Organization of the Murine and Human Interleukin-7 Receptor Genes: Two mRNAs Generated by Differential Splicing and Presence of a Type I-Interferon-Inducible Promoter

CHRISTOPHER M. PLEIMAN,† STEVEN D. GIMPEL, LINDA S. PARK, HISASHI HARADA, TADATSUGU TANIGUCHI, and STEVEN F. ZIEGLER

Departments of Molecular Biology and Biochemistry, Immunex Corporation, Seattle, Washington 98101, and Institute for Molecular and Cellular Biology, Osaka University, Suita-shi, Osaka 565, Japan

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To better understand the regulation of interleukin-7 receptor (IL-7R) expression, we have pursued a detailed analysis of the structure of the murine and human IL-7R genes. The genes consist of eight exons, the sizes of which are conserved in mouse and human cells, spread out over 24 kbp (murine) and 19 kbp (human). A differential splicing event results in an mRNA encoding a secreted form of the human IL-7R gene. Primer extension and S1 nuclease analysis show a single transcriptional start site for the murine IL-7R gene. The 5'-flanking region of the murine IL-7R gene contains TATA- and CAAT-like sequences. The promoter region also contains a functional interferon regulatory element, to which the interferon-induced nuclear factors IRF-1 and IRF-2 are capable of binding and which is able to confer interferon-inducible expression on a heterologous gene. There are also potential binding sites for the transcription factors AP-1 and AP-2 as well as multiple glucocorticoid response elements. A fusion gene containing 2.5 kb of murine IL-7R 5' regulatory sequence linked to the bacterial chloramphenicol acetyltransferase gene directed expression of chloramphenicol acetyltransferase activity in murine pre-B-cell line 70Z/3 but not in the mouse fibroblast cell line NIH 3T3. Comparison of the murine and human IL-7R exon/intron boundaries with those of other hematopoietin receptor superfamilies whose exon/intron boundaries are also known reveals a conserved evolutionary structure.

Interleukin-7 (IL-7) is a 25-kDa glycoprotein, involved in regulation of hematopoiesis, which acts primarily on cells of lymphoid lineage (18). IL-7 was originally identified and characterized as a pre-B-cell growth factor capable of stimulating the proliferation of surface immunoglobulin-negative (sIg−) B220+ cells derived from long-term bone marrow cultures, although IL-7 was unable to stimulate the proliferation of mature B cells or to induce the differentiation of B-cell precursors to sIg+ cells (27, 33). It has also been shown that cells of the T-cell lineage will proliferate in response to IL-7. Resting fetal and adult thymocytes will proliferate when exposed to IL-7 in a manner independent of IL-2, IL-4, or IL-6 (7, 45). In addition, adult peripheral T cells respond to IL-7 in the presence of suboptimal mitogen concentrations (1a, 5, 31). Recent results have shown that IL-7 can also exert effects on cells of the myeloid lineage. IL-7-stimulated human peripheral blood monocytes express several inflammatory mediators, including IL-1α and -β, IL-6, and MIP-1β (1). These data suggest a role for IL-7 in inflammatory immune responses.

Complementary DNA clones encoding the human and murine IL-7R receptor (IL-7R) have been obtained by using a direct expression cloning system (17). Transfection of these cDNAs into COS-7 cells resulted in the cell surface expression of IL-7R capable of binding IL-7 with the same properties displayed by native receptors. In addition to cDNAs encoding membrane-bound IL-7R, cDNAs capable of encoding a soluble form of the human receptor were isolated (17). These soluble IL-7R cDNA clones, when expressed in COS-7 cells, encoded a protein able to bind IL-7 efficiently in solution. Thus, the IL-7R, like the IL-4R (32), can be expressed in both soluble and membrane-bound forms.

To elucidate the mechanism generating membrane-bound and soluble forms of the IL-7R, and to investigate the sequences responsible for regulation of IL-7R gene expression, the structures of the murine and human IL-7R genes were determined. We demonstrate that the murine and human IL-7R genes consist of eight exons, spanning 24 and 19 kb, respectively. Comparison of the murine and human IL-7R exon/intron boundaries with those of other hematopoietin receptor superfamilies (16, 44, 49) reveals a common evolutionary structure. Exon 6, which encodes the transmembrane region, is differentially spliced to generate two IL-7R mRNAs, encoding the membrane-bound and soluble receptors.

