TUMOR NECROSIS FACTOR α STIMULATES THE GROWTH OF THE CLONOGENIC CELLS OF ACUTE MYELOBLASTIC LEUKEMIA IN SYNERGY WITH GRANULOCYTE/MACROPHAGE COLONY-STIMULATING FACTOR

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Proliferation of human hemopoietic cells in culture depends upon the presence of exogenous growth factors such as granulocyte/macrophage colony-stimulating factor (GM-CSF)[1] (1, 2), granulocyte CSF (G-CSF) (3, 4), and IL-3 (5), which exert their mitogenic effects either singly or in synergy with IL-1 (6–9) or IL-6 (10–13). Negative growth control in the hemopoietic system is, however, less understood. Recent studies indicated that transforming growth factor β (TGF-β) (14, 15), TNF-α (16–21), and interferons (22, 23) may restrain hemopoietic cell proliferation after binding to specific cell surface receptors.

Autocrine stimulation and absence of negative growth control may represent possible events leading to cancer cell proliferation. There is accumulating evidence to support the concept of autocrine stimulation in cancer (reviewed in reference 24). To this effect, it has been shown that the leukemic cells of acute myeloblastic leukemia (AML) are capable of autostimulation when cultured at high cell density (25). The importance of negative growth control in normal cell proliferation and neoplasia has recently been more widely acknowledged. Thus, Kimchi et al. (26) have shown that retinoblastomas lack TGF-β receptors and growth inhibitory responses while normal fetal retina are sensitive to the antimitogenic effects of TGF-β. We investigated the possibility of whether AML blasts may also have altered responsiveness to the growth inhibitory cytokines. Our previous work (27) indicated that AML blasts are sensitive to the inhibitory effects of TGF-β. Here, it is shown that TNF-α, another growth inhibitory cytokine, fails to support growth arrest under specific conditions. Thus, TNF-α enhances the cell density–dependent and GM-CSF-dependent proliferation of AML blasts while it antagonizes the mitogenic effects of G-CSF and

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[1] *Abbreviations used in this paper:* AML, acute myeloblastic leukemia; G-CSF, granulocyte CSF; GM-CSF, granulocyte/macrophage CSF; IMDM, Iscove's modified Dulbecco's medium; TGF, transforming growth factor.

IL-3 on the same cells. TNF-α, therefore, acts as a bimodulator of AML cell proliferation, with the unique capacity to either support or inhibit cell proliferation depending on the growth signaling context, while on normal hemopoietic progenitors, TNF-α appears to support only growth arrest.

Materials and Methods

Source of Cells and Growth Factors. AML blasts were isolated by centrifugation of peripheral blood cells on a Ficoll-Hypaque gradient (Pharmacia Fine Chemicals, Uppsala, Sweden). The cells were cryopreserved in Iscove's modified Dulbecco's medium (IMDM) (Gibco Laboratories, Grand Island, NY) containing 10% DMSO and 90% FCS (Gibco Laboratories) and stored in liquid nitrogen until use. A programmed freezing rate of 1°/min ensures a 90% recovery in viable cells upon thawing. Patient data are shown in Table I. The numbering system is the same as in references 6 and 10. Normal bone marrow cells were obtained from patients undergoing hip replacement (Dr. C. Godin, Hotel Dieu Hospital, Montreal) and separated on a Ficoll-Hypaque gradient.

Purified recombinant TNF-α was obtained from Amgen Biologicals (Arlington Heights, CA). A concentration of 60 pM corresponds to 1 ng/ml or 10 U/ml. rG-CSF, purified rIL-3, rIL-6, and rGM-CSF were kindly provided by Drs. G. G. Wong and S. C. Clark (Genetics Institute, Cambridge, MS); the specific activity of rGM-CSF was 8×10^6 U/mg. A protein concentration of 100 pM (2.5 ng/ml) corresponds to a biological activity of 20 U/ml. Purified rIL-1α and the neutralizing antibody against IL-1β were generous gifts from Dr. A. Shaw (Biogen, Geneva, Switzerland), and rErythropoietin was from Dr. D. Hankins (Armed Force Radiobiological Institute, Bethesda, MD). Serum-free conditioned medium from the cell line 5637 (5637 CM) was harvested 1 wk after confluence as detailed previously (6).

Culture Conditions. Culture conditions were chosen in order to minimize colony formation in the absence of growth factors. (*a*) AML blasts were plated at a concentration of 7,000/well, unless otherwise stated, in 100 μl of IMDM (Gibco Laboratories) viscified with methylcellulose (Fluka, Switzerland), and supplemented with 10% FCS (Gibco Laboratories) as described previously (6). KG-1 cells, obtained from the American Type Culture Collection (Rockville, MD) were cultured under the same conditions, except that the cell concentration was 1,000/well. (*b*) Nonadherent normal bone marrow cells were plated in the same culture medium as above supplemented with BSA and human transferrin (Behring Diagnostics, San Diego, CA) at a concentration of 5×10^4 cells/ml as detailed elsewhere (6).

Thymidine Suicide Determinations. The number of AML clonogenic cells that was in cycle in response to growth factors was determined by an adaption of the thymidine suicide technique (28), as described by Pébusque et al. (29). Briefly, cells were exposed to TNF-α or GM-CSF for 16 h, washed twice, and exposed to [^{3}H]thymidine (Amersham Corp., Arlington Heights, IL) (25 Ci/mmol, sp act; 200 μCi/ml) for 20 min. The reaction was stopped by the addition of cold thymidine and the cells were washed twice in medium containing FCS before plating. Control cells were exposed to the whole procedure without radioactive thymidine.

Northern Blots and Hybridization Conditions. RNA was extracted in the presence of guanidium isothiocyanate (Bethesda Research Laboratories, Gaithersburg, MD), subjected to electrophoresis in 1% agarose in the presence of glyoxal (30), and transferred to a Nytran membrane (Schleicher & Schuell, Inc., Keene, NH). The probe was the Pst-1 fragment of the human IL-1β cDNA (6) and was labeled by random priming with the Klenow (31) to a specific activity of 3×10^8 cpm/μg. Prehybridization and hybridization were done in the presence of heparin (Sigma Chemical Co., St. Louis, MO) (100 and 500 μg/ml, respectively) as detailed previously (6).

IL-1β ELISA. The IL-1β ELISA kit was from Cistron Biotechnology (Pinebrook, NJ). All reactions were carried out at room temperature. The specificity of the assay was confirmed by the lack of reactivity with TNF-α, GM-CSF, and IL-6. Conditioned media from AML blasts were harvested 24 h after initiation of the cultures either in the presence of GM-CSF alone or both GM-CSF and TNF-α at the indicated concentrations.

TABLE I

Clinical Data on the AML Patients Included in the Study

Patient	FAB classification	Percent blasts
AML 1	M2	68
AML 2	M4	60
AML 3	M5b	38
AML 5	M3	90
AML 6	M1	40
AML 8	M2	80
AML 9	M4	85
AML 10*	M2	49

The proportion of blasts is expressed as a percentage of total nucleated cells in the peripheral blood.

* Sample AML 10 contains, in addition, 35% immature granulocytic cells.

Results

When tested in primary cultures of AML blasts from different patients, TNF-α stimulated colony formation in synergy with GM-CSF (Fig. 1 *A*). As documented elsewhere (32–34), the level of stimulation observed in the presence of GM-CSF alone (intersects with the *y*-axes) was suboptimal with wide patient to patient variations. Addition of TNF-α at increasing concentrations nearly reconstituted or, in two instances, doubled the maximal level of stimulation attained with our standard conditioned medium 5637, which contained a mixture of GM-CSF, G-CSF, and IL-1, and was so far optimal in supporting the growth of AML clonogenic cells (6). The synergy with GM-CSF occurred at concentrations of TNF-α as low as 6–60 pM, in the same range as the dissociation constant of TNF-α reported for AML blasts and myeloblastic cell lines (29–94 pM; reference 21). Further, these concentrations required for stimulation of AML blasts were in the same range as that described for other hemopoietic growth factors. No inhibitory effect was observed, even at concentrations that were 1,000-fold in excess (60 nM). Nevertheless, in five of eight pa-

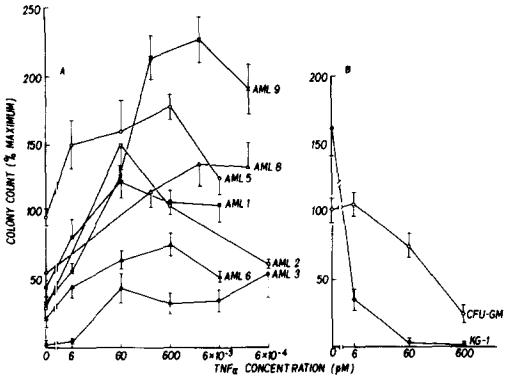

FIGURE 1. Effects of TNF-α on AML blast clonogenic cells and normal granulocyte/monocyte precursors in the presence of GM-CSF. (*A*) Purified rGM-CSF was added at a final concentration of 40 pM to cultures containing the indicated concentrations of rTNF-α. Data shown represent the mean of five replicate cultures. Values were normalized to the colony counts obtained in the presence of our standard conditioned medium 5637: 44 (AML 1); 35 (AML 2); 56 (AML 3); 33 (AML 5); 222 (AML 6); 70 (AML 8); and 143 (AML 9). There was no colony formation in the absence of exogenous growth factors. (*B*) Growth factors were added at the same concentrations as above. Colony counts in the presence of 5637-CM were 37 (KG-1) and 72 (CFU-GM). Colony counts in the absence of growth factors were 4 (KG-1) and 0 (CFU-GM).

tient samples, the degree of synergy with GM-CSF was lower at this high concentration of TNF-α.

In contrast to its growth-promoting effects on AML clonogenic cells, TNF-α antagonized the mitogenic effects of purified GM-CSF on normal granulo-monopoietic progenitors (CFU-GM) and on the human leukemic cell line KG-1 (Fig. 1 *B*), in the same concentration range as documented by others (18–20). These studies (18, 20) also reported an inhibitory effect of TNF-α on AML or CML patient cells cultured in the presence of crude conditioned media from mitogen-stimulated leukocytes or cell lines that contained a mixture of growth factors (1–13). We have observed the same inhibition when TNF-α was added to cultures of AML blasts stimulated with either 5637-CM, IL-3, or G-CSF (Table II). However, TNF-α does not affect cell proliferation in the presence of IL-1α (Table II). Cultures with IL-1α were done at higher cell concentration, as we have shown previously that IL-1α can support blast colony formation by itself, only at higher cell concentration (6).

It has been reported by Nara and McCulloch (25) that AML blasts plated at higher cell concentration are capable of autocrine stimulation. Under such conditions, TNF-α supported colony formation from AML blasts without further addition of other exogenous growth factors (Table II and Fig. 2 *A*). Taken together, the data suggested that TNF-α promoted growth of AML clonogenic cells in the presence of either GM-CSF or an endogenous source of growth stimulators.

To determine whether TNF-α recruits the same population of progenitors as GM-CSF or a different population, AML blasts were exposed to GM-CSF, followed by thymidine suicide, a procedure based on the lethal incorporation of [^{3}H]TdR by cells undergoing DNA synthesis. Surviving cells were plated in the presence of either GM-CSF, TNF-α, or 5637-CM and compared with cells that have not been exposed to thymidine (Table III). Thymidine suicide of cells pre-incubated with GM-CSF resulted in a complete suppression of the population of cells responsive to TNF-α and GM-CSF, while a significantly high number of clonogenic cells stimulated by 5637-CM survived the exposure to radioactive thymidine. Comparable results were observed when cells were pre-exposed to TNF-α instead of GM-CSF (data not shown).

TABLE II

TNF-α Inhibits the IL-3- and G-CSF-dependent Growth of AML Clonogenic Cells

Cells	Growth factors	Colony counts	
		− TNF-α	+ TNF-α
AML 6*	None	0	17 ± 4
	IL-3	47 ± 5	8 ± 3
	G-CSF	24 ± 5	0
	5637 CM	77 ± 1	46 ± 3
AML 8‡	None	9 ± 3	51 ± 6
	GM-CSF	45 ± 8	79 ± 6
	IL-1	54 + 7	69 ± 10

Growth factor concentrations were optimal on separate dose response curves: TNF-α (600 pM), IL-3 (50 U/ml), and G-CSF (50 U/ml). Data are mean of five replicate cultures, with AML 6 at 7,000 cells/well and AML 8 at 10^4 cells/well. Results shown are typical of two independent experiments.

