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Cloning of cDNA for human T-cell replacing factor (interleukin-5) and comparison with the murine homologue

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ABSTRACT

We have cloned cDNA for T-cell replacing factor (interleukin-5), which replaces T-cell helper function for normal B cells which secrete immunoglobulin, from human T cell leukemia line, ATL-2, using mouse interleukin-5 cDNA as probe. Total nucleotide sequence of the cDNA (816 base pairs) was determined and compared with that of mouse interleukin-5 cDNA. The cloned cDNA encoded the interleukin-5 precursor of 134 amino acids containing an N-terminal signal sequence. Although the human interleukin-5 precursor is one amino acid longer than the murine homologue, the sizes of the mature proteins appear similar. The nucleotide and amino acid sequence homologies of the coding regions of human and murine interleukin-5 are 77% and 70%, respectively. Human interleukin-5 synthesized by the direction of the cloned cDNA induced immunoglobulin synthesis in human B cells stimulated by Staphylococcus aureus mitogen.

INTRODUCTION

Soluble factors have been shown to be involved in maturation and proliferation of B lymphocytes (1, 2). Recent molecular cloning and characterization of cDNAs encoding IgG_1 induction factor (3, 4) and T-cell replacing factor (5) have clearly demonstrated that at least two distinct protein molecules secreted from murine T-cell lines exert different activities regulating maturation and differentiation of B cells. These studies have also elucidated the fact that several different biological activities are ascribed to a single protein molecule (3-5).

IgG_1 induction factor (6) which induces an elevated IgG_1 synthesis in B cells activated by lipopolysaccharide is identical to B-cell stimulatory factor (7) which stimulates DNA synthesis in anti-IgM-stimulated B cells. Since this lymphokine was also shown to stimulate proliferation of mast cells and T cells (3, 4, 8), the name of interleukin (IL)-4 was proposed for this molecule. T-cell replacing factor (9, 10), which induces IgM secretion from BCL_1 leukemic B-cell line and secondary IgG synthesis in antigen-primed B cells, was shown to be identical to B-cell growth factor II (5). T-cell

replacing factor may also have the eosinophil differentiation factor activity (11). We therefore proposed to call this lymphokine IL-5 (5).

In spite of extensive studies on human factors involved in B-cell proliferation and maturation (12) none of their complete structures have been elucidated. It was not clear whether murine IL-4 and IL-5 have human counterparts which have homologous structures and similar activities. We report here isolation and characterization of cDNA encoding human IL-5, which is 70% homologous to murine IL-5 and active in induction of IgM synthesis in stimulated human B cells.

EXPERIMENTAL PROCEDURES

Cells

ATL-2 cells, a human T-cell line derived from adult T cell leukemia patients (13), were cultured in RPMI1640 medium with 10% fetal calf serum in a humidified atomosphere of 5% CO_2 at 37°C. Human B cell-enriched fractions were obtained from heparinized venous blood of healthy adult volunteers and from human spleen cells as described (14).

Enzymes and other materials

Restriction endonucleases were purchased from Takara-Shuzo (Kyoto), and Toyobo (Osaka) and used according to manifactures' recommendation. T4 ligase was from Takara-Shuzo, and E. coli ligase, RNase H, DNA polymerase I and terminal transferase were from Pharmacia (Uppsala). Reverse transcriptase was from Midwest Bioproducts, Inc. (Covinton). pSP65 plasmid and SP6 RNA polymerase were from Pharmacia. M13 sequence kit was from Amersham or Takara-Shuzo. Staphylococcus aureus, Cowan I (SAC I) was purchased from Wako Chemicals (Osaka).

α-^{32}P-dCTP (spec. act. 3,000 Ci/mmole) was purchased from Amersham or New England Nuclear (Boston). IgM quantitation test kits (EIA) using ß-D-galactosidase-coupled antibody were purchased from Medical & Biological Laboratories Co. (Nagoya). Nitrocellulose filters (pore size 0.45 µm) for Southern and Northern blots were purchased from Schleicher & Schüel (Dassel) and those for cDNA library screening from Toyo Roshi (Tokyo).

