*Proc. Natl. Acad. Sci. USA*
Vol. 86, pp. 302–306, January 1989
Immunology

# Human interleukin 7: Molecular cloning and growth factor activity on human and murine B-lineage cells

(cDNA sequence/pre-B cells)

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*Communicated by Lewis H. Sarett, October 20, 1988*

ABSTRACT **A cDNA encoding biologically active human interleukin 7 was isolated by hybridization with the homologous murine clone. Nucleotide sequence analysis indicated that this cDNA was capable of encoding a protein of 177 amino acids with a signal sequence of 25 amino acids and a calculated mass of 17.4 kDa for the mature protein. Recombinant human interleukin 7 stimulated the proliferation of murine pre-B cells and was active on cells harvested from human bone marrow that are enriched for B-lineage progenitor cells. Analysis of RNA by blot hybridization demonstrated the presence of two size classes of interleukin 7 mRNA in human splenic and thymic tissue.**

Study of immunoglobulin gene rearrangement and expression, as well as expression of specific cell-surface antigens, has led to the definition of the various developmental stages in the formation of mature functional B lymphocytes (1–4). The earliest identified cells committed to the B lineage are pro-B (or pre-pre-B) cells, which contain the immunoglobulin heavy- and light-chain genes in the germ-line configuration (5). These cells do not express the B-lineage antigen B220 and express only low levels of the T-lineage antigen Thy-1. Pro-B cells differentiate into $B220^+$ pre-B cells, which subsequently rearrange and express cytoplasmically the immunoglobulin heavy-chain genes. Following rearrangement and expression of light-chain genes, these cells give rise to mature B cells, which express surface immunoglobulin.

A great deal of research has gone into the molecular characterization of the factors responsible for the growth and differentiation of various hematopoietic cells of the myeloid and lymphoid lineages. Factors that have been reported to affect the development of pre-B cells include interleukin 1, interleukin 3, interleukin 4, interferon-$\gamma$, and transforming growth factor type $\beta$ (reviewed in ref. 6). In addition, a humoral factor found in the serum of New Zealand Black (NZB) mice has been shown to enhance the maturation of B-cell precursors, and one or more factors found excreted from cyclic neutropenic patients are able to stimulate the formation of pre-B and B cells in cultures of human or mouse bone marrow (6). Finally, murine bone marrow stromal cells have been shown to support the growth of pre-B cells (7, 8). Recently, we described a factor derived from a stromal cell line that is capable of supporting the growth of pre-B cells *in vitro* in the absence of any stromal elements (9). We subsequently reported (10) the molecular cloning of a cDNA encoding this factor, which we termed interleukin 7 (IL-7). We were able to show that recombinant murine IL-7 was able to replace murine bone marrow stromal cells in supporting the extended growth of both pre-B cells and pro-B cells (10).

We have used the murine IL-7 cDNA to probe a cDNA library derived from a human hepatoma cell line and now report the isolation of a human clone, the sequence of which is highly homologous to murine IL-7.* When expressed in COS cells, the human cDNA directed the synthesis of a protein capable of supporting the proliferation of murine pre-B cells. In addition, we have used this recombinant protein to develop an assay for IL-7 by using human bone marrow cells enriched for B-lineage cells.

## MATERIALS AND METHODS

**Genomic and cDNA Library Construction and Screening.** A human genomic library (11) was screened (12) with nick-translated insert from the murine IL-7 cDNA 1046 (10). Filters were washed twice in 6× SSC (standard saline citrate; 1× SSC = 0.15 M NaCl/0.015 M sodium citrate) and 0.1% $NaDodSO_4$ at 63°C followed by autoradiography at −70°C. Double-stranded cDNA was synthesized (13) using $poly(A)^+$ RNA isolated from SK-HEP-1 cells. The cDNA was modified with *Eco*RI linkers, cloned into λgt10 (Stratagene), packaged *in vitro*, and used to infect *Escherichia coli* strain C600hfl$^-$ as described by Huynh *et al.* (14). Replicate nitrocellulose filters (Schleicher & Schuell) were screened (12) with oligonucleotides 5′ end-labeled using T4 polynucleotide kinase and [$\gamma$-$^{32}$P]ATP. Conditions used for hybridization were as described (15). After hybridization the filters were washed twice in 1× SSC/0.1% $NaDodSO_4$ at 55°C followed by autoradiography at −70°C. Sequencing was performed after subcloning into pGEMBL18 as described (16, 17).

