Cytokine Regulation of the Human Burst-Forming Unit-Megakaryocyte

By Robert A. Briddell and Ronald Hoffman

The human burst-forming unit-megakaryocyte (BFU-MK) is a primitive megakaryocytic progenitor cell. A marrow cell population enriched for BFU-MK ($CD34^+DR^-$) was obtained by monoclonal antibody labeling and fluorescence-activated cell sorting. $CD34^+DR^-$ cells were assayed in a serum-depleted, fibrin clot culture system. Recombinant granulocyte-macrophage colony-stimulating factor (rGM-CSF), recombinant interleukin-3 (rIL-3), and megakaryocyte colony-stimulating factor (MK-CSF), partially purified from human plasma, were each individually capable of promoting BFU-MK–derived colony formation. Recombinant erythropoietin, rG-CSF, rIL-4, rIL-6, and thrombocytopoiesis stimulating factor, partially purified from human embryonic kidney cell conditioned media, had no stimulatory effect on BFU-MK–derived colony formation when added alone or in various combinations with either GM-CSF, IL-3, or MK-CSF. GM-CSF and IL-3, GM-CSF and MK-CSF, but not IL-3 and MK-CSF had additive actions in promoting BFU-MK–derived colony formation. rIL-1α had no influence alone on BFU-MK cloning efficiency, but had a dose-dependent, synergistic effect with IL-3, but not with GM-CSF or MK-CSF. The synergistic relationship between IL-1α and IL-3 was abrogated by addition of an IL-1α neutralizing antibody but not by a GM-CSF neutralizing antiserum, suggesting that IL-1α acts directly on the BFU-MK and not by stimulating marrow auxiliary cells to secondarily release additional cytokines. Information presented here indicates that the regulatory influences, acting on the different stages of megakaryocyte development, are stage-specific and accomplished by multiple cytokines.

A HIERARCHY of megakaryocyte (MK) progenitor cells recently has been shown to exist.[1-4] The burst-forming unit-MK (BFU-MK) is the most primitive progenitor cell committed to the MK lineage identified to date.[3,4] Properties that allow for the human BFU-MK to be readily distinguished from the more differentiated MK progenitor cell, the colony-forming unit-MK (CFU-MK), have been reported.[4] BFU-MK is resistant to in vitro pretreatment with 5-fluorouracil; CFU-MK is not.[4] The two MK progenitor cells are physically separable using counterflow centrifugal elutriation.[4] Both BFU-MK and CFU-MK express the CD34 antigen, while only the CFU-MK expresses detectable quantities of the HLA-DR locus.[4] BFU-MK–derived colonies require longer incubation periods to develop, are composed of multiple foci of development, and contain larger numbers of MK elements than CFU-MK–derived colonies.[4]

A growing number of purified and recombinant cytokines have been shown by a number of laboratories, including our own, to affect CFU-MK proliferation.[5-13] In a previous report we showed that recombinant granulocyte-macrophage colony-stimulating factor (rGM-CSF) and recombinant interleukin-3 (rIL-3) were each individually capable of promoting BFU-MK–derived colony formation, whereas recombinant erythropoietin (Epo), rG-CSF, rIL-1α, rIL-4, rIL-6, and partially purified thrombocytopoiesis stimulating factor (TSF) did not possess such abilities.[4] Recently, IL-6 has been shown to play a pivotal role in MK development.[6-10] Ishibashi et al[7] have presented data to indicate that IL-6 is a direct promotor of MK maturation, while Lotem et al[8] have suggested that the MK-inducing properties of IL-3 are actually due to the production of IL-6 by bone marrow cells. However, Long et al[12] have reported that IL-6 fails to synergize with either IL-3 or GM-CSF to augment CFU-MK–derived colony formation. Therefore, it is important to determine the role of IL-6 in the development of the BFU-MK. We report here the results of investigations to further define the cytokine requirements of the human BFU-MK. These studies indicate that cytokine regulation of the BFU-MK is relatively complex, with the actions of a number of cytokines being additive, while others synergistically interact to affect BFU-MK–derived colony formation.

MATERIALS AND METHODS

Bone marrow aspirates were obtained under local anesthesia from the posterior iliac crests of hematologically normal volunteers. Informed consent was obtained from the donors according to guidelines previously established by the Human Investigations Committee of the Indiana University School of Medicine, which adheres to the principles of the Declaration of Helsinki.

BFU-MK purification procedure. Bone marrow aspirates were immediately diluted 1:1 with Iscove's modified Dulbecco's media (GIBCO, Grand Island, NY) containing 20 U/mL sodium heparin. This mixture was passed through a 150-μm screen. Low-density mononuclear cells (LDMC) were obtained according to the method previously described by Bruno et al.[11] LDMC were further separated by counterflow centrifugal elutriation to obtain those cells eluting at flow rates between 12 and 14 mL/min (FR 12-14) according to the methods described by Brandt et al.[14] Rotor speed and temperature were maintained at 1,950 rpm and 10°C throughout the elutriation. Seventy-five milliliters of effluent was collected at flow rates of 12 and 14 mL/min using a buffer containing 5% fetal bovine serum vol/vol (Hyclone, Logan, UT), 0.01% EDTA wt/vol, and 1 g/L D-glucose in phosphate-buffered saline at pH 7.4. To further purify FR 12-14 for BFU-MK, we used fluorescence-activated cell sorting according to previously established methods.[4,14-16] Cell populations containing high densities of CD34 and no detectable density of HLA-DR ($CD34^+DR^-$) were used for our experiments. The purity of sorted cells was determined by reanalyzing aliquots of the sorted subset on the Coulter Epics 753 (Coulter, Hialeah, FL) immediately after the sort was finished. The $CD34^+DR^-$ cell fraction consistently contained greater than 85% $CD34^+$ cells and less than 5% HLA-DR

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positive cells. Next, aliquots of the $CD34^+DR^-$ cell fraction were analyzed on the FACScan (Coulter) to determine the percentage of T cells contained in this cell population. Cells were tagged with mouse anti-human monoclonal antibodies (MoAbs) against CD2, CD4, CD5, CD7, CD8, and both the α and β chains of T-cell receptors, which are all common markers found on human T cells. Cells were also tagged with mouse anti-human MoAbs against CD16 and CD56, which are both common markers found on human natural killer cells. The eight aforementioned mouse anti-human MoAbs conjugated with either FITC or PE were purchased from Becton Dickinson Immunocytometry Systems (Mountain View, CA). The $CD34^+DR^-$ cell fraction was determined to contain nondetectable densities of CD2, CD4, CD5, CD7, CD8, both the α and β chains of T-cell receptors, CD16, and CD56.

Human hematopoietic cytokines. (1) Epo: specific activity (sp act) $>10^4$ U/mg determined by the ex-hypoxic, polycythemic mouse assay (Amgen, Thousand Oaks, CA). (2) G-CSF: sp act = 2.0×10^8 U/mg protein determined by GM colony formation from nonadherent human bone marrow cells in semisolid media (Amgen). (3) GM-CSF: sp act $>5.0 \times 10^7$ U/mg protein determined by GM colony formation from human bone marrow cells in soft agar (Genzyme, Boston, MA). (4) IL-1α: sp act = 10^8 U/mg protein determined by proliferative effects on D-10 cells (Genzyme). (5) IL-3: sp act = 10^8 U/mg protein based on its ability to promote mixed colonies composed of granulocytes, macrophages, MK, and eosinophils from human bone marrow cells (Genzyme). (6) IL-4: sp act = 10^8 U/mg protein determined by a coproliferation assay using human tonsillar B cells and anti-human immunoglobulin G (IgG) (Genzyme). (7) IL-6: sp act = 10^7 U/mg protein determined by that amount required to augment Ig production by the CESS cell line to the half-maximal level (Genzyme). (8) Partially purified MK-CSF: sp act = 9.7×10^2 U/mg protein partially purified from human plasma according to Hoffman et al.[17] (9) TSF: step III partially purified from human embryonic kidney cell conditioned media kindly provided by Dr Ted McDonald.[18]

