[CANCER RESEARCH 50, 345–349, January 15, 1990]

Tumor Growth Inhibitory Activity of a Lymphocyte Blastogenesis Inhibitory Factor[1]

Kazuhisa Sugimura,[2] Takeshi Ohno, Shigeharu Fukuda, Yumiko Wada, Tatsuji Kimura, and Ichiro Azuma

Institute of Immunological Science, Hokkaido University, Sapporo 060 [K. S., T. O., Y. W., T. K., I. A.], and Hayashibara Biochemical Laboratories, Inc., 675-1, Fujisaki, Okayama 702 [S. F.]

ABSTRACT

A lymphocyte blastogenesis inhibitory factor (LBIF) has been characterized as an immunoregulatory molecule, especially on the T-lymphocyte proliferation. Using fast protein liquid chromatography-purified LBIF, we examined the effect of LBIF on the proliferation of various 18 tumor cell lines *in vitro* in comparison with those of interferon-α, interferon-γ, tumor necrosis factor-α, transforming growth factor-β1, or interleukin 1α/β. We showed here that LBIF strongly inhibited the proliferation of various tumor cell lines irrespective of cell lineage or species. LBIF was effective on a wider spectrum of tumor cell lines than other cytokines tested here. The inhibition resulted from cytotoxic or cytostatic effects, depending on individual characteristics of tumor cell lines. Five cell lines showed insensitivity against LBIF activity, suggesting a plausible involvement of LBIF receptor molecules to transduce LBIF signals. These results suggest that LBIF may play important roles in regulating cell growth.

INTRODUCTION

A LBIF[3] is constitutively produced from a human macrophage-like cell line, U937 (1–4). We have purified LBIF to homogeneity by FPLC (3, 4). LBIF is a single polypeptide chain and has a molecular weight of 45,000 by the estimation of SDS-PAGE. LBIF shows approximately pI 4.5 upon chromatofocusing. Amino acid sequencing analysis showed that LBIF was a new immunoregulatory factor.[4] The function of LBIF has been characterized (3, 4). (*a*) LBIF inhibits the proliferation of lymphocytes stimulated with lectins, IL-1, IL-2, or antigens. (*b*) LBIF selectively inhibits the expression of IL-2 receptor, p75, but neither the induction of IL-2 receptor, p55, nor the IL-2 production of lectin-stimulated T-lymphocytes.[4] (*c*) LBIF action is cytostatic and reversible. (*d*) LBIF functions to both human and murine lymphocytes. (*e*) LBIF arrests lectin-stimulated T-lymphocytes at early G1. (*f*) Lectin stimulation is a prerequisite process in order for LBIF to be active on lymphocytes. These results have suggested that LBIF may play important roles in cell growth control of various cell lineage. To examine this question, we first studied the effect of LBIF on cell growth of various tumor cell lines *in vitro* in comparison with those of IFN-α, IFN-γ, TNF-α, TGF-β1, or IL-1 α/β all of which are known as growth-inhibitory cytokines (5–11). We showed here that firstly, LBIF strongly inhibits the proliferation of tumor cell lines of various lineage. Secondly, LBIF is effective as a growth inhibitory factor on a wider spectrum of tumor cell lines than other cytokines tested here. Thirdly, LBIF sensitivity of tumor cell lines are categorized in two fashions, *i.e.*, cytotoxic and cytostatic. Fourth, there are tumor cell lines that are resistant to growth inhibitory activity of LBIF. Thus this study has suggested that LBIF functions at crucial steps of cell cycle progression irrespective of specific cell lineage. The existence of LBIF-resistant cell lines conversely suggested a plausible involvement of LBIF receptor molecules to transduce LBIF signals. Furthermore this study showed a potential activity of LBIF to inhibit the growth of tumors *in vivo* and to be applied to clinical investigation.

MATERIALS AND METHODS

Cell Lines. A human T-cell leukemia, Jurkat, human Burkitt's lymphomas, Manaca and Raji, an Epstein-Barr virus-transformed B-cell line, EBV-BLCL (obtained from Dr. de Vries, University Hospital, Leiden), a human histiocytic lymphoma, U937 (obtained from American Type Culture Collection, or provided by Dr. Fujiwara, Habikino Hospital, Osaka), a human neuroblastoma, SK-N-MC (obtained from Dr. I. Saiki, Hokkaido University), a human melanoma, A375 (provided by Hayashibara Biochemical Lab., Inc., Okayama) and G-361 (provided by Japanese Cancer Research Resources Bank, JCRRB, Tokyo), a gibbon ape T-lymphoma, MLA144, murine macrophage cell lines, WEHI3 and P388D1, a murine mastocytoma cell line, P815, human adult T-cell leukemia cell lines, MT-2 and TL-Mor (kindly provided by Dr. Sugamura, Tohoku University), a human epithelial carcinoma cell line, HeLa (provided by JCRRB) were maintained in RPMI 1640 supplemented with 100 units/ml of penicillin, 100 μg/ml of streptomycin, 0.29 mg/ml of L-glutamine (M. A. Bioproducts Inc.), 40 μM of 2-mercaptoethanol, and 7.5% heat-inactivated FBS. A human melanoma cell line, Mewo, VMRC-MELG, and C32TG (provided by JCRRB) and a murine-transformed fibroblast cell line, L929 (provided by Dr. Saiki, Hokkaido University), were maintained in MEM supplemented with 100 units/ml of penicillin, 100 μg/ml of streptomycin, 20 μl/ml of vitamin solution (100 ×, Boehringer Mannheim), 1 mM sodium pyruvate (Wako pure chemical, Ltd., Osaka), 20 μl/ml of nonessential amino acid (GIBCO), 0.29 mg/ml of L-glutamine, and 10% of heat-inactivated FBS.

