RESEARCH COMMUNICATION

Two differentially expressed interleukin-11 receptor genes in the mouse genome

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Interleukin-11 (IL-11) is a multifunctional cytokine involved in the regulation of cell proliferation and differentiation in a variety of cell types and tissues in vitro and in vivo. The effects of IL-11 were shown to be mediated by the IL-11 receptor (hereafter referred to as IL-11Rα), which is a ligand-binding subunit and provides ligand specificity in a functional multimeric signal-transduction complex with gp130. Here we show that the mouse genome contains a second gene encoding an IL-11-binding protein, referred to as IL-11Rβ. The structure of the IL-11Rβ gene is highly similar to that of IL-11Rα, and IL-11Rβ exhibits 99% sequence identity with IL-11Rα at the amino acid level. IL-11Rβ is co-expressed with IL-11Rα, albeit at lower levels, in embryos and in various adult tissues. IL-11Rβ transcripts are abundant in testis, and, in contrast with IL-11Rα, absent from skeletal muscle. IL-11Rβ expressed in vitro binds IL-11 with high affinity, suggesting that the mouse genome contains a second functional IL-11R.

INTRODUCTION

Interleukin-11 (IL-11) is a multifunctional cytokine that exhibits a wide spectrum of biological activities in vitro, including stimulation of plasmacytoma cell multiplication [1–3], induction of acute-phase protein expression by hepatocytes [4], inhibition of adipocyte [5,6], neuronal [7] and osteoprogenitor cell differentiation [8], and osteoestrogen-mediated bone resorption [9]. In vivo, IL-11 was shown to trigger acute-phase proteins [4], increase platelet count [4,10] and induce loss of body fat and hyperactivity [11].

Recently, we [12] and others [13] have isolated cDNAs (designated Etl2 and NR-1 respectively) that encode a specific receptor for IL-11, hereafter designated IL-11Rα. The biological effects of IL-11 are mediated by association of the ligand with IL-11Rα and the signal-transducing receptor gp130 [13,14]. Sequence comparisons show that IL-11Rα, like gp130, is a member of the cytokine family of receptors [15], with greatest similarity in sequence to IL-6R and CNTF-R. Although IL-11Rα exhibits 99% sequence identity with IL-11Rβ at the amino acid level. IL-11Rβ binds IL-11 with high affinity, is expressed at high levels in testis, and at low levels co-expressed with IL-11Rα in embryos and adult tissues, but in contrast with IL-11Rα it is not expressed in skeletal muscle.

MATERIALS AND METHODS

Isolation and characterization of phage clones and genomic DNA

A genomic library, constructed from partially digested DNA of the D3 ES cell line, was screened with a cDNA probe containing exons 2–12 of the IL-11R as described previously [12]. DNA from positive clones was isolated and characterized by restriction mapping and was subcloned in Bluescript vector for sequencing. High-molecular-mass DNA was isolated from D3 ES (129Sv195) cells or mouse tissues and was analysed by Southern-blot hybridizations as described previously [12].

DNA isolation and reverse transcription (RT)–PCR analysis

Total RNA from tissues and embryos from 129Sv195 mice was isolated as described previously [12]. cDNA synthesis and PCR were done with the SuperScript preamplification system (Gibco–BRL) according to the manufacturer’s instructions, in a Robocycler 40 (Stratagene). For the simultaneous amplification of the IL-11Rα and IL-11Rβ cDNAs, primers (I) 5′-TGTGAGCTGG-GCTGCCACACAG-3′ and (II) 5′-CTGCCCCGCTTCCTTTCTTGC-3′ were used in a first PCR, and primers (III) 5′-GGATGCTGAGCAGGAACG-3′ and (IV) 5′-GGACAGTCTTGCACTAAAGGA-3′ were used for a nested PCR reaction (conditions: 2 min at 94 °C; 1 min at 94 °C, 2 min at 58 °C, 2 min at 74 °C, 30 cycles; 5 min at 74 °C). IL-11Rβ-specific PCR products were identified by digesting the ethanol-precipitated PCR products with BamHI, followed by analysis on 2% agarose gels. RT-PCR specific for IL-11Rα was done with primers (IV) 5′-GGAGAGAATTTCTTCAGGC-3′ (which binds in exon 1b) and (V) 5′-GGCTGTGGGCAGACATAAGTGAC-3′ (which binds in exon 4), and PCR for IL-11Rβ was done with primers (VI) 5′-GAGACATCTTGCTCTAAAGGA-3′ (which binds in exon 1) and (VII). As a control, primers for β-actin (5′-TGGAGAAATACCTGGCACACC-3′ and 5′-AATGGTGTAGACCTGGCCGT-3′) were used. PCR conditions were as described above. PCR products were analysed by Southern-blot hybridizations with gene-specific end-labelled oligonucleotides.

Abbreviations used: IL-11, interleukin-11; IL-11R, interleukin-11 receptor; RT, reverse transcription.

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The nucleotide sequence data reported here have been deposited in the EMBL Nucleotide Sequence Database under the accession numbers X94162 and X94163 for the IL-11Rα gene, X94157–X94161 for the IL-11Rβ gene and X98519 for the IL-11Rβ cDNA.
(VII) TGGCACCTACGTCACTGTGA (for IL-11Rα) and (VIII) GGCTTTCAGGAATGAAGCC (for IL-11Rβ).

Cloning of IL-11Rβ cDNAs

A testis cDNA library in λZAP (Stratagene) was screened with an IL-11R cDNA probe containing 1.2 kb of the coding sequence, using standard procedures [18]. Positive phages were eluted in 1 ml of SM [100 mM NaCl, 10 mM MgSO_4, 50 mM Tris/HCl, pH 7.5, and 0.01% (w/v) gelatin], and 1 μl of the phage eluate was used as template for PCR reactions with primers (I) and (II). PCR products were ethanol precipitated, dissolved in 10 mM Tris/HCl (pH 7.5), and digested with BamHI; then they were analysed on 1.5% agarose gels. Phages that gave rise to a BamHI-cleavable PCR product were isolated plaque pure and were analysed further.

Expression of IL-11Rβ protein and binding studies

The extracellular portion of IL-11Rβ cDNA was cloned into the pIG-1 vector [14], and expression and binding assays were performed as described previously [14].

RESULTS

The mouse genome contains a gene highly homologous to the IL-11Rz gene

Restriction mapping of genomic phage clones that were isolated with IL-11Rz cDNA probes suggested that they derived from two different genomic loci. Southern-blot hybridizations of genomic DNA from various mouse strains and from D3 ES cells confirmed that the clones represent two genuine loci present in the mouse genome, which we refer to as the IL-11Rz and IL-11Rβ genes (Figure 1A). All subcloned regions in IL-11Rz and IL-11Rβ that hybridized to IL-11Rz cDNA probes were identified by Southern-blot hybridizations and were sