Proliferation-Inducing Effects of Recombinant Human Interleukin-7 and Interleukin-3 in B-Lineage Acute Lymphoblastic Leukemia *

M. Eder [1], O. G. Ottmann [1], T. E. Hansen-Hagge [2], C. R. Bartram [2], S. Gillis [3], D. Hoelzer [1], and A. Ganser [1]

Introduction

Acute lymphoblastic leukemia (ALL) is a clonal disorder characterized by derangements of self-renewal and differentation of lymphoid precursor cells in the bone marrow. Corresponding to the inconsistent stimulatory effects of the recombinant hematopoietic growth factors studied to date on B-lineage ALL blasts in vitro [1], a reproducible culture assay that supports proliferation and maturation of ALL blasts has not yet been reported.

Recently, a new cytokine has been defined by its stimulatory effects on DNA synthesis in murine pre-B cells from Whitlock-Witte culture [2]. This stromal cell-derived cytokine, termed interleukin-7 (IL-7), has been purified and molecularly cloned, and the recombinant murine and human proteins are now available [3, 4]. Since IL-7 also stimulates murine pre-B cells from bone marrow [5], murine thymocytes, and, as comitogen, mature T cells [6] and induces proliferation of human T cells [7], we investigated whether IL-7 could stimulate DNA synthesis in B-lineage ALL blasts in suspension culture and also the capacity of IL-7 to induce blast cell maturation in vitro.

* This work was supported by grants Ga 333/1-2 and Ba 770/2-3 from the Deutsche Forschungsgemeinschaft and by a grant from the Deutsche Krebshilfe.

[1] Department of Hematology, University of Frankfurt, Frankfurt, FRG.
[2] Department of Pediatrics II, Section of Molecular Biology, University of Ulm, Ulm, FRG.
[3] Immunex Corporation, Seattle, WA, USA.

Methods

Low-density peripheral blood ($n = 10$) or bone marrow ($n = 4$) cells were separated by Ficoll-Hypaque density centrifugation and were classified as common ALL (cALL; including pre-pre-B-lineage all and pre-B-lineage ALL; $n = 10$; HLA-DR+/CD 10+/CD 19+/sIg−) or B-lineage ALL ($n = 4$; HLA-DR+/CD 10− or +/CD 19+/sIg+) [8]. All samples were depleted of adherent cells and then incubated with OKT4, OKT8, and OKM1 and rabbit complement in order to eliminate mature myeloid and T-lymphoid cells. Cells were cultured in Iscove's modified Dulbecco's medium (IMDM), 20% fetal calf serum (FCS), 1% L-glutamine and 1% penicillin/streptomycin supplemented by IL-7 (50 U/ml, Immunex) or IL-3 (50 ng/ml, Behring) and incubated in 96-well plates at $1-2.5 \times 10^5$ cells per well (quadruplicate values). After 7 days of liquid culture, samples were pulsed with [^{3}H]thymidine (1 μCi/well) for 4 h and harvested on nitrocellulose filters. Thymidine uptake was defined by liquid scintillation counting. Cells of responsive samples were further characterized by four-parameter flow cytometry using a panel of monoclonal antibodies (moAb) and by immunogenotyping in order to monitor individual leukemic cell populations prior to and after suspension culture. All moAbs were from Becton and Dickinson (anti-CALLA CD10, Leu12 CD19, Leu16 CD20, anti-κ, anti-λ, anti-HLA-DR, LeuM9 CD33, anti-HPCA1 CD34), Coulter Clone (My7 CD13) and Medac (goat anti-mouse IgG for indirect

Haematology and Blood Transfusion Vol. 35
Modern Trends in Human Leukemia IX
R. Neth et al. (Eds.)

immunofluorescence staining). After culture, one sample was incubated with Leu 12 and propidium iodide (PI) in order to examine viability and CD 19 expression in different cell populations characterized by their light scatter properties. Southern blot analysis was performed as previously described [9]. *Eco*RI and *Hin*dIII digests were hybridized to a 2.4-kb *Sau* 3 a JH probe and *Bam*HI and *Hin*dIII digests to a 1.3-kb *Eco*RI Cμ, as well as Ck probe to demonstrate Ig gene rearrangements. To analyze configuration of T-cell receptor genes *Eco*RI, *Bam*Hi and *Hin*dIII digests were hybridized to a TCRβ probe and to a TCRγ probe, and *Hin*dIII- and *Bgl*II-digested DNA was hybridized to TCRδ probe $J\delta_{S16}$.