**Isolation and Analysis of RNA.** Total RNA from SK-HEP-1 (ATCC HTB 52) cells was isolated by a Nonidet P-40 lysis protocol described by Pennica *et al.* (18). Total RNA from the human tissues was isolated by a guanidinium hydrochloride procedure (19). For blot analysis, 15 $\mu$g of $poly(A)^+$ RNA was fractionated on a 1.1% agarose-formaldehyde gel and blotted onto Hybond (Amersham) as recommended by the manufacturer. The blot was probed for IL-7 mRNA with an antisense $^{32}$P-labeled transcript prepared by SP-6 RNA polymerase transcription of pGEMBL containing human IL-7 cDNA 3. The blot was hybridized, washed, and exposed as described (19).

**Bioassays.** The murine IL-7 bioassay was performed as described earlier (9). A unit of activity is defined as the amount of IL-7 required to stimulate a half-maximal [$^3$H]thymidine incorporation in the respective assay system. The human bioassay was carried out with cells recovered from human bone marrow. Marrow obtained from the iliac crest of a normal healthy donor was layered over 30 ml of 60% isotonic Percoll and centrifuged at 500 × *g* for 20 min in a Sorvall RT6000 centrifuge. The interface was collected and washed with 5 vol of phosphate-buffered saline, and the cells

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Abbreviation: IL-7, interleukin 7.

*The sequence reported in this paper is being deposited in the EMBL/GenBank data base (accession no. J04156).

were pelleted by centrifugation. The cells were suspended in 10 ml of phosphate-buffered saline and layered onto a 35-ml preformed linear Percoll gradient (85–30%) and centrifuged at 500 × *g* for 20 min in a Sorvall RT6000 centrifuge. Two-milliliter fractions were collected and diluted with 8 ml of phosphate-buffered saline, and the cells were recovered by centrifugation. The B-lineage progenitor population, as defined by the binding of the B-lineage-restricted monoclonal antibody B4 (20), was found to sediment at a density of ≈1.045 g/ml. This fraction routinely contains 5–10% of the nucleated cells in the starting population and contains 40–50% B4-positive cells. The cells collected at this density of Percoll were washed twice in phosphate-buffered saline and resuspended to 5 × $10^5$ cells per ml in Iscove's medium containing 0.5% fetal bovine serum, penicillin (50 units/ml), streptomycin (50 μg/ml), and 5 × $10^{-5}$ M 2-mercaptoethanol. One hundred microliters of the cell suspension (5 × $10^4$ cells per well) was added to serial dilutions of the samples in microtiter trays and incubated for 96 hr in a humidified atmosphere as described earlier. The cells were pulsed with 2 μCi of [$^3$H]thymidine per well (1 Ci = 37 GBq) during the last 6 hr of incubation, harvested, and data were quantified as described (9).

## RESULTS

**Isolation of Human IL-7 Clones.** We began our search for a human IL-7 cDNA by screening a human genomic library (kindly provided by T. Maniatis) using nick-translated murine IL-7 cDNA as a probe. Approximately 400,000 plaques were screened, from which a single hybridizing clone (λM1) was isolated and restriction mapped. As diagrammed in Fig. 1*A*, blot analysis of this recombinant phage DNA indicated that hybridization of the murine probe occurred primarily within a 1.2-kilobase (kb) *Eco*RI fragment. After subcloning and sequencing of this fragment, a comparison of the human genomic sequence with that of the murine cDNA revealed the homology shown in Fig. 1*B*. The region of homology present in the human genomic clone corresponds to the 5′ noncoding region of the murine IL-7 cDNA and extends to the first 10 nucleotides of the coding region. At that point, the human genomic sequence diverges from that of the murine IL-7 cDNA because of the presence of an intron.