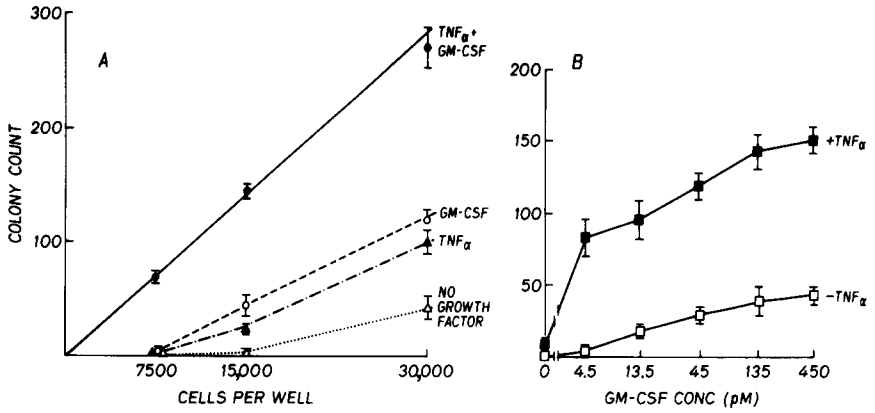

FIGURE 2. Synergistic effects of TNF-α and GM-CSF in the stimulation of AML blast clonogenic cells. (*A*) Effects of growth factors at different cell concentrations. Growth factors were TNF-α (600 pM) and GM-CSF (100 pM). Cells were from AML 6. (*B*) GM-CSF titration in the presence of TNF-α (600 pM). The cell concentration was 10^4/well (AML 6).

The data suggested that TNF-α supported cell proliferation in the subpopulation of progenitors that also appeared to be responsive to GM-CSF. The difference between the surviving fractions of cells stimulated with 5637-CM and GM-CSF could be attributed to other growth factors present in the conditioned medium, such as G-CSF, which has been shown to act on different target cells than GM-CSF (29).

We further investigated the question of whether the stimulatory effects of GM-CSF and TNF-α were additive or synergistic. The two growth factors were added either singly or in combination to cultures containing different cell numbers (Fig. 2 *A*). At the lowest cell concentration, either growth factor alone failed to support significant colony formation; however, high numbers of colonies were observed in the presence of both GM-CSF and TNF-α. Further, when TNF-α was added at an optimal concentration to cultures containing varying concentrations of GM-CSF, the response of AML clonogenic cells to GM-CSF was significantly enhanced (Fig.

TABLE III

Growth of AML Clonogenic Cells after Exposure to GM-CSF in Suspension Culture Followed by Thymidine Suicide

Growth factor added to methylcellulose cultures	Colonies per 10^4 cells	
	Control	Exposed to [^{3}H]Tdr
5637 CM	126 ± 20	75 ± 5
TNF-α	43 ± 4	0
GM-CSF	33 + 3	0
TNF-α + GM-CSF	124 ± 14	5 ± 3

Cells (AML 8) were exposed to GM-CSF (100 pM) in suspension cultures for 16 h, at a concentration of 2 × 10^5 cells/ml. The cells were then washed and exposed to [^{3}H]TdR before plating. Methyl cellulose cultures were done at 10^4 cells/well, with either 5637 CM (10%), or TNF-α (600 pM) or rGM-CSF (100 pM). Data shown are typical of two distinct experiments.

2 *B*). For example, at a suboptimal concentration of GM-CSF (4 pM), only a few colonies were observed. However, when both growth factors were present, there was a 40-fold increase in the number of colonies. Taken together, the results indicated that the stimulatory effects of the two cytokines on AML clonogenic cells are synergistic, rather than additive.

TNF-α has been reported to modulate the production of IL-1 by monocytes (35). In parallel, AML blasts have been shown to express IL-1β mRNA (36) and to respond to the synergistic effects of exogenously added IL-1 and GM-CSF (6). We therefore addressed the question of whether the observed synergy between TNF-α and GM-CSF could be attributed to an endogenous production of IL-1β by AML cells. Cultures were done at higher cell concentration (10^4/ml) to favor cell interac-

