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INTERLEUKIN 1α mRNA IN VIRUS-TRANSFORMED T AND B CELLS

Takafumi Noma, Tomoyuki Nakamura*, Michiyuki Maeda**, Masafumi Okada*, Yoshihisa Taniguchi*, Yutaka Tagaya*, Yoshio Yaoita, Junji Yodoi*, and Tasuku Honjo[1]

Department of Medical Chemistry, Institute for Immunology*,
Kyoto University Faculty of Medicine,
Chest Disease Research Institute**, Kyoto University,
Sakyo-ku, Kyoto 606, Japan

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SUMMARY: IL-1α cDNA clone was isolated from a T cell line infected by the human T lymphocropic retrovirus type-I (HTLV-I/ATLV). We found significant amounts of mRNA hybridizing to IL-1α cDNA not only in HTLV-I-transformed T cells but also in Epstein-Barr Virus-transformed B cells. A part of IL-2 receptor inducing activity in Adult T cell leukemia (ATL) cell line seems to be due to IL-1α.

Interaction between interleukin-2 (IL-2) and the IL-2 receptor plays an essential role in antigen-stimulated proliferation of T lymphocytes (1). Temporal induction of the IL-2 receptor on T lymphocytes stimulated with antigens or mitogens (2) provides the molecular basis for positive selection of antigen-specific T lymphocyte clones. By contrast, abnormal regulation of IL-2 receptor expression might be associated with leukemogenesis of T lymphocytes which are infected with HTLV-I as an excessive number of IL-2 receptors are expressed constitutively on leukemic cells of ATL (3). Recently, we have found that culture supernatants of ATL cell lines contain the activity that induces the IL-2 receptor expression on a natural killer-like cell line, YTC3 (4) and an HTLV-I(+) T cell line, ED, which is dependent on IL-2 for its proliferation (5). We proposed a hypothesis that this activity might be involved in the constitutive expression of the IL-2 receptor on ATL cells in an autocrine manner. The putative factor for this activity was tentatively named ATL-derived factor (ADF), which had no IL-2 activity (6).

[1]Address correspondence to Dr. Tasuku Honjo, Department of Medical Chemistry, Kyoto University Faculty of Medicine.

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Another lymphokine, interleukin-1 (IL-1) is a cytokine known to have a wide range of biological activities (7), including co-mitogenicity on the thymocyte proliferation, stimulation of B-lymphocyte maturation and proliferation, fibroblast growth factor activity and induction of acute-phase protein synthesis. Recent molecular cloning of cDNA encoding IL-1α and IL-1β (8-10) has lead us to ask an obvious question whether IL-1 is different from ADF although IL-1 is shown to be produced by activated macrophages and monocytes (11).

Here we report that IL-1α mRNA was produced not only in many human T cell lines transformed by HTLV-I but also in some of human B cell lines transformed by Epstein-Barr virus (EBV). Comparison of biological activities of ADF and recombinant IL-1α indicates that at least a part, if not all, of the crude ADF activity assayed by YTC3 cells is due to IL-1α.

MATERIALS AND METHODS

Enzymes and reagents. Restriction endonucleases were purchased from Takara-Shuzo, Ltd., T4 DNA ligase was from Takara-Shuzo, Ltd. and *E.coli* ligase, RNaseH, DNA polymerase I and terminal transferase were from PL-Biochemicals. Reverse transcriptase was from Midwest Bio-Products, Inc. M13 sequence kit was from Takara-Shuzo, Ltd. α-[^{32}P]-dCTP (3000 Ci/mmole) was purchased from Amersham. Nitrocellulose filters (pore size 0.45 μm) for Southern and Northern blots were purchased from Schleicher & Schüel and those for cDNA library screening from Toyo-Roshi, Ltd. All the other reagents used were analytical grade.

Cells. ATL derived cell lines, ATL-2, ATL-2 (D), ED, ED (D), and ATL-6 (D), were established as described (6, 12). In brief, leukemic cells obtained from ATL patients were cultured in the presence IL-2. IL-2 dependent T cell lines indicated by (D) after notation were established from the culture and remained dependent on IL-2 for more than 8 months. Occasionally, IL-2 independent subclones were obtained, which had the same rearrangement profiles of the T cell receptor gene with parental IL-2 dependent lines (12). Origins of other cells are as described in literature; MOLT4 (6), HL-60 (13), CESS (14), FLEB-14 (15), YTC3 (4), MT-1 (16), and RPMI-8866 (6). Cells were cultured in RPMI-1640 medium with 10% fetal calf serum (FCS) in humidified atomosphere of 5% CO_2 in air at 37°C. Recombinant IL-2 produced by *E.coli* was kindly provided by Takeda Chemical Co. Human monocyte-like cell line, HL-60, was differentiated with phorbol-12-myristate-13-acetate (PMA) (500ng/ml) and retinoic acid (500ng/ml), and then IL-1 mRNA was induced by the addition of LPS(10μg/ml), PMA (500ng/ml) and cycloheximide (1μg/ml).

