Brief Definitive Report

AUTOCRINE STIMULATION OF INTERLEUKIN 1β IN ACUTE MYELOGENOUS LEUKEMIA CELLS

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Involvement of IL-1 in tumor cell growth was first observed in subclones of a murine T cell lymphoma (1). On EBV-transformed B cell lines, including VDS-O, both IL-1α and IL-1β have been reported to share the same receptor (2). This suggests that IL-1 plays a role in autocrine control of VDS-O cell proliferation, since these cells produced IL-1 and proliferated after its addition, though the effects of anti-IL-1 antibodies on proliferation of these cells were not studied (2, 3). Subsequent isolation of the genes coding for IL-1 has enabled studies on the effects of rIL-1 free of contaminating materials, and measurement of the genes' intracellular expression (4, 5). In this study, the effects of rIL-1 and other recombinant hematopoietic factors on proliferation and CD25 antigen expression by acute myelogenous leukemia (AML) cells were examined.

Materials and Methods

Patients. Five patients with AML were examined, and diagnosed according to the French-American-British (FAB) classification (6, 7). Electron microscopic studies of platelet peroxidase (PPO) activity were performed according to the method of Breton-Gorius et al. (8).

Cell Culture. Leukemic blasts were obtained from either peripheral blood or bone marrow by Ficoll-Conray density gradient centrifugation. The relative proportion of blasts in each cell suspension always exceeded 90%. Purified leukemic cells (10^6 cells/ml) were cultured at 37°C in the presence or absence of various reagents, in complete medium (RPMI 1640 medium supplemented with 100 U/ml penicillin G, 100 μg/ml streptomycin, and 10% FCS) and a humidified atmosphere of 5% CO_2 in air. Human rIL-2, rIL-1α, rIL-1β, granulocyte/macrophage colony-stimulating factor (GM-CSF), and gibbon rIL-3 were obtained from Takeda Pharmaceutical Co. Ltd., Dainippon Pharmaceutical Co. Ltd., Otsuka Pharmaceutical Co. Ltd., and the Genetics Institute, respectively. Specific rabbit antibodies against IL-1α and IL-1β were provided by Dainippon Pharmaceutical Co. Ltd. and Otsuka Pharmaceutical Co. Ltd., respectively.

[^{3}H]TdR incorporation of leukemic cells was measured as described previously (9).

Cell Surface Marker Analysis. Cell surface markers were analyzed by laser flow cytometry (Spectrum III; Ortho Diagnostic Systems Inc., Westwood, MA). mAbs used were MCS-2 (CD13), anti-Tac (CD25) (kindly provided by Dr. T. Uchiyama at Kyoto University,

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Kyoto, Japan), phycoerythrin (PE)-conjugated anti-IL-2-R antibody (CD25) (Becton-Dickinson Monoclonal Center, Mountain View, CA), anti–platelet-associated glycoprotein IIb/IIIa (CDw41) (Immunotech, Marseille, France), T11 (CD2), and B4 (CD19) (Coulter Immunology, Hialeah, FL). To prevent nonspecific binding of mAb to cell surface Fc receptors, mAb was always incubated in the presence of 50 μl heat-inactivated human AB serum (9). Two-color fluorescence analysis was performed on cells sequentially stained with anti-CD13 mAb and FITC-conjugated goat anti–mouse IgG followed by PE-conjugated anti-CD25 mAb, and the data were analyzed by a DS-1 computer equipped with a program developed at Ortho Diagnostic Systems Inc. according to the original program (10).

IL-1 Activity in Culture Supernatants. Crude conditioned media were prepared as described elsewhere (11). IL-1 activity in the culture supernatants was examined by thymocyte comitogenic activity, though with minor modifications (11).

Detection of IL-1 Gene Expression. Northern blot analysis was performed according to the established methods (12). IL-1β mRNA was detected using a 0.6-kb segment of IL-1β cDNA (kindly provided by Dr. J. Yodoi at Kyoto University), which had been labeled to a specific activity of 10^9 cpm/μg with oligonucleotide primers and [^{32}P]dCTP, as previously described (13). Hybridization was performed by immersion of the membranes with the radiolabeled IL-1β probe at 42°C for 6 h. The washed membranes were dried and then exposed to x-ray film at −80°C. U937 and HPB-ALL cell lines were also used as positive and negative controls, respectively.

Results

Surface Markers and Proliferation of Cells. Leukemic cells from five patients with AML (one with M1, one with M2, one with M5b, and two with M7) were examined. Surface marker analysis with mAbs CD2 and CD19 revealed that contamination with normal lymphocytes in cell suspensions was <10%.