Human hematopoietic cytokine neutralizing antisera and antibodies. (1) Rabbit polyclonal antiserum to human GM-CSF; anti-GM-CSF; 80% IgG, 1.0 mg of antiserum neutralizes 20.0 ng of human GM-CSF as determined by radioimmunassay based on anti-GM-CSF/GM-CSF binding (Genzyme). This antiserum has been shown in our laboratory to neutralize the ability of 200 pg/mL of GM-CSF to promote MK colony formation (unpublished observation, February 1990). (2) Rabbit polyclonal antibody to human IL-1α; anti-IL-1α; 90% IgG, 1.25 mg of antibody neutralizes 10.0 ng of IL-1α as determined by the phytohemagglutinin-induced thymocyte proliferation assay (Genzyme). This antibody has been previously shown to neutralize numerous biologic activities of IL-1α in our laboratory.[19]

Assay system. $CD34^+DR^-$ cells/mL, 5×10^3, were assayed for their ability to produce BFU-MK–derived colonies in a serum-depleted fibrin clot culture system as described by Bruno et al.[11] Varying doses, alone or in combinations, of recombinant or purified growth factors were used as a source of MK colony-stimulating activity (MK-CSA). Based on our determination of the optimum in vitro time of appearance of BFU-MK–derived colonies previously reported by our laboratory, cultures were incubated for 21 days at 37°C in a 100% humidified atmosphere of 5% CO_2 in air.[4] After incubation, fibrin clots were fixed in situ in methanol:acetone (1:3) for 20 minutes, washed with 0.01 mol/L phosphate-buffered saline pH 7.2, and air-dried. Fixed plates were stored at −20°C until immunofluorescent staining was performed.

Immunofluorescent identification. Immunofluorescent staining was performed as previously described by our group.[4,11] Cultures were scored in situ to enumerate fluorescein positive colonies. The 35-mm Petri dishes were inverted, and the base area completely scanned with a fluorescent microscope at 100×(Carl Zeiss, New York, NY). BFU-MK–derived colonies were identified by criteria established by Long et al.[3] These colonies appeared in human marrow cultures as clusters ≥42 fluorescent cells distributed in at least two foci of development, and are identified after 21 days of incubation.[4] Human BFU-MK–derived colonies are distinguished from CFU-MK–derived colonies by duration of incubation required for their appearance in vitro (21 days *v* 12 days, respectively), colony size (108.6 ± 4.4 cells/colony *v* 11.2 ± 1.2 cells/colony, respectively), and foci of development (2.3 ± 0.4 foci/colony *v* 1.2 ± 0.1 foci/colony, respectively).[4]

RESULTS

The effect of no additions, GM-CSF, IL-3, and MK-CSF on BFU-MK–derived colony formation by 5×10^3 $CD34^+DR^-$ cells is shown in Fig 1. No BFU-MK–derived colonies or nonfluorescing colonies appeared in the absence of an exogenous cytokine. Using titration curves generated in preliminary experiments, we determined the suboptimal and optimal concentrations of GM-CSF, IL-3, and MK-CSF for promoting BFU-MK–derived colony formation. The optimal concentration of IL-3 (1.0 ng/mL) promoted the appearance of more than twice the number of BFU-MK–derived colonies (5.6 ± 0.4) than the optimal concentration of GM-CSF (200.0 pg/mL, 2.6 ± 0.4). The suboptimal and optimal concentrations of MK-CSF (12.5 μg/mL and 25.0 μg/mL) promoted the appearance of similar numbers of BFU-MK–derived colonies as the suboptimal and optimal concentrations of GM-CSF.

The ability of combinations of suboptimal and optimal concentrations of GM-CSF, IL-3, and optimal concentrations of MK-CSF to promote BFU-MK–derived colony formation by 5×10^3 $CD34^+DR^-$ cells is shown in Fig 2. GM-CSF and IL-3, GM-CSF and MK-CSF, but not IL-3 and MK-CSF had additive actions in promoting BFU-MK–derived colony formation. These additive actions are observed when one compares Figs 1 and 2: (1) an optimal concentration of GM-CSF plus an optimal concentration of IL-3 promoted 10 ± 1.8 BFU-MK derived colonies; (2) an optimal concentration of GM-CSF plus an optimal concentra-