We have also examined the 5' regulatory region of the murine IL-7R gene and have found it to contain consensus binding sequences for several known transcription factors. Most striking was the presence of an interferon (IFN) regulatory element (IRE). This sequence was shown to be capable of conferring IFN responsiveness in L929 cells on a chimeric construct consisting of the chloramphenicol acetyltransferase (CAT) gene linked to a portion of the murine IL-7R promoter. A second fusion gene linking 2.5 kb of murine IL-7R 5' regulatory sequence to the CAT gene directed expression in pre-B-cell line 70Z/3 but not in fibroblast cell line NIH 3T3.

MATERIALS AND METHODS

Isolation and characterization of genomic clones for the murine and human IL-7R genes. Genomic libraries from
murine liver (Clonetech Laboratories, Palo Alto, Calif.) and human placenta (Promega Biological Research, Madison, Wis.) were screened with cDNA clones for the murine and human IL-7R genes (17), respectively. Additional clones for the human IL-7R gene were isolated from a library generated from peripheral blood whole mononuclear cells (Stratagene Cloning Systems, La Jolla, Calif.). Restriction maps of the bacteriophage clones containing IL-7R gene sequences were constructed (Fig. 1A). The maps were confirmed by hybridization using oligonucleotides generated from the cDNA sequence. Specific restriction fragments encompassing coding regions isolated from the phage were subcloned into pBluescriptSK (Stratagene) and sequenced by the dideoxy-chain termination method (42).

**FIG. 1.** Molecular characterization of the murine and human IL-7R gene and promoter. (A) Restriction map of the murine IL-7R gene constructed from the overlapping bacteriophage clones depicted beneath the map. (B) Restriction map of the human IL-7R gene constructed from four overlapping phage clones depicted beneath the map. The positions of the exons and translational start and stop sites are marked. R, EcoRI; B, BamHI; S, SstI. The size of each exon (in nucleotides) is given. The sizes of exon 8 in the murine gene and exon 1 in the human gene are undetermined.

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- 1 Kb

**PCR.** Polymerase chain reaction (PCR) was performed as previously described (38). Total RNA (10 μg) or polyadenylated RNA (1 μg) was used to direct oligo(dT)-primed first-strand cDNA synthesis according to standard procedures (19). The primers used for amplification were (i) an oligonucleotide from the coding nucleotides 676 to 697 of the murine IL-7R cDNA M1 and 649 to 670 of the human IL-7R cDNA M1 and 649 to 670 of the human IL-7R cDNA clone H20 and (ii) an oligonucleotide complementary to nucleotides 967 to 990 of the murine IL-7R cDNA M1 and 939 to 961 of the human IL-7R cDNA clone H20 (17). Amplification was performed for 25 cycles, using the conditions specified by Perkin-Elmer-Cetus. The reaction products were then separated on a 3% NuSieve agarose gel, transferred to nylon filters, and probed with random-primed full-length IL-7R cDNAs. Reaction products were also subcloned, and the inserts were subjected to sequence analysis.

**Primer extension analysis.** Primer extension was performed by using an oligonucleotide primer complementary to a sequence in the 5' untranslated region of the murine IL-7R mRNA (CD25; see Fig. 3). End-labeled oligonucleotide primer (10 3 cpm) was annealed to total RNA (20 μg) or poly(A)- RNA (1 μg) and extended with reverse transcriptase (Life Sciences, Inc., St. Petersburg, Fla.) as previously described (38). The samples were then electrophoresed under denaturing conditions on a 6% polyacrylamide sequencing gel alongside a set of dideoxy sequencing reactions to permit single-base resolution of the extension products.