CD25 antigen was detected on cells from two M7 AML patients, while studies into induction of its expression by, and effects on cell proliferation of IL-1α and IL-1β (1–100 U/ml) found that both marked proliferation and CD25 antigen expression were stimulated only in cultures of cells from one M7 AML patient (HN) (Table I). Since the spontaneous proliferation rate measured by [^{3}H]TdR incorporation was greater among HN cells (48,800 cpm) than among any of the others (5,500–32,800 cpm), and because CD25 antigen expression increased after cultivation with medium alone, as described below, the involvement of IL-1 in HN cell proliferation and CD25 antigen expression was suspected. Addition of rabbit anti-IL-1β antibody to these cells specifically inhibited proliferation and expression of CD25 antigen (Table I).

To test whether other well known hematopoietic growth factors also regulated proliferation and expression of CD25 antigen on HN cells, the effects of IL-2 (10–100 U/ml), GM-CSF (10–100 U/ml), and IL-3 (10–100 U/ml) were studied, but were found to influence neither.

Freshly isolated patient HN leukemic cells showed the following percent cell surface marker expression: 40.3% CD13, 18.9% CD25, 56.5% CD7, 54.2% CDw41, 1.3% CD2, and 0.6% CD19, and after cultivation for 24 h in complete medium, their surface markers were as follows: 88.6% CD13, 54.5% CD25, 32.4% CD7, 76.5% CDw41, 1.9% CD2, and 0.8% CD19. Thus, contamination with normal T or B cells in cell suspensions was <5%, before and after cultivation, suggesting that CD25 antigen was expressed on the leukemic cells. To clarify this, cells that had been stored in liquid nitrogen were examined by two-color

TABLE I

Effect of IL-1 and Anti-IL-1 Ab on CD25 Ag Expression and Proliferation of Leukemic Cells

Antibody	Concentration or dilution	CD 25 Ag expression*	[^{3}H]TdR uptake‡
			cpm
Medium	—	54.5	48.8 ± 6.6
IL-1α	1 U/ml	NT	65.2 ± 10.2
	10 U/ml	67.2	158.8 ± 10.4
	100 U/ml	70.1	155.4 ± 11.5
IL-1β	1 U/ml	NT	47.2 ± 7.3
	10 U/ml	69.7	115.7 ± 9.7
	100 U/ml	72.1	180.4 ± 30.6
anti-IL-1α	1:10^3	55.2	49.2 ± 3.1
anti-IL-1β	1:10^5	53.2	47.5 ± 4.1
	1:10^4	48.4	39.0 ± 3.3
	1:10^3	32.9	28.1 ± 3.0
NRS	1:10^3	55.8	50.5 ± 5.1

* Percentage of cells positive for CD25 antigen. NT, not tested.
‡ Data represent mean ± SD of quadruplicate samples (× 10^{-3} cpm).

TABLE II

IL-1 Activity in Culture Supernatants

Sources	Concentration or final dilution	[^{3}H]TdR uptake*	Suppression‡		
			Anti-IL-1α	Anti-IL-1β	NRS
		cpm		%	
Medium	1:4	830 ± 60			
IL-1α	100 U/ml	24,930 ± 2,010	92.5	−2.1	−3.1
IL-1β	100 U/ml	27,740 ± 1,090	−1.1	88.6	0.6
M7 AML (HN)	1:4	36,200 ± 1,520	10.9	85.9	9.6
	1:12	18,280 ± 1,530	−12.0	89.1	−10.6
	1:36	7,120 ± 680	9.2	87.5	−11.5

* Mean ± SD of quadruplicate samples.
‡ Anti-IL-1 antibodies and preimmune normal rabbit sera (NRS) were used at final dilutions of 1:1,000. Percent suppression was calculated as: 100 × {1 − [(cpm in the presence of antibody)/(cpm in the absence of antibody)]}.

fluorescence, since fresh leukemic cells were no longer available. Only 33.7% of medium-cultured frozen leukemic cells expressed both CD13 and CD25 antigens, and its further induction by IL-1β (100 U/ml) was not observed. However, the relative proportions of cells bearing both markers decreased to 24.7% when cultured with anti-IL-1β antibody.

IL-1 Activity in Culture Supernatants. The mouse thymocyte comitogenic proliferation assay detected IL-1 activity in HN leukemic cell culture supernatants. This activity was specifically blocked by anti-IL-1β antibody in a dose-dependent manner, but not by either anti-IL-1α antibody or preimmune normal rabbit sera (NRS) (Table II).