A source of human IL-7 mRNA from which we could isolate cDNA clones was identified by using $^{32}$P-labeled oligonucleotides complementary to the human IL-7 sequence (boxed in Fig. 1*B*) to probe blots of RNA from various human cell lines. Autoradiograms of these hybridized blots revealed that the human liver adenocarcinoma cell line SK-HEP-1 contained IL-7 transcripts (data not shown). An oligo(dT)-primed cDNA library was constructed in λgt10 utilizing polyadenylylated RNA derived from SK-HEP-1 cells, and it was screened with these $^{32}$P-labeled oligonucleotides. Approximately 150,000 plaques were screened yielding a single positive clone (cDNA 1), which was isolated and its insert was sequenced. This cDNA was then labeled by nick-translation and used to screen ≈$10^6$ additional λgt10 clones, resulting in the isolation of five additional IL-7 cDNAs. Fig. 2 shows a schematic diagram and the nucleotide sequence of the human IL-7 cDNA 3, which encodes a protein active in the pre-B-cell growth assay (see below) and the region spanned by three additional cDNA clones. The site of initiation of translation and the first amino acid of the mature protein were inferred by comparison with the murine IL-7 sequence. The human IL-7 cDNA 3 contains a 5′ untranslated region of 384 nucleotides and a 3′ noncoding region of 658 nucleotides, which is terminated by a string of adenine residues. This cDNA contains a coding region of 534 nucleotides capable of encoding a protein of 177 amino acids, including a signal sequence of 25 amino acids. As shown in Fig. 2*B*, the deduced protein sequence contains three potential N-linked glycosylation sites. The predicted molecular mass of the unglycosylated mature protein is 17.4 kDa. By comparison, cDNA 1 lacks nucleotides 613–744 found in cDNA 3, thus deleting 44 amino acids found in cDNA 3 but maintaining the same reading frame. Isolation of additional genomic clones for human IL-7 revealed that this deletion was due to alternative splicing, resulting in the loss of an entire exon (data not shown). The significance or relative abundance of this alternative transcript is presently unknown. cDNA 4 also lacks sequences found in cDNA 3; in this case, nucleotides 799–802 are missing, which results in the alteration of the translational reading frame and the subsequent termination of translation four amino acids after this deletion. The sequence of cDNA 6 exactly matches that of cDNA 3 but begins at nucleotide 412 and thus is lacking all of the 5′ untranslated region and part of the coding sequence.

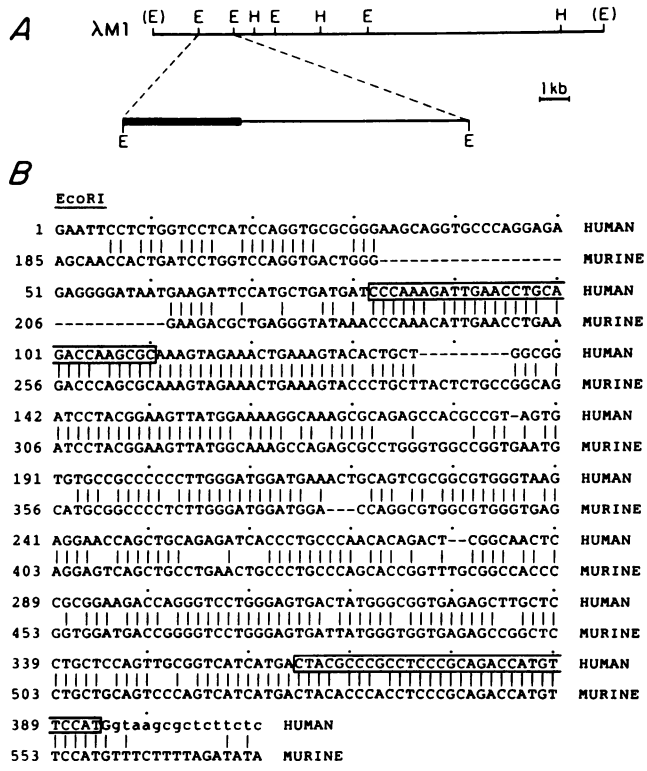
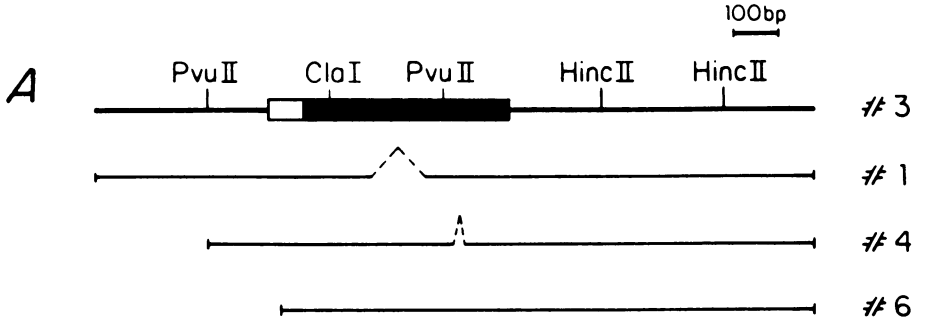



