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Effects of IL-7 on Human Lymphocytes

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Interleukin-7 (IL-7) is known as a growth factor for pre B-cell and mature T-cells in human. But in leukemic cells, IL-7 effect is variously reported. To investigate the effect of IL-7 on the cells of childhood acute leukemia we used 3H-Thymidine assay. Twelve Acute lymphoblastic leukemia (ALL), seven T-ALL and three Acute myelogenous leukemia (AML) were involved in this study. Two out of twelve ALL and three out of seven T-ALL bone marrow (BM) cells were stimulated by IL-7 in 3H-Thymidine incorporation. In normal and AML BM cells, IL-7 had no stimulatory activity as in various leukemic cell lines. Two normal peripheral blood T-cells responded to IL-7 dose dependently. We have seen the effect of IL-7 to stimulate T-linege cells but, for precise conclusion, further study using more purified samples will be needed.

Key Words: *Interleukin-7, Leukemia, Lymphocyte, 3H-Thymidine incorporation*

INTRODUCTION

Interleukin 7 (IL-7), initially termed lymphopoietin-1 (LP-1). (Namen et al, 1988[a]) is a 25 KDa protein found in supernatants of a stromal cell line (Namen et al, 1988[a, b]: Lee et al, 1988). Its gene is located at the proximal long arm of chromosome 8 (Sutherland et al, 1989). IL-7 has growth factor activity for pre B cells, early thymocytes (Henney, 1989) and mature T-cells (Henney, 1989; Morrissey et al, 1989) in mice and it also has growth factor activity for pre B-cells (Goodwin et al, 1989) and mature T-cells in human (Welch et al, 1989). The effect of IL-7 on human leukemic cells has been reported recently in other studies. B-cell growth factor (BCGF) is the major stimulant for precursor-B ALL cell proliferation but IL-7 had no stimulatory activity (Makrynikola et al, 1991). While some authors found that leukemic cells were responded to IL-7 with enhanced DNA synthesis (Skjonsberg et al, 1991) and others showed that IL-7 induced proliferation of the cells from mature leukemia (Digel et al, 1991) and T-lineage ALL (Dibirdik et al, 1991). To investigate the effect of IL-7 on the cells from child leukemia we have seen DNA synthesis using 3H-Thymidine assay. In addition we attempted to evaluate the effect of IL-7 on normal BM mononuclear cells as control.

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MATERIALS AND METHODS

Subjects

Following informed consent, 22 pediatric patients (12 B-precursor ALL, 7 T-ALL and 3 AML) were studied at the time of diagnosis or at relapse. The diagnosis and classification of leukemia were based on the morphological, cytochemical and immunological surface profiles of bone marrow cells. BM cells from 5 ALL patients in continuous complete remission and off therapy at least for 3 months were used as control. Peripheral blood cells were obtained from a normal adult. To compare the IL-7 effect with the counterpart of normal and leukemic cells, various cell lines were tested. They were 4 pre B ALL, Burkitt's lymphoma (Raji), erythroleukemia (K562), AML (870) and T-ALL (KT). Bone marrow and peripheral blood mononuclear cells were isolated by centrifugation on Ficoll-Hypaque (1.077 g/ml), washed twice in HBSS and resuspended at 10^6/ml in RPMI 1640 containing 5% fetal calf serum and 15% calf serum. The cells were incubat-

ed in plastic flasks for one hour at 37°C. Nonadherent cells were obtained by gently washing the flasks.

T-cell depletion

An immunomagnetic separation procedure for depletion or positive selection of T-cells from BM or peripheral blood was done using the method described by Zhou et al (1989). Briefly, mononuclear cells were incubated with anti-CD_3 (Leu 4) antibody for 30 min, then the cells were incubated with immunomagnetic beads coated with sheep antimouse IgG (Dynabeads M-450, Dynal AS, Norway) for 30min. Separation was performed by placing the culture tube in a magnet (Dynal) for one to two minutes. The procedure was performed on 5 ALL BM, 1 AML BM, 1 off therapy BM and 2 peripheral blood.

Immunophenotyping

Immunologic surface marker analysis of BM blast was performed using either direct or indirect immunofluorescence and flow cytometry (FACS, Becton Dickinson). A panel of monoclonal antibodies specific for normal or malignant B, T and myeloid cells was used (Becton Dickinson, San Jose, CA).

Lymphokines

Recombinant IL-7 was generously provided by Immunex Corp. (Seattle, Wa). The concentration of the undiluted preparation was 10,000 U/ml. Low molecular weight B cell growth factor (LMW-BCGF) was obtained from Cellular Production Inc. (Buffalo, NY). Recombinant IL-2 was purchased from AMGEN (Thousand Oaks, CA).