Methods

High molecular weight DNA was extracted from AKR mouse liver or normal human placenta as described (15). Crude total cellular RNA was extracted from ATL-2 cells, and poly(A)$^+$ RNA was purified by oligo(dT)-cellulose column chromatography as described (16). cDNA library containing 2 X 10^5 independent cDNA clones was constructed from 3 µg poly(A)$^+$ RNA from ATL-2 cells using pCD expression vector according to Okayama and Berg (17) and

screened by the method of Hanahan and Meselson (18). Southern and Northern blot transfer and hybridization were done as described (19, 20), using as probe DNA fragments labeled with ^{32}P by nick-translation. Nucleotide sequences were determined by dideoxy method using pUC18 vector as described (21). In vitro transcription of human IL-5 cDNA was carried out as described (22, 23). The culture supernatants of Xenopus oocytes injected with IL-5 mRNA synthesized in vitro were used as human recombinant IL-5. Stimulation of IgM synthesis of human B cells was tested as described (14). IgM secreted into culture supernatants from human B cells was measured by the enzyme immuno-assay. IgM-secreting cells were measured as described (6).

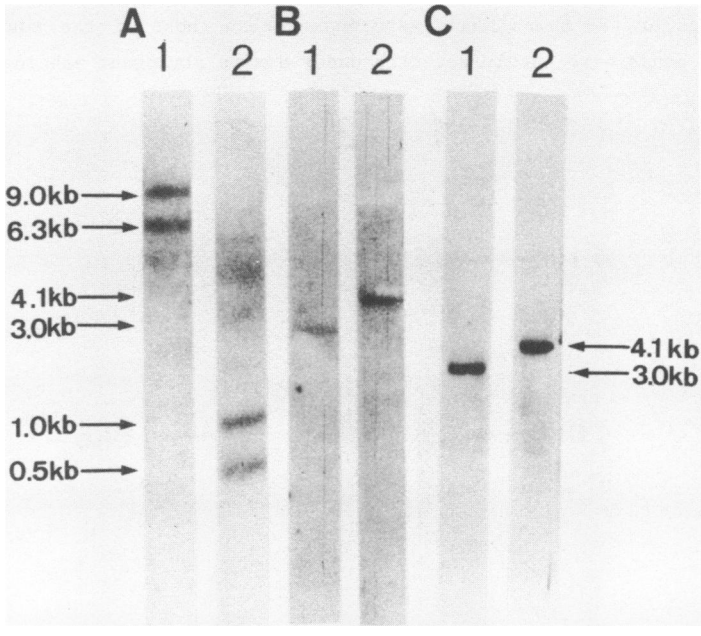

Fig. 1. Detection of the human IL-5 sequence using mouse IL-5 cDNA as probe. Two µg of AKR mouse liver DNA (A) and human placenta (B and C) were digested with PstI (lane 1) or PvuII (lane 2) and electrophoresed in a 0.6% agarose gel. After transfer to nitrocellulose filters, filters were hybridized to 32P-labeled BamHI-AccI fragment (654 bp) of mouse IL-5 cDNA pSP6K-mTRF23 (A and B) or to 32P-labeled PstI fragment (515 bp) of ph·IL-5-30 described in Fig. 3 (C). Washing conditions were as follows; in 0.1 X SSC (SSC is 0.15 M NaCl-0.015 M Na citrate)-0.1% Na dodecyl sulfate (SDS) at 65°C for 45 min for (A) and (C); in 2 X SSC-0.1% SDS at 50°C for 45 min for (B). Exposure of autoradiogram was carried out for one day in (A) and (C), and for 4 days in (B).

RESULTS AND DISCUSSION

Detection of the human counterpart to the mouse IL-5 gene

In order to test whether human genomic DNA contains sequences homologous to the mouse IL-5 gene, Southern blot hybridization of human DNA was carried out using murine IL-5 cDNA as probe. To remove slightly repetitive sequences of the mouse IL-5 cDNA mTRF23 (5), we isolated the BamHI-AccI fragment (654 bp) which contained the entire coding region and a small portion of the 3' untranslated region. Mouse DNA digested with either PstI or PvuII contained two fragments hybridizing with the murine IL-5 cDNA probe as shown in Fig. 1A.

By contrast, this probe detected the 3.0-kb and 4.1-kb bands in human placenta DNA digested with PstI and PvuII, respectively, under a relatively mild washing condition (in 2 X SSC at 50°C for 45 min) (Fig. 1B). The intensities of the human bands were weaker than those of the mouse bands. The human bands were hardly detected under a more stringent washing condi-

