Antitumor Effects of Human Recombinant Interleukin-6 on Acute Myeloid Leukemia in Mice and in Cell Cultures

By Tamar Givon, Shimon Slavin, Nechama Haran-Ghera, Rita Michalevicz, and Michel Revel

Interleukin-6 (IL-6) has been shown to inhibit growth and induce differentiation of several myeloid leukemia cell lines. In this work, two in vivo models of acute myeloid leukemia (AML) in mice have been used to test the therapeutic potential of recombinant human IL-6. In mice inoculated by a transplantable AML tumor, IL-6 injections inhibited the development of leukemia and increased survival. The effect was related to dose and length of treatment. In a model of radiation-induced leukemogenesis in SJL/J mice, administration of low-dose IL-6 for 10 days, 4 months after irradiation, reduced the incidence of leukemia observed during 1 year, whereas granulocyte-macrophage colony-stimulating factor (GM-CSF) increased the incidence of leukemia. In vitro liquid cultures of leukemic blood cells obtained from AML patients showed that IL-6 slowed growth and decreased the proportion of blasts with an increase in more mature myeloid elements in 72% of M1, M2, M4 AML cases. In contrast, GM-CSF less often produced differentiation but stimulated leukemic cell growth in liquid cultures, without synergism by IL-6.

THE MULTIFUNCTIONAL cytokine interleukin-6 (IL-6) mediates responses of the organism to infections and inflammation, such as induction of liver acute-phase proteins, potentiation of hematopoitic activity with production of mature myeloid and megakaryocytic cells, stimulation of B- and T-lymphocyte differentiated functions with increased antibody secretion, and T-cell cytotoxicity.[1-3] As found with other cytokines, IL-6 affects the growth of various cell types in opposite ways. IL-6 promotes growth, sometimes in an autocrine mode, of some plasmacytomas and myelomas,[4] Epstein-Barr virus (EBV)-transformed B cells,[5] T- and B-cell lymphomas,[6] Kaposi sarcoma-derived cells,[7] or keratinocytes.[8] Growth inhibition by IL-6 was observed in several breast carcinoma cell lines.[9-11] IL-6, alone or in combination with other cytokines, induces growth-arrest and resumption of terminal differentiation in myeloleukemic cells. Murine IL-6 is, in fact, the monocytic-granulocytic differentiation factor for normal and leukemic progenitors, known as MGI-2.[12] Clones of the murine myeloleukemia M1 line exposed to IL-6 undergo growth-arrest and macrophage differentiation with induction of surface markers (Mac-1, Mac-3, Fc-receptors, c-fms/macrophage colony-stimulating factor [M-CSF] receptor, major histocompatibility complex [MHC] class I) and of typical cellular activities (nonspecific esterase, lysozyme, 2′-5′ A synthetase, phagocytosis).[13-16]

Among human myeloid cell lines, growth of U937 histiocytic lymphoma cells is inhibited by IL-6[9,13,17,18] and differentiation is induced by IL-6 combined with IL-1,[17] with interferon-γ (IFN-γ),[13] or with granulocyte-macrophage CSF (GM-CSF),[18] which all synergize with IL-6. In human ML-1 myeloblastic cells, IL-6 synergizes the differentiation-inducing effects of tumor necrosis factor (TNF).[19] In THP-1 acute promonocytic leukemia cells, some differentiation is induced by IL-6 plus IL-1,[20] and in HL-60 promyelocytic leukemia IL-6 synergizes with GM-CSF to suppress colony growth in agar.[18] AML-193 monoblastic leukemia cells, which grow slowly with IL-6, undergo some granulocytic differentiation.[21] In an M4 AML line OCI/AML, IL-6 reduces the G-CSF–dependent growth, inducing some differentiation.[22]

Despite the above effects of IL-6 on myeloleukemic cell lines, the in vivo activity of IL-6 on acute myeloid leukemia (AML) has not been reported. It was argued that use of IL-6 in AML may be detrimental because of its synergism with IL-3, GM-, or G-CSF for growth of granulocyte/monocyte progenitors, which is also seen in AML blast colony formation from blood cells of certain (but not all) AML patients.[21-24] In this study, we have used two models of murine AML to investigate the in vivo effects of human recombinant IL-6 (rIL-6). The results indicate antitumor activity of IL-6 on AML, as has been recently found also for murine sarcoma metastases[25] and erythroleukemia models.[26] The possibility of using IL-6 in human AML is discussed by reexamining, in both liquid and semisolid cultures, the in vitro action of IL-6 on growth and differentiation of AML blast cells from individual patients.

MATERIALS AND METHODS

Cytokines. Recombinant human IL-6 (rhIL-6) was from Chinese hamster ovary (CHO) cells prepared and purified as described.[27] The specific activity determined in comparison with standard 88/514 (National Institute for Biological Standards and Control, Potters Bar, UK) by a plasmacytoma growth assay, was 10^7 reference units/mg protein. Certain experiments were also performed using *Escherichia coli* produced rIL-6 as described.[13] Both rIL-6 contained less than 0.1 ng endotoxin per milligram protein.

rGM-CSF, 1.2×10^7 U/mg, was a gift from Sandoz (Basel, Switzerland). rG-CSF, 2×10^8 U/mg, was a gift from Dr M. Moore (Memorial Sloan-Kettering Cancer Center, New York, NY) and from Amgen (Thousand Oaks, CA). rM-CSF, 10^8 U/mg was from Genzyme. Human IFN-β, 5×10^8 U/mg, and IFN-γ, 10^8 U/mg (both CHO-derived) were prepared and purified as previously described.[28,29]

From the Department of Bone Marrow Transplantation Center, Hadassa Medical School, Jerusalem; the Departments of Chemical Immunology and Molecular Genetics-Virology, Weizmann Institute of Science, Rehovot; and the Department of Hematology, Ichilov Hospital, Tel Aviv, Israel.

Submitted August 12, 1991; accepted December 27, 1991.

Supported by InterPharm and Ares-Serono.

Address reprint requests to Michel Revel, MD, PhD, Department of Molecular Genetics and Virology, Weizmann Institute of Science, Rehovot 76100, Israel.

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

0006-4971/92/7909-0005$3.00/0

Transplantable AML in mice. SJL/J mice were injected in the tail vein with 5×10^3 transplantable mAML-1 cells (described in the text) and treated by rIL-6 in the indicated doses and schedules. Control mice received similar phosphate-buffered saline (PBS) injections. Blood was drawn weekly (weeks 6 to 10) from the retro-orbital vein using heparinized glass capillaries, and red blood cells (RBCs) were lysed with 2% acetic acid. White blood cells (WBCs) were counted, and values over 20×10^6/mL were considered as overt leukemia. AML incidence and survival of the mice was followed. Statistical probabilities were calculated by the two-tailed Student's *t*-test.

Radiation-induced AML in mice. Three-month-old female SJL/J mice (Jackson Labs, Bar Harbor, ME) were exposed to 350 rads total body irradiation, and received 1 to 3 hours later a single subcutaneous (s.c) injection of dexamethasone (0.5 mg), as detailed.[30-32] Starting at day 140 after irradiation, cytokines were injected for 2 courses of 5 days per week. Incidence of overt leukemia was followed for 8 months after treatment.

Human AML cell studies in vitro. We studied a group of 23 untreated AML patients with French-American-British (FAB) classification and percentage of blasts in the blood samples indicated in the text. Liquid cultures of peripheral blood mononuclear cells (PBMC) were performed at 0.5 to 1×10^6 cells/mL in Iscove-modified Dulbecco medium (IMDM; GIBCO, Grand Island, NY) with 10% fetal calf serum and the indicated cytokines. After 5 days at 37°C, cells were counted and slides were Giemsa-stained. The number of blasts, promyelocytes, metamyelocytes, bands, and segmented myeloid elements and monocytes were compared after culture with and without cytokines. The few lymphocytes were not included in the calculations. The growth of blast colonies in methyl-cellulose was performed as described.[23]

RESULTS

Effects of IL-6 on transplantable AML in mice. In this first model, SJL/J mice were inoculated intravenously (IV) by mAML-1 tumor cells freshly taken from leukemic mice. These cells were originally obtained from radiation-induced AML in SJL/J mice[30,31] and were maintained by passage in vivo. The newly injected mice develop overt leukemia (ie, WBC counts over 20×10^6/mL) in about 6 to 7 weeks (Table 1). We first tested several doses of rIL-6 (CHO-produced) administered from day 1 to 5 after mAML-1 cell inoculation. Daily doses of 1, 5, 25, and 120 μg per mouse (or PBS vehicle) were divided in three intraperitoneal (i.p.) injections administered 8 hours apart. WBC counts showed that with PBS or 1, 5, and 25 μg/d rIL-6 in this 5-day treatment, AML developed rapidly: at 7 weeks, there were three to four leukemic mice out of four in each of these groups (Table 1). However, none of the mice treated by 120 μg/d developed leukemia at 7 weeks, and three of four were still disease-free at 9 weeks (Table 1). Survival followed for 12 weeks (Fig 1) was prolonged in mice receiving 120 μg/d rIL-6, with a significant difference in mean day of death ($P < .05$) and 100% survival at 72 days compared with 25% in the control and 25 μg/d groups.

The effective dose and the first ineffective dose (120 and 25 μg/d) were retested on larger groups of 32 mice and with a more prolonged treatment, ie, 3 courses of 5 d/wk for the 3 first weeks after mAML-1 inoculation. At 8 weeks, 75% of the control mice had leukemia, and both doses of rIL-6 reduced the incidence of disease, by 50% ($P < .025$) at 25 μg/d and by 62.5% ($P < .02$) at 120 μg/d (Table 2). At 10 weeks, there was still a 35% reduction of leukemia incidence at both doses ($P < .05$, not shown). The 3-course treatment seems essential for response at 25 μg rIL-6/d (compare the two experiments in Table 2). Follow-up for 115 days shows (Fig 2) that with the longer treatment schedule, rIL-6 at 25 μg/d prolonged survival ($P = .02$) as well as at 120 μg/d ($P < .01$). Similar reductions in AML incidence were seen whether rIL-6 was administered i.p. or s.c., or if the treatment was started only in the second week after mAML-1 cell inoculation and continued for 2 weeks (not shown).

Table 1. Effect of IL-6 Injections on Transplanted AML Development in SJL/J Mice

Treatment		Peripheral Blood WBC × 10^{-6}/mL at: 6 wk	7 wk	8 wk	9 wk
PBS		72	237	220	D
		19	94	216	D
		15	68	206	152
		9	20	6	15
IL-6	1 μg/d	39	254	230	D
		26	244	210	D
		12	214	167	353
		10	70	151	263
IL-6	5 μg/d	20	192	D	D
		18	190	431	D
		8	185	219	179
		7	10	8	19
IL-6	25 μg/d	126	D	D	D
		12	33	148	243
		10	24	126	238
		10	14	31	201
IL-6	120 μg/d	13	16	29	167
		9	13	14	10
		9	12	11	9
		8	5	6	7

Mice treated with the indicated daily dose of rIL-6 divided in 3 i.p. injections, from day 1 to 5 after IV inoculation of mAML-1 cells. Mean WBC in PBS *v* rIL-6 120 μg/d: 105 ± 81 *v* 11.5 ± 4 ($P < .05$) at 7 weeks; 162 ± 90 *v* 15 ± 8.5 ($P < .025$) at 8 weeks.

Abbreviation: D, death from leukemia.

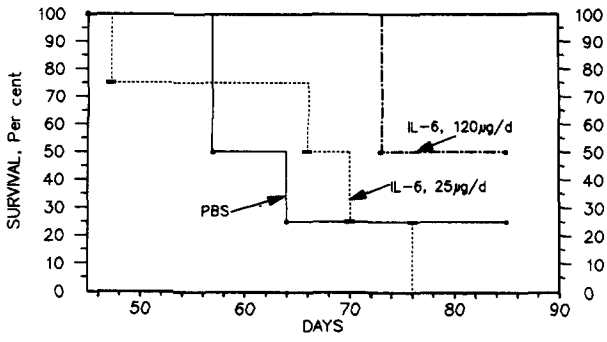

Fig 1. Survival of SJL/J mice inoculated by mAML-1 and treated 5 days by IL-6. Mice (four per group) were treated with rIL-6, 25 μg or 120 μg per day divided in three i.p. injections, from day 1 to 5 after IV inoculation of mAML-1 cells. For time of leukemia onset, see Table 1. Survival was followed for 80 days after treatment. Mean day of death: control and 25 μg/d rIL-6, 65 ± 11; 120 μg/d rIL-6, 79 ± 6 days ($P < .05$).

Table 2. Effect of IL-6 Injections on Transplanted AML Incidence in SJL/J Mice

Treatment	AML Incidence at 8 wk (leukemic mice/total) 5 d Treatment* (%)	3 × 5 d Treatment† (%)	
PBS	3/4 (75)	24/32 (75)	
IL-6 25 μg/d	4/4 (100)	12/32 (37.5)	$P < .025$
IL-6 120 μg/d	1/4 (25)	9/32 (28)	$P < .02$

*Mice treated with indicated daily dose of rIL-6 divided in 3 i.p. injections from days 1-5 after IV mAML-1 cell inoculation.

†Mice treated with indicated daily dose of rIL-6 divided in 2 i.p. injections for days 1-5, 8-12, 15-19 after IV mAML-1 cell inoculation.

We also tested a pretreatment in which mice received rIL-6 (25 and 120 μg/d in three i.p. injections) from day −3 to −1 before being injected IV with mAML-1 cells. At 7 weeks, eight of eight mice in both IL-6 groups had developed leukemia versus seven of eight in the control group. There was also no beneficial effect of rIL-6 on survival and all mice died at 65 to 70 days (not shown). Therefore, rIL-6 pretreatment is not inducing a stable resistance to AML before the tumor cells are present.

Effects of rIL-6 on radiation-induced AML. A model of myeloid leukemogenesis[30] was used to test the effect of rIL-6 administration on the slow de novo development of AML in mice. In this model, young SJL/J mice subjected to x-irradiation at 350 rads develop radiation-induced AML (RI-AML) with an incidence of about 20% at 1 year. This incidence can be increased to about 50% with a mean latency of 280 days, when a single injection of dexamethasone is given with the irradiation.[30,31] With this protocol, the incidence of disease is high enough to allow testing of antitumor agents. The RI-AML that develops has been defined as myelomonocytic, and the leukemia cells are all characterized by deletions affecting a delimited region in one chromosome 2.[30,31] Cells with such deletions appear early, long before overt leukemia is observed: at 4 months after the coleukemigenic stimulus, cells carrying chromosome 2 deletions form 20% to 30% of the bone marrow

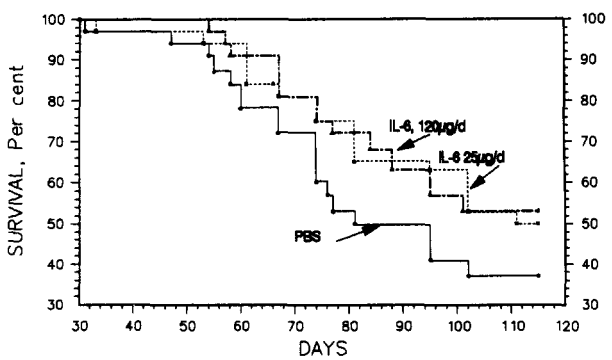

Fig 2. Survival of SJL/J mice inoculated by mAML-1 and treated for 3 weeks by IL-6. Mice (32 per group) were treated with 25 μg or 120 μg rIL-6 per day divided in two i.p. injections, at days 1 through 5, 8 through 12, and 15 through 19 after IV inoculation of mAML-1 cells. See Table 2 and text for leukemia incidence. Survival was monitored for 95 days after treatment. Mean day of death: control 70.8 ± 17; rIL-6 25 μg/d, 85 ± 24 ($P = .02$), 120 μg/d, 86 ± 21 ($P < .01$).