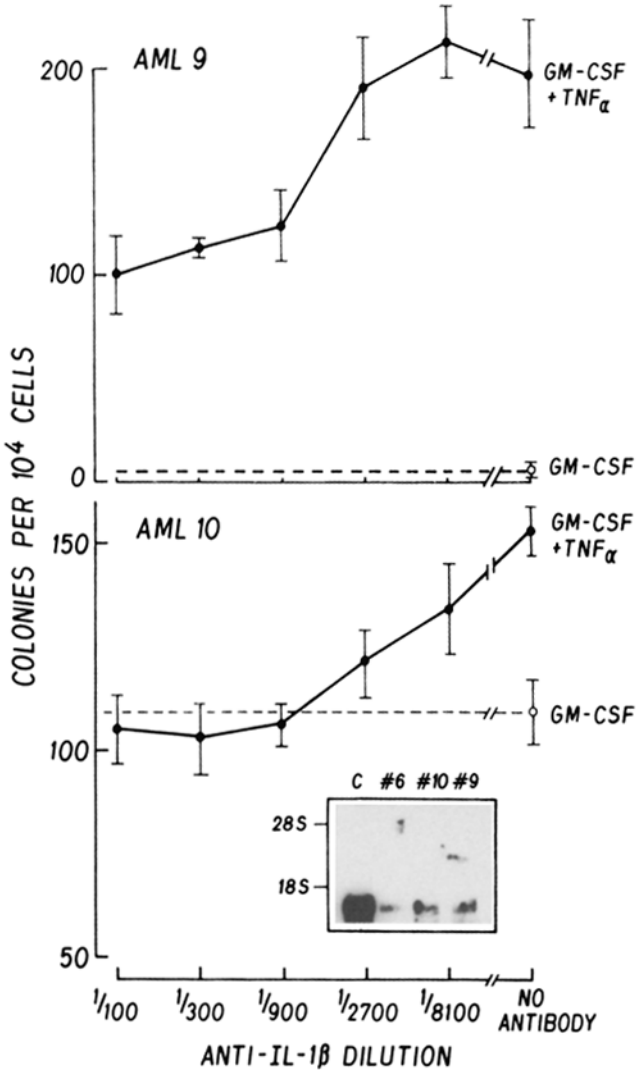

FIGURE 3. Effects of the neutralizing antibody against IL-1β on the growth of blast clonogenic cells in the presence of TNF-α and GM-CSF. Cultures were done at 10^4 cells/well. Data shown in the lower panel are typical of independent cultures with AML 8 and AML 10; results are shown for 10 (concentrations of growth factors as in Fig. 2 *A*). Colony counts in the absence of growth factors were 0 (AML 9) and 25 (AML 10); and in the presence of TNF-α alone were 0 (AML 9) and 47 (AML 10). (*Inset*) Expression of IL-1β mRNA by AML blasts. Total RNA (20 μg/lane) was denatured and analyzed by RNA blotting with the IL-1β cDNA probe. Shown are RNA from AML 6, AML 10, AML 9, and 5637 cells (*C*).

tion and endogenous growth factor production (Fig. 3). Addition of anti-IL-1β to such cultures reduced the synergistic action of TNF-α and GM-CSF on AML blasts with a dose-dependent effect. The reduction in colony count was not due to a nonspecific toxicity of the antibody, since it does not affect the synergistic stimulation of blast progenitors by G-CSF, GM-CSF, and IL-6 (Table IV).

There was, however, a marked difference between the three samples tested, AML 8, 9, and 10 (Fig. 3). AML 8 and 10 behaved similarly, in that addition of anti-IL-1β to cultures containing both GM-CSF and TNF-α brought colony counts to the levels of stimulation observed with GM-CSF alone. In contrast, there was only a partial neutralization of the synergistic effects of the two growth factors on AML 9 cells upon addition of anti-IL-1β. The endogenous production of IL-1β was confirmed by Northern blotting analyses, revealing the presence of IL-1β mRNA in the same cell populations (Fig. 3 *inset*). As the intensities of the IL-1β bands are comparable between AML 9 and 10, the levels of IL-1β produced by the two cell types may also be comparable. Consequently, the difference between a complete and partial neutralization effect of anti-IL-1β on the two cell types may not be due to differences in IL-1 concentrations, but may perhaps be attributed to the involvement of additional mechanisms in the synergy between TNF-α and GM-CSF in the case of AML 9 blasts.