Cytokines and LBIF. Human recombinant IL-1α (2×10^7 units/mg) and β (2×10^7 units/mg) were kindly provided by Dr. Hirai (Ohtsuka Pharmaceutical Co., Ltd., Tokyo) and Dainippon Pharmaceutical, respectively. Human natural TNF-α (0.3 mg/ml, 5×10^8 units/mg), human natural IFN-γ (2.1 mg/ml, 1×10^7 IU/mg), murine natural IFN-γ (2.8×10^6 IU/mg), and human natural IFN-α (3.5×10^7 IU/ml, 2×10^8 units/mg) were provided by Hayashibara Biochemical Lab., Inc. (12, 13). Human recombinant TGF-β1 was a gift by Mitsubishi Chemical Ind., Co., Ltd. (Tokyo). Lymphocyte blastogenesis inhibitory factor, LBIF, was purified from crude supernatant of a human histiocytic lymphoma U937 as described (3, 4). Briefly, U937 cells were cultured at 1×10^6 cells/ml in serum free RPMI 1640 medium. The

Received 6/8/89; revised 8/29/89; accepted 10/10/89.

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

[1] Supported by Grants-in-Aid for Cancer Research from the Japanese Ministry of Education, Science and Culture; from the Japanese Ministry of Health and Welfare for Comprehensive 10-Year Strategy for Cancer Control; from the Japanese Ministry of Education, Science and Culture for Scientific Research and for Developmental Scientific Research (No. 62870023); and from the Japanese Ministry of Education, Science and Culture for Scientific Research. It is also supported by the Osaka Foundation for Promotion of Clinical Immunology; by Yamanouchi Foundation for Research on Metabolic Disorders; by a Grant-in-Aid for Special Project Research from Hokkaido University; by Grants-in-aid from the Mochida Memorial Foundation for Medical and Pharmaceutical Research, and also by the Akiyama Foundation.

[2] To whom requests for reprints should be addressed, at Institute of Immunological Science, Hokkaido University, Sapporo 060, Japan.

[3] The abbreviations used are: LBIF, lymphocyte blastogenesis inhibitory factor; HPLC, high performance liquid chromatography; FPLC, fast protein liquid chromatography; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; IFN, interferon: TNF, tumor necrosis factor; FBS, fetal bovine serum; TGF, transforming growth factor; MEM, minimal essential medium; IL, interleukin; PBL, peripheral blood lymphocytes.

[4] K. Sugimura, Y. Ueda, T. Ohno, Y. Wada, T. Uede, K. Sugamura, N. Nakamura, K. Nagase, M. Taniai, M. Suzuki, O. Ando, and I. Azuma, manuscript in preparation.

serum free supernatant is concentrated by an ultrafiltration membrane. The crude concentration is fractionated by TSK gel DEAE-5PW (21.5 mm × 15 cm, TOSOH, Tokyo) equilibrated with 20 mM Tris-HCl, pH 7.7. The separation was done by a linear gradient from 0 to 0.5 M NaCl. LBIF activity was tested as described in "LBIF assay" (3, 4). The active fractions were subsequently fractionated by Mono P chromatofocusing column (HR5/20, Pharmacia). The purity of LBIF preparation was assessed by SDS-PAGE and estimated to be about 75–80%. These results were described in previous paper (2, 4). All functional assays on LBIF were carried out by using this FPLC-purified LBIF. One unit of LBIF was defined as the amount of LBIF preparation required to induce a half-maximum response of LBIF assay as described. Approximately 10–20 ng of LBIF samples corresponds to 1 unit.