Tritiated thymidine incorporation assay

DNA synthesis was measured by 3H-thymidine incorporation. Cells (2.5×10^4) were cultured in 0.25ml RPMI 1640 media plus serum (described above). Cells were cultured with or without the addition of IL-7 (1,000U/ml) and/or LMW-BCGF (0.1U/ml) in 96 well flat-bottomed microtiter plates for 72 hours. At this concentration they showed maximum effect. Twenty four hours before harvesting, 0.5μCi tritiated thymidine (84.2 Ci/mmol, NEN Inc., Wilmington, DE) was added. Cells were harvested on nitrocellulose paper using a semi-automated cell harvester (Skatron Inc., Sterling, Va). Radioactivity was determined with a liquid scintillation analyzer (2200 Ca, Packard Instrument Company). All experiments were performed in triplicate. Data was expressed as percentage of DNA synthesis to control CPM (media alone).

RESULTS

Effect of IL-7 and BCGF on ALL cells

Effect of IL-7 and BCGF on ALL BM lymphocytes of 12 ALL cases was analyzed. Of which 1 was B-ALL, 1 was biphenotypic type of acute leukemia (CALLA (−), BA 97%, My4 80%) and 10 were pre B ALL. FAB morphology was L_1 except 1 B-ALL (L_3) case, Percentage of bone marrow blast was between 76% and 98%. All except one case of relapse were at the time of initial diagnosis (Table 1).

The response to IL-7 both in the presence or absence of BCGF was analyzed in a thymidine incorporation assay. IL-7 stimulated the proliferation of leukemic cells from 1 of 5 T-cell depleted cases (Fig. 1) and 1 of 10 cases without T-cell depletion (Fig. 2). This IL-7 responsive case after T-cell depletion was biphenotypic leukemia. BCGF stimulated 2 of 5 T-cell depleted cases. One of these was cell from B-ALL and this case showed additive response to combination of IL-7 and BCGF.

Effect of IL-7 and BCGF on T-ALL cells

The seven T-ALL showed blast percentage in BM between 28 to 98%. One case was mixed L_1 and L_2. Others showed L_1 morphology (Table 2). BM cells from