Northern Blot Analysis. To investigate the regulation of IL-1β gene expression in HN cells, transcription was examined by Northern blot analysis of RNA extracted from fresh or cultured cells. IL-1β gene expression was observed in fresh HN cells and in PMA-stimulated U937 cells, but not in HPB-ALL cells.

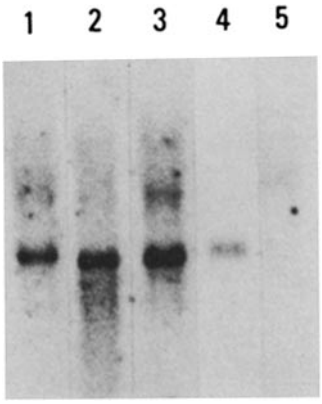

FIGURE 1. Northern blot analysis of IL-1β gene expression in AML cells and cell lines. RNAs were isolated from M7 AML (HN) cells. (*1*) Cells freshly obtained, (*2*) cells cultured in complete medium, and (*3*) cells cultured with IL-1β. (*4*) PMA-treated U937 cells, and (*5*) HPB-ALL cells. Equal amounts of each RNA (10 μg/lane) were analyzed by Northern blot hybridization, using a ^{32}P-labeled IL-1β probe.

Furthermore, levels of IL-1β gene expression were higher in IL-1-treated HN cells than in those cultivated in medium alone (Fig. 1).

Discussion

This paper presented the results of a study in which leukemic cells from an M7 AML patient proliferated in the presence of IL-1β, which in addition, induced the cells to express CD25 antigen. The leukemic cells' culture supernatants contained IL-1β activity, and expression of their IL-1β genes was confirmed by Northern blot hybridization.

Recently, Young et al. detected GM-CSF transcription in leukemic cells of 11 of 22 patients with AML, and found biologically active CSF activity in AML cell-conditioned media. Furthermore, autocrine stimulation of GM-CSF expression in AML cells has also been reported by this group (14, 15). It is also known that IL-1 induces production of GM-CSF by endothelial cells (16). Thus, we initially thought that IL-1 might induce GM-CSF in AML cells, which would in turn promote AML cell proliferation. However, in these experiments we were unable to detect any GM-CSF effects on proliferation or CD25 antigen expression.

Proliferation and induction of IL-1β gene transcription in HN cells by IL-1β, and production of IL-1β by these cells, strongly suggests that autocrine stimulation of IL-1β plays a role in proliferation of these cells. Infusions of IL-1 have been reported to cause neutrophilia in mice (17), while recently, it was also reported (18, 19) that IL-1 constituted one component of hemopoietin-1, which is believed to induce mouse stem cells to become responsive to other CSFs. The leukemic cells in our study possessed CD7, CD25, and CDw41 antigens, suggesting that they originated from pluripotent stem cells. Thus our findings described here suggest that IL-1 directly promotes proliferation of human myeloid progenitor cells. However, the leukemic cells from the other M7 patient, which had surface markers similar to those of the HN cells, neither responded to IL-1 nor produced it, suggesting that the involvement of IL-1 in AML is not universal and showing that we were unable to define the exact relationship between the stages of this leukemia and IL-1 production. Nevertheless, our study suggests that IL-1 is involved in proliferation of some myeloid leukemia cells, and that interference with IL-1 activity might be beneficial in treatment of AML.

Summary

A significant increase in CD25 antigen–positive cells by IL-1 was observed in cells of a patient with M7 acute myelogenous leukemia. Basal proliferation and expression of CD25 antigen by the M7 leukemic cells were inhibited by addition of anti-IL-1β antibody in a dose-dependent manner, but not by rabbit anti-IL-1α antibody. Culture supernatants of these leukemic cells contained IL-1 activity, which was specifically inhibited by addition of anti-IL-1β antibody, and Northern blot analysis detected intracellular IL-1β mRNA. These results indicated that autocrine secretion of IL-1β was involved in proliferation of some myelogenous leukemic cells.

We would like to thank Drs. S. Fukuda and K. Takahashi for the PPO analysis. We are grateful to Dr. J. Yodoi for the gift of IL-1β cDNA. We also thank Dr. Y. Hirai of Otsuka Pharmaceutical Co. Ltd. for provision of IL-1β and anti-IL-1β antibody; Dainippon Pharmaceutical Co. Ltd. for IL-1α and anti-IL-1α antibody; and the Genetics Institute for IL-3 and GM-CSF.

Received for publication 20 July 1987.

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