To show the purity of FPLC-purified LBIF, this sample was further resolved by reversed-phase high-performance liquid chromatography on RP-304 column (Bio-Rad) under a linear gradient from 0 to 90% acetonitrile containing 0.1% trifluoroacetic acid at flow rate of 0.5 ml/m. Fig. 1*a* shows the resolution pattern of this chromatography and Fig. 1*b* shows the result of LBIF fraction by SDS-PAGE (15% polyacrylamide gel) analysis (14). No IFN activity nor TNF activity was detected in FPLC-purified LBIF which was used in this study. IFN activity was assayed by the inhibition of cytopathic effect of sindbis virus in FL cells using NIH international IFN-α reference (Ga-23-901-531) (5, 12). TNF activity was assayed by the dye uptake microtiter method using L929 cells treated with actinomycin D as described (5, 13). Taking into consideration the minimum quantity of protein required to be detected, IFN assay (1 IU/100 pg) and TNF assay (1 unit/2 pg) have much higher sensitivities than LBIF assay has (1 unit/10 ng).

Cell Culture. Cells were cultured at 5×10^3 cells/well/200 μl in a flat-bottomed 96-well culture plate (Falcon 3072) in the presence or absence of two- to threefold diluted cytokines. L929 cells were cultured in MEM containing 2% FBS. Three days later, cells were pulsed with 0.5 μCi/well of [^{3}H]thymidine (Amersham, UK) for the last 4 h of the culture and collected by a harvester (Labo Science Co., Ltd.). When adherent cell lines such as SK-N-MC, A375, G-361, WEHI3, Hela, Mewo, VMRC-MELG, C32TG, or L929, were harvested, culture supernatants were removed by a cell harvester, and 50 μl of 0.25% trypsin-0.02% EDTA (GIBCO) were added to each well. After the cells were detached, they were harvested on the same filter by the harvester. The radioincorporation was measured by the liquid-scintillation counter (Beckman LS3801). The results were calculated by:

$$\% \text{ inhibition} = \left[1 - \left(\frac{\text{cpm of experimental group}}{\text{cpm of control group}}\right)\right] \times 100$$

Cell proliferation was always monitored by visual observation or counting viable cell number directly by trypan blue dye exclusion test.

LBIF Assay. LBIF assay was carried out as described previously (3, 4). Briefly, thymocytes from 5–6-week-old BALB/c mice were cultured for 72 h at 1.5×10^6 cells/well of 96-well culture plates. Cells were stimulated with 50 units/ml of human recombinant IL-1β in the presence or absence of LBIF. Cells were pulsed with 0.5 μCi/well of [^{3}H] thymidine for the last 4 h. The radioincorporation was measured by the liquid-scintillation counter.

RESULTS

Effect of LBIF on the Proliferation of Various Tumor Cell Lines *in Vitro*. In order to determine whether LBIF inhibits the constitutive proliferation of various tumor cell lines *in vitro*, cells were cultured at 5×10^3 cells per well in the presence or absence of 4 units/ml of LBIF. Three days later, cell proliferation was monitored by pulsing cells with [^{3}H]thymidine for the last 4 h. In all experiments, visual observation was done to confirm the reduction of cell number and in some cases, viable cell number was directly counted. These results primarily gave the same results as those of [^{3}H]thymidine uptake experiments. The results were shown in Table 1 and Fig. 2. The proliferation of seven cell lines, MLA-144, Manaca, EBV-BLCL, A375, Mewo, VMRC-MELG, or C32TG was strongly inhibited at over 90% suppression. Six cell lines, Raji, U937, WEHI3, G361, SK-N-MC, and MT-2 showed sensitivity to LBIF activity at 40–70% suppression. Five cell lines, P815, P388D1, B16F1, HeLa, and TL-Mor were resistant to LBIF activity upon their proliferation.

Thus these results demonstrated that first, LBIF had the ability to control the constitutive proliferation at certain spectrum of tumor cell lines, secondly, LBIF appeared to be neither specie specific (*i.e.*, LBIF inhibited the growth of human, ape, and murine cell lines) nor tissue specific (*i.e.*, LBIF inhibited the proliferation of lymphomas, melanomas, or neuroblastomas). It was of note that LBIF inhibited cell proliferation by two characteristically different features. LBIF showed lethal effect on MLA144. MLA144 cells were fragmented and disappeared at day 3 in the presence of LBIF (4 units/ml). On the other hand, LBIF appeared to show cytostatic effects on other cell lines. Analyses to determine whether these cells can be arrested at any specific phase of cell cycle or that the inhibitory activity may be reversible will be required.

Dose Response of LBIF Activity. Various behaviors of tumor cells against LBIF could have resulted from their sensitivity to LBIF. To answer this question, the inhibitory activity of LBIF