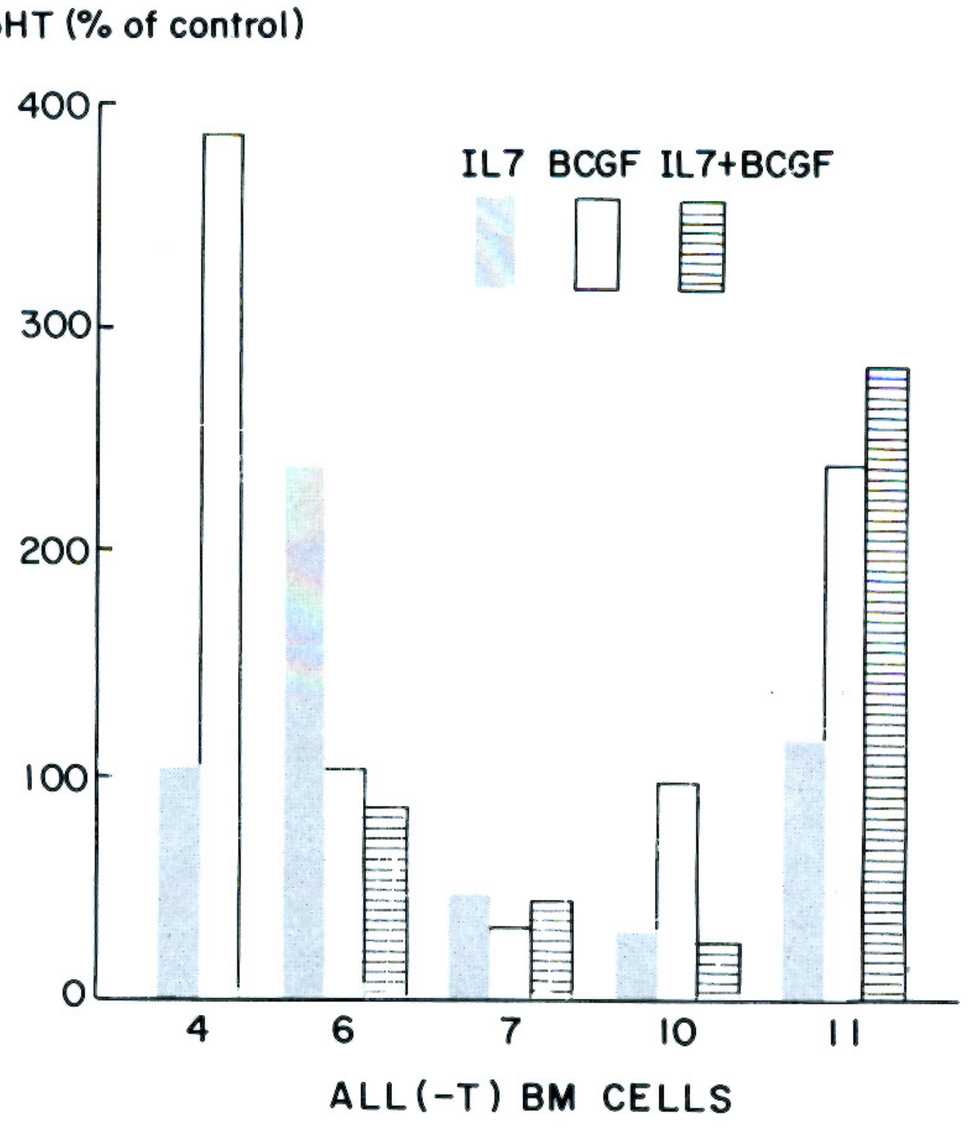

Fig. 1. Proliferative response of T-cell depleted bone marrow cells from non-T ALL patients to IL-7, BCGF and IL-7 plus BCGF (mean control CPM: 1268)

Table 1. Patient's characteristics of non-T Acute Lymphoblastic Leukemia

PtNo	Age/Sex	FAB	state of Ds	Blast (%)	CALLA	B4	Slg	Leu1+9/Leu4
1	11/F	L1	R	80	66	72	15	13/38
2	6/F	L1	I	86	80	86	9	11/8
3	3/M	L1	I	97	79	66	NT	12/10
4	3/M	L1	I	84	72	77	19	10/14
5	3Mo/M	L1	I	97	85	68	25	5/8
*6	3Mo/F	L1	I	76	-	73	10	10/5
7	8/F	L1	I	89	74	69	6	2/7
8	1/M	L1	I	98	94	93	3	5/5
9	13/F	L1	I	96	93	93	3	6/2
10	4/F	L1	I	89	85	85	5	10/10
11	9/F	L3	I	27.5	72	82	77	11/12
12	4/M	L1	I	85	70	66	59	21/25

* Biphenotypic BA1 97%, B4 73%, My4 80%
R: relapse
I: initial diagnosis
NT: not tested

Table 2. Patient's characteristics of T-Acute Lymphoblastic Leukemia

PtNo	Age/Sex	FAB	state of Ds	Blast (%)	CALLA	B4	Slg	Leu1+9/Leu4
1	7/M	L1	I	96	-	1	2	98/14
2	9/M	L1	I	28	-	8	44	61/20
3	5/M	L1+L2	I	70	63	5	4	90/88
4	4/F	L1	I	95	-	14	-	32/NT
5	14/F	L1	I	98	-	NT	1	90/5
6	3/M	L1	R	93	-	NT	1	90/NT
7	4/F	L1	I	81	-	2	4	92/5

Table 3. Patient's characteristics of Acute Myelocytic Leukemia

PtNo	Age/sex	FAB	state of Ds	Blast	CALLA	My4	My7	My9	Leul+9/Leu4
1	12/F	M4orM5	i	38	-	32	52	52	7/11
2	8/M	M5a	I	98	-	98	97	98	-/1
3	4/M	M2	I	20	-	59	36	38	1/13

these patients were incubated with and without IL-7, LMW-BCGF or a combination of IL-7 and BCGF. IL-7 was able to stimulate DNA synthesis in cells from 3 out of 7 T-ALL patients. Only one case showed increased response to LMW-BCGF and in this case both factors had additive effect (Fig. 3).

Effect of IL-7 and BCGF on cell lines, off Therapy BM and AML BM cells

Three AML BM cells were characterized as shown in Table 3. Four off therapy BM cells from ALL cases were tested. These cells showed no definite IL-7 stimulatory response compared to controls (media alone). BCGF showed stimulatory effect to off therapy BM cells 3 times compared to controls (Fig. 4). Both unfractionated and T-depleted (-T) BM cells showed no stimulatory response to IL-7 in 1 off Therapy BM and 1 AML BM cells (data not shown).

Eight cell lines showed no stimulatory response to IL-7, LMW-BCGF or a combination of IL-7 and BCGF.