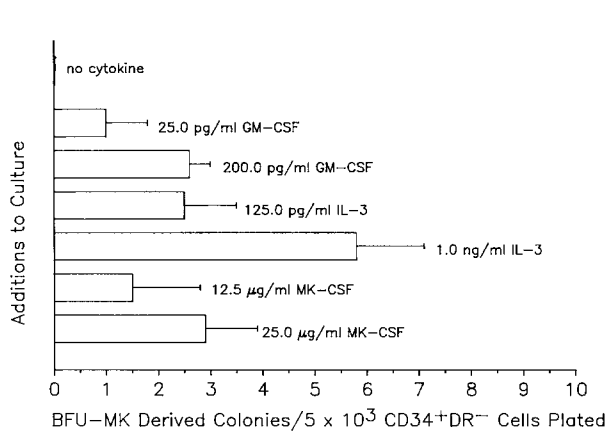

Fig 1. Effect of no additions, GM-CSF, IL-3, and MK-CSF on BFU-MK–derived colony formation by $CD34^+DR^-$ cells. Both suboptimal and optimal concentrations of each cytokine were assayed. Bars indicate the mean ± SE of the mean of pooled data obtained from experiments performed in duplicate on at least four separate occasions.

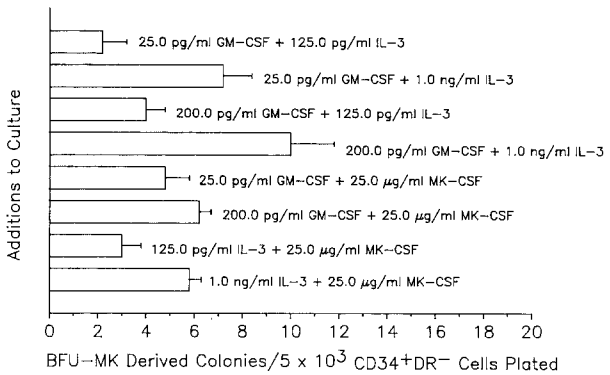

Fig 2. Effect of combinations of suboptimal and optimal concentrations of GM-CSF, IL-3, and MK-CSF on BFU-MK–derived colony formation by $CD34^+DR^-$ cells. Bars indicate the mean ± SE of the mean of pooled data obtained from experiments performed in duplicate on at least two separate occasions.

tion of MK-CSF promoted 6.2 ± 0.5 BFU-MK–derived colonies; (3) an optimal concentration of IL-3 plus an optimal concentration of MK-CSF promoted only 5.8 ± 0.5 BFU-MK–derived colonies.

We have previously reported that Epo, G-CSF, IL-1α, IL-4, and TSF alone did not promote BFU-MK–derived colony formation by FR 12-14 cells.[4] In Table 1 we provide data to indicate that Epo, G-CSF, IL-4, IL-6, and TSF are incapable of promoting BFU-MK–derived colony formation by $CD34^+DR^-$ cells. In addition, the ability of Epo, G-CSF, IL-4, IL-6, and TSF to potentiate the MK-CSA of either GM-CSF, IL-3, or MK-CSF is shown. None of these cytokines possessed such activity.

Figure 3 illustrates the effect of varying doses of IL-1α on the ability of the suboptimal and optimal dose of IL-3 to promote BFU-MK–derived colony formation by 5 × 10^3 $CD34^+DR^-$ cells. IL-1α had a dose-dependent, synergistic effect with IL-3. The optimal synergistic effect of IL-1α and IL-3 (10.5 ± 0.5) on BFU-MK cloning efficiency from 5 × 10^3 $CD34^+DR^-$ cells was equivalent to the additive effect of optimal concentrations of GM-CSF and IL-3 (10.0 ± 1.8). IL-1α had no effect when cultured with suboptimal or optimal doses of GM-CSF or the optimal dose of MK-CSF on BFU-MK cloning efficiency from 5 ×10^3 $CD34^+DR^-$ cells (data not shown).

In Table 2 we provide data to further indicate that IL-1α acts alone to augment the BFU-MK-CSA of IL-3. When cultured together, the synergism between IL-1α and IL-3 leads to the formation of 9.4 ± 0.6 BFU-MK–derived colonies/5 × 10^3 $CD34^+DR^-$ cells plated. The addition of anti–IL-1α to assays containing IL-1α and IL-3 resulted in the abrogation of the synergistic relationship between IL-1α and IL-3 in promoting BFU-MK–derived colony formation (Table 2). This synergistic relationship between IL-1α and IL-3 in promoting BFU-MK–derived colony formation was retained in those assays to which anti–GM-CSF was added. These studies indicate that IL-1α does not affect the BFU-MK by promoting GM-CSF release from auxiliary marrow cells.