FIG. 1. Genomic clone of human IL-7. (*A*) Restriction map of the genomic clone designated λM1. Enlarged is the 1.2-kb *Eco*RI fragment that contained the majority of sequence hybridizable with the murine IL-7 cDNA. Heavy line, region of IL-7 exon sequences. E, *Eco*RI; H, *Hin*dIII; (E), artificial *Eco*RI sites introduced in the construction of the genomic library. (*B*) Partial sequence of the exon containing 1.2-kb *Eco*RI fragment and alignment of the human genomic and murine IL-7 cDNA sequences. Vertical lines, identical nucleotides; horizontal lines, gaps introduced to provide maximum alignment, determined by using a program designed by the University of Wisconsin Genetics Computer Group (21). Boxed nucleotides indicate the sequences from which complementary oligonucleotides were synthesized to use as probes in screening Northern blots and cDNA libraries. The lowercase nucleotides at the end of the human sequence represent intron sequence.

Both cDNAs 1 and 3 contain *Eco*RI sites at their 5′ termini that are not derived from the linkers used in construction of the cDNA library. As shown in Fig. 1, an *Eco*RI site also occurs at this same position in the genomic sequence, suggesting that cleavage at this site occurred during construction of the cDNAs. Thus, the methylation reaction used to protect the internal *Eco*RI sites from cleavage in construction of the library was ineffective. By comparison with the murine IL-7 cDNA, which contains a 5′ untranslated se-




B

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GAATTCCTCTGGTCCTCATCCAGGTGCGCGGGAAGCAGGTGCCCAGGAGAGAGGGGATAATGAAGATTCCATGCTGATGATCCCAAAGATTGAACCTGCA
                                                  50                                                  100

GACCAAGCGCAAAGTAGAAACTGAAAGTACACTGCTGGCGGATCCTACGGAAGTTATGGAAAAGGCAAAGCGCAGAGCCACGCCGTAGTGTGTGCCGCCC
                                                 150                                                  200

CCCTTGGGATGGATGAAACTGCAGTCGCGGCGTGGGTAAGAGGAACCAGCTGCAGAGATCACCCTGCCCAACACAGACTCGGCAACTCCGCGGAAGACCA
                                                 250                                                  300

                                                                                          -25
                                                                                  Met Phe His Val
GGGTCCTGGGAGTGACTATGGGCGGTGAGAGCTTGCTCCTGCTCCAGTTGCGGTCATCATGACTACGCCCGCCTCCCGCAGACC ATG TTC CAT GTT
                                                 350

    -20                                 -10                                  1
Ser Phe Arg Tyr Ile Phe Gly Leu Pro Pro Leu Ile Leu Val Leu Leu Pro Val Ala Ser Ser Asp Cys Asp Ile
TCT TTT AGG TAT ATC TTT GGA CTT CCT CCC CTG ATC CTT GTT CTG TTG CCA GTA GCA TCA TCT GAT TGT GAT ATT
  400                                                                 450

                    10                                  20
Glu Gly Lys Asp Gly Lys Gln Tyr Glu Ser Val Leu Met Val Ser Ile Asp Gln Leu Leu Asp Ser Met Lys Glu
GAA GGT AAA GAT GGC AAA CAA TAT GAG AGT GTT CTA ATG GTC AGC ATC GAT CAA TTA TTG GAC AGC ATG AAA GAA
                                  500

 30                                  40                                  50   ▽
Ile Gly Ser Asn Cys Leu Asn Asn Glu Phe Asn Phe Phe Lys Arg His Ile Cys Asp Ala Asn Lys Glu Gly Met
ATT GGT AGC AAT TGC CTG AAT AAT GAA TTT AAC TTT TTT AAA AGA CAT ATC TGT GAT GCT AAT AAG GAA GGT ATG
  550                                                                 600