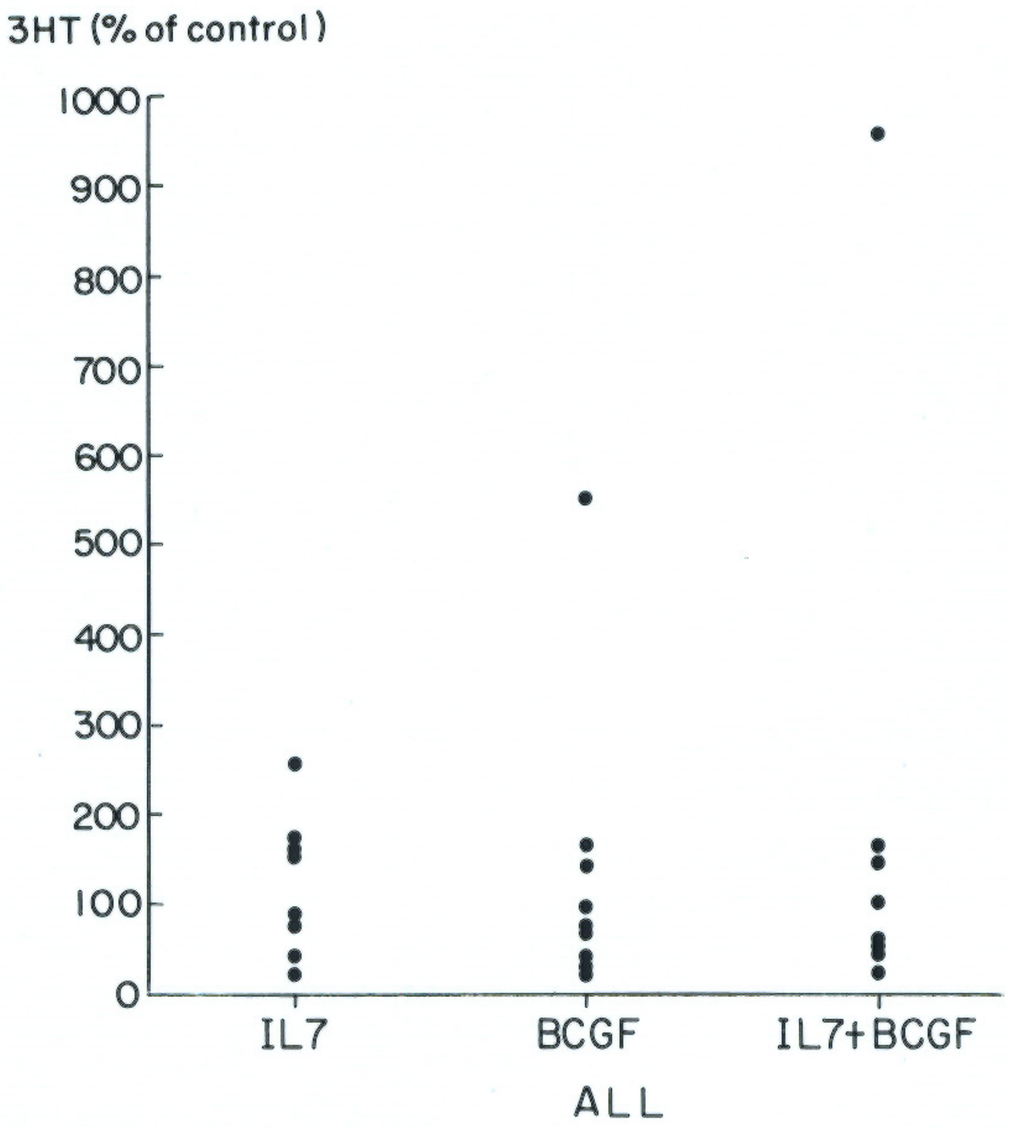

Fig. 2. The response to IL-7, BCGF and IL-7+BCGF in 10 non-T ALL BM cells without T-cell depletion. (3H-thymidine incorporation) IL-7 stimulated the proliferation of leukemic cells in 1 of 10 cases (more than 200%). This case was stimulated with BCGF also and combination of IL-7 and BCGF showed additive response. (mean control CPM: 697)