Results and Discussion

The stimulation indices (SI), defined as counts per minute (cpm) of the sample/cpm of control are given in Table 1. With an arbitrary cut-off of a SI > 5,4 of 10 cALL samples (5, 7, 8, 9) and 1 of 4 B-ALL samples (14) were stimulated by IL-7. IL-3 stimulated DNA synthesis in 5 of 9 cALL and 3 of 4 B-ALL samples. In two of the cALL samples responsive to IL-7, IL-7 was more potent than IL-3 (8, 9), while IL-3 was more effective than IL-7 in all B-ALL samples examined.

To further define the nature of proliferating cells and the maturation stage of the leukemic blasts, samples 6, 8, 9, and 14 were analyzed by four-parameter flow cytometry and Southern blot analysis prior to and after liquid culture. Table 2 summarizes the results of immunophenotyping; Fig. 1 presents the analysis of sample 14. Analysis gates were fitted to light scatter properties of PT-negative cells in order to gate preferentially viable cells after liquid culture. Sample 6, which was Ph^+, revealed a marked increase in the percentage of $CD33^+$ and $CD13^+$ cells at day 7, suggesting that non-lymphoid cell populations preferentially proliferated during suspension culture. In sample 8, a decrease in CD 34 and CD 19 expression and in the percentage of CD 10/CD 19 double-positive cells were detectable, while the percentage of $CD20^+$ cells was unchanged. Sample 9 revealed a decrease in $CD34^+$ cells, but CD 19 expression was unchanged and CD 10/CD 34 double-positive cells were detectable after 7 days of liquid culture. A net increase in $CD19^+$ cells (data not

Table 1. Stimulation indices of ALL blasts stimulated by IL-7 and IL-3

Sample	Diagnosis	IL-7 (50 U/ml)	IL-3 (50 ng/ml)
1	cALL	1.5	1.5
2	cALL	2.6	nd
3	cALL	3.3	0.6
4	cALL	1.3	1.8
5	cALL	5.1	6.7
6	cALL Ph	2.3	6.8
7	cALL	15.8	25.5
8	cALL	5.9	1.5
9	cALL	53.6	37.1
10	cALL	3.4	45.1
11	B-ALL	1.8	3.5
12	B-ALL	2.6	9.2
13	B-ALL	1.5	15.6
14	B-ALL	8.9	18.3

Mean SI of quadruplicate cultures (cpm sample/cpm control). Control cpm were < 850 in all cases examined.

Table 2. Surface marker analysis prior to and after suspension culture of ALL samples

Sample[a]	Antigen	% positive cells	
		Day 0	Day 7
6 cALL	CD 34	11	54
	CD 33	3	43
	CD 13	< 1	75
8 cALL	CD 34	24	9
	CD 19	69	45
	CD 20	36	41
	CD 10/CD 19	69	37
9 cALL	CD 34	96	75
	CD 19	68	64
	CD 20	6	4
	CD 10/CD 19	63	47
	CD 10/CD 34	92	42
14 B-ALL	CD 34	< 1	< 1
	CD 19	14	57
	CD 20	72	82
	sIgκ	36	< 1

[a] Samples 8, 9, and 14 were stimulated by IL-7 (50 U/ml) and sample 6 by IL-7 (50 U/ml) combined with IL-3 (50 ng/ml).

shown), combined with a lack of surface-bound Ig κ light chain, was found in sample 14, suggesting proliferation of B-lineage restricted cells during culture. The lack of surface-bound Ig light chain and the consistent pattern of Ig recombination prior to and after liquid culture (see below) suggest that leukemic transformation occurred at a maturation level preceding B-cell stage. Maturation induction, e.g., expression of surface-bound Ig after liquid culture, was not detectable in any of the examined cases.