                    60                                  70
Phe Leu Phe Arg Ala Ala Arg Lys Leu Arg Gln Phe Leu Lys Met Asn Ser Thr Gly Asp Phe Asp Leu His Leu
TTT TTA TTC CGT GCT GCT CGC AAG TTG AGG CAA TTT CTT AAA ATG AAT AGC ACT GGT GAT TTT GAT CTC CAC TTA
                                  650

 80                                  90                  ▽               100
Leu Lys Val Ser Glu Gly Thr Thr Ile Leu Leu Asn Cys Thr Gly Gln Val Lys Gly Arg Lys Pro Ala Ala Leu
TTA AAA GTT TCA GAA GGC ACA ACA ATA CTG TTG AAC TGC ACT GGC CAG GTT AAA GGA AGA AAA CCA GCT GCC CTG
  700                                                                 750

                    110                                 120
Gly Glu Ala Gln Pro Thr Lys Ser Leu Glu Glu Asn Lys Ser Leu Lys Glu Gln Lys Lys Leu Asn Asp Leu Cys
GGT GAA GCC CAA CCA ACA AAG AGT TTG GAA GAA AAT AAA TCT TTA AAG GAA CAG AAA AAA CTG AAT GAC TTG TGT
                                  800

130                                 140                                 150
Phe Leu Lys Arg Leu Leu Gln Glu Ile Lys Thr Cys Trp Asn Lys Ile Leu Met Gly Thr Lys Glu His End
TTC CTA AAG AGA CTA TTA CAA GAG ATA AAA ACT TGT TGG AAT AAA ATT TTG ATG GGC ACT AAA GAA CAC TGA AAA
  850                                                                 900

ATATGGAGTGGCAATATAGAAACACGAACTTTAGCTGCATCCTCCAAGAATCTATCTGCTTATGCAGTTTTTCAGAGTGGAATGCTTCCTAGAAGTTACT
                                  950                                 1000

GAATGCACCATGGTCAAAACGGATTAGGGCATTTGAGAAATGCATATTGTATTACTAGAAGATGAATACAAACAATGGAAACTGAATGCTCCAGTCAACA
                                 1050                                 1100

AACTATTTCTTATATATGTGAACATTTATCAATCAGTATAATTCTGTACTGATTTTTGTAAGACAATCCATGTAAGGTATCAGTTGCAATAATACTTCTC
                                 1150                                 1200

AAACCTGTTTAAATATTTCAAGACATTAAATCTATGAAGTATATAATGGTTTCAAAGATTCAAAATTGACATTGCTTTACTGTCAAAATAATTTTATGGC
                                 1250                                 1300

TCACTATGAATCTATTATACTGTATTAAGAGTGAAAATTGTCTTCTTCTGTGCTGGAGATGTTTTAGAGTTAACAATGATATATGGATAATGCCGGTGAG
                                 1350                                 1400

AATAAGAGAGTCATAAACCTTAAGTAAGCAACAGCATAACAAGGTCCAAGATACCTAAAAGAGATTTCAAGAGATTTAATTAATCATGAATGTGTAACAC
                                 1450                                 1500

AGTGCCTTCAATAAATGGTATAGCAAATGTTTTGACATGAAAAAAGGACAATTTCAAAAAAATAAAAT (A)n
                                 1550
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FIG. 2. (*A*) Schematic representation of human IL-7 cDNA 3 and the corresponding regions contained in additional cDNA isolates. Open box, leader sequence; solid box, region encoding mature IL-7. Dotted lines in cDNAs 1 and 4 represent those sequences that are lacking in these clones when compared with cDNA 3. (*B*) The nucleotide sequence and deduced amino acid sequence of cDNA 3. Numbers above the sequence refer to the amino acid positions with position 1 being the predicted amino-terminal residue of the mature protein. Numbers below the sequence refer to the corresponding nucleotides. Inverted triangles mark the ends of the deletion in cDNA 1. Additional potential initiation codons in the 5′ noncoding region are underlined. Potential N-linked glycosylation sites are designated by the boxed areas. The four nucleotides lacking in cDNA 4 are indicated by the double underline.

quence of 548 base pairs (bp), the human transcript may contain an additional 200 bp of 5′ untranslated sequence. However, the exact position of the 5′ termini of the IL-7 mRNAs has yet to be determined.