DISCUSSION

The BFU-MK is the most primitive MK progenitor cell that has been assayed from human bone marrow.[3,4] Our laboratory has developed a serum-depleted culture system

Table 1. Effect of Cytokine Combinations on BFU-MK–Derived Colony Formation

Cytokine(s)	BFU-MK–Derived Colonies 5 × 10^3 $CD34^+DR^-$ Cells Plated
Epo	
500.0 ng/mL	0.0 ± 0.0*
+ A_1†	1.5 ± 0.6
+ A_2	1.8 ± 0.5
+ B_1	2.8 ± 0.2
+ B_2	5.8 ± 0.2
G-CSF	
250.0 pg/mL	0.0 ± 0.0
+ A_1	0.2 ± 0.2
+ A_2	1.8 ± 0.5
+ B_1	3.2 ± 0.5
+ B_2	4.5 ± 1.0
IL-4	
300.0 pg/mL	0.0 ± 0.0
+ A_1	0.8 ± 0.2
+ A_2	2.0 ± 0.4
+ B_1	2.2 ± 0.5
+ B_2	5.0 ± 1.2
IL-6	
10.0 ng/mL	0.0 ± 0.0
+ A_1	1.5 ± 0.6
+ A_2	3.0 ± 1.4
+ B_1	2.8 ± 1.0
+ B_2	6.0 ± 1.8
+ C	3.2 ± 0.5
30.0 ng/mL	0.0 ± 0.0
+ A_1	1.8 ± 1.0
+ A_2	2.8 ± 0.5
+ B_1	3.0 ± 0.8
+ B_2	6.8 ± 1.0
+ C	2.2 ± 0.5
60.0 ng/mL	0.0 ± 0.0
+ A_1	1.2 ± 1.0
+ A_2	2.5 ± 1.3
+ B_1	3.2 ± 1.2
+ B_2	5.2 ± 2.6
TSF	
100.0 ng/mL	0.0 ± 0.0
+ A_1	2.0 ± 0.4
+ A_2	2.5 ± 0.6
+ B_1	4.0 ± 0.6
+ B_2	6.0 ± 0.5

*Each point represents the mean ± SE of the mean of pooled data obtained from experiments performed in duplicate on at least two separate occasions.

†A_1 = 25.0 pg/mL GM-CSF (1.0 ± 0.8); A_2 = 200.0 pg/mL GM-CSF (2.6 ± 0.4); B_1 = 125.0 pg/mL IL-3 (2.5 ± 1.0); B_2 = 1.0 ng/mL IL-3 (5.8 ± 0.4); C = 25.0 μg/mL MK-CSF (2.9 ± 1.0).

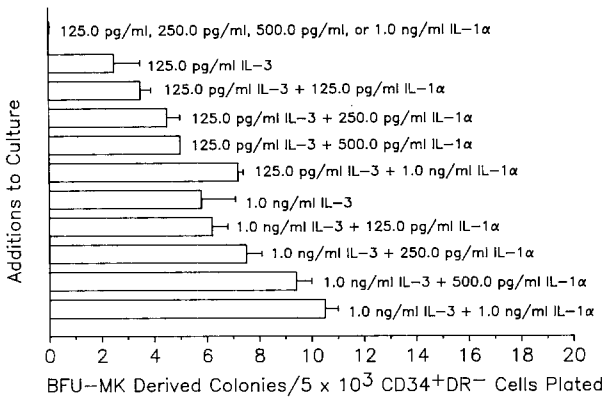

Fig 3. Effect of varying doses of IL-1α and suboptimal and optimal concentrations of IL-3 on BFU-MK–derived colony formation by $CD34^+DR^-$ cells. Bars indicate the mean ± SE of the mean of pooled data obtained from experiments performed in duplicate on at least four separate occasions.

for this purpose, and has also devised methods for isolating marrow populations enriched for BFU-MK using MoAb staining and fluorescence-activated cell sorting.[4,11] Such technical advances have permitted us to define further the cytokine requirements necessary for the proliferation and differentiation of the human BFU-MK. Our group has previously reported the effect of various exogenous cytokines alone on BFU-MK–derived colony formation by FR 12-14 cells.[4] This report describes the effect of various cytokines, alone and in combinations, on the ability of $CD34^+DR^-$ cells, which are more highly enriched for BFU-MK, to promote BFU-MK–derived colony formation.