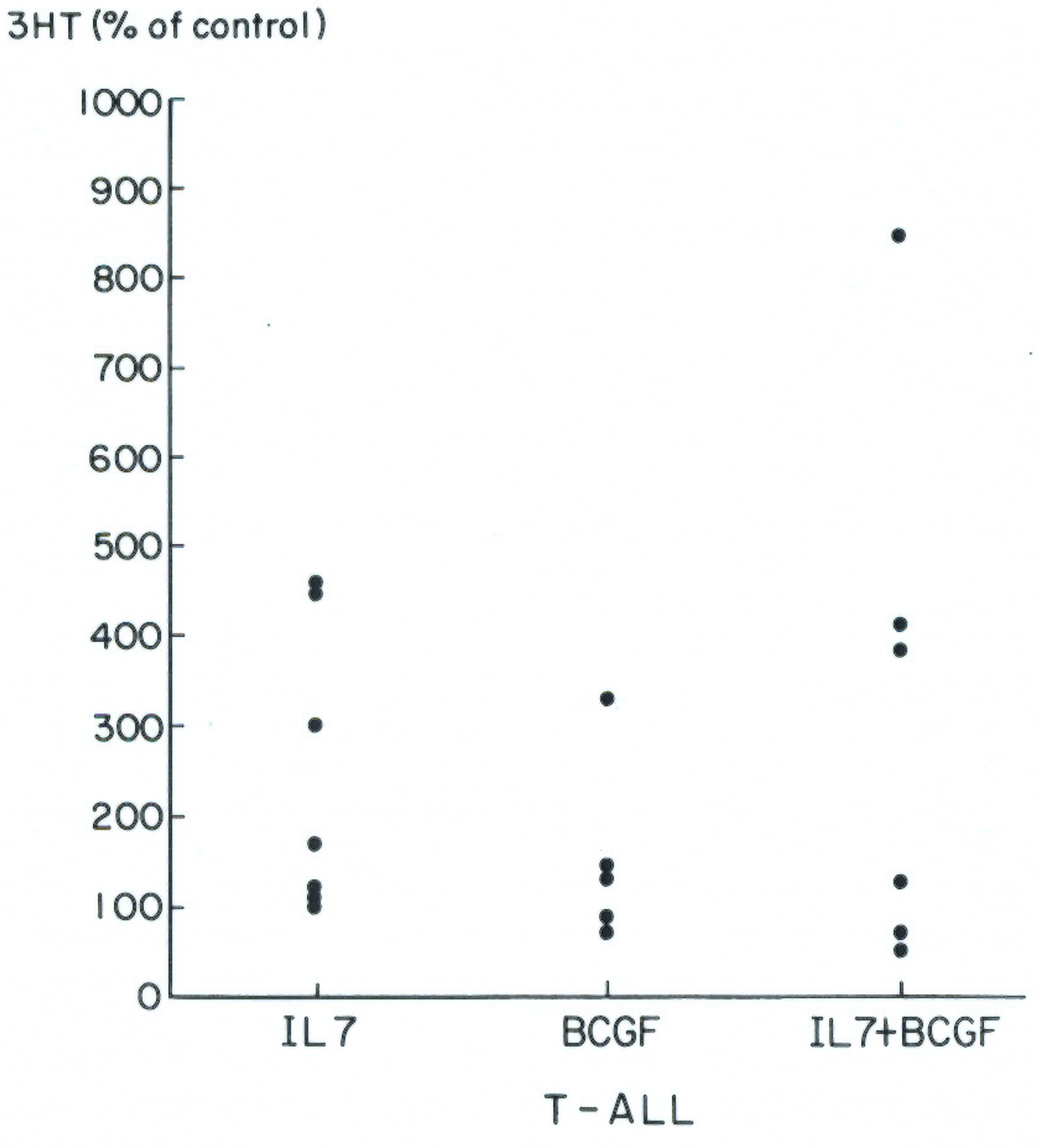

Fig. 3. 3H-Thymidine incorporation activity in T-ALL BM cells in response to IL-7, BCGF or IL-7+BCGF (mean control CPM: 417)