population in over 80% of the mice. Transplantation studies show that these bone marrow cells taken at 4 months are able to produce overt AML of donor origin, at high incidence, in F1 recipient mice.[30,31]

We chose to administer rIL-6 therapy at 4 months after the coleukemogenic stimulus, because in the development of RI-AML this is the time when preleukemic clones of cells (defined as above) are readily detected in the bone marrow and spleen. This 4-month delay also insured that transient changes in endogenous cytokines produced by irradiation or dexamethasone[32] would have no interference with the rIL-6 treatment. On day 140, one group of preleukemic mice (20 animals) received rIL-6 (CHO) at 1 μg/d/mouse in two i.p. injections for 10 days (5 days per week for 2 consecutive weeks). Another group received rGM-CSF (0.15 μg/d) with the same schedule and a third group (25 mice) was used as control. In bone marrow collected from the rIL-6–treated mice, 14 days after the first injection, karyotyping[30] already indicated a significant reduction in the percentage of cells carrying chromosome 2 deletions (9% ± 7% with rIL-6 *v* 28% ± 12% in the control group, $P < .001$). No rIL-6–treated mouse had more than 30% cells with the deletion, whereas this was the case in 40% of the control mice (not shown). Therefore, rIL-6 treatment leads to a rapid decrease in the putative preleukemic cells. Figure 3 shows the cumulative incidence of overt myeloid leukemia during the 8 months after the short cytokine treatment. In the rIL-6–treated mice, the incidence of overt of leukemia at 1 year after leukemogenesis was 23% versus 48% in the control group ($P < .05$) and the mean time of leukemia onset was delayed ($P = .01$). In contrast, with rGM-CSF the incidence of leukemia was increased to 77% ($P < .025$ *v* control) and the mean leukemia onset was earlier ($P < .001$). In other experiments, M-CSF was found, like GM-CSF, to accelerate the rate of RI-AML and increase its incidence, with both CSF acting most probably by enhancing growth of preleukemic AML clones.

Effects of IL-6 on growth of human AML blasts in vitro. In view of the inhibitory effect of rIL-6 on the two murine AML in vivo models, it was important to consider if IL-6 could be used at all in human AML. IL-6 has been suspected to promote growth of human AML blast by synergism with GM-CSF,[23,24] but IL-6 also contributes to differentiation of a number of myeloleukemic cell lines.[12-22] To evaluate what rIL-6 does to fresh ex vivo human AML blasts, we performed not only blast colony assays in methylcellulose as done before[22,23] but also liquid cultures of PBMC from AML patients with 33% to 89% of undifferentiated blasts. The number of leukemic cells and their differentiation status were measured after 5 days of liquid cultures without or with rIL-6, GM-CSF, or IFNs (example shown in Table 3). Table 4 gives the statistical analysis of growth: in 22 AML cases studied with rIL-6, average growth was 15% lower than in cultures without cytokine ($P < .001$), similar to what is seen with IFN-β. In contrast, rGM-CSF stimulated growth, yielding an average 50% cell increase ($P < .001$). In liquid cultures, the combination of rIL-6

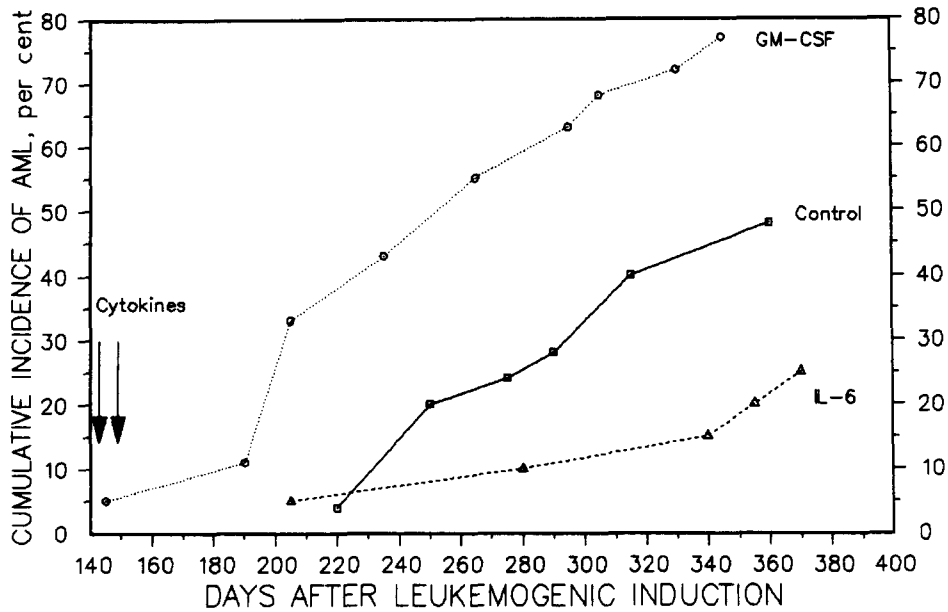

Fig 3. Cumulative incidence of AML in irradiated SJL/J mice treated by IL-6 or GM-CSF. Mice (3-month-old females, 20 to 25 per group) were subjected to 350 rads of x-ray total body irradiation, and 1 to 3 hours later received 0.5 mg dexamethasone s.c. Treatment by rIL-6 (1 μg per day divided in two i.p. injections) was started at 140 days and administered for 2 consecutive weekly courses of 5 days each (arrows). rGM-CSF (0.15 μg/d) was administered in the same schedule. The incidence of myeloid leukemia was monitored by WBC counts for 1 year after leukemogenesis. Mean day of disease: control, 288 ± 44; rIL-6, 310 ± 60 ($P = .01$); rGM-CSF, 245 ± 56 ($P < .001$).

with rGM-CSF did not increase the growth effect of GM-CSF (Table 4, last column).