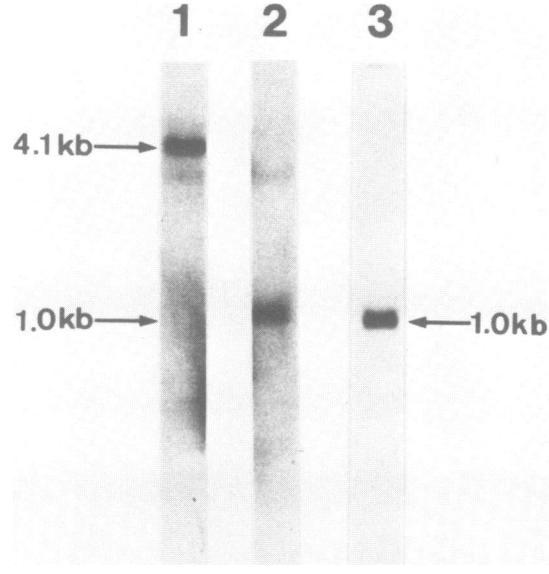

Fig. 2. Detection of the human IL-5 cDNA clones and mRNA using mouse IL-5 cDNA as probe. Two µg of the total library DNA of ATL-2 poly(A)$^+$ RNA was digested with SalI (lane 1) or BamHI (lane 2), electrophoresed in a 0.6% agarose gel, and transferred to a nitrocellulose filter. The filter was hybridized to the 32P-labeled BamHI-AccI fragment of pSP6K-mTRF23. Washing condition was in 2 X SSC-0.1% SDS at 50°C for 45 min. In lane 3, 5 µg of ATL-2 poly(A)$^+$ RNA was electrophoresed in a 1% agarose gel and blotted to a nitrocellulose filter. The filter was hybridized with 32P-labeled PstI fragment of ph·IL-5-30 (Fig. 3).

tion (in 0.1 X SSC at 65°C for 45 min) (data not shown), indicating that the mouse IL-5 and human counterpart genes are significantly similar but obviously not exactly homologous. The results also indicate that the murine IL-5 probe is useful for isolation of the human homologue.

Cloning of cDNA for human IL-5

We tested whether any candidates for human IL-5 cDNA clones were present in the cDNA library of a human T-cell line. This cDNA library was constructed previously from ATL-2 poly(A)$^+$ RNA using pCD expression vector and contained 2 X 10^5 independent cDNA clones. For this purpose, the murine IL-5 cDNA probe was hybridized with the total mixture of cDNA clones digested with SalI which gave one cut to the vector sequence or with BamHI which cleaved the vector at proximities of both ends of the inserted cDNA. As shown in Fig. 2, a discrete band of cDNA clone containing about 1-kb insert was detected as the plasmid vector is about 3.1 kb long. The results indicate that this library contained the human counterpart to the mouse IL-5 cDNA.

After screening about 5 X 10^4 clones, we obtained 29 clones hybridizing to mouse IL-5 cDNA probe. All the clones isolated were indistinguishable from each other on the basis of the endonuclease cleavage map and Southern hybridization analyses of digested fragments. A clone designated ph·IL-5-30 was chosen arbitrarily and characterized further. The restriction endonuclease cleavage map of the insert was constructed by conventional procedure and summarized in Fig. 3. The nucleotide sequence of ph·IL-5-30 was determined according to the strategy shown.

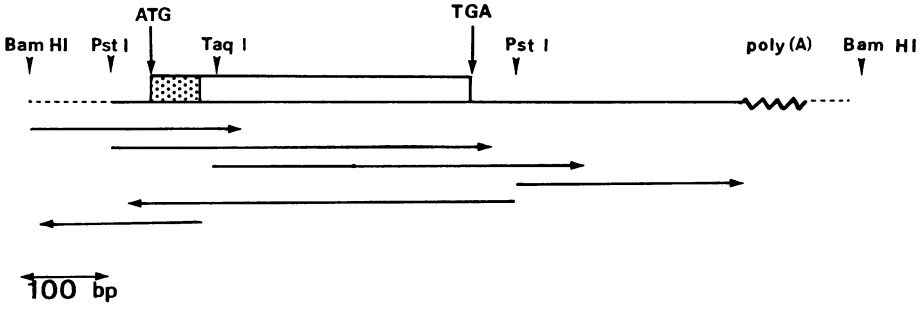

Fig. 3. Restriction map and sequencing strategy of human IL-5 cDNA clone ph·IL-5-30. The structure of human IL-5 cDNA and restriction enzyme cleavages site are shown at the top. Solid and broken lines, and rectangles show the untranslated, vector and protein coding sequences, respectively. A dotted rectangle indicates a putative signal peptide. Ranges and directions of sequences read are shown below by arrows.