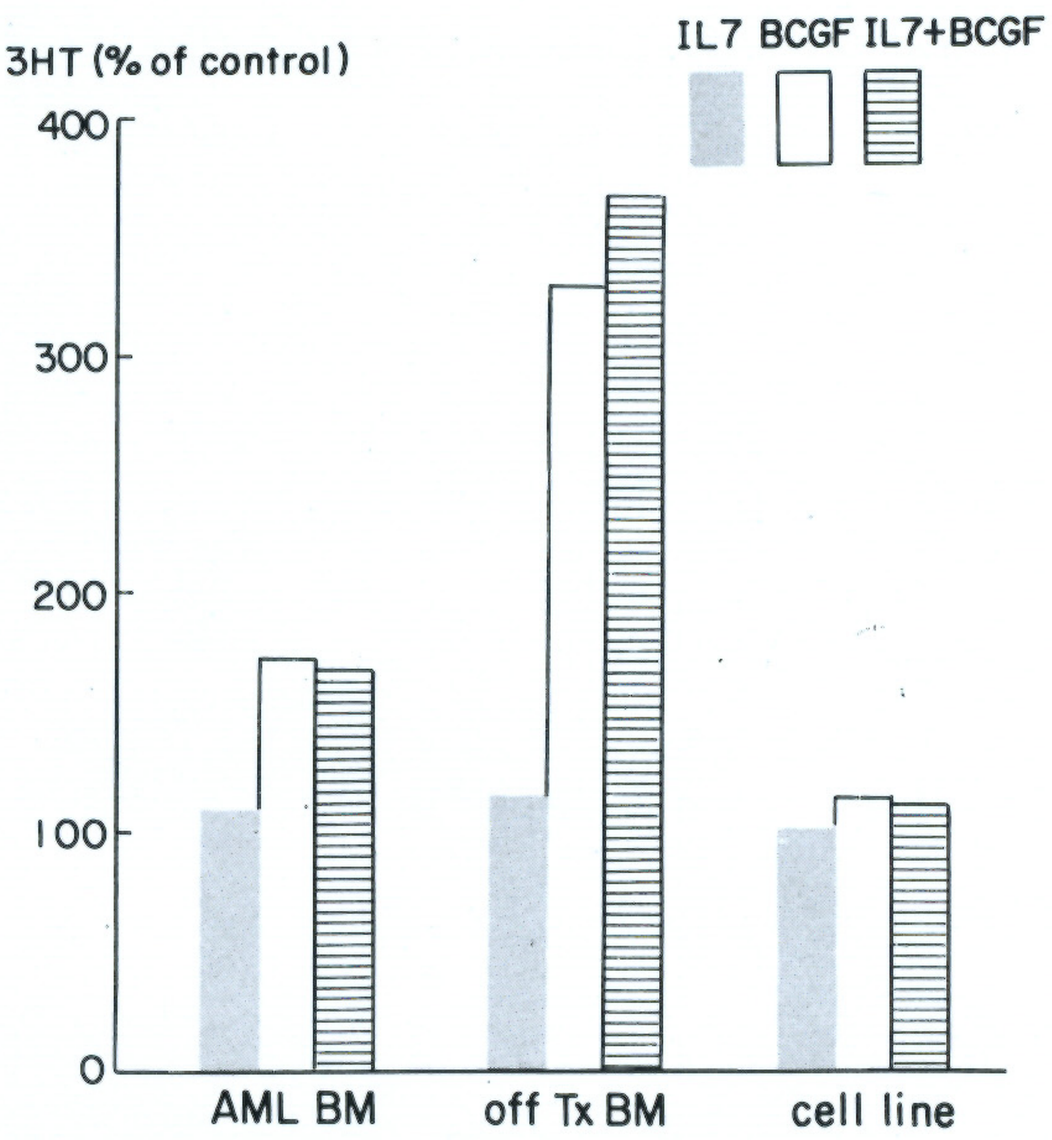

Fig. 4. 3H-Thymidine incorporation activity in BM cells of 3 cases of AML, 4 cases of ALL off therapy and in cell lines (mean control CPM AML: 6062 off Tx BM: 6318 Cell line: 9425)

IL-7 effect on peripheral blood T-cells

Peripheral blood T-cells after positive selection with immunomagnetic beads were incubated with and without IL-7, IL-2, BCGF or combination of IL-7 and IL-2, and IL-7 and BCGF. IL-7 exerted a marked stimulatory effect on PB T-cells in 1 case.

The other case showed a moderate response (Fig. 5). Compared to the cells without T-cell separation, IL-7 effect to T-cells exerted 17 times and 3 times increased 3H-Thymidine incorporation in each case (data not shown). IL-2 was able to stimulate DNA synthesis more than IL-7 and combination of IL-2 and IL-7 showed additive effect implying the possibility that IL-2 and IL-7 respond in different ways. BCGF alone has no stimulatory effect.

Dose response of cells to IL-7

The response of 1 peripheral blood T-cell and 1 T-ALL BM cell to different doses of IL-7 was determined using the DNA synthesis assay. These cells were incubated with IL-7 at a concentration of 30 u/ml to 5000 u/ml. As little as 30 u/ml IL-7 was able to stimulate DNA synthesis of these cells (Fig. 6). CPM increased dose dependently until IL-7 concentration of 1000 u/ml where the curve made plateau.