The proportion of blasts compared with more differentiated cells (myelocytes, bands, and segmented, see Table 3) changed during the 5-day liquid cultures. We calculated a differentiation index (percent of myelomonocytes divided by that of blasts) and the ratio of this index in cultures with cytokines over cultures without them. Table 5 shows these ratio values, with the FAB class and percentage of blasts (before culture) of the AML patients studied. Considering first only M1, M2, M4 AML, ratios of 2 to 10 (ie, significant decreased blasts *v* more differentiated elements) were produced by rIL-6 in 72% of cases (13 of 18), by IFN-β in 73% (8 of 11), by both in 78% (7 of 9), but with rGM-CSF in only 28% (5 of 18, $P = .003$ *v* rIL-6). When GM-CSF was added to IL-6, the differentiation responses were 27% (4 of 15), much lower than with IL-6 alone (67%, 10 of 15) in the same cases (Table 5). We also scored cases with ratios < 1 (indicating increased blast proportion): in M1, M2, M4 AML there were 11% (2 of 18) with rIL-6, and 33% with rGM-CSF alone or with rIL-6 (6 of 18 and 5 of 15), indicating that IL-6 does not enhance the risk of GM-CSF to increase blasts. The same holds true if one includes the M5 and M0 (stem cell) AML (Table 5). M5 AML showed no differentiation with rIL-6. From these liquid cultures it appears that GM-CSF but not IL-6 stimulates AML cell growth, and is more likely than IL-6 to increase blasts. IL-6 does not synergize these GM-CSF effects. While the differentiation effects of IL-6 are often prevented by GM-CSF, the two together are not worse than GM-CSF alone.

In the AML blast colony assay on methylcellulose, rGM-CSF was also a potent growth factor, whereas rIL-6 alone was not (Table 6). But, unlike the liquid cultures, the semisolid colony assay indicates that rIL-6 can sometimes increase the GM-CSF effect. In line with previous studies,[22,23] such synergism was apparent only in some patients; in this study it was in three out of eight AML cases (Table 6).

Table 3. Effects of In Vitro Incubation With IL-6, GM-CSF, and IFNs on Growth and Differentiation in PBMC From Three AML Patients

Additions	Cell No. $\times 10^{-6}$	Blasts %	Myelocytes Pro-, Meta- %	Bands Segmented %	Monocytes %
None	2.3	53	28	18	1
IL-6	1.4	31	15	53	1
GM-CSF	4.0	48	26	23	1
GM-CSF + IL-6	3.9	57	35	7	1
IFN-γ	1.2	29	28	41	2
IFN-γ + IL-6	1.1	26	39	31	4
IFN-β	1.4	33	26	40	8
IFN-β + IL-6	1.4	31	25	38	6

PBMC from M2 AML patient were seeded at 10^6 cells/mL and incubated 5 days with rIL-6 (CHO) 15 ng/mL, rGM-CSF 10 U/mL, rIFN-β or rIFN-γ, 100 U/mL. Cells were counted and smears analyzed for cytology.

DISCUSSION

In vivo antitumor activity of human rIL-6 on AML in mice was observed in two experimental settings. First, in

Table 4. Effects of In Vitro Incubation With IL-6, GM-CSF, and IFN-β on Growth of PBMC From AML Patients

	Ratio of No. of Cells Over Control Culture				
	IL-6	IFN-β	IL-6 + IFN-β	GM-CSF	GM-CSF + IL-6
Mean	0.85	0.82	0.87	1.47	1.31
(SEM)	(0.038)	(0.023)	(0.065)	(0.108)	(0.093)
N	22	14	13	18	17
P	<.001	<.001	=.05	<.001	<.01

PBMC from patients with AML were incubated at 0.5-1 × 10^6 cells/mL for 5 days with rIL-6, 5 ng/mL, rIFN-β 100 U/mL, rGM-CSF 5 U/mL. Viable cell counts were divided by the number of cells in the 5-day control incubation without cytokine additions. Mean of the indicated number (N) of patients is shown with standard error of the mean (SEM). Same patients as in Table 5.

mice inoculated with transplantable murine mAML-1 cells, treatment with rIL-6 during 1 to 3 weeks after inoculation reduced the incidence of leukemia development and increased survival. The effect appears to be dose-dependent. At high doses (120 μg/d) a 5-day treatment was efficient, but at lower doses (25 μg/d) the treatment had to be prolonged. IL-6 therapy could be started 1 week after AML inoculation but pretreatment was not efficient, suggesting that IL-6 acts when the leukemic cells are growing in the animal. The second model used was in vivo leukemogenesis, in which high-incidence AML develops slowly after irradiation combined with a single dexamethasone injection.[30,31] rIL-6 was administered 4 months later, when preleukemic cells, with chromosome 2 deletions, are known to accumulate in the bone marrow and lymphoid organs.[30] In this model, a low-dose rIL-6 treatment (1 μg/d for 10 days) reduced the rate and incidence of overt leukemia in the year following the leukemogenic stimulus. It is likely that this long-term decrease in leukemia development is related to the decrease in cells with chromosome 2 deletions, which was already observed in the bone marrow a few days after rIL-6 treatment.