As indicated in Table 3, the immunogenotype corresponded to the immunophenotype in the cases examined. In order to monitor individual leukemic cell populations, defined by specific molecular genetic markers, cells were analyzed prior to and after liquid culture. In cases 9 and 14, IL-7 and IL-3 induced almost exclusively proliferation of the leukemic cell clone as concluded from the consistent pattern of Ig recombination prior to and after liquid culture. In cases 6 and 8, nonleukemic cell proliferation associated with the generation of $CD33^+$ and $CD13^+$ cells (case 6) and detection of burst-forming unit-erythroid (BFU-E) and colony-forming unit-granulocyte-

Table 3. Immunogenotype of ALL samples

Sample	Phenotype	Rearrangement	Proliferative population[a]
6	cALL Ph	IgH, TCR δ	Nonleukemic
8	cALL	IgH, TCR γ, TCR δ	Nonleukemic
9	cALL	IgH, TCR γ, TCR δ	Leukemic
14	B-ALL	IgH, Ig κ	Leukemic

[a] Proliferation of the leukemic cell clone was concluded from the consistent pattern of Ig recombination observed prior to and after liquid culture.

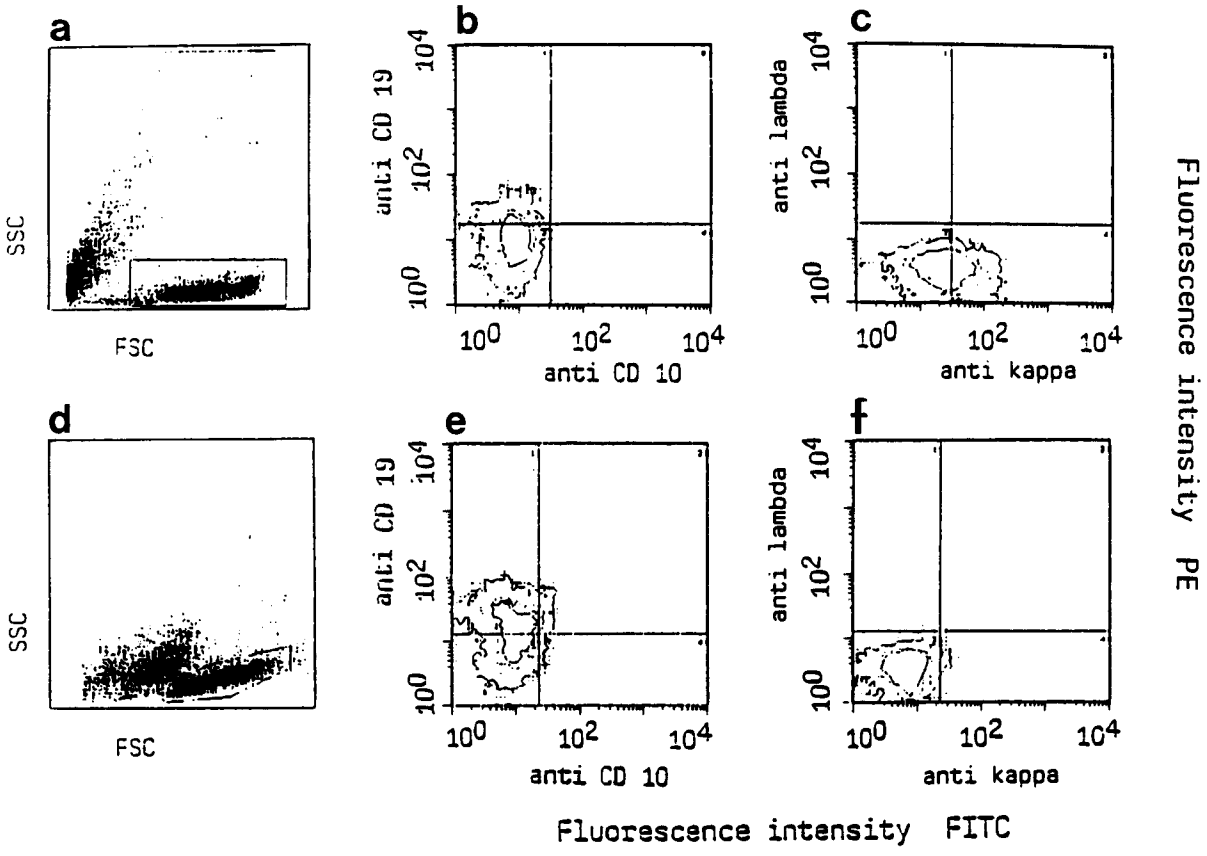

Fig. 1a–f. Four-parameter flow cytometry of sample 14 prior to and after 7 days of liquid culture. Cells were analyzed prior to (**a–c**) and after 7 days of liquid culture (**d–f**). **a**, **d** Dot plot diagrams of foreward (FSC) and sideward scatter (SSC) properties. Viability of gated cells was 93% (**a**) and 98% (**d**). **b**, **c**, **e**, **f** Contour graphs from two-color FACS analysis. Samples were stained as indicated. Quadrants were chosen corresponding to controls stained by unspecific MsIgG-FITC and MsIgG-PE

macrophage (CFU-GM) after liquid culture (case 8; data not shown) was observed when cultures were stimulated by IL-7 or IL-3. The detection of non-leukemic cells generated during suspension culture stimulated by IL-7 or IL-3 underlines the necessity to define exactly the nature of proliferating cells in responsive samples.

We conclude that IL-7 and IL-3 stimulate proliferation of leukemic cells in a subset of B-lineage restricted ALL without evidence of concurrent maturation induction. However, additional growth factors may be required to improve the in vitro culture of ALL blasts.

