

EXPRESSION OF DIFFERENT COMBINATIONS OF
INTERLEUKINS BY HUMAN T CELL LEUKEMIC
CELL LINES THAT ARE CLONALLY RELATED

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Recent molecular studies on structures and functions of many lymphokines have shown that each lymphokine has multiple effects on diverse cell types (1-3). Furthermore, a single T lymphocyte is able to produce multiple lymphokines. The whole picture of the immune regulation by T lymphocytes seems to be more complicated than expected because combinatorial effects of various lymphokines may synergize or counteract their effects with each other.

An attractive hypothesis that functional subsets of T lymphocytes may correlate with particular combinations of lymphokines produced prompted Mosmann et al. (4, 5) to propose that murine helper T cell clones are classified into Th1 and Th2, which can be distinguished by production of IL-2/IFN- γ and IL-4/IL-5, respectively, in addition to commonly expressed IL-3 and granulocyte/macrophage CSF (GM-CSF). These classifications depend on two assumptions that have not been fully verified. First, they assume that the collected T cell clones should represent the majority of T cells, although the murine T cell clones established could have been selected by stimuli such as antigens and IL-2 which were used for their proliferation in vitro (4). A second assumption is that expression of a particular combination of lymphokines is a stable phenotype of each T cell.

We have investigated the profile of lymphokines produced in human T cell clones from patients with adult T cell leukemia (ATL) that had been transformed by human T lymphotropic retrovirus type I (HTLV-I). We did not find any particular combination patterns of seven lymphokines produced by 19 ATL clones. Furthermore, we found that different combinations of lymphokines were produced by four clonally related leukemic lines established independently from the same patient. The results indicate that a particular lymphokine production profile may not be a fixed phenotype of a T cell but rather variable depending on the states of the T cell.

Materials and Methods

Cells. ED4015, ED4015A, ED40810, and ED41214B cell lines were independently established from leukemic cells of a patient with ATL at three different times (15 May, 15 May, 10 August, 14 December, 1984, respectively) by Maeda et al. (6, 7). These ED-designated lines are clonally related with the original leukemic cells because they have the same rear-

rearrangement pattern of the TCR- β gene and a common integration site of HTLV-I (6, 7). The rearrangement patterns of their β genes were confirmed to be identical recently. SITF, Su9FT (provided by N. Arima of Kagoshima University, Kagoshima, Japan) and ED41214B are the cell lines derived from the patients 1, 2, and 4, respectively (7). The other ATL-derived cell lines were newly established except for ATL2 (6) and MT1 (8). These cell lines have been maintained at least for 1 yr as described (6). Stimulation of ED40810 and MLA144 cells was done by culturing with PHA-P (0.1%) and TPA (10 ng/ml) for 17 h.

Preparation of mRNA and Northern Blotting. Crude RNAs were extracted from various cell lines using guanidinium thiocyanate (9). Poly (A)⁺ RNAs were purified by oligo(dT)-cellulose (Type 3; Collaborative Research, Lexington, MA) column chromatography. Poly(A)⁺ RNA (5 μ g) of each cell line was glyoxalated and electrophoresed in a 1% agarose gel, then transferred to a nitrocellulose filter (Schleicher & Schuell, Dassel, FRG) as described (10). Hybridization was carried out as described (11).