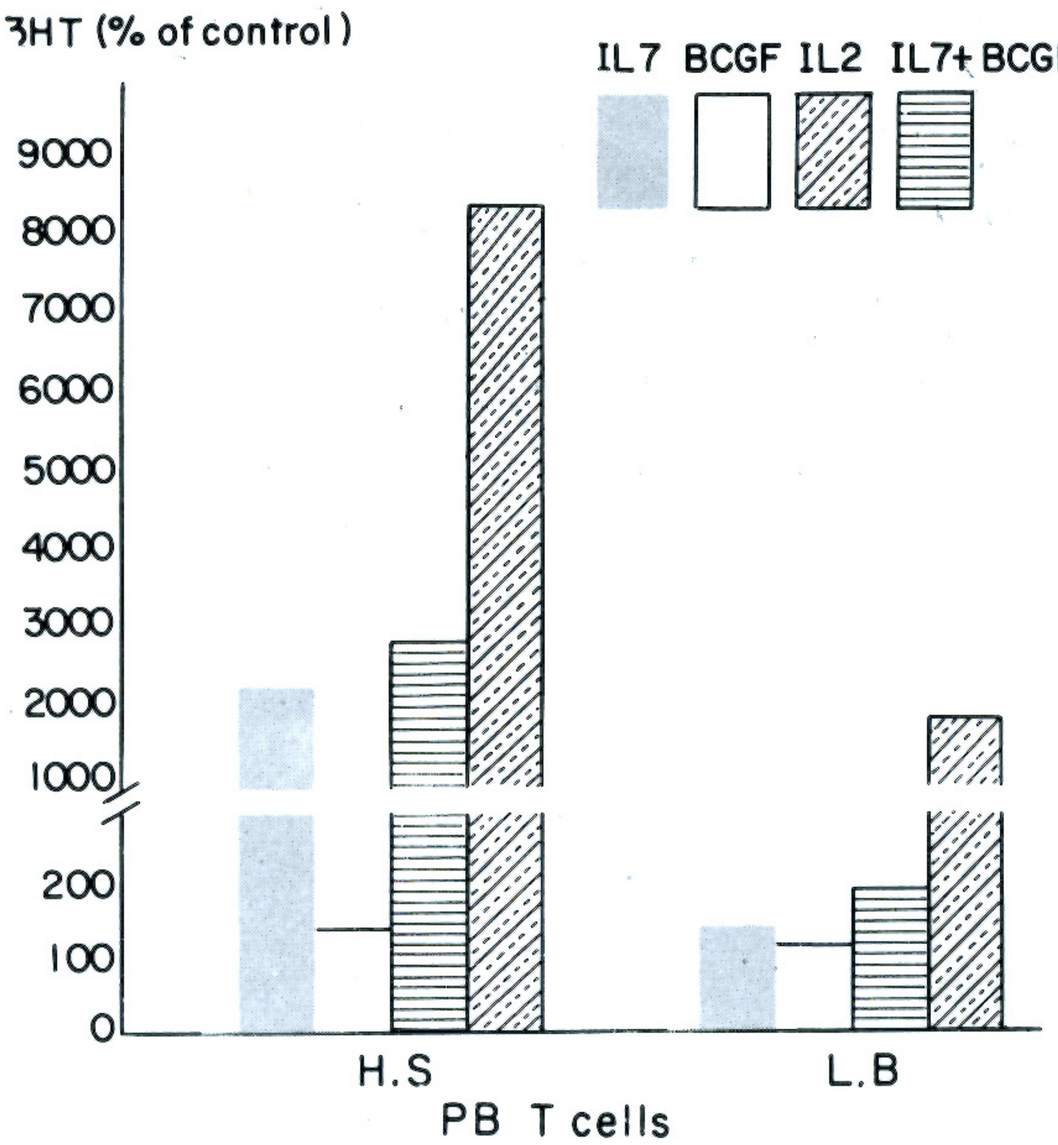

Fig. 5. 3H-Thymidine incorporation activity in peripheral blood T cells (mean control CPM: 154)

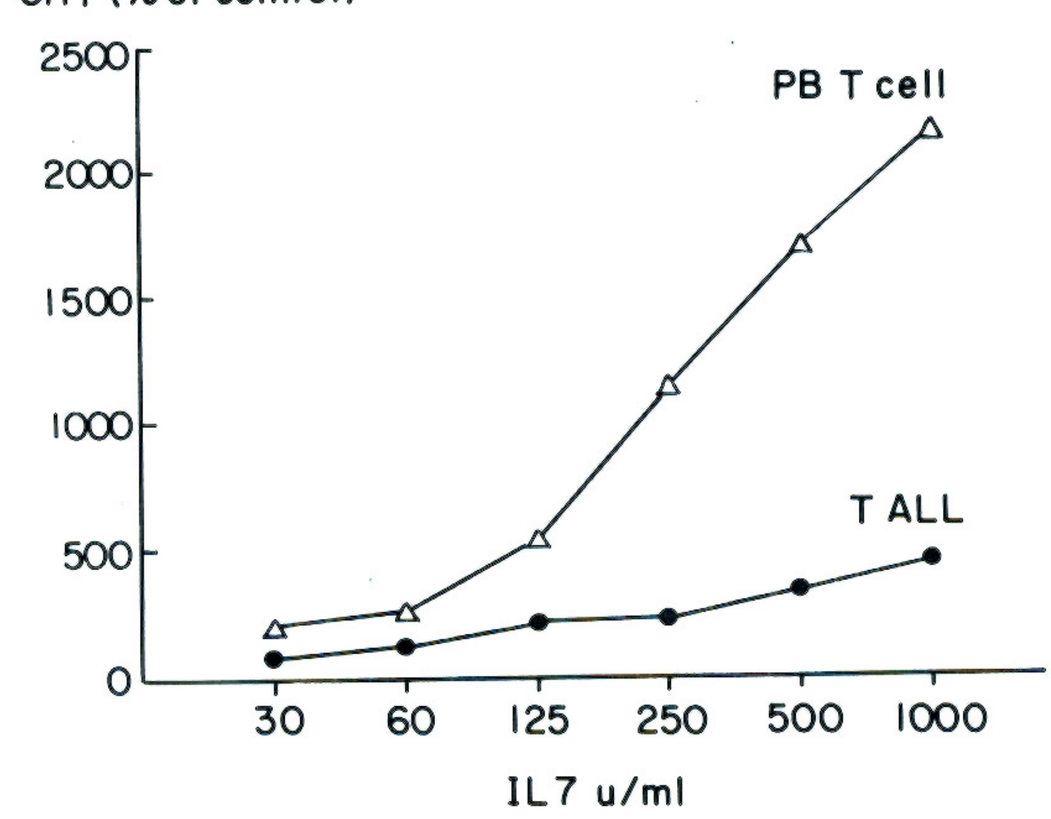

Fig. 6. Effect of IL-7 on peripheral blood T-cell or T-ALL proliferation.