Table 5. In Vitro Response of Leukemia Cells From 23 AML Patients to Cytokine Induced Differentiation

AML Type	Blasts %	Ratio of Differentiation Index Relative to Control Cultures: IL-6	IFN-β	IL-6 + IFN-β	GM-CSF	GM-CSF + IL-6
M1	40	3.5	ND	ND	0.7	0.8
M1	89	3.2	6.2	3.2	6.5	6.8
M1	83	1.3	2.6	ND	0.6	0.4
M1	74	2.0*	ND	ND	0.8	ND
M2	74	3.5	4.1	ND	1.5	2.1
M2	50	2.2	2.0	2.0	1.0	0.7
M2	44	4.5	7.1	12.2	10.5	4.8
M2	50	0.6	ND	ND	2.1	1.2
M2	75	2.8	2.1	1.3	2.1	2.0
M2†	36	3.7*	ND	ND	0.4	ND
M2	87	5.4*	ND	ND	1.0	1.4
M2	70	10.4*	ND	ND	1.1	ND
M4	60	6.0	ND	ND	0.6	0.8
M4	70	2.1	1.9	2.1	1.4	1.5
M4	81	1.5	1.4	3.4	4.7	1.9
M4	50	1.7	1.1	1.5	0.5	0.9
M4	33	2.5	4.3	3.1	1.0	1.5
M4	82	0.9	2.3	2.8	1.4	1.1
M5	88	0.6	2.7	3.1	3.7	3.2
M5	15	1.2	1.9	1.9	0.8	1.1
M5	81	1.8	1.3	1.0	1.5	3.8
MO	60	1.6	2.9	2.7	3.2	2.0
MO†	85	5.6	4.4	ND	3.9	6.1

PBMC from AML patients with indicated FAB type were incubated for 5 days with rIL-6, 5 ng/mL (CHO-produced unless otherwise indicated), rIFN-β 100 U/mL, rGM-CSF 5 U/mL, or without cytokines. The percentage of blasts before incubation is shown (mean 64% ± 20%). The differentiation index is the percentage of myelomonocytes divided by that of blast, computed from data as in Table 3. The ratio of the differentiation index after incubation with the indicated cytokine over that in control cultures is shown (ratio >2 is defined as differentiation response).

**E coli* IL-6 used.

†Bone marrow cells instead of PBMC.

Table 6. Blast Colony Formation on In Vitro Semisolid Culture of AML Cells With IL-6 and GM-CSF

AML Type	No. of Blast Colonies With Additions: None	IL-6	GM-CSF	GM-CSF + IL-6	IFN-β	IFN-β + IL-6
M1	0	0	7	21	ND	ND
M2	55	38	87	89	40	41
M2	0	0	8	22	ND	ND
M2	240	193	300	286	235	183
M4	6	5	48	78	3	2
M4	0	3	4	4	4	1
M5	51	36	95	95	39	31
M5	0	3	8	5	0	2

Monocyte– and T-cell–depleted PBMC from AML patients with indicated FAB type were grown for 15 days in methyl cellulose with rIL-6, 5 ng/mL, rGM-CSF 5 U/mL or rIFN-β 100 U/mL.

Several mechanisms could be invoked to explain these in vivo effects of rIL-6 on murine AML, including direct effects on tumor cells and indirect effects on host defenses, such as immune effects related to IL-6 being a coactivator of T and B lymphocytes.[2] An involvement of T cells was found to be required for the antimetastatic effects of IL-6 on sarcoma.[25] Similarly, rIL-6 induced specific $CD8^+$ cytotoxic lymphocytes (CTL) against murine erythroleukemic FBL-3 cells, and maintained survival 100 days after tumor inoculation, with the mice showing immunity to the tumor.[26] However, in this study IL-2 also induced this CTL response, but was much less efficient than IL-6 to prolong survival. This may indicate that CTL induction is not the only mechanism involved in IL-6 action, or may be due to the toxic effects of IL-2. Whereas the mice appear to tolerate high doses of rIL-6, such treatment with IL-2 can cause vascular leak syndrome, neurologic toxicity, and often death.[25]

It is more difficult to assess whether rIL-6 in vivo could also decrease AML cells by some effect on their differentiation and growth control as observed in several human and murine myeloleukemic cell lines with rIL-6 alone or in combination with other cytokines.[12-22] If the outcome of IL-6 treatment in vivo was influenced by the growth response of the leukemic cells, one would expect that leukemias of B cells for which IL-6 is a growth factor[4-6] would not respond to in vivo IL-6. Indeed, we found that rIL-6 did not delay leukemia in mice inoculated with murine B-lymphocytic leukemia BCL-1 cells (T.G. and S.S., unpublished observation, November 1990). Similarly, in AKR mice thymectomized at age 1 to 3 months, rIL-6, administered at 1 year (1 μg/d for 10 days) increased the incidence of B-cell leukemia from 23% to 61% at 140 days after IL-6, and in the same system IL-2 produced 100% leukemia within 70 days (N.H.-G. and A. Peled, unpublished observation, March 1990). Moreover, the fact that GM-CSF accelerated notably the rate of RI-AML also supports some relation between in vivo effects on AML and leukemia cell growth control.