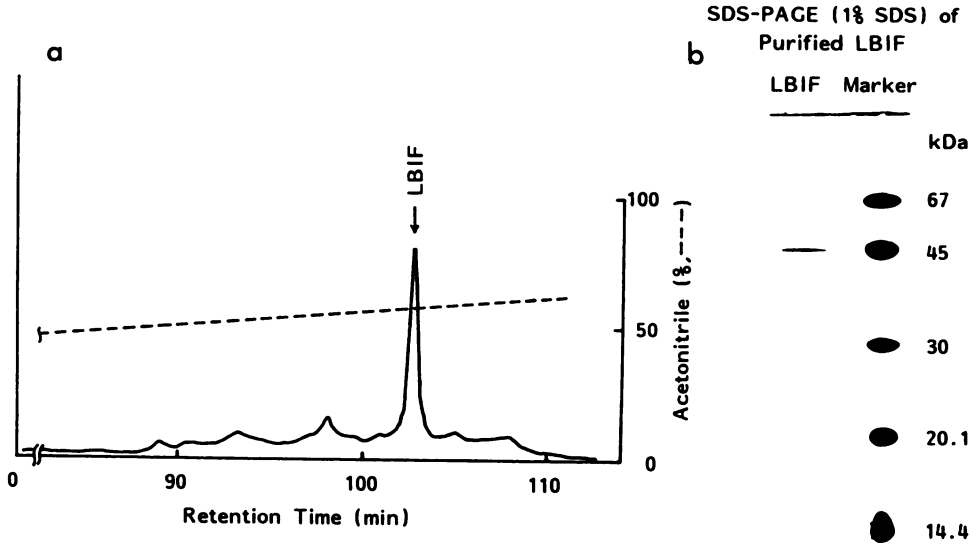

Fig. 1. *a*, purification of LBIF by reversed-phase HPLC on RP-304; *b*, SDS-PAGE analysis of LBIF. See "Materials and Methods."

Table 1 *Effect of LBIF on the proliferation of various tumor cell lines*

Cells were cultured at 5×10^3 cells/well in 96-well culture plate in the presence or absence of LBIF (4 units/ml). Three days later, cell proliferation was monitored by pulsing [^{3}H]thymidine for the last 4 h.

Cell line	Origin	Specie	[^{3}H]Thymidine uptake (cpm ± SD)		% inhibition[a]
			None	LBIF (4 units/ml)	
Jurkat	T-lymphoma	Human	1.2×10^{6}[b]	1.4×10^{5}[b]	
MLA144	T-lymphoma	Ape (gibbon)	44,664 ± 9,746	251 ± 108	99[c]
TL-Mor	Adult T-cell leukemia	Human	22,194 ± 2,503	26,228 ± 1,049	No[d]
MT-2	Adult T-cell leukemia	Human	40,924 ± 941	17,334 ± 1,247	58
Raji	B-cell lymphoma	Human	7,617 ± 306	2,345 ± 13	69
Manaca	B-cell lymphoma	Human	133,362 ± 9,911	7,133 ± 239	94[c]
EBV-BLCL	Epstein-Barr-virus transformed B-cell line	Human	8,001 ± 591	506 ± 84	94[c]
U937	Histiocytic lymphoma	Human	63,233 ± 5,233	23,410 ± 7,020	63
WEHI 3	Macrophage cell line	Mouse	3,971 ± 967	2,297 ± 110	42
P388D1	Macrophage cell line	Mouse	5,459 ± 149	4,659 ± 406	No
P815	Mastocytoma	Mouse	6,287 ± 437	6,504 ± 437	No
Hela	Epidermal carcinoma	Human	43,622 ± 2,866	31,306 ± 2,090	No
Mewo	Melanoma	Human	20,370 ± 980	1,785 ± 369	91[c]
VMRC-MELG	Melanoma	Human	6,615 ± 530	529 ± 252	92[c]
A375	Melanoma	Human	79,731 ± 1,234	1,056 ± 208	99[c]
G361	Melanoma	Human	13,299 ± 3,578	3,808 ± 890	71
C32TG	Melanoma	Human	8,623 ± 913	754 ± 224	91[c]
B16F1	Melanoma	Mouse	89,156 ± 1,001	86,101 ± 5,130	No
SK-N-MC	Neuroblastoma	Human	20,033 ± 1,231	5,676 ± 227	72

[a] % inhibition, see "Materials and Methods."
[b] Jurkat cells were cultured at 5×10^4 cells/well in 24-well culture plate in the presence or absence of LBIF (4 units/ml). Three days later, cell proliferation was monitored by directly counting the number of viable cells by trypan blue dye exclusion test.
[c] Over 90% inhibition.
[d] No, less than 40% inhibition.