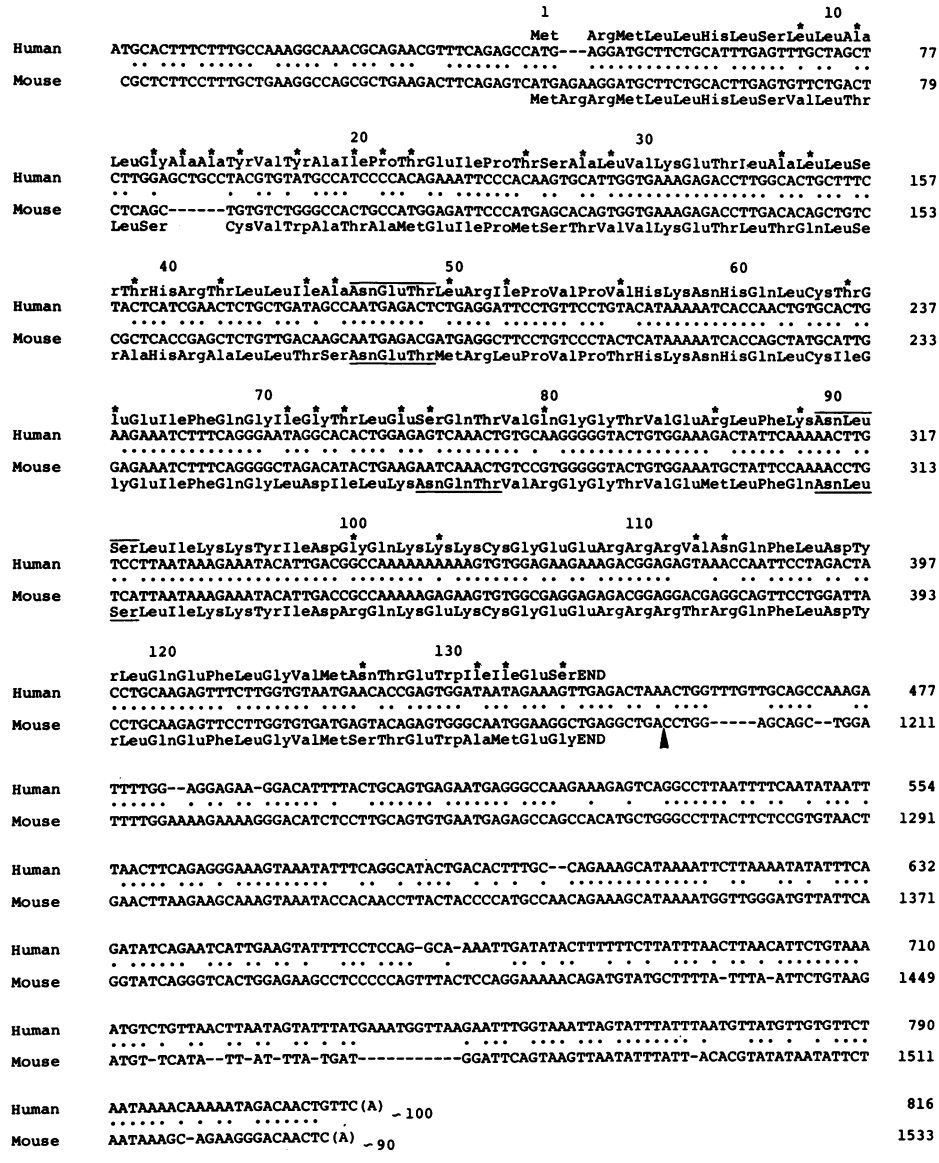

Fig. 4. Nucleotide and predicted amino acid sequences of human IL-5 and their comparison with murine IL-5 sequences. Numbers above amino acid residues indicate positions of the human amino acid sequence. Nucleotide positions are shown at right. Matched nucleotides are shown by (•). Mismatched amino acids are shown by asterisks above the human residues. Possible N-glycosylation sites are shown by horizontal lines. Deletions are shown by hyphens. An arrow head in the mouse sequence indicates the insertion of the 744-bp, sequence, which is not shown.

Structure of human IL-5 and comparison with murine IL-5

The total nucleotide sequence of the ph•IL-5-30 insert and the deduced amino acid sequence are shown in Fig. 4. The cDNA insert is 816 base-pair long excluding the poly (A) tail flanking 19 bp 3' to a typical poly (A) addition sequence (AATAAA) (30). Comparison of the human and murine IL-5 sequences allowed us to identify the coding region of human IL-5. The precursor of human IL-5 has 134 amino acid residues including a putative signal sequence of the N-terminal 22 hydrophobic amino acids. The molecular weight of the secreted core polypeptide of human IL-5 would be 12,840 assuming that the N-terminal 22 residues were cleaved off. Human IL-5 precursor is one amino acid longer than mouse IL-5 precursor. Since the difference is located in the N-terminal hydrophobic region, the length of the secreted core polypeptides of man and mouse IL-5 may be identical.