**Construction of murine IL-7R/CAT chimeric plasmids and analysis by electroporation.** A 2.5-kb HindIII-HindII DNA fragment containing 5'-flanking sequence from the murine IL-7R gene (shown in Fig. 3) was cloned into pCAT/Basic (Promega). The construct was analyzed in the murine B-lineage cell line J02Z3 (36) and in the murine fibroblast line NIH 3T3. Electroporations were carried out by using a Gene-Pulsor (Bio-Rad, Richmond, Calif.) with 10 3 cells and 30 μg of plasmid DNA at a voltage of 300 V and a capacitance of 960 μF. Cellular extracts were prepared by Tris buffer extraction 48 h later, and CAT assays were carried out as previously described (39, 43).
A second murine IL-7R/CAT fusion plasmid was constructed by using an EcoRV-HincII fragment from the murine IL-7R promoter (see Fig. 3) linked to the CAT gene. This construct was electroporated into murine L929 cells, using the settings specified above. Twenty-four hours later IFN-α/β (1,000 U/ml) was added, and 12 h after that poly(I-C) (100 μg/ml) was added. Cells were harvested after an additional 12 h of culture and extracted, and CAT assays were performed as described above.

**Results**

*Isolation and characterization of IL-7R genomic clones.* A murine genomic library in bacteriophage λEMBL3 was screened by using the full-length murine IL-7R cDNA clone M1 (17) as a probe. Four overlapping phage clones were obtained, ranging in size from 14.2 to 16.4 kb in length, which in sum contained the complete murine IL-7R gene. Detailed restriction mapping, Southern blot analysis, and DNA sequencing revealed that the murine IL-7R gene spans approximately 24 kb of genomic DNA and consists of eight exons and seven introns (Fig. 1A). The complete restriction map of the murine IL-7R gene was confirmed by genomic Southern blot analysis (data not shown). Restriction fragments within the phage genomic clones that hybridized with the cDNA clone were subcloned, and the nucleotide sequence was determined to delineate exon boundaries. The nucleotide sequence found at each of the exon/intron junctions comprised consensus splice donor or acceptor sequences (data not shown) (4). The first exon includes the 5′ untranslated region, signal peptide, and amino terminus of the mature protein. Exons 2 to 6 encode the remainder of the extracellular region. Exon 6 also encodes the transmembrane and part of the cytoplasmic region, with exons 7 and 8 encoding the rest of the cytoplasmic region. Exon 8 also encodes the entire 3′ untranslated region.

A similar analysis was performed on phage clones isolated from a human genomic library, using the human IL-7R cDNA H20 (17) as a probe. Two phage clones, 3-1 and 22-1, which contained inserts of 19.4 and 13.4 kb, respectively, were isolated. Restriction maps of the two inserts and locations of the exons within the inserts are shown in Fig. 1B. Clone 3-1 contains the 3′ terminus of intron 1 through the 5′ terminus of intron 5. Clone 22-1 contains sequence from the 3′ terminus of intron 4 to approximately 15 kb downstream of the stop codon.

Since these clones did not contain the entire human IL-7R gene, a second library was screened, using a probe derived from the human IL-7R cDNA clone containing only sequences from exons 1 and 2. One phage clone (8-3) was isolated which contained a 15.6-kb insert. The 5′ end of this clone extended approximately 500 bp upstream of the initiation ATG. As was true with the murine gene, all sequences at exon/intron boundaries conform to canonical sequences (4, 36). The locations of the exon/intron junctions within the human IL-7R gene are identical to those found in the murine IL-7R gene (data not shown).

**FIG. 2.** Evidence that differential splicing generates mRNA encoding membrane-bound and soluble forms of the human IL-7R. (A) Schematic representation of the splicing of the human IL-7R gene and the sequences of the membrane-bound and soluble forms of the human IL-7R mRNAs. (B) Sequence of the membrane-bound form of the murine IL-7R mRNA. (C) PCR product generated from W126VA4 cells (W126), human peripheral blood lymphocytes (PB), and human splenic tissue (Spleen) first-strand cDNA and hybridized to human IL-7R cDNA H20 (17). (D) PCR product generated from T02/3, HAFTL-1, and murine splenic tissue first-strand cDNA and hybridized to murine IL-7R cDNA M1 (17). T. M., transmembrane domain.