DNA Probes. DNA fragments used as probes are as follows: Human IL-1 α cDNA, 2-kb Bam HI fragment of pCDIL-1 α (11); human IL-1 β cDNA, 1-kb Pst I-Acc I fragment of pA-26 (12) (provided by Dr. Webb of Massachusetts Institute of Technology, Cambridge, MA); human IL-2 cDNA, 0.8-kb Pvu II-Pst I fragment of pHIG5-3 (13) (provided by Dr. Onoue of Kumamoto University); human IL-3 cDNA, synthetic 31-mer oligonucleotide 5'-GTTGAATGCCTCCAGG-TTTGGCCTTCGAAGG-3' corresponding to antisense strand between positions 222 and 252 of the published sequence (14), which is identical to gibbon IL-3 cDNA sequence; human IL-4 cDNA, 0.3-kb Nhe I-Eco RI fragment (supplied by Ono Pharmaceutical Co., Osaka, Japan); human IL-5 cDNA, 1-kb Bam HI fragment of ph-IL-5-30 (15); human IL-6 cDNA, 1.1 kb Bam HI-Eco RI fragment of pBSF2-38 (16) (provided by Dr. T. Hirano of Osaka University); β -actin gene, 0.4-kb Hinf I fragment which encodes the 4th exon and its 3' intron (17). These fragments, except for human IL-3 synthetic oligonucleotide, were labeled to obtain the specific activity of 600–1,200 cpm/pg using random primers (18) and α -[³²P]dCTP (3,000 Ci/mmol, Amersham Corp., Arlington Heights, IL). The oligonucleotide for IL-3 cDNA was labeled by the 5' end labeling method (19) using γ -[³²P]dATP (5,000 Ci/mmol; Amersham Corp.).

Results and Discussion

Expression of seven interleukin genes was tested in 15 ATL clones by Northern blot analysis of their poly(A)⁺ RNA. The amounts of total RNAs on the filters were quantitated by hybridizing with human β actin probe after the signals were washed or decayed out. We estimated the amount of each interleukin mRNA expressed in ATL clones semiquantitatively by comparison of intensities of hybridized bands on autoradiograms with those of the most abundantly expressing cells and categorized them into four groups: (–) no band was visible; (+) <10% of the strongest; (++) >10% of the strongest; (+++) the strongest. The results of 15 ATL clones summarized in Table I revealed diverse patterns of lymphokine production in the ATL cell lines, which were divided into at least seven groups.

We then focused on the four cell lines (ED40515, ED40515A, ED40810, and ED41214B) that are clonally related and most likely the progenies of a single leukemic cell clone because they have the same rearrangement pattern of the TCR β chain gene and share a common HTLV-I integration site (6, 7). As shown in Fig. 1, none of them expressed the same combination of lymphokines. The semiquantitative comparison of lymphokine mRNAs among all the clones tested in the present study is summarized in Table II. It is of particular interest that the IL-2 producer (ED40515A) and the IL-4 producer (ED40810) are clonally related. We did not find deletion of lymphokine genes that were not expressed.

The expression of particular lymphokines in each clone seems stable, as the stimulation of ED40810 by PHA and TPA did not change the combination of lymphokines

TABLE I
Summary of mRNA Expression of Interleukins in ATL Cell Lines

Cell	Growth factor	Cell type CD4/CD8 phenotype	Expression of mRNA*						
			IL-1 α	IL-1 β	IL-2	IL-3	IL-4	IL-5	IL-6
SITF	IL-2	CD4	-	-	-	-	-	-	+
MT1	None	CD4	-	-	-	-	-	-	+
K2	IL-2	CD4	-	-	-	NT	-	-	+
ATL21C	None	CD4	+	-	-	-	-	-	++
SezM3	None	CD4	++	-	-	-	-	-	+
Su9FT	IL-2	CD4	+	-	-	-	-	-	+
ATL6	IL-2	CD4	+	-	-	-	-	-	++
ATL16T	None	CD4	++	-	-	-	+++	+++	+++
ATL2	None	CD4	++	-	-	-	-	+	++
H5	IL-2	CD4	+	-	-	NT	-	++	+
ED50823	IL-2	CD4	+	-	-	-	-	++	-
ATL35	None	CD4	+++	-	+++	-	-	++	-
H4	IL-2	CD8	-	-	-	-	-	+	+
H6	IL-2	CD8	-	-	-	-	-	++	++
K1	IL-2	CD8	-	-	-	NT	-	+	+
MLA144†	None	-	-	-	++	+++	-	-	-

* Amounts of mRNA are shown by semiquantitative estimation as described in the text. NT, not tested.
† Stimulated with PHA and TPA.

produced (Fig. 2). The expression of lymphokine mRNA was also stable for reasonable time intervals, as the presence or absence of IL-1 α mRNA in ATL-2, ATL-6, ED40810, and MT-1 cells was unchanged for almost 2 yr (11). However, we found that IL-1 α mRNA expression in ED40515 had been lost after we detected its mRNA almost 2 yr ago (11).