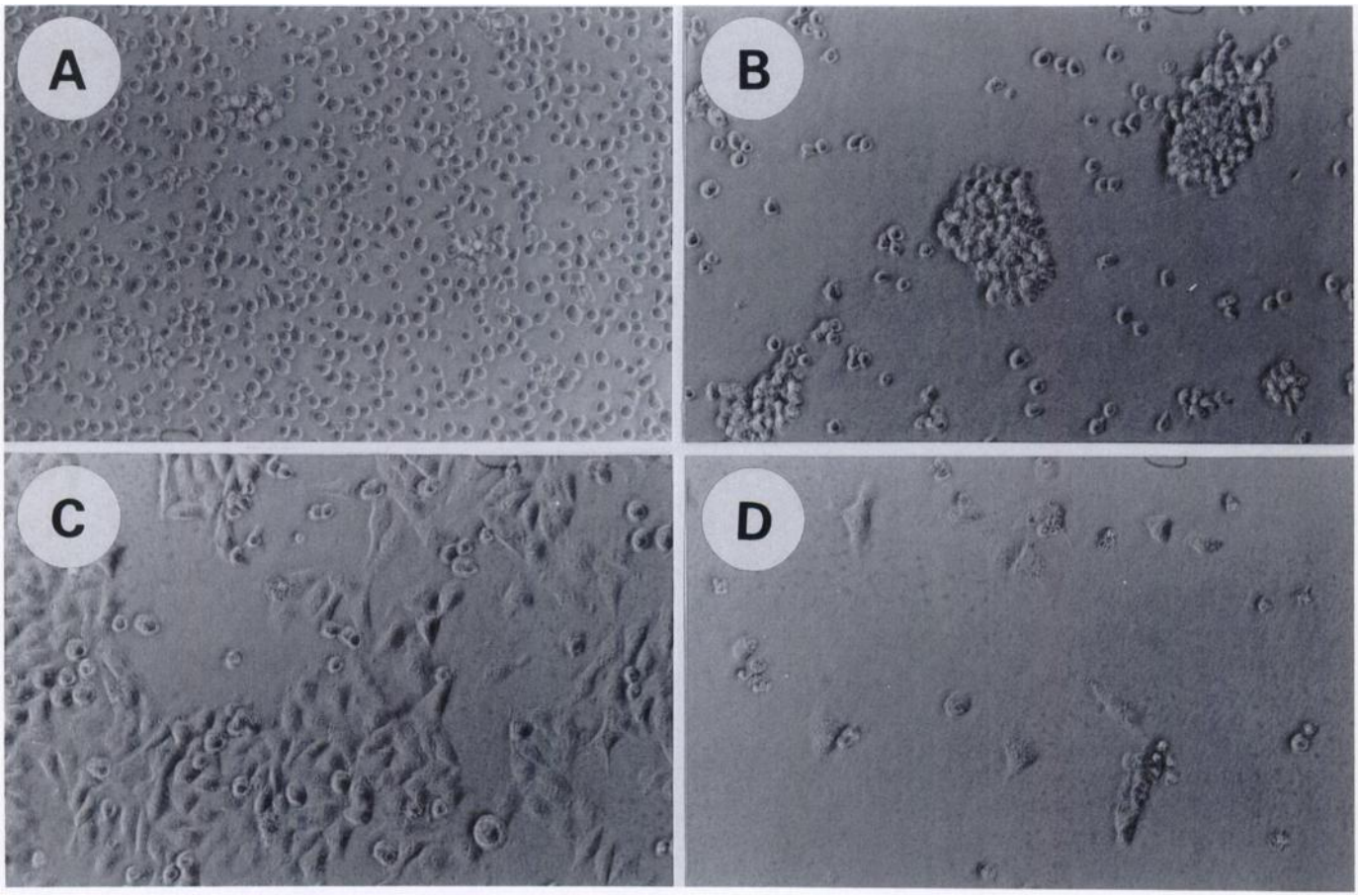

Fig. 2. Morphological view of Manaca (*A*, medium; *B*, LBIF) and A375 (*C*, medium; *D*, LBIF). Cells were cultured for 3 days in the presence or absence of LBIF (5 units/ml). × 200.

was quantitatively assessed. Since the proliferation of PBL stimulated with phytohemagglutinin was completely inhibited at around 1.6 units/ml of LBIF, the highest dose of LBIF was varied up to 20 units/ml in these quantitative analyses. A series of experiments has made clear that tumor cell lines are classified into four groups regarding their sensitivity to LBIF (Fig. 3). The first group includes TL-Mor, HeLa, P815, P388DI, and B16F1 of which growth are refractory to LBIF activity over all