The soluble form of the human IL-7R is generated by differential splicing. A comparison of the nucleotide sequence of the human IL-7R gene with that of cDNA clones predicted to encode soluble IL-7R shows that the cDNA clones lack sequences from exon 6, which encodes the transmembrane domain (Fig. 2). The mRNA for the soluble IL-7R has joined exons 5 and 7, apparently by splicing out exon 6. To investigate this finding further, oligonucleotide primers from sequences in exons 5 and 7 were synthesized (see Materials and Methods) and used to amplify cDNA, derived from a variety of sources, by using PCR. Expected PCR products from human mRNA would be 330 or 234 bp (containing or lacking exon 6), while the corresponding products from murine mRNA would be 331 and 235 bp. Figure 2C shows the results of PCR reactions performed on RNA isolated from the human fibroblast cell line W126VA4, from which the IL-7R cDNA was originally cloned (17), peripheral blood T cells, and whole spleen. In each case, two PCR products were generated, corresponding to the membrane-bound form containing exon 6 and the soluble form lacking exon 6. These results were confirmed by subcloning the two PCR products and obtaining their nucleotide sequences (data not shown). However, no PCR product corresponding to an mRNA encoding a soluble form of the murine IL-7R was detected (Fig. 2D). Similarly, no cDNA clones have been isolated that could encode a soluble murine IL-7R (data not shown).

To determine whether a soluble IL-7R was secreted from cells, conditioned medium from W126VA4 cells was collected, concentrated, and analyzed for its ability to bind IL-7. It was found to be capable of binding to both 125I-IL-7
and a monoclonal antibody specific for the human IL-7R (data not shown).

Determination of the transcriptional start site of the murine IL-7R gene. To facilitate analysis of the 5' regulatory region of the murine IL-7R gene, a 4.8-kb HindIII fragment from bacteriophage clone 1-1 was subcloned and mapped, and the nucleotide sequence from the 3' end of exon 1 to the end of the fragment was obtained (Fig. 3). To determine the 5' end of the murine IL-7R mRNA, primer extension and S1 nuclease analyses were performed. An oligonucleotide complementary to nucleotides 477 to 502 of the 5' untranslated region of the murine IL-7R gene (CD25; Fig. 3) was used as a primer on mRNA prepared from murine spleen and from the B-lineage cell lines HAFTL-1 ( lane 10) and 70Z/3 (36). The oligonucleotide was also used as a primer in nucleotide sequencing of the 4.8-kb HindIII fragment. The sequencing reactions were run with the primer extension products for single-base resolution (Fig. 4). A single fragment of 502 bp was detected in murine spleen (Fig. 4, lane 1), HAFTL-1 (lane 2), and 70Z/3 (lane 3) cell mRNA but not in murine liver mRNA (data not shown). These results were confirmed in an S1 nuclease protection assay (data not shown).

Characterization of murine 5'-flanking regions. Nucleotide sequence analysis of DNA 5' of the transcriptional start site reveals a potential TATA-like sequence (TITAAA) at nucleotides −30 to −35 and a CAAT sequence (CAAT) at nucleotides −146 to −150 (Fig. 3). The murine gene also contains a potential AP-2 binding site, on the antisense strand, at nucleotides −190 to −198 (5'-T/C G/G CC A/C N C/G C/G C/G-3') (25) and a potential AP-1 site at nucleotides −1376 to −1383 (5'-TGAGTCA-3') (28). There are four regions in this 5'-flanking sequence which conform to the consensus binding site for the glucocorticoid receptor (5'-TTGTCTC-3') (26): at nucleotides −767 to −772, −1059 to −1064, −1472 to −1477, and −2224 to −2229 (31). The murine IL-7R gene 5' regulatory region also contains a sequence at nucleotides −249 to −262, that resembles an IRE (Fig. 3). A search of the 5'-flanking region failed to reveal other known binding sites for sequence-specific DNA-binding proteins implicated in the regulation of transcription.