**Comparison of Human and Murine IL-7.** Comparison of the human cDNA 3 nucleotide sequence with murine IL-7 shows that the highest homology occurs in the coding region (81%) and is only slightly less in the 5′ noncoding (73%) and 3′ noncoding (63%) regions (data not shown). As was found with murine IL-7, the human IL-7 transcripts also contain a large 5′ untranslated region with eight potential initiation codons upstream from the presumptive authentic initiation codon (Fig. 2*B*). Although the *in vivo* significance of this region is unknown, we previously demonstrated that removal

of these upstream sequences from the murine IL-7 cDNA resulted in a substantial increase in expression in transfected COS cells (10). A comparison of the human and murine IL-7 amino acid sequences again shows a strong degree of homology (60%) (Fig. 3), with all six cysteines conserved. However, it is evident (Fig. 3) that human IL-7 contains an insert of 19 amino acids (residues 96–114) not present in murine IL-7. This additional sequence is present in all four of the described human cDNAs and results from the presence of an additional exon in the human IL-7 gene (data not shown).

**Induction of Proliferation of Progenitor B Cells by IL-7.** Utilizing long-term bone marrow cultures as a source of pre-B cells, we previously described an assay to measure the proliferation of pre-B cells by murine IL-7 (9). We subsequently isolated a cDNA for murine IL-7 by assaying COS cell supernatants after transfection with cDNAs cloned into a mammalian expression vector pDC201 (10). To determine whether any of the human IL-7 cDNAs described above was active in the murine pre-B cell proliferation assay, cDNAs 1, 3, and 4 were inserted into pDC201 and transfected into COS-7 cells. Assay of these COS cell supernatants revealed that only cDNA 3 was able to encode a protein active in the murine pre-B-cell growth assay (Table 1). Comparison of the activity produced by the human cDNA with that obtained using the murine IL-7 cDNA (clone 1046) revealed that both yielded roughly equivalent amounts of activity. Presumably the deletions in the coding regions of cDNAs 1 and 4 (Fig. 2), which resulted in altered translation products, were responsible for their loss of pre-B-cell growth-promoting activity.

Although human IL-7 is active on murine pre-B cells, it was of interest to determine whether human IL-7 possessed an equivalent activity on human precursor B cells. Utilizing recombinant human IL-7, we developed a bioassay using human bone marrow cells that were fractionated on Percoll gradients so as to enrich for B-lineage cells. As in the murine assay, the proliferation of these cells, caused by the addition of IL-7, was measured by incorporation of labeled thymidine. Again, only cDNA 3 was able to encode a protein that was active in the human bone marrow proliferation assay (Table 1). Recombinant murine IL-7 was unable to induce the proliferation of these human cell populations. Because human IL-7 contains an insertion of 19 amino acids not found in the murine cDNA (Fig. 3), it was of interest to determine whether the removal of these additional amino acids would affect the proliferative activity of IL-7 on human bone marrow cells. To aid in construction of a cDNA lacking these additional amino acids, cDNA 3 was cloned into a yeast expression vector that utilizes the ADH2 promoter (22), and amino acids 96–114 were removed by *in vivo* mutagenesis (23). The human IL-7 expressed in yeast was then assayed in both the murine and human proliferation assays. The results of these experiments indicated that removal of the additional amino acids failed to abolish IL-7 activity in either assay (Table 1). Thus, the failure of the murine IL-7 to induce proliferation of human bone marrow cells is not due to its lacking the additional 19 amino acids found in human IL-7.

Table 1. Biological activity of human and murine IL-7

| IL-7 cDNA expressed | Murine IL-7 assay, units/ml | Human IL-7 assay, units/ml |
|---|---|---|
| Human cDNA 1* | 0 | 0 |
| Human cDNA 4* | 0 | 0 |
| Human cDNA 3* | 7,264 | 2150 |
| Murine cDNA* | 11,334 | 0 |
| Human cDNA 3 lacking amino acids 96–114† | 13,983 | 6945 |
| Human cDNA 3† | 17,508 | 2662 |

*IL-7 activity recovered after transfection of COS-7 cells utilizing the expression vector pDC201.
†IL-7 activity recovered from expression of the relevant cDNA in yeast.