Several studies have been concerned with the role that IL-6 could play in human AML. First it was observed that IL-6 is produced by human AML cells,[24] although only the more differentiated M4 and M5 AML cells produce significant amounts, and the production is not constitutive in fresh ex vivo cells but only seen after in vitro incubation.[33] Nevertheless, IL-6 was suspected to act as a growth factor for AML blasts, which would limit its use in human AML. In fact, as confirmed here, IL-6 alone does not significantly stimulate growth of AML blast colonies in semisolid medium, although it can synergize the growth activity of GM-CSF, G-CSF, IL-3, or IL-4.[22,23,34] However, this synergistic action of IL-6 on blast colony growth is found only in a subset of AML cases, while in others the effect is absent or even suppression is observed.[22] Recently, it was reported that $CD34^-$ AML rarely show synergism of IL-6 with IL-3 or IL-4, whereas it is seen in greater than 80% of $CD34^+$ AML.[34] In our group of AML patients, three of eight showed some synergism of IL-6 with GM-CSF. In addition, our study shows that in liquid cultures IL-6 does not enhance the growth stimulatory effect of GM-CSF. Like IFNs, IL-6 was even able to induce a decrease in the percentage of blasts with an increase in partially differentiated myeloid elements after 5 days of liquid culture, in over 70% of M1, M2, M4 (but not M5) AML cases. GM-CSF produced similar effects in only 28% of cases and often inhibited IL-6 differentiative action.

These in vitro activities of IL-6 will have to be taken into account when considering how to use rIL-6 in human AML. Liquid cultures and blast colony assays may be useful to select subsets of patients for treatment. However, considering the beneficial effects of rIL-6 in the experimental AML models in mice, it appears that the administration of rIL-6 alone in human AML should not be prohibited by the risk of enhancing blast colony growth through some synergistic action. The mechanism by which rIL-6 exerts in vivo an antitumor effect on murine AML could be complex, and our data suggest that trials of IL-6 in the treatment of certain human AML cases may be warranted.

ACKNOWLEDGMENT

The collaboration of Yael Koren, InterPharm Ltd, Nes-Ziona, Israel, is gratefully acknowledged.

REFERENCES

1. Revel M: Host defense against infections and inflammations: Role of the multifunctional IL-6/IFN-β2 cytokine. Experientia 45:549, 1989

2. Kishimoto T: The biology of interleukin-6. Blood 74:1, 1989

3. Sehgal PB: Interleukin 6 in infection and cancer. Proc Soc Exp Biol Med 195:183, 1990

4. Kawano M, Hirano T, Matsuda T, Taga T, Horii Y, Iwato K, Asaoku H, Tang B, Tanabe O, Tanaka H, Kuramoto A, Kishimoto T: Autocrine generation and requirement of BSF-2/IL-6 for human multiple myelomas. Nature 332:83, 1988

5. Tosato G, Tanner J, Jones KD, Revel M, Pike SE: Identification of interleukin-6 as an autocrine growth factor for Epstein-Barr virus immortalized B cells. J Virol 64:3033, 1990

6. Yee C, Biondi A, Wang XH, Iscove NN, DeSousa J, Aarden LA, Wong GG, Clark SC, Messner HA, Minden MD: A possible autocrine role for interleukin-6 in two lymphoma cell lines. Blood 74:798, 1989

7. Miles SA, Rezai AR, Salzar-Gonzales JF, Meyden MV, Stevens RH, Logan DM, Mitsuyasu RT, Taga T, Hirano T, Kishimoto T, Martinez-Maza O: AIDS Kaposi sarcoma-derived cells produce and respond to interleukin-6. Proc Natl Acad Sci USA 87:4068, 1990

8. Grossman RM, Kreuger J, Yourish D, Granelli-Piperno A, Murphy DP, May LT, Kupper TS, Sehgal PB, Gottlieb A: Interleukin-6 is expressed in high levels in psoriatic skin and stimulates proliferation of cultured human keratinocytes. Proc Natl Acad Sci USA 86:6367, 1989

9. Chen L, Mory Y, Zilberstein A, Revel M: Growth inhibition of human breast carcinoma and leukemia/lymphoma cell lines by recombinant interferon-β2. Proc Natl Acad Sci USA 85:8037, 1988

10. Tamm I, Cardinale I, Krueger J, Murphy JS, May LT, Sehgal PB: Interleukin-6 decreases cell-cell association and increases motility of ductal breast carcinoma cells. J Exp Med 170:1649, 1989

11. Revel M, Chen L, Novick D, Shulman LM: Effects of recombinant human IL-6 on breast cancer cells, in Oppenheim JJ, Powanda MC, Kluger MJ, Dinarello CA (eds): Molecular and Cellular Biology of Cytokines, Progress in Leukocyte Biology Vol 10A. New York, NY, Wiley-Liss, 1990, p 509

12. Shabo Y, Lotem J, Rubinstein M, Revel M, Clark SC, Wolf SF, Kamen R, Sachs L: The myeloid blood cell differentiation-inducing protein MGI-2A is interleukin-6. Blood 72:2070, 1988

