purifying sufficient numbers of primary adult bone marrow–derived progenitors. The investigations at the biochemical level were often confined to immortalized cell line models, thus not offering much information how signaling events are linked to the biological functions that are important to normal blood physiology. Since in a number of studies the specificity of the inhibitors we used was confirmed, the blockade of signaling pathways by their respective chemical inhibitors gave us also the opportunity to link the particular signaling activity to the biological response of the hematopoietic progenitor cells. Although this approach did not show a definite link between the particular cytokine that triggers a particular signal molecule activity, similar strategy using the inhibition of signaling pathways for the assessment of signaling molecules activated in normal hematopoietic progenitors was previously also reported (17–19). Thereby, beside pointing to different regulatory pathways that appear to be activated in myeloid and erythroid progenitor cells, the data presented demonstrated constitutive expression of signaling molecules in committed hematopoietic progenitors, suggesting their participation in the regulation of normal steady-state hematopoiesis.

The results concerning the involvement of PTKs inhibitors in progenitor cell growth, suggested that the erythrocyte linage was a more sensitive target for the tested PTKs compounds. The differential sensitivity of erythroid and myeloid progenitors to the action of PTKs' specific inhibitors is in line with the important role of the tyrosine kinases in the Epo signal transduction, since Epo along with

its receptor-EpoR is the crucial cytokine regulator of the red blood cell development. The vital role of Jak2 in mediating signals downstream of EpoR (15, 16) explains the significant inhibitory effect of AG490 on erythroid progenitors' growth. Similar opposite effects of AG490 on the *in vitro* proliferation and differentiation of human myeloid and erythroid progenitor cells were reported by Lewis *et al*. (17). However, besides linage-specific differences, in our study differences in the susceptibility of the early and late erythroid progenitors to the action of AG490 were also observed. The more profound effect of AG490 on the CFU-E formation is probably related to the number of Epo receptors expressed on the erythroid progenitors, because it is well known that EpoR is primarily expressed on erythroid cells between the CFU-E and the pronormoblast stage of erythroid development, with the highest number seen on the CFU-E (15). The observed involvement of Src tyrosine kinases in erythroid progenitor cell proliferation and/or differentiation is consistent with the recent data demonstrating that Src family tyrosine kinases are also activated by the EpoR and are implicated as secondary kinases in Epo signaling (3, 16, 20). As regarding the EGFR tyrosine kinase, at present there is little evidence on its activation and subsequent intracellular signaling in hematopoietic progenitor cells, but it is well known that a wide range of biologically active ligands can bind and activate this receptor and that pleiotropic cell responses induced by EGFR activation include cell proliferation, migration, and differentiation, as well as homeostatic functioning (21, 22). However, the observed effects of Genistein, the inhibitor of EGFR-kinase, on the hematopoietic progenitors' growth, suggested an important role of this receptor PTK in proliferation and/or differentiation of erythroid progenitor cells.