Expression of different lymphokine genes is unlikely to be due to integration of

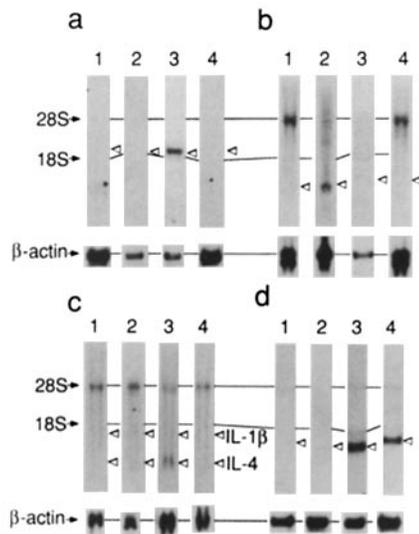


FIGURE 1. Expression of interleukin mRNAs in ATL-derived T cell lines. 5 μ g each of poly(A)⁺ RNA from ED series cell lines was used. Expected positions of probed mRNAs are shown by open triangles on the right sides of the lanes. Marker positions of 28S and 18S ribosomal RNA on the filters are indicated by lines. cDNA probes used were: (a) IL-1 α ; (b) IL-2; (c) IL-1 β and IL-4; and (d) IL-6. (Lanes 1, 2, 3, and 4) mRNAs from ED40515, ED40515A, ED40810, and ED4124B, respectively. After autoradiogram, all the filters were washed to remove hybridized probes, and then rehybridized with β -actin probe to estimate the amount of blotted RNA.

TABLE II
Production of Lymphokines in Clonally Related ATL Cell Lines

Cell line	Date of establishment	Growth factor	Expression of mRNA*						
			IL-1 α	IL-1 β	IL-2	IL-3	IL-4	IL-5	IL-6
ED40515	1984/05/15	IL-2	-	-	-	-	-	-	-
ED40515A	1984/05/15	IL-2	-	-	+	NT	-	-	-
ED40810	1984/08/10	None	+	-	-	-	+	+	++
ED41214B	1984/12/14	None	-	-	-	-	-	-	++

* Amounts of mRNA are shown by semiquantitative estimation as described in the text by comparing all the T cell clones tested in the present study. NT, not tested.

HTLV-I near promoter regions of lymphokine genes because the number and location of retroviral integration sites do not correlate with the number and type of lymphokines produced (6, 7). Since mRNAs of aberrant sizes were not detected by the Northern blot analysis, the detected lymphokine mRNAs are likely to be functional and initiated from the regular promoters. In fact, biological activities of IL-1 α , IL-2, and IL-4 were detected in the culture supernatants of ATL2, ATL35, and ATL16T, respectively. MLA144 stimulated with PHA and TPA is known to produce IL-2 and IL-3, in agreement with mRNA synthesis.

HTLV-I infection has been shown to activate genes for various lymphokines and their receptors, such as IL-2 and the IL-2 receptor light chain (Tac antigen) in Jurkat cells (20). However, only a few ATL clones examined expressed IL-2, indicating that lymphokine gene expression is not solely regulated by the viral genome, which is in agreement with recent findings that multiple protein factors are involved in the regulation of a single gene. Therefore, it is likely that lymphokine production profiles of ATL cell lines represent more or less their physiological phenotypes, which were fixed probably by the viral integration.