Cloning of IL-1α cDNA. cDNA library was constructed from 3μg of poly(A)+ RNA of ATL-2 cells using pCD vector according to Okayama and Berg (17). *E.coli* HB101 transformants (2 x 10^5 clones) were obtained and the plasmid DNA prepared from the transformed HB101 was digested with *Sal*I endonuclease and size-fractionated to collect the 4- to 6-kilobase pair (kb) recombinant plasmid DNA. The fractionated

DNA was recyclized with T4 DNA ligase and used to transform HB101. ATL-2 cDNA library containing about 20,000 independent transformants was obtained. Two regions (Ser^{113}-Val^{122}, Thr^{262}-Ala^{271}) of the published IL-1α cDNA sequence (10) were synthesized and used as probe for screening the ATL-2 cDNA library as described (18).

Other Methods. Poly(A)+ RNAs were prepared from various cell lines as described (18). Nucleotide sequences were determined by dideoxy method using plasmid PUC18 and PUC19 as vectors (19). DNA transfection was performed by the calcium phosphate precipitation method as described (18). IL-2 receptors on YTC3 cells were stained with fluoresceinated (FITC) anti-Tac or with combination of anti-Tac and FITC-goat anti-mouse-Ig antibody, and the fluorescence intensity of the stained cells was analyzed by flow cytometry using Ortho Spectrum III (Ortho Pharmaceutical Co., NJ). ADF activity was determined as described (5). In brief, a unit of ADF activity (IL-2 receptor inducing activity) was defined as the amount required for a half maximal induction of the Tac antigen on YTC3 cells. IL-2 receptor-inducing activities in various culture supernatants were titrated using YTC3 cells and units of samples were determined. Southern blot hybridization was carried out as follows. High molecular weight DNA extracted from each cell line and peripheral blood lymphocytes of ATL patients were digested with restriction enzymes, and the digests were electrophoresed in 0.5% agarose gels. The gels were blotted to nitrocellulose filters. Hybridization (15hr, 65°C) with ^{32}P-labelled probes were carried out as described (18). Filters were washed with 0.015M NaCl-0.0015M Na citrate with 0.1% sodium dodecyl sulfate. Northern blot hybridization was carried out as described (18). The filter was hybridized with *Hin*dIII - *Hin*cII (661bp) fragment of IL-1α cDNA as probe. DNA probe was labelled as described (18).

RESULTS and DISCUSSION

We have constructed a library of cDNA complementary to mRNA of ATL-2 cells, culture supernatants of which contain the strong ADF activity. The library was screened by two known oligonucleotides of the IL-1α cDNA sequence. Two hybridizing clones with inserts of 1,800 base pair (bp) and 1,600bp were isolated, restriction maps of which overlapped with each other. Partial nucleotide sequences (655 bp in total) of the longer clone matched completely with the published sequence of IL-1α cDNA (9, 10) except that the longer clone contained an extra adenine base at the 5' end and ended at 19 bases after the first poly(A) addition signal (10).

Using the IL-1α cDNA as probe we have tested the presence of IL-1α mRNA in various T and B cells. As shown in Fig. 1, there was a significant amount of mRNA hybridizing to IL-1α cDNA in a T cell line ATL-2 and a B cell line CESS. The amounts of IL-1α mRNA in these cells are less than but comparable to that in activated HL-60, a macrophage cell line (13). Less but significant amounts of IL-1α

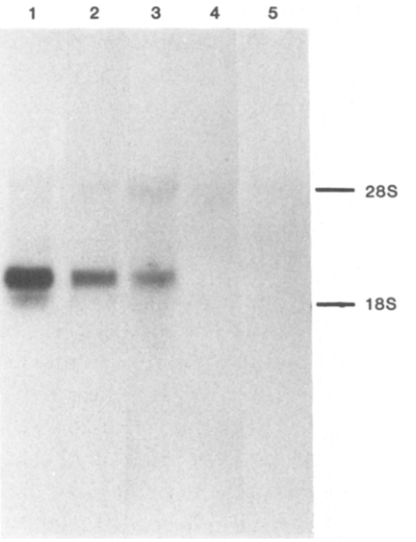

Figure 1. Northern blot analysis of IL-1α mRNA. 5μg each of poly(A)+ RNA isolated from induced HL60 cells (lane 1), ATL-2(lane 2), CESS (lane 3), MOLT-4 (lane 4), and RPMI-8866 (lane 5) were hybridized with IL-1α cDNA probe (*Hind*III - *Hinc*II 661bp) labelled as described (18).

mRNA were found in other T cells transformed by HTLV-I; ATL-2(D), ED (D), ATL-6, and ED as shown in Table I. MT-1, a HTLV-I-infected T cell did not contain a detectable amount of IL-1α mRNA. A T cell line MOLT-4, which was not infected with HTLV-I, did not contain a detectable amount of IL-1α mRNA. Two B cell lines transformed with Epstein-Barr virus contained significant amounts of IL-1α mRNA in agreement with a previous report that the IL-1 activity was found in EB virus-transformed cell line (20).