The presence of TATA- and CAAT-like sequences directly upstream of the transcriptional start site suggested that this region might contain the promoter for the murine IL-7R gene. A fusion gene containing a 2.5-kb HindIII-HincII fragment from the 5'-flanking region of the murine gene (Fig. 3) linked to the bacterial CAT gene (pM7RCAT) was prepared. This construct was electroporated into NIH 3T3 and 70Z/3 cells to test for promoter activity. Parallel cultures were electroporated with plasmid p652CAT, containing the CAT gene driven by the cytomegalovirus immediate-early promoter (28a). NIH 3T3 cells electroporated with pM7RCAT showed no detectable CAT activity, while cells electroporated with p652CAT contained CAT activity (Fig. 5). However, when pM7RCAT was electroporated into the murine pre-B-cell line 70Z/3, CAT activity was detectable. These results are in agreement with RNA analyses of these cell lines showing that 70Z/3 cells express IL-7R mRNA while NIH 3T3 cells do not (17; data not shown). Thus, it appears that the 5'-flanking sequence from nucleotides −2495 to +5 contains promoter sequences that are active in lymphoid cells.

The murine IL-7R promoter contains a functional IRE. A specific nucleotide sequence, present in the 5' regulatory regions of type I IFN genes and virus- and IFN-inducible genes, known as an IRE (13), is capable of binding two regulatory factors (12, 21). These factors, IRF-1 and -2, can exert both positive and negative effects on transcription when bound to this sequence (12, 20). A sequence was found in the 5' regulatory region of the murine IL-7R gene which closely resembled the consensus IRE sequence. Table 1 shows a comparison of IRE binding sites from a variety of virus- and IFN-inducible genes. The sequence in the murine IL-7R gene most closely resembles the IRE found in the IFN-α1 gene.

Complementary 50-mer oligonucleotides containing the IRE-like sequence from the murine IL-7R gene were annealed and used as a probe to determine whether this sequence was capable of binding IRF-1 and -2. End-labeled probe was incubated with Escherichia coli-produced IRF-1 and -2 (see reference 21 for details of the purification), and the resulting protein-DNA complexes were analyzed by gel retardation. Both IRF-1 and IRF-2 were capable of binding to the 50-mer, demonstrated by the gel-shifted bands in Fig. 6A. The specificity of this interaction was determined by the ability of a probe containing three repeats of the sequence 5'-(AAGTGA)-3' to compete for binding with the 50-mer, while other probes containing the binding sites for the transcriptional activators AP-1, AP-2, and Sp1 were not (Fig. 6A and data not shown). The competing sequence was chosen because of its ability to strongly bind both IRF-1 and -2 (12). These data show that the IRE-like sequence in the murine IL-7R promoter is able to specifically bind the transcription factors IRF-1 and -2.

As a further test of the virus and IFN inducibility of the murine IL-7R promoter, a truncated version of the promoter was analyzed. A 350-bp EcoRV-HincII fragment, derived from the 2.5-kb HindIII-HincII fragment used previously (Fig. 3), was fused to the CAT gene and transfected into murine L929 cells. This cell line was chosen because of its sensitivity to double-stranded RNA (dsRNA) and IFN treatment (20). The cells were treated with IFN-α/β (1,000 U/ml) 24 h following electroporation, and 12 h later poly(I-C) (100 μg/ml) was added. The cells were harvested after 12 h of coinubation and extracted, and CAT assays were performed. As shown in Fig. 6B, the untreated cells electroporated with the construct had barely detectable CAT activity. However, the IFN-poly(I-C) treatment caused a five- to sixfold increase in the levels of CAT activity. IFN treatment alone was also capable of inducing CAT activity from this construct (data not shown). These data show that there is a functional IRE in the murine IL-7R promoter.

DISCUSSION

IL-7 has been shown to promote the growth of mature T cells as well as B-cell progenitors (18). Also, recent data suggest a role for IL-7 in inflammatory immune responses (1). However, the mechanism by which IL-7 exerts its effects is unknown. In an attempt to better understand IL-7 function, cDNAs encoding the human and murine IL-7R have been cloned and characterized (17). The factors that regulate the expression of these receptors remain obscure. In this report we show the structure of the murine and human IL-7R genes and demonstrate that an mRNA encoding a soluble form of the human IL-7R is generated by differential splicing. Also, we have shown that a promoter for the murine IL-7R gene lies within 2.5 kb 5' to the start of transcription. Contained with this promoter is a functional IRE.