Acknowledgements. We gratefully appreciate the excellent technical assistance by P. Reutzel, P. Sauer, S. Ströcker-Pels, C. Tell und U. Mehr.

References

1. Touw I, Groot-Loonen J, Broeders L, van Agthoven T, Hählen K, Hagemeijer A, Löwenberg B (1989) Recombinant hematopoietic growth factors fail to induce a proliferative response in precursor B acute lymphoblastic leukemia. Leukemia 3:356–362
2. Namen AE, Schmierer AE, March CJ, Overell RW, Park LS, Urdal DL, Mochizuki DY (1988) B cell precursor growth-promoting activity. Purification and characterization of a growth factor active on lymphocyte precursors. J Exp Med 167:988–1002
3. Namen AE, Lupton S, Hjerrild K, Wignall J, Mochizuki DY, Schmierer A, Mosley B, March CJ, Urdal D, Gillis S, Cosman D, Goodwin RG (1988) Stimulation of B cell progenitors by cloned murine interleukin-7. Nature 333:571–573

4. Goodwin RG, Lupton S, Schmierer A, Hjerrild KJ, Jerzy R, Clevenger W, Gillis S, Cosman D, Namen AE (1989) Human interleukin 7: molecular cloning and growth factor activity on human and murine pre-B lineage cells. Proc Natl Acad Sci USA 86:302–306
5. Lee G, Namen AE, Gillis S, Ellingsworth LR, Kincade PW (1989) Normal B cell precursors responsive to recombinant murine IL-7 and inhibition of IL-7 activity by transforming growth factor-β. J Immunol 142:3875–3883
6. Henney CS (1989) Interleukin 7: effects on early events in lymphopoiesis. Immunol Today 5:170–173
7. Armitage RJ, Namen AE, Sassenfeld HM, Grabstein K (1990) Regulation of human T cell proliferation by IL-7. J Immunol 144:938–941
8. Thiel E, Hoelzer D, Dörken B, Löffler H, Messerer C, Huhn D (1987) Clinical relevance of blast cell phenotype as determined with monoclonal antibodies in acute lymphoblastic leukemia in adults. Hamatol Blood Transfus 30:95–103
9. Ragavachar A, Bartram CR, Ganser A, Heil G, Kubanek B (1986) Acute undifferentiated leukemia (AUL): implications for cellular origin and clonality suggested by analysis of cell surface markers and immunoglobulin gene rearrangement. Blood 68:658–662