Relative amounts of IL-1α mRNA were estimated by densitometry tracing of autoradiograms with those of β-actin mRNA as reference. The amounts of IL-1α mRNA in T and B cells were compared with ADF activities of their culture supernatants as assayed by the IL-2 receptor induction on YTC3 cells (Table I). Cells producing ADF always contained IL-1α mRNA. However, the relative abundance of IL-1α mRNA is not directly related with the ADF activity, especially in FLEB-14 and CESS. IL-1β mRNA was not detected by hybridization using cDNA probe (data not shown).

Table I

Comparison between the amounts of IL-1α mRNA and ADF activities of various cell lines

cell lines	cell types	virus genomes	IL-1α mRNA*	ADF activity† (U/10^5 cells)	ADF/IL-1α
HL60	macrophage	none	35.4		
ATL-2	T cell	HTLV-I	5.82	25	4.3
ATL-2(D)	T cell	HTLV-I	1	5.8	5.8
ED(D)	T cell	HTLV-I	0.34	7.1	20.9
ATL-6	T cell	HTLV-I	0.24	5.2	21.7
ED	T cell	HTLV-I	0.18	N.D.	
MT-1	T cell	HTLV-I	N.D.	N.D.	
MOLT-4	T cell	none	N.D.	N.D.	
FLEB-14	B cell	EBV	2.8	3.7	1.3
CESS	B cell	EBV	2.3	0.7	0.3
RPMI-8866	B cell	none	N.D.	N.D.	

*Relative amounts of IL-1α mRNA were estimated by densitometry tracing with β-actin mRNA as reference. Exon 4 of β-actin gene was used as probe. The amount of IL-1α mRNA in ATL-2(D) was taken as a unit.

†ADF activities of culture supernatants were assayed by the IL-2 receptor induction on YTC3 cells (5). The supernatant of HL60 was not assayed since phorbol-12-myristate-13-acetate (PMA) used for induction of IL-1 induced the IL-2 receptor on YTC3 cells. N.D., not detectable.

We then tested whether recombinant IL-1α synthesized by the direction of the cloned IL-1α cDNA has the activity to induce expression of the IL-2 receptor on YTC3 cells. Since the plasmid vector used for cloning IL-1α cDNA contains the simian virus 40 (SV40) early promoter, the cDNA clone was directly introduced into COS7 cells by DNA transfection. The addition of culture supernatants of transfected COS7 cells to YTC3 cell culture (5%) increased the percentage of the IL-2 receptor-positive cells from 7% to 51% as shown in Table II. Supernatant of ATL-2 showed comparable activities for induction of the IL-2 receptor. IL-1α cDNA was recloned in the pSP65 vector (21) and IL-1α mRNA was synthesized *in vitro*. The IL-2 receptor induction on YTC3 cells was also demonstrated by IL-1α produced in *Xenopus* oocytes injected with IL-Iα mRNA synthesized *in vitro* (data not shown).

A shorter cDNA, which has an extra out-of frame ATG codon 5' to the in-frame ATG codon, showed the IL-2 receptor-inducing activity though much weaker when

Table II

IL-2 receptor inducing activity of recombinant IL-1α

origins of supernatants	supernatants added (%)	IL-2 receptor positive YTC3 cells† (%)
COS 7 transfected with IL-1α cDNA*	5	51
	50	61
COS7	5	7
	50	14
ATL-2	25	72

*The cloned IL-1α cDNA was introduced into COS7 cells by DNA transfection (18).
†ADF activity was assayed by the addition of culture supernatants of cells indicated to YTC3 cell culture.

intruduced into COS7 cells. The 5' ATG frame has a termination codon about six codons 3' to the in-frame ATG, which may support reinitiation at the 3' in-frame ATG.

We have shown that not only macrophages but also virus-transformed T and B cells can produce IL-1α although we do not know whether IL-1α expression in virus-transformed cells is associated with transformation *per se*. We have tested the possibility that the retrovirus genome integrated in the proximity of the IL-1α gene, resulting in activation of this gene. No rearrangement of the IL-1α gene was found by Southern blot analysis of DNA of ATL cell lines and leukemic cells, which were digested with *Hind*III, *Sac* I, *Eco*RI, and *Bam*HI (data not shown). It is likely that some trans-acting mechanisms are involved in activation of the IL-1α gene in many cell lines transformed with HTLV-I or EBV. The product of the pX gene of HTLV-I could be a candidate for the trans-actining molecule as suggested by several investigators (22, 23).

The results indicate that at least a portion of the crude ADF activity in ATL cell lines is due to IL-1α. However, not all the ADF activities are explained by IL-1α. In fact, recombinant IL-1α had little IL-2 receptor-inducing activity on ED(D) whereas crude ADF showed a significant activity on ED (D) (Okada, M., *et al.*, submitted). Furthermore, ADF induced the IL-2 receptor in leukemic cells of a patient with

myeloid leukemia but recombinant IL-1α and macrophage derived IL-1α did not (24). The results suggest that the crude ADF preparation might contain an additional factor which has a broader specificity. Alternatively, the crude ADF preparation may contain several derivatives of IL-1α with different target specificities, which were produced by differential processing of IL-1α precursors, as suggested by Oppeheim et al (7). Further purification of ADF protein is required to clarify these questions.

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