DISCUSSION

The IL-7 gene is located at the long arm of chromosome 8 (Sutherland et al, 1989), and its m-RNA is expressed in the thymus and in the spleen (Goodwin et al, 1989; Conlon et al, 1989). It stimulates DNA synthesis of early B-cells (Lee et al, 1988; Namen et al, 1988[b]; Goodwin et al, 1989; Takeda et al, 1989; Lee et al, 1989; Sudo et al, 1989) and early thymocytes (Henney, 1989; Watson et al, 1989; Conlon et al, 1989) in both murine and human systems. Mature human T-cells respond to IL-7 directly (Welch et al, 1989) whereas murine T-cells respond to IL-7 after stimulation with mitogen or antigen (Henney, 1989; Morrisey et al, 1989; Chazen et al, 1989). There was no evidence of differentiation with IL-7 (Henney, 1989). IL-7 effect was independent from IL-2, IL-4, and IL-6 (Henney, 1989; Morrissey et al, 1989; Chazen et al, 1989) and it's effect was blocked by TGF β (Lee et al, 1988; Lee et al, 1989).

In B-cell lineage, IL-7 is effective to the pro B and pre B cell population. If the cells express SIg (Surface immunoglobulin), they are no longer responsive to IL-7 (Henney, 1989). In stromal cell dependent B-cell development, mature pre-B stage cells only proliferated in response to IL-7 (Sudo et al, 1989).

In this study, most of the cells from ALL patient were not responsive to IL-7, only 2 of 12 ALL BM lymphocytes were responsive.

They were one case of pre-B ALL and one case of biphenotypic of leukemia (CALLA (−), blast 76%). Since AML BM cells showed no effects to IL-7, such effects of biphenotyphic type may be due to mature pre B cell response to IL-7, but we could not determine whether this effect is from normal or leukemic B-cells.

BM blast of the other case was 96% and 93% was CALLA (+). IL-7 stimulatory effect to these cells was 2.5 times to control and BCGF effect was 5.5 times to control. Though we did not separate the T-cell population, we could suspect that the IL-7 effect in this case was due to leukemic BM cells. In one case of B-ALL, there was no effect from IL-7.

In T-ALL cells, three IL-7 responsive cases had BM blast between 70 to 96%, and Leu 1+9 were more than 90%. Of these, blast percentage of the least responsive case was only 70% and leu 4 was 88%. So it looks like leukemic T-cells responded to IL-7 more effectively than normal BM cells. However one of the non reponsive cases had 98% blast and 90% Leu 1+9. According to these results we could not predict the responsiveness of leukemic cells to IL-7 definitely. Although Goodwin (1989) and Lee (1989) suggested the good relationship between neoplasia and IL-7, we could not conclude with the result of this study that the IL-7 response to ALL BM and T-ALL BM was related to the malignant cell itself. We could not see any effect to normal BM cells from IL-7. This is a little different from the results of Goodwin et al. B4 (+) cells which were included in 40-50% in their experiment were lacked in our study. We considered that this discrepancy between two experiment leads to somewhat different conclusion and also we could rule out contamination of other malignant cells because all the patients were in remission state for at least more than 3

months. But further study is needed to determine more precise conclusion for this difference.

In AML BM, nonresponsiveness to IL-7 may be due to high percentage of surface immunoglobulin, but In one case after T cell selection, T-cells also showed no response from IL-7.

In cell lines, there was no effect from IL-7. This result is similar to that of Lee et al (1989) as in murine B-cell line.

In peripheral blood T-cells, the IL-7 effect was most prominent as was in the results of Welch et al (1989). LMW-BCGF did not show any effect. IL-2 showed more stimulatory effect. The fact that IL-2 showed more prominent stimulatory effect and combination of IL-2 and IL-7 had additive effect is implying IL-7 and IL-2 responds in a different way. Welch et al (1989) showed in their study that IL-7 effect is more prominent than IL-2. We used a higher concentration of IL-2 than in Welch's experiment, but their stimulatory effect to IL-7 presents in suboptimal dose (100 u/ml). As shown in the dose response curve. IL-7 effect was more prominent in peripheral blood T-cells than T-ALL cells.

We have seen that IL-7 stimulates 2 out of 12 ALL, 3 out of 7 T-ALL BM cells and normal peripheral blood T-cells. IL-7 did not stimulate off therapy BM and AML BM cells and various leukemic cell lines. In conclusion we have demonstrated the effect of IL-7 to stimulate DNA snthesis of mature T-cells and T-lineage leukemic cells. For some of the cell from non T ALL cases were stimulated by IL-7, further study using more purified cells will be needed.

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