Two recombinant cytokines, GM-CSF and IL-3, have been shown individually by a large number of laboratories to affect MK colony formation by the more differentiated MK progenitor cells.[4,5,11,20-27] Studies by Lu et al,[15,16] using populations of marrow cells enriched for CFU-MK, have suggested that these cytokines act directly on the CFU-MK.

Table 2. Effect of Cytokine CsA Neutralizing Antisera or Antibodies on the Ability of IL-1α to Augment the BFU-MK of IL-3

Cytokine(s)	Antisera/Antibody Addition	BFU-MK–Derived Colonies/5 × 10^3 $CD34^+DR^-$ Cells Plated
None	None	0.0 ± 0.0*
500.0 pg/mL IL-1α	None	0.0 ± 0.0
1.0 ng/mL IL-3	None	5.8 ± 0.4
500.0 pg/mL IL-1α + 1.0 ng/mL IL-3	None	9.4 ± 0.6
500.0 pg/mL IL-1α + 1.0 ng/mL IL-3	5.0 μg/mL anti-GM-CSF	9.5 ± 0.6
500.0 pg/mL IL-1α + 1.0 ng/mL IL-3	62.5 μg/mL anti-IL-1α	5.0 ± 0.9

*Each point represents the mean ± SE of the mean of pooled data obtained from experiments performed in duplicate on at least two separate occasions.

A lineage-specific MK growth factor has been detected in human, rodent, and canine thrombocytopenic plasma and serum, and has been shown to promote CFU-MK cloning efficiency in vitro.[17,28-33] In addition, several groups have detected this growth factor in human urine obtained from thrombocytopenic individuals.[5,34-36] This factor has been termed MK-CSF, but has not to date been purified to hemogeneity or genetically cloned using recombinant DNA technology.[5]

We have demonstrated that GM-CSF, IL-3, and MK-CSF are each individually capable of promoting BFU-MK–derived colony formation by $CD34^+DR^-$ marrow cells. The optimal dose of IL-3 and MK-CSF, needed to promote BFU-MK–derived colony formation (1.0 ng/mL and 25.0 μg/mL respectively), were identical to that previously reported by our laboratory for CFU-MK. By contrast, the optimal dose of GM-CSF needed to promote BFU-MK–derived colony formation (200.0 pg/mL) was fourfold higher than that required by the CFU-MK (50.0 pg/mL). Bruno et al[37] had previously shown that the MK-CSA actions of GM-CSF and IL-3 directed against the CFU-MK were additive at suboptimal but not at optimal concentrations of these cytokines. The effects of these two cytokines on the BFU-MK are somewhat different in that this additive relationship was observed at both their suboptimal and optimal concentrations. A similar additive relationship exists between MK-CSF and both suboptimal and optimal concentrations of GM-CSF. Surprisingly, the colony-stimulating effect of MK-CSF was not additive to that of either suboptimal or optimal concentrations of IL-3.

Several laboratories, including our own, have shown that IL-6 is capable of promoting CFU-MK–derived colony formation by unfractionated marrow cells and LDMC.[6,8] Lotem et al[8] have presented data which suggests that the MK-CSA of IL-3 is actually due to the production of IL-6 by marrow auxiliary cells. Such studies indicate that IL-6 plays a pivotal role in the regulation of megakaryocytopoiesis. In our studies, IL-6 was incapable of promoting BFU-MK–derived colony formation alone and did not have a synergistic interaction with any of the other cytokines evaluated. These studies indicate that the biologic properties of IL-6, as it relates to megakaryocytopoiesis, are quite distinct from those of IL-3 and suggests that it is unlikely that the action of IL-3 at the level of the BFU-MK was due to the secondary elaboration of IL-6. The fact that the MK-CSA of IL-3 was an indirect one is especially unlikely since a population of marrow cells, which is largely devoid of marrow auxiliary cells, was used as the target cell population for these studies.