Using specific chemical inhibitors for the main groups of distinctly regulated MAPK cascades in this study, we have demonstrated that MAPK pathways play a crucial role in the growth of normal murine erythroid progenitor cells, with no major influence on the formation of myeloid progenitors. In addition, we have shown differential involvement of MEK/ERK signaling pathway in erythroid progenitor cell growth depending on progenitors' maturity. The obtained results are consistent with the numerous reports that the activation of p38 and JNK MAPK, but not ERK, is essential for Epo-induced erythroid differentiation (18, 23–26). Although ERK MAPK activation was shown to be induced by Epo in various erythroblastic cells, the lack of involvement of ERK kinases in Epo-induced differentiation was reported (23–26). Based on these studies, it was even suggested that the ERK/MAPK pathway might play a critical role in erythroid cell proliferation but have a negative role in erythroid differentiation, and/or that ERK1/2 activity might be down-regulated at later stages of erythroid differentiation (25, 27). These assumptions are in line with our finding regarding differential involvement of MEK/ERK MAPK signaling pathway in the proliferation and/or differentiation of early and late murine erythroid progenitor cells. Similar observations on the differential requirement of ERK MAPK signaling in erythroid progenitors of different maturity were reported for the avian erythropoiesis, because the MEK-1/ERK pathway was strictly required for the self-renewal in early, but not late, avian erythroid progenitors (19). Besides this differential involvement depending on progenitors' maturity, lineage-specific effects of ERK MAPK cascade were also reported. While not required for the erythroid differentiation, the activation of ERK MAPK pathway was shown to promote the monocytic and megakaryocytic cell differentiations (24, 26). However, as the growth of early erythroid progenitors BFU-E in our colony assays was the result of cooperative effects of several cytokines, including Epo and SCF, it cannot be excluded that the involvement of ERK MAPK, observed only in BFU-E colony formation, was associated with the synergy in signal transduction. The synergistic activation of ERK1/2 MAPK by Epo and SCF was proposed as the intracellular mechanism by which Epo and SCF coordinate their well-documented synergistic effect on the production of erythroid cells (28–30).

Numerous studies emphasized the important role of PI3-kinase/Akt signaling pathway in the regulation of erythropoiesis and myelopoiesis (17, 31–34). In this report, PI-3 kinase was also identified as an important signaling intermediate involved in the proliferation and differentiation of murine myeloid and erythroid progenitor cells, since dose-dependent inhibition of progenitor cell growth was obtained with three structurally and mechanistically distinct PI-3K inhibitors. Our results also demonstrated that the appropriate regulation of NF-κB activity is required for normal hematopoiesis and maintenance of hematopoietic progenitor cell function. This finding is consistent with the established role of NF-κB as secondary messenger for a number of cytokines known to regulate hematopoiesis, such as IL-3, Epo, IL-6, SCF, GM-CSF (35–39), as well as with the data demonstrating the requirement of NF-κB for human CD34+ bone marrow cell clonogenic function and survival (35, 39).

Accumulating evidence suggests that a number of different mechanisms may determine the cell specificity of signaling pathways (40–43). The data presented above demonstrated differential involvement of signal transduction intermediates in the growth of erythroid and myeloid progenitor cells and indicated that the lineage-specificity, at least partly, is determined by the activation of various sets of downstream molecules expressed in each progenitor cell type examined. These data are consistent with one of the proposed mechanisms that the control of specificity in cell signaling is achieved through tissue/lineage-specific activation/expression of signal transduction intermediates. As different concentrations of signal inhibitors were needed to induce the inhibition of colony formation (100-fold higher concentrations were required for the inhibition of BFU-E than CFU-E colony growth in the presence of p38 MAPK

and PI-3K inhibitors; while 2500-fold higher concentrations were required for the inhibition of CFU-GM than BFU-E in the presence of JNK MAPK inhibitor), the results obtained also pointed to different threshold levels, another proposed mechanism by which the signaling specificity may be achieved. Moreover, since most of the signal transduction inhibitors were developed as potential therapeutic agents, the data concerning their inhibitory potential point to another important aspect of their action: the impact of these chemicals on normal bone marrow. Although a different threshold of sensitivity between normal hematopoietic progenitors and neoplastic cells cannot be ruled out, the observed susceptibility of normal hematopoietic progenitors to specific inhibitors suggests that proposed strategies involving signal transduction inhibitors as an adjunct to cancer chemotherapy should be approached with caution, because of the potentially important effects that signal transduction inhibitors may have on hematopoietic progenitor proliferation and differentiation processes.

In summary, the results of this study demonstrated different signal transduction intermediates that regulate erythroid and myeloid progenitor cell proliferation and differentiation, as well as differential requirement for particular signaling molecules in early and late normal murine erythroid progenitors. In addition, the results indicated that committed hematopoietic progenitors express a certain level of constitutive PTKs, MAPKs, and PI-3K signaling activities that participate in the regulation of normal steady-state hematopoiesis.

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