Studies on mRNA expression for the seven lymphokines in 19 human ATL cell clones did not allow us to classify human T cells on the basis of combination of lymphokines produced. Especially, the four clonally related leukemic lines produced

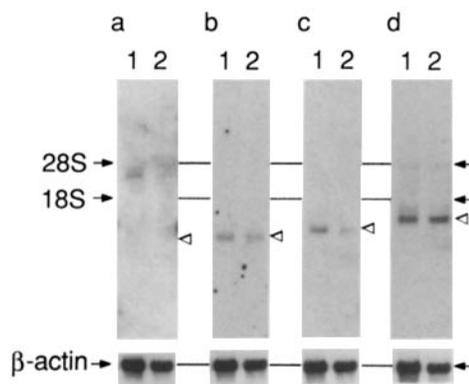


FIGURE 2. Lymphokine expression in ED40810 cells stimulated by PHA-P and TPA. Experiments were done as described in the legend to Fig. 1. cDNA probes used were: (a) IL-2; (b) IL-4; (c) IL-5; and (d) IL-6. mRNAs from stimulated and unstimulated cells were run in lanes 1 and 2, respectively. Positions of mRNAs estimated from positions of 28S and 18S ribosomal RNA are shown as described in the legend to Fig. 1.

different combinations of lymphokines. These results contradict the concept that each mature T cell clone has a fixed profile of lymphokine production, and further imply that the combination of lymphokines produced may not serve as appropriate criteria for classification of T cell subsets (4, 5). It is likely that the profile of lymphokine production including Th1 and Th2 phenotypes may not be a fixed stable phenotype of each T cell, but variable, given different stimuli probably through different receptors.

During preparation of this manuscript, Paliard et al. (21) reported studies on lymphokine production profiles of human T cell clones. They found that IL-2 and IL-4 could be coexpressed by some T cell clones and concluded that the classification proposed by Mosmann and his colleagues (4, 5) is not valid for human T cells.

Summary

We have analyzed expression patterns of 7 lymphokine mRNAs by Northern blot analyses in 19 different human T cell clones derived from patients with adult T cell leukemia. However, we were not able to reveal particular combinations of lymphokine production that allowed classification of human T cells. Especially, four clonally related leukemic lines that were established independently from the same patient with adult T cell leukemia expressed different combinations of lymphokine mRNAs, indicating that the expression of various lymphokines is not fixed but rather variable even among progenies of a single T cell clone.

Received for publication 10 January 1989 and in revised form 9 February 1989.

References

1. Noma, Y., P. Sideras, T. Naito, S. Bergstedt-Lindquist, C. Azuma, E. Severinson, T. Tanabe, T. Kinashi, F. Matsuda, Y. Yaoita, and T. Honjo. 1986. Cloning of cDNA encoding the murine IgG₁ induction factor by a novel strategy using SP6 promoter. *Nature (Lond.)* 319:640.
2. O'Garra, A., S. Umland, T. DeFrance, and J. Christiansen. 1988. 'B-cell factors' are pleiotropic. *Immunol. Today* 9:45.
3. Sideras, P., T. Noma, and T. Honjo. 1988. Structure and function of interleukin 4 and 5. *Immunol. Rev.* 102:189.
4. Mosmann, T. R., H. Cherwinski, M. W. Bond, M. A. Giedlin, and R. L. Coffman. 1986. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J. Immunol.* 136:2348.
5. Cherwinski, H. M., J. H. Schumacher, K. D. Brown, and T. R. Mosmann. 1987. Two types of mouse helper T cell clone. III. Further differences in lymphokine synthesis between Th1 and Th2 clones revealed by RNA hybridization, functionally monospecific bioassays, and monoclonal antibodies. *J. Exp. Med.* 166:1229.
6. Maeda, M., A. Shimizu, K. Ikuta, H. Okamoto, M. Kashihara, T. Uchiyama, T. Honjo, and J. Yodoi. 1985. Origin of human T-lymphotropic virus I-positive T cell lines in adult T cell leukemia. Analysis of T cell receptor gene rearrangement. *J. Exp. Med.* 162:2169.
7. Maeda, M., N. Arima, Y. Daitoku, M. Kashihara, H. Okamoto, T. Uchiyama, K. Shirono, M. Matsuoka, T. Hattori, K. Takatsuki, K. Ikuta, A. Shimizu, T. Honjo, and J. Yodoi. 1987. Evidence for the interleukin-2 dependent expansion of leukemic cells in adult T cell leukemia. *Blood* 70:1407.
8. Miyoshi, I., I. Kubonishi, M. Sumida, S. Hiraki, T. Tsubota, I. Kimura, K. Miyamoto, and J. Sato. 1980. A novel T-cell line derived from adult T-cell leukemia. *Gann* 71:155.