**RNA Blot Analysis.** To analyze the IL-7 transcripts expressed in SK-HEP-1 cells and in various human tissues, polyadenylylated RNA was isolated and examined by blot hybridization (Fig. 4). SK-HEP-1 cells express two size classes of IL-7 transcripts of ≈1.8 and 2.4 kb, while RNA derived from splenic tissue contains only the 2.4-kb transcript. No IL-7 message is visible in this exposure of RNA from human thymic tissue (Fig. 4). However, longer exposures did reveal the presence of small amounts of both size classes of IL-7 transcripts in RNA derived from thymic tissue.

## DISCUSSION

In this report, cross-species hybridization with a murine cDNA has enabled us to isolate several human IL-7 cDNAs. By expression of these cDNAs in transfected COS-7 cells,

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   -20        -10          1          10          20
MFHVSFRYIFGLPPLILVLLPVASSDCDIEGKDGKQYESVLMVSIDQLLD  Human
MFHVSFRYIFGIPPLILVLLPVTSSECHIKDKEGKAYESVLMISID-ELD  Murine
   -20        -10          1          10          20

   30         40          50          60          70
SMKEIGSNCLNNEFNFFKRHICDANKEGMFLFRAARKLRQFLKMNSTGDF  Human
KMTGTDSNCPNNEPNFFRKHVCDDTKEAAFLNRAARKLKQFLKMNISEEF  Murine
    30         40          50          60          70

   80         90         100         110         120
DLHLLKVSEGTTILLNCTGQVKGRKPAALGEAQPTKSLEENKSLKEQKKL  Human
NVHLLTVSQGTQTLVNCTSK------------------EEKNVKEQKK-  Murine
    80         90                                100

  130        140        150
NDLCFLKRLLQEIKTCWNKILMGTKEH  Human
NDACFLKRLLREIKTCWNKILKGSI--  Murine
   110        120
```

FIG. 3. Alignment of amino acid sequences (single-letter code) encoded by human (cDNA 3) and murine IL-7 cDNAs. Vertical lines, identical amino acids; horizontal lines, gaps introduced to provide maximum alignment, which was determined by using a program designed by the University of Wisconsin Genetics Computer Group (21).

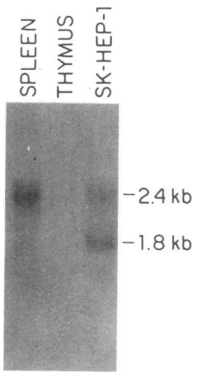


FIG. 4. RNA blot analysis of human IL-7 mRNA. The sizes of the IL-7 mRNAs were estimated by comparison with RNA standards obtained from Bethesda Research Laboratories.

one clone (cDNA 3) was shown to encode a protein capable of inducing the proliferation of murine pre-B cells (Table 1). We were further able to demonstrate that this recombinantly expressed human IL-7 was similarly able to induce the proliferation of human bone marrow cells.

The isolated human IL-7 cDNA has been used to probe blots of RNA from the human liver cell line SK-HEP-1 as well as from human splenic and thymic tissue, revealing the presence of two size classes of IL-7 transcripts (Fig. 4). The cDNAs described in this report all terminated with a string of adenines and thus presumably are derived from the smaller (1.8-kb) IL-7 transcript. The larger transcripts may result from alternative polyadenylylation resulting in a longer 3′ untranslated region as found with murine IL-7 (10), but this has not been established. The hybridized RNA blot also revealed that the human splenic tissue expressed considerably more IL-7 message than does the thymic tissue. This is in sharp contrast to the mouse, where the highest expression of IL-7 message was observed in the thymus (10). The significance of this difference is presently unknown.

The molecular cloning of the human cDNA encoding IL-7 represents a starting point for a variety of further studies. For example, engineering of the cDNA into an appropriate expression system should allow for the production and purification of significant quantities of recombinant human IL-7. This will enable the pursuit of a variety of *in vitro* experiments aimed at determining the role of IL-7 in hematopoiesis and regulation of immune function. It is possible that disregulation of the expression of IL-7 or its receptor may be involved in the generation of neoplasia in lymphoid-related leukemias or in the production of autoimmune diseases. Production of substantial quantities of recombinant IL-7 should also enable investigators to pursue the mechanism of action and to characterize the receptor and the cells responsive to this regulator of lymphocyte growth and development.

We wish to thank Dirk Anderson and Janis Wignall for their assistance and advice throughout this work, Jeff Hesselberg for oligonucleotide synthesis and purification, Steve Gimpel for DNA sequence determination, and Linda Troup for manuscript preparation.

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