The production of IL-1β by AML blasts was confirmed by ELISA. No production was detectable when cells were cultured in GM-CSF alone. However, in the presence of TNF-α and GM-CSF, the production of IL-1β was in the order of 100 pg/ml (Table V). Taken together, our data suggest that the growth-promoting effects of TNF-α on AML blasts could be attributed in part to induction of an endogenous

TABLE IV

The Antibody against IL-1β Does not Affect the Synergistic Effects of G-CSF and GM-CSF or IL-6 with G- and GM-CSF on AML Blasts

Cells	Growth factor added	Antibody dilutions	Colony counts
AML 10	0	-	0
	GM-CSF	-	35 ± 5
	G-CSF	-	2 ± 1
	GM-CSF + G-CSF	-	57 ± 3
	GM-CSF + G-CSF	1:8,100	68 ± 10
	GM-CSF + G-CSF	1:2,700	65 ± 2
	GM-CSF + G-CSF	1:900	59 ± 8
	GM-CSF + G-CSF	1:300	60 ± 1
	GM-CSF + G-CSF	1:100	65 ± 15
AML 8	GM-CSF + G-CSF	-	91 ± 7
	GM-CSF + G-CSF + IL-6	-	140 ± 4
	GM-CSF + G-CSF + IL-6	1:100	136 ± 6
	IL-3	-	83 ± 11
	IL-3	1:100	78 ± 1

The anti-IL-1β was a sheep antibody raised against purified IL-1β. At a concentration of 1:10,000, it neutralized 50 pM of IL-1β. Cultures of AML blasts were stimulated with optimal concentrations of growth factors. 75 pM (GM-CSF), 10 U/ml (G-CSF), 750 pM (IL-6), and 75 pM (IL-3).

TABLE V
Production of IL-1β by AML Blasts Determined by ELISA

Growth factor		Concentration	OD
		pg/ml	
Negative control	0	-	0.059
	TNFα	4,000	0.040
	GM-CSF	2,000	0.043
	IL-6	2,000	0.046
Positive control	IL-1β	10	0.100
		33	0.168
		100	0.377
		330	0.766
		1,000	1.558
AML 9-conditioned media	AML-CM 1	1:3	0.150
		Undiluted	0.494
	AML-CM 2	1:3	0.041
		Undiluted	0.053

The IL-1β ELISA was performed as described in Materials and Methods. Growth factors were diluted in IMDM/10% FCS. Culture medium with no added growth factor served as a negative control. Cells were from AML 9 stimulated with GM-CSF (AML-CM 2) or TNF-α and GM-CSF (AML-CM 1) at the following concentrations: 80 pM (2 ng/ml, GM-CSF) and 240 pM (4 ng/ml, TNF-α).

production of IL-1β. Further, TNF-α did not significantly affect colony formation in the presence of exogenously added IL-1 (Table II), suggesting that the two molecules may have overlapping growth signaling pathways. In fact, it has been shown that TNF-α, IL-1α, and IL-1β induce the production of the same enzymes while other pleitropic cytokines such as interferons and TGF-β behave differently (37).

Discussion

The presence of a single class of high affinity receptors for TNF-α on normal hemopoietic cells, AML blasts, and human leukemic cell lines was reported recently by Munker et al. (21). Unlike TGF-β on retinoblastomas, lack of a growth inhibitory response to TNF-α was not related to the absence of TNF-α receptors on several TNF-α-resistant cell lines such as K562. These results would suggest that the peculiar response of AML blasts to TNF-α and GM-CSF, as compared with normal cells, is probably not due to differences in TNF-α receptors.

The mechanism underlying the synergistic stimulation of AML blasts by TNF-α and GM-CSF may be complex. Our results suggest that it can be explained in part by an induction of endogenous IL-1β production by the cells. However, patient sample AML 5 was refractory to exogenously added IL-1, as observed in our previous study (6), while the other patient samples are responsive. The synergy between GM-CSF and TNF-α in the case of AML 5 is likely due to other mechanisms. Further, there was only a partial neutralization of the synergy between TNF-α and GM-CSF in the stimulation of AML 9 blasts upon addition of anti-IL-1β, suggesting the involvement of additional mechanisms. Thus, at the cellular level, TNF-α appears to recruit the same population of progenitors as GM-CSF and to increase the sensitivity

of the cells to GM-CSF. As receptor modulation has been reported in other systems to explain the synergistic effects between growth factors (38), it is possible that TNF-α exerts a modulation of GM-CSF receptors. In the case of AML blasts, however, TNF-α enhanced the stimulatory effects of GM-CSF while antagonizing that of IL-3, a growth factor that appears to share common biologic properties with GM-CSF and has the unique capacity to compete for GM-CSF binding to target cells (39). It is possible that the molecular mechanism of synergy with GM-CSF is more complex than mere receptor modulation, involving perhaps post-receptor events.