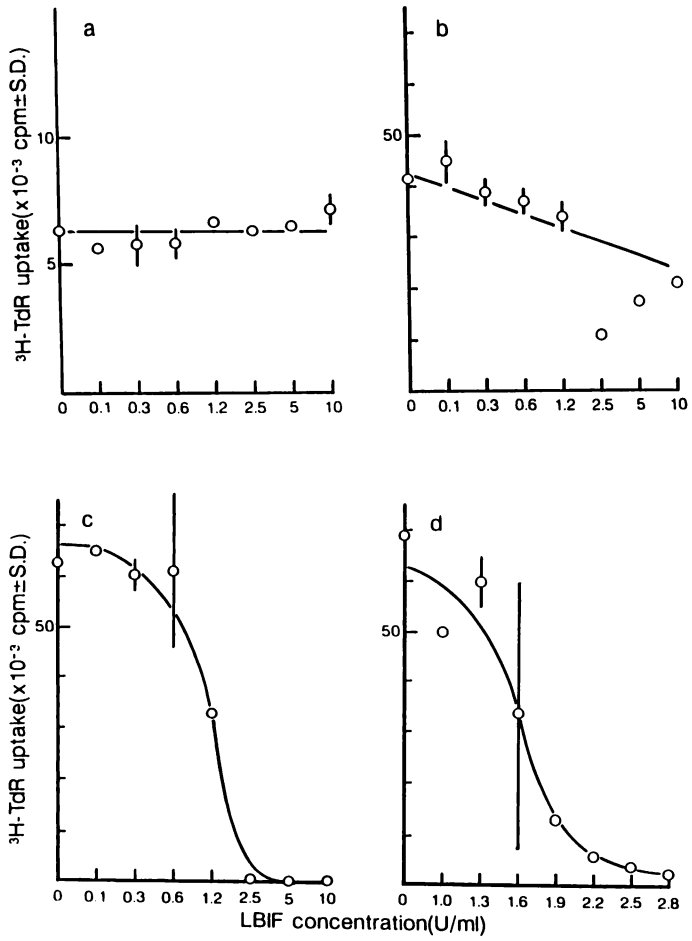

Fig. 3. Dose-response of LBIF activity: Cells were cultured as described in Table 1. *a*, P815; *b*, MT-2; *c*, MLA144; *d*, Manaca. SD was not shown if the value was below 1,500 cpm.

doses tested (Tables 1 and 2 and Fig. 3*a*). Raji, U937, and MT-2 belong to the second group of which growth was gradually decreased in dose-dependent manner. The IC_{50} value was variable among cell lines and usually higher than 1.5 units/ml (Fig. 3*b* and Table 2). The third group includes Manaca, EBV-BLCL, Mewo, VMRC-MELG, A375, G361, C32TG, and SK-N-MC which were highly sensitive to LBIF activity (Fig. 3*d*). The IC_{50} value of this group was at 0.5–1.6 units/ml and almost complete inhibition was attained at 2.5 units/ml. These values are almost compatible to those of lectin-stimulated PBL. By day 3, we didn't observe a cytocidal effect of LBIF on this group. The effects appeared to be cytostatic although it has not been proved in the present study. Visual observation and direct counting of viable cell number confirmed the results obtained by [^{3}H]thymidine uptake experiments. The fourth case is MLA144 which were killed completely by day 3 (Fig. 3*c*). The IC_{50} value was 1.25, similar value to those of the third group (Table 2) and lectin-stimulated T-lymphocytes (4).

Comparative Study of LBIF with Other Cytokines on Their Growth Inhibitory Activities. Several cytokines are reported as tumor growth inhibitory factors although their spectrums are different from each other. To compare LBIF with these cytokines, we examined the dose-response of TGF-β1, IL-1α/β, TNF-α, IFN-γ, and IFN-α on the growth of LBIF-sensitive tumor cell lines (Table 2). TGF-β1 showed the inhibitory activity on p388D1, A375, C32TG, or G-361. IL-1β showed the inhibitory activity on a subline of A375 only. TNF-α showed the inhibitory activity of MLA144, U937, G361, and L929. IFN-γ inhibited the growth of VMRC-MELG and A375. IFN-α inhibited the growth of MLA144, Manaca, and A375 but not Raji which was one of the LBIF-sensitive tumor cell lines. Thus, these results indicate that LBIF is effective at a wider range of spectrum of tumor cell lines in comparison with that of other cytokines, suggesting that the functional mode of LBIF may be different from those of other cytokines tested here.

DISCUSSION

A lymphocyte blastogenesis inhibitory factor (LBIF) has been characterized as an immunoregulatory factor, especially on the T lymphocyte proliferation (1–4). As LBIF selectively inhibited the expression of IL-2 receptor, p75[4], it was suggested that the functional mode of LBIF might be closely linked to a molecular mechanism involved in cell proliferation. In the present study, we have demonstrated that LBIF possesses strong anti-proliferation activity against various tumor cell lines *in vitro*. LBIF drastically inhibited the proliferation of a T cell lymphoma: MLA144, a Burkitt's lymphoma: Manaca, EBV-BLCL, melanomas, Mewo, VMRC-MELG, A375 or C32TG and a neuroblastoma: SK-N-MC irrespective of cell-lineage and specie-specificity.