Previously we reported that a partially purified preparation of TSF, although lacking any MK-CSA activity at the level of the CFU-MK, was capable of potentiating the MK-CSA of IL-3.[37] We have shown here that TSF alone or in combination with suboptimal or optimal concentrations of IL-3 had no effect on BFU-MK proliferation. Such observations would be consistent with the hypothesis that TSF affects more differentiated MK elements, thus acting primarily as an MK maturation factor.[5]

A number of cytokines have been identified recently that

alone do not possess in vitro hematopoietic CSA but rather potentiate the CSF responsiveness of other cytokines.[5,10,37-45] For instance, IL-1α and IL-6 are capable of potentiating the colony-stimulating ability of IL-3 directed against early hematopoietic precursors.[43-45] IL-1α has been shown to be devoid of MK-CSA alone at the level of both the CFU-MK and BFU-MK.[4,6] However, Peschel et al[46] reported that IL-1α enhanced the MK-CSA of IL-3 in promoting CFU-MK–derived colonies, but their findings were not confirmed by Bruno et al.[37] We report that IL-1α, but not IL-6, augments the ability of IL-3 to promote BFU-MK–derived colony formation. This synergistic effect occurs in a dose-related fashion with regard to IL-1α concentration, and is observed at both suboptimal and optimal concentrations of IL-3. These observations are in no way subtle, in that optimal concentrations of both IL-1α and IL-3 promote similar numbers of BFU-MK–derived colonies as does the combination of optimal concentrations of GM-CSF and IL-3. Such synergistic interactions were not observed when IL-1α was added to GM-CSF–or MK-CSF–containing cultures. McNiece et al[47] have reported that although G-CSF contains no MK-CSA, it was capable of increasing the number of CFU-MK–derived colonies formed in the presence of IL-3 as compared with assays containing IL-3 alone. No such synergistic interaction between G-CSF and GM-CSF or IL-3 was observed with regard to BFU-MK–derived colony formation. Such studies indicate the specificity of the IL-1α and IL-3 interaction in promoting BFU-MK–derived colony formation.

The effect of IL-1α on hematopoiesis has been attributed in part to its ability to cause either stromal cells, fibroblasts, or endothelial cells to elaborate a number of cytokines, including G-CSF, GM-CSF, or IL-6, which might then directly influence hematopoietic progenitor cell proliferation.[48-52] To determine if IL-1α's effect on IL-3–promoted BFU-MK–derived colony formation was due to the secondary release of cytokines by marrow auxiliary cells, cytokine neutralizing antisera or antibodies were added to assays containing IL-1α and IL-3. The IL-1α potentiating effect on IL-3–promoted BFU-MK–derived colony formation was abrogated by the addition of an IL-1α neutralizing antibody but not by a GM-CSF neutralizing antiserum, suggesting that the IL-1α effect is not due to the secondary release of GM-CSF by marrow auxiliary cells. It also seems unlikely that this potentiating effect would be due to the ability of IL-1α to promote the release of either G-CSF or IL-6 by marrow auxiliary cells since neither the addition of G-CSF or IL-6 enhanced the response of the BFU-MK to IL-3. Based on these results, we concluded that IL-1α directly promotes BFU-MK–derived colony formation. The elucidation of mechanisms by which IL-1α and IL-3 interact at the level of the BFU-MK requires further investigation. The time of appearance in vitro of the BFU-MK–derived colonies as compared with the CFU-MK–derived colonies suggests that the BFU-MK is a more quiescent cell than the CFU-MK. Because IL-3 by itself has MK-CSA at the level of the BFU-MK, IL-1α is apparently not required for this biologic activity of IL-3. Rather, IL-1α might serve to activate additional cytokine receptors possessed by the BFU-MK, thus triggering the BFU-MK into a more active phase of the cell cycle, thereby increasing IL-3 responsiveness, or alternatively by activating a previously IL-3–unresponsive population of BFU-MK. Further study of the mechanism by which the IL-1α/IL-3 synergism occurs will require isolation of homogenous populations of BFU-MK, performance of ligand-receptor interactions, and careful cell cycle analysis of this cell population. Unfortunately, at present the means to isolate sufficient numbers of BFU-MK for the performance of such studies does not exist.

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