Human IL-5 contains two possible N-glycosylation sites (Asn-X-Thr/Ser) at residues 47-49 and 90-92, which correspond to the first and the third N-glycosylation sites of murine IL-5. The second N-glycosylation site of murine IL-5 is not conserved. Of three cysteine residues in murine IL-5, the C-terminal two were conserved in human IL-5. The nucleotide and amino acid sequences of the coding regions of the human and murine IL-5 cDNA are 77% and 70% homologous, respectively. In contrast, the 3' untranslated regions of two IL-5 are highly diverged in sequence as well as in length. The human sequence has a large deletion of 744 bp in the 3' untranslated region. The homology of human and murine IL-5 is more significant in the C-terminal half (80% homology) than in the N-terminal half (66% homology) of the putative mature peptide.

The cloned cDNA for human IL-5 hybridized with the same PstI and PvuII fragments of human DNA as had previously been detected by mouse IL-5 cDNA (Fig. 1C). Northern blot hybridization of ATL-2 poly(A)$^+$ RNA revealed a single 1-kb band hybridizing to the cloned human IL-5 cDNA (Fig. 2 lane 3). These results indicate that the cloned human IL-5 cDNA is derived from a unique human counterpart to the mouse IL-5 gene and that the single species of mRNA is transcribed from the human IL-5 gene. Since lengths of poly(A) are variable it is reasonable that the IL-5 cDNA of about 920 bases is close to the full length cDNA, although a small portion of the 5' untranslated region might be missed. It is, however, unlikely that the coding sequence extends further upstream from the comparison with the murine IL-5 cDNA.

Human IL-5 induces IgM secretion from normal B cell stimulated with SAC I

To test the biological activity of ph•IL-5-30, its BamHI fragment con-

taining the whole cDNA insert and a small portion of the vector DNA (Fig. 3) was recloned in pSP65 vector. mRNA was synthesized in vitro and injected into Xenopus oocytes. The oocytes were incubated for 48 hrs and supernatants were separated, concentrated and used as human recombinant IL-5.

The biological activity of human IL-5 was tested by measuring the amount of IgM in culture supernatants and the number of IgM-secreting cells of human B cells stimulated with B cell mitogen, SAC I. Human B cells purified from peripheral blood mononuclear cells and spleen cells were cultured with SAC I in the presence or absence of human recombinant IL-5. Both the amounts of IgM in culture supernatants and the numbers of IgM-secreting cells of human B cells increased 2-3 folds by the addition of recombinant IL-5 (Table 1). Addition of IL-2 augmented the effect of IL-5 as had been

Table 1. Human IL-5 stimulates IgM synthesis.

	Lymphokines added		IgM (ng/well)	IgM-secreting cells (cells/well)
	rIL-5	IL-2		
Expt. I	0	0	66.2	--
	0	50 U/ml	43.6	--
	5%	0	88.8	--
	15%	0	108.2	--
	15%	50 U/ml	131.3	--
Expt. II	0	0	--	62
	1.5%	0	--	129
	3%	0	--	186
	6%	0	--	221

The BamHI fragment of ph•IL-5-30 (Fig. 3) was recloned in pSP65. The plasmid was cleaved by HindIII and used for template of SP6 RNA polymerase. The culture supernatant of Xenopus oocytes injected with human IL-5 mRNA which was obtained by in vitro transcription as described, was used as human recombinant IL-5 (rIL-5) after 4-fold concentration by Centricon 10 (Amicon). Human B cells were stimulated with SAC I (0.0025%). Human recombinant IL-2 and rIL-5 were added where indicated. In experiment I, B cells (1×10^5/100 µl) from peripheral blood were cultured for 6 days and amounts of IgM in culture supernatants were measured by the enzyme immunoassay. In experiment II, B cells (5×10^4/200 µl) from spleen cells were cultured for 3 days and numbers of IgM-secreting cells were measured.

shown in the murine T-cell replacing factor (IL-5) (24, 25). A relatively weak activity of human IL-5 as compared with murine IL-5 might be partially due to inefficient translation of the transcripts of the BamHI fragment which contains the vector sequence at the 5' flanking region.

Human factors showing the similar activity were studied previously and called B-cell differentiation factor (12, 26-28) or T-cell replacing factor (14). However, the N-terminal 13 residues (29) determined for a B-cell differentiation factor is not found in the sequence deduced by the present study. It is possible that there is another lymphokine affecting human B cells. It remains to be seen whether human IL-5 has a variety of biological activities including proliferation stimulation of B cells as shown for murine IL-5 (5).

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