9. Chirgwin, J. M., A. E. Przybyla, R. J. MacDonald, and W. J. Rutter. 1979. *Biochemistry*. 18:5294.
10. McMaster, G. K., and G. G. Carmichael. 1977. Analysis of single- and double-stranded nucleic acids on polyacrylamide and agarose gels by using glyoxal and acridine orange. *Proc. Natl. Acad. Sci. USA*. 74:4835.
11. Noma, T., T. Nakamura, M. Maeda, M. Okada, Y. Taniguchi, Y. Tagaya, Y. Yaoita, J. Yodoi, and T. Honjo. 1986. Interleukin 1 α mRNA in virus-transformed T and B cells. *Biochem. Biophys. Res. Commun.* 139:353.
12. Auron, P. E., A. C. Webb, L. J. Rosenwasser, S. F. Mucci, A. Rich, S. M. Wolff, and C. A. Dinarello. 1984. Nucleotide sequence of human monocyte interleukin 1 precursor cDNA. *Proc. Natl. Acad. Sci. USA*. 81:7907.
13. Maeda, S., N. Nishino, K. Obaru, S. Mita, H. Nomiyama, K. Shimada, K. Fujimoto, T. Teranishi, T. Hirano, and K. Onoue. 1983. Cloning of interleukin 2 mRNAs from human tonsils. *Biochem. Biophys. Res. Commun.* 115:1040.
14. Yang, Y. C., A. B. Carletta, P. A. Temple, M. P. Chung, S. Kovacs, J. S. Witek-Gianotti, A. C. Leary, R. Kriz, R. E. Donahue, G. G. Wong, and S. C. Clark. 1986. Human IL-3 (multi-CSF); identification by expression cloning of a novel hemopoietic growth factor related to murine IL-3. *Cell*. 47:3.
15. Azuma, C., T. Tanabe, M. Konishi, T. Kinashi, T. Noma, F. Matsuda, Y. Yaoita, K. Takatsu, L. Hammarstrom, C. I. Edvard Smith, E. Severinson, and T. Honjo. 1986. Cloning of cDNA for human T-cell replacing factor (interleukin-5) and comparison with the murine homologue. *Nucleic Acids Res.* 14:9149.
16. Hirano, T., K. Yasukawa, H. Harada, T. Taga, Y. Watanabe, T. Matsuda, S. Kashiwamura, K. Nakajima, K. Koyama, A. Iwamatsu, S. Tsunasawa, F. Sakiyama, H. Masui, Y. Takahara, T. Taniguchi, and T. Kishimoto. 1986. Complementary DNA for a novel human interleukin (BSF-2) that induced B lymphocytes to produce immunoglobulin. *Nature (Lond.)*. 324:73.
17. Nakajima-Iijima, S., H. Hamada, P. Reddy, and T. Kakunaga. 1985. Molecular structure of the human cytoplasmic β -actin gene: Interspecies homology of sequences in the introns. *Proc. Natl. Acad. Sci. USA*. 82:6133.
18. Feinberg, A., and B. Vogelstein. 1984. Addendum to "A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity." *Anal. Biochem.* 137:266.
19. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. 545 pp.
20. Maruyama, M., H. Shibuya, H. Harada, M. Hatakeyama, M. Seiki, T. Fujita, J. Inoue, M. Yoshida, and T. Taniguchi. 1987. Evidence for aberrant activation of the interleukin-2 autocrine loop by HTLV-I encoded by p40x and T3/Ti complex triggering. *Cell*. 48:493.
21. Paliard, X., Waal Malefijt, H. Yssel, D. Blanchard, I. Chrétien, J. Abrams, J. de Vries, and H. Spits. 1988. Simultaneous production of IL-2, IL-4 and IFN- γ by activated human CD4⁺ and CD8⁺ T cell clones. *J. Immunol.* 141:849.