Common gene structure of members of the hematopoietin receptor family. Recently, cDNA clones encoding the receptors for several cytokines and hematopoietic growth factors
FIG. 3. Nucleotide sequence of the murine IL-7R 5′ HindIII to start of translation. The G which is the major transcriptional start site is marked +1. The TATA- and CAAT-like sequences are overlined. The putative AP-1 site is marked by a double underline, the putative AP-2 is marked by a single underline, and the four putative glucocorticoid receptor regulatory elements are marked by a broken underline. The IRE is boxed. Relevant restriction sites and the primer used for primer extension (CD25) are indicated.
FIG. 4. Primer extension analysis of murine IL-7R mRNA. Oligonucleotide CD25 (Fig. 3) was used to prime murine spleen (lane 1), HAFTL-1 (lane 2), and 70Z/3 (lane 3) mRNAs. CD25 was also used as a primer to sequence the HindIII-Xbal fragment from the murine IL-7R gene, and the sequencing reactions were run with the primer extension reactions (lanes A, C, G, and T).

have been isolated. These include the receptors for IL-2 (β chain) (22), IL-3 (24), IL-4 (32), IL-6 (48), erythropoietin (9), granulocyte-macrophage colony-stimulating factor (15), and granulocyte colony-stimulating factor (14). An inspection of the deduced amino acid sequences of these receptors, as well as the receptors for prolactin and growth hormone, has shown considerable homology and has led to these receptors being referred to as the hematopoietin receptor family (8).

The gene structures for several members of the hematopoietin receptor family have been determined (16, 44, 46, 49). A comparison of the portion of each gene encoding the hematopoietin receptor family domain with that of the murine and human IL-7R genes shows that there has been evolutionary conservation of the exon/intron boundaries. The extracellular domain of these receptors is encoded by five exons, the exception being the human IL-2R β-chain gene, which contains an extra intron splitting exon 3 (numbering as described here for the IL-7R gene). These receptors contain two pairs of conserved cysteine residues, each pair encoded in a separate exon. Also, the fibronectin type III repeat-like sequence of each receptor is encoded by two exons, similar to other genes containing this type of domain (37).

As described above for the IL-7R genes, the transmembrane region of each of these genes is encoded by a single exon. The structural homology extends through the entire gene, although the amino acid sequence homology is largely limited to the extracellular domain (8). The intracellular portion of each receptor, as well as the 3′ untranslated sequences, is encoded on two exons (Fig. 1; 16, 44, 46, 49).

The structural similarities between these genes strongly suggest that they are evolved from a common ancestor. This belief has also been held for the genes encoding the ligands of these receptors, the interleukins and colony-stimulating factors, based on similar exon/intron structures, sizes of the

FIG. 5. Evidence that a 2.5-kb HincII-HindIII fragment from the murine IL-7R gene functions as a promoter. Transient transfection experiments in NIH 3T3 and 70Z/3 cells were carried out with a plasmid bearing the bacterial CAT gene under the control of the murine IL-7R promoter (mIL-7R; see Materials and Methods for details), the cytomegalovirus-based promoter (CMV), or no promoter (Mock). CAT assays were performed as previously described (39, 43).

FIG. 6. (A) Gel retardation assay using purified IRF-1 and IRF-2. Complementary 50-mer oligonucleotides (nucleotides −286 to −237 of the murine IL-7R promoter) containing the IRE sequence were end labeled and used in gel retardation assays using purified IRF-1 and IRF-2. The protein-DNA complexes were separated on polyacrylamide gels and subjected to autoradiography. The complexes either were (+) or were not (−) competed against an oligonucleotide containing the sequence (5′-AAGTGA-3′). P refers to the probe with no added protein. (B) Assays showing that the murine IL-7R promoter is IFN-α/β and dsRNA inducible. A 295-bp EcoRV-HincII fragment containing the IRE (Fig. 3) was fused to the CAT gene and electroporated into murine L929 cells. The electroporated cells were treated with IFN-α/β and poly(I-C) as described in Materials and Methods. CAT assays were then performed, using equal amounts of protein in each assay. The acetylated forms of chloramphenicol were scraped from the thin-layer chromatography plate and counted. IFN and poly(I-C) treatment resulted in a sixfold increase in CAT activity.