13. Chen L, Novick D, Rubinstein M, Revel M: Recombinant interferon-β2 (interleukin-6) induces myeloid differentiation. FEBS Lett 239:299, 1988

14. Miyaura C, Onozaki K, Akiyama Y, Taniyama T, Hirano T, Kishimoto T, Suda T: Recombinant human interleukin-6 (B cell stimulatory factor 2) is a potent inducer of differentiation of mouse myeloid leukemia cells (M1). FEBS Lett 234:17, 1988

15. Chiu CP, Lee F: IL-6 is a differentiation factor for M1 and WEHI-3B myeloid leukemic cells. J Immunol 142:1209, 1989

16. Gothelf Y, Raber J, Chen L, Schattner A, Chebath J, Revel M: Terminal differentiation of myeloleukemic M1 cells induced by IL-6: Role of endogenous interferon. Lymphokine Cytokine Res 10:369, 1991

17. Onozaki K, Akiyama Y, Okano A, Hirano T, Kishimoto T, Hashimoto T, Yoshizawa K, Taniyama T: Synergistic regulatory effects of interleukin-6 and interleukin-1 on the growth and differentiation of human and mouse myeloid leukemic cell lines. Cancer Res 49:3602, 1989

18. Maekawa T, Metcalf D, Gearing DP: Enhanced suppression of human myeloid leukemic cell lines by combinations of IL-6, LIF, GM-CSF and G-CSF. Int J Cancer 45:353, 1990

19. Samal B, Stearns G, Crandall C, Arakawa T, Boone T: Identification of IL-6 as a synergistic factor for the differentiation-inducing effects of TNF on leukemic ML-1 cells. Leuk Res 14:575, 1990

20. Onozaki K, Akiyama Y, Okano A, Hirano T, Kishimoto T, Hashimoto T, Yoshizawa K, Taniyama T: Synergistic regulatory effects of BSF-2/IL-6 and IL-1 on the growth and differentiation of human and mouse myeloid leukemic cell lines into macrophage-like cells. Lymphokine Res 7:304, 1988 (abstr)

21. Caracciolo D, Clark SC, Rovera G: Human interleukin-6 supports granulocytic differentiation of hematopoietic progenitor cells and acts synergistically with GM-CSF. Blood 73:666, 1989

22. Suzuki T, Morio T, Tohda S, Nagata K, Yamashita Y, Imai Y, Aoki N, Hirashima K, Nara N: Effects of interleukin-6 and

granulocyte colony stimulating factor on the proliferation of leukemic blast progenitors from acute myeloblastic leukemia patients. Jpn J Cancer Res 81:979, 1990

23. Hoang T, Haman A, Goncalves O, Wong GG, Clark SC: Interleukin-6 enhances growth factor-dependent proliferation of the blast cells of acute myeloblastic leukemia. Blood 72:823, 1988

24. Oster W, Cicco NA, Klein H, Hirano T, Kishimoto T, Lindermann A, Mertelsmann RH, Hermann F: Participation of the cytokines interleukin-6, tumor necrosis factor-alpha and interleukin 1-beta secreted by acute myelogenous leukemia blasts in autocrine and paracrine leukemia growth control. J Clin Invest 84:451, 1989

25. Mule JJ, McIntosh JK, Jablons DM, Rosenberg SA: Antitumor activity of recombinant interleukin-6 in mice. J Exp Med 171:629, 1990

26. Kitahara M, Kishimoto S, Hirano T, Kishimoto T, Okada M: The in vivo antitumor effect of human recombinant interleukin-6. Jpn J Cancer Res 81:1032, 1990

27. Novick D, Eshhar Z, Revel M, Mory Y: Monoclonal antibodies for affinity purification of IL-6/IFN-β2 and for neutralization of HGF activity. Hybridoma 8:561, 1989

28. Chernajovsky Y, Mory Y, Chen L, Marks Z, Novick D, Rubinstein M, Revel M: Efficient constitutive production of human fibroblast Interferon by hamster cells transformed with the IFN-β_1 gene fused to an SV40 early promoter. DNA 3:297, 1984

29. Mory Y, Ben-Barak J, Segev D, Cohen B, Novick D, Fischer DG, Rubinstein M, Kargman S, Zilberstein A, Vigneron M, Revel M: Efficient constitutive production of interferon-γ in Chinese hamster ovary cells. DNA 5:181, 1986

30. Resnitzky P, Estrov Z, Haran-Ghera N: High incidence of acute myeloid leukemia in SJL/J mice after x-irradiation and corticosteroids. Leuk Res 12:1519, 1985

31. Haran-Ghera N: Radiation induced deletion of chromosome 2 in myeloid leukemogenesis. Curr Top Microbiol Immunol 149:35, 1989

32. Haran-Ghera N, Resnitzky P, Krautghamer R, Tartakovsky B: Multiphase process involved in radiation-induced murine AML. Leuk Res (in press)

33. van der Schoot CE, Jansen P, Poorter M, Wester MR, von dem Borne AEG, Aarden LA, van Oers RHJ: Interleukin-6 and interleukin-1 production in acute leukemia with monocytoid differentiation. Blood 74:2081, 1989

34. Akashi K, Harada M, Shibuya T, Eto T, Takamatsu Y, Teshima T, Niho Y: Effects of interleukin-4 and interleukin-6 on the proliferation of $CD34^+$ and $CD34^-$ blasts from acute myelogenous leukemia. Blood 78:197, 1991