We noticed that the behavior of tumor cell lines against LBIF was characterized into four different patterns. Firstly, it is of note that there is a group of LBIF-nonsensitive cell lines including TL-Mor, Hela, P815, P388D1, and B16F1. If LBIF directly inhibits the basic molecular mechanism for cell-cycle progression undergoing in the intracytoplasm or nucleus, LBIF should affect the proliferation of all cell lines. These results may suggest that LBIF function through putative LBIF receptors expressing on the cell surface. The same implication was also suggested by studies on T lymphocyte proliferation i.e., lectin stimulation is a prerequisite process for LBIF to be active on lymphocytes (3, 4). Secondly, in the case of a very-sensitive group, it appears that responses to LBIF begin abruptly at the concentration of about 1–2 U/ml LBIF. The molecular basis for such steep or even switchlike responses to graded signals is also an important question. Purified recombinant LBIF will be needed to investigate the existence of putative LBIF receptors and to study the relationship between specific receptor binding and biological activity. Thirdly, the difference on molecular mechanisms between a very-sensitive group (Fig. 3*d*) and a less-sensitive group (Fig. 3*b*) may raise more complicated issues. Simply a coexistence of LBIF-resistant subline in a less-sensitive group is conceivable. Alternatively, not only the number but also the structure of LBIF receptor might be related to the difference of LBIF-sensitivity.

In the case of very-sensitive group (Fig. 3*d*) and less sensitive group (Fig. 3*b*), we have not investigated whether these inhibitory activities are cytostatic/reversible or cytotoxic/lethal. It was not made clear by our observation during 3 day-culture. In contrast, the lethal effect of LBIF on MLA144, a cell of the 4th group (Fig. 3*c*), was remarkable. MLA144 expresses IL-2 receptor, p75 but not p55 molecules and produce a large amount of IL-2 (15). MLA144 is able to proliferate by accepting growth signal through p75 molecules with low/medium-affinity to IL-2 (Kd = 200 pM) in autocrine manner (16, 17). As LBIF selectively inhibited the expression of p75 on lectin-stimulated T lymphocytes[4], it is possible that LBIF can be lethal against the growth of MLA144.

Thus, these results suggest that LBIF may play important roles in regulating cell growth in general, not only in the regulation of immune response. LBIF may be a useful tool to

Table 2 *Comparative study of LBIF with other cytokines on their growth inhibitory activities*

Target	IC_{50}[a]					
	LBIF (units/ml)	TGF-β1 (ng/ml)	IL-1β (units/ml)	TNF-α (units/ml)	IFN-γ (IU/ml)	IFN-α (IU/ml)
MLA144	1.25	>100	$>10^4$	$>10^5$ [g]	$>10^4$	0.06
MT-2	1.25	>100	$>10^4$	$>10^5$	$>10^4$	ND
TL-Mor	>20	>100	$>10^4$	$>10^5$	$>10^4$	ND
Raji	1.5	>100	$>10^3$	$>10^5$	$>10^3$	$>10^4$
Manaca	1.6	>100	$>10^3$	$>10^5$	$>10^4$	8.7
EBV-BLCL	0.5	>100	$>10^{4b,e}$	$>10^5$	$>10^{4h}$	ND
U937	4[f]	>100	$>10^3$	250	$>10^{3m}$	ND
WEHI3	ND[c]	ND	ND	$>10^5$	$>10^4$	ND
P388D1	>10	1	$>10^4$	$>10^5$	$>10^4$	ND
Mewo	0.7	>100	$>3 \times 10^{3i}$	$>10^{5j}$	$>10^{4k}$	ND
VMRC-MELG	0.7	>100	$>10^{4b}$	$>10^{5b}$	45	ND
A375	1.25	0.1	$>10^4$ (0.7)[d]	$>10^5$	180	10
C32TG	0.63	0.06	$>10^4$	$>10^5$	$>10^{4l}$	ND
G-361	0.63	0.08	$>3 \times 10^{3e}$	100	$>10^4$	ND
P815	>10	>100	$>10^4$	$>10^5$	$>10^4$	ND
Hela	>10	>100	$>10^4$	$>10^5$	$>10^{4m}$	ND
L929	ND	ND	ND	1×10^3	ND	ND

[a] Cells were cultured at 5×10^3 cells/well in 96-well culture plate in the presence or absence of two- or three-fold diluted cytokines. After a sigmoid curve of cytokine activity had been generated, IC_{50} was defined as the concentration of a factor required to induce a half-maximum response of control proliferation. Murine cell lines were tested by murine IFN-γ. LBIF, 13 ng/1 unit; IL-1α or β, 50 pg/1 unit; TNF-α, 2 pg/1 unit; human IFN-γ, 100 pg/1 IU; murine IFN-γ, 357 pg/1 IU; IFN-α, 5 pg/1 IU.

[b] Proliferative response was augmented but not inhibited.

[c] ND, not determined.

[d] A375 subline shows high sensitivity against IL-1β inhibitory activity.

[e] IL-1α was used.

[f] Purified LBIF shows 4 units of IC_{50}, however, crude supernatant containing LBIF does not show the inhibitory activity on U937 proliferation.

[g] 30% inhibition at 300 units/ml.

[h] 28% inhibition at 30 IU/ml.

[i] 33% inhibition at 30 units/ml.

[j] 28% inhibition at 10^3 units/ml.

[k] 25% inhibition at 10^4 IU/ml.

[l] 38% inhibition at 300 IU/ml.

[m] 48% inhibition at 30 IU/ml.