TABLE 1. Comparison of IRF-1 and IRF-2 binding sites of the murine IL-7R gene, the type I IFN genes, and the murine H-2Db gene

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<td>Human IFN-α1 (41)</td>
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<td>Human IFN-β1 (12)</td>
<td>5′-GAGAAATGGAAAGT-3′ (−79 to −67)</td>
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<td>Mouse H-2Dβ (21)*</td>
<td>5′-AGAAATGGAAACCT-3′ (−152 to −140)</td>
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* Numbers in parentheses refer to distance from sequence to the start of transcription.

* From antisense strand.
proteins, and common sequence motifs regulating gene expression (2, 8). Taken together, these data suggest that the hematopoietic growth factor/hematopoietin receptor system has evolved from single genes encoding a primordial growth factor and a primordial receptor. The present complex array of factors and receptors may reflect the evolution of the immune system as a whole.

Two forms of the human IL-7R generated by differential splicing. Our data show that an mRNA encoding a soluble form of the human IL-7R is generated by differential splicing (Fig. 2). The generation of this mRNA appears to be common, as every cell line or tissue source that expressed IL-7R mRNA coexpressed both mRNA species (Fig. 2 and data not shown). The presence of IL-7 binding activity in the culture medium of WI26VA4 cells indicates that a soluble IL-7R is expressed and secreted. The function of this soluble IL-7R is at present unclear. One possible function may be to act as a sponge to aid in clearing IL-7 from a site of action. Alternatively, the soluble receptor may serve to deliver IL-7 to a particular site or to increase the local concentration of IL-7 at a site. Further experimentation is needed to determine the function of the soluble IL-7R.

There is considerable evidence that soluble forms of cytokine receptors exist in vivo. A soluble, high-affinity IL-4-binding protein has been found in the biological fluids of mice (11). The relationship between this protein and the cDNA clone capable of encoding a soluble IL-4R (32) is unclear. Also, several soluble cytokine-binding proteins have been purified from human urine (34). Among the binding proteins purified was a soluble form of the IL-6R (34), a member of the hematopoietin receptor family. Several other soluble cytokine-binding proteins were found, including receptors for IFN-γ, IL-2R (α subunit), and tumor necrosis factor (34). It was not clear from these studies whether these soluble receptors represented shed forms of membrane-bound receptors or whether they were encoded by distinct mRNAs.

Murine IL-7R gene expression is induced by type I IFN and dsRNA. A surprising aspect of this study was the finding that the murine IL-7R promoter contains a functional Ire capable of conferring type I IFN and dsRNA inducibility. These results suggest a role for IL-7, acting through its receptor, in responses to viral infection. In support of this notion is the recent finding that human peripheral blood monocytes respond to IL-7 stimulation by secreting IL-6 and other inflammatory cytokines (1). We have also shown that IFN-α/β treatment of murine splenic B cells induces IL-7R, which then renders the cells capable of responding to IL-7 stimulation by secreting IL-6 (10a). Thus, in two separate systems, IL-7 treatment induces the expression of inflammatory cytokines. These data indicate that IL-7 is involved in cellular responses to viral infection and inflammation.

IRF-1 and IRF-2 exert their effects on IFN-induced gene expression through competition for binding to the same site (20). IRF-1 functions as a transcriptional activator, while IRF-2 appears to inhibit the effect of IRF-1 (12, 13, 20, 21). Yamada et al. (47) have generated transgenic mice expressing IRF-1 under the control of the μ enhancer. These mice have dramatically reduced levels of mature B cells and no detectable CFU-IL-7 in their bone marrow (47), indicating that B-cell progenitors in these mice are unable to respond to IL-7. It is unclear whether this is a direct effect of IRF-1 or whether IRF-1 is inducing the expression of a negative regulator, such as IRF-2, which is inhibiting the expression of IL-7R. These data strongly suggest that inappropriate expression of IRF-1 can have dramatic effects on the ability of B cells to respond to IL-7. Although the mechanism for the lack of IL-7 responsiveness is as yet obscure, this observation strengthens the linkage between the responses mediated by IFN and IL-7. It will be interesting to examine the expression of IL-7R on different cell types in these mice.

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REFERENCES


MURINE AND HUMAN IL-7 GENES

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