TNF-α in most biological systems acts as a growth inhibitory cytokine. However, a growth-enhancing effect was observed on fibroblasts and epithelial cells (reviewed in reference 40), and a growth-stimulating effect was observed on the malignant cells of chronic B cell leukemias (41). On AML blasts, TNF-α can either support or inhibit cell proliferation, depending on the other growth factors present. Thus, much in the same way as TGF-β (40), TNF-α appears to be multifunctional, depending on the cell type and the growth-signaling context. We have not directly addressed the question of cell type responding to TNF in the present study. Nonetheless, several observations suggest that blast clonogenic cells are able to respond to the growth-promoting effects of TNF-α. First, GM-CSF and TNF appear to recruit the same population of cells. Second, AML blasts have been shown to express TNF-α receptors (21) with dissociation constants that are in the same range as the concentrations required for half-maximal stimulation of blast colony formation. Taken together, the data suggest that TNF-α can efficiently function as a bimodulator of AML cell proliferation. It will be important, however, to document by additional cell separation techniques whether an accessory cell function is required for the stimulation of AML blasts by TNF-α.

Whether the peculiar response of AML blasts to TNF-α and GM-CSF would bear some physiological significance is a matter of debate. So far, analysis of AML blasts at the cellular and molecular levels and of clinical outcome in AML have disclosed significant patient to patient heterogeneity (42). Our previous studies on GM-CSF, IL-1, and IL-6 responsiveness also revealed significant variations (6, 10). For example, AML 1-4 responded to IL-1 while AML 5 was refractory (6). Similarly, AML 1, 2, and 7 were IL-6 responsive, while AML 3 and 5 were not (10). However, all these AML samples displayed a comparable response to the combined stimulatory effects of GM-CSF and TNF-α. In striking contrast with the other biological properties, the uniformity of response to TNF-α and GM-CSF in the eight AML samples studied so far would suggest that this property may bear some physiological significance. Of interest, TNF-α is capable of supporting colony formation in the absence of other growth factors when tested at high cell density, that is, under conditions that favor autocrine stimulation. It is possible that the synergistic effects of TNF-α with an autocrine growth signal would allow the cells to escape from negative regulation and acquire a proliferative advantage.

Our study also brings out the importance of primary cultures in studying some intrinsic biologic properties of leukemic cells. Thus, the GM-CSF responsive cell line KG-1 was inhibited by TNF-α (11), indicating that it failed to exhibit the same response to TNF-α as freshly isolated AML blasts. The availability of large numbers of cells that are not selected by culture conditions provides a unique opportunity to study mechanisms involved in both normal and leukemic cell proliferation.

Summary

TNF-α has been shown to antagonize the proliferative effects of growth factors present in crude conditioned media from PHA-stimulated leukocytes or cell lines on the clonogenic cells of acute myeloblastic leukemia (AML) (19, 21). In the present study, we investigated the responses of AML blasts to TNF-α in the presence of defined growth factors (recombinant granulocyte/macrophage-CSF [rGM-CSF], recombinant granulocyte-CSF [rG-CSF], rIL-3, and rIL-1) and under conditions described for autocrine stimulation (32). While TNF-α antagonized the stimulatory effects of G-CSF and IL-3 on blast progenitors, TNF-α did not affect blast colony formation in the presence of IL-1. Unexpectedly, TNF-α significantly enhanced blast proliferation in the presence of GM-CSF. Further, TNF-α also acted synergistically with an endogenous source of growth stimulatory signal to promote proliferation of blast clonogenic cells. Thus, on human leukemic cells, TNF-α appears to be a molecule that is at least bifunctional, having the ability to either support or inhibit cell proliferation, depending on the other growth factors present. It is postulated that the proliferative response of blast progenitors to TNF-α under conditions that favor autocrine stimulation may represent one property that allows the cells to escape from negative regulation and proliferate in AML.

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