[n] 47% inhibition at 100 IU/ml.

analyze the molecular mechanism of cell cycle control in eukaryotes. Furthermore this study has demonstrated a potential activity of LBIF to inhibit the growth of tumors *in vivo* and to be applied to clinical investigation.

ACKNOWLEDGMENTS

We thank Mihoko Sato for her expert editorial assistance.

REFERENCES

1. Fujiwara, H., and Ellner, J. J. Spontaneous production of a suppressor factor by the human macrophage-like cell line U937. I. Suppression of interleukin 1, interleukin 2, and mitogen-induced blastogenesis in mouse thymocytes. J. Immunol., *136:* 181–185, 1986.
2. Fujiwara, H., Toosi, Z., Ohnishi, K., Edmonds, K., and Ellner, J. J. Spontaneous production of a suppressor factor by a human macrophage-like cell line U937. II. Suppression of antigen- and mitogen-induced blastogenesis, IL-2 production and IL-2 receptor expression in T lymphocytes. J. Immunol., *138:* 197–203, 1987.
3. Sugimura, K., Tsukahara, K., Ueda, Y., TaKeda, K., Habu, Y., and Azuma, I. Fast protein liquid chromatography of lymphocyte blastogenesis inhibitory factor (LBIF) produced by a human macrophage-like cell line U937. J. Chromatogr., *440:* 131–140, 1988.
4. Sugimura, K., Ueda, Y., Takeda, K., Fukuda, S., Tsukahara, K., Habu, Y., Fujiwara, H., and Azuma, I. A lymphocyte blastogenesis inhibitory factor (LBIF) arrests mitogen-stimulated T lymphocytes at early G1 phase with no influence on IL-2 production and IL-2 receptor light chain expression. Eur. J. Immunol., in press, 1989.
5. Fukuda, S., Ando, S., Sanou, O., Taniai, M., Fujii, M., Masaki, N., Nakamura, K., Ando, O., Torigoe, K., Sugimoto, T., and Kurimoto, M Simultaneous production of natural human tumor necrosis factor-α, -β and interferon-α from BALL-1 cells stimulated by HVJ. Lymphokine Res., *7:* 175–185, 1988.
6. Gajiwski, T. F., Goldwasser, E., and Fitch, F. W. Anti-proliferative effect of IFN-γ in immune regulation II. IFN-γ inhibits the proliferation of murine bone marrow cells stimulated with IL-3, IL-4 or granulocyte-macrophage colony-stimulating factor. J. Immunol., *141:* 2635–2642, 1988.
7. Sugarman, B. J., Aggarwal, B. B., Hass, P. E., Figari, I. A., Palladino, M. A., and Shepard, H. M. Recombinant human tumor necrosis factor-α: Effects on proliferation of normal and transformed cells *in vitro.* Science (Wash. DC), *230:* 943–945, 1985.
8. Onozaki, K., Matsushima, K., Aggarwal, B. B., and Oppenheim, J. J. Human interleukin 1 is a cytocidal factor for several tumor cell lines. J. Immunol., *135:* 3962–3968, 1985.
9. Darzynkiewicz, Z., Williamson, B., Carswell, E. A., and Old, L. J. Cell cycle-specific effects of tumor necrosis factor. Cancer Res., *44:* 83–90, 1984.
10. Besancon, F., Silbermann, F., Dron, M., Tovey, M. G., Thang, M. N., and Bourgeade, M-F. Relationship between inhibition of cell growth and of transferrin receptor expression by interferon (IFN)α: Studies in IFN-sensitive and IFN-resistant Daudi cells. J. Gen. Virol., *68:* 2647–2654, 1987.
11. Keski-Oja, J., Leof, E. B., Lyons, R. M., Coffey, R. J., Moses, H. L. Transforming growth factors and control of neoplastic cell growth. J. Cell. Biochem., *33:* 95–107, 1987.
12. Johnston, M. D., Finster, N. B., and Young, P. A. Dye uptake method for assay of interferon activity. Methods Enzymol., *78:* 394–399, 1981.
13. Eifel, P. J., Walker, S. M., and Lucas, Z. J. Standardization of a sensitive and rapid assay for lymphotoxin. Cell Immunol., *15:* 208–221, 1975.
14. Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (Lond.), *227:* 680–685, 1970.
15. Tsudo, M., Kozak, R. W., Goldman, C. K., and Waldmann, T. A. Demonstration of a non-Tac peptide that binds interleukin 2: a potential participant in a multichain interleukin 2 receptor complex. Proc. Natl. Acad. Sci. USA, *83:* 9694–9698, 1986.
16. Smith, K. A. T-cell growth factor and glucocorticoids: opposing regulatory hormones in neoplastic T-cell growth. Immunobiology, *161:* 157–173, 1982.
17. Smith, K. A. The bimolecular structure of the interleukin 2 receptor. Immunol. Today, *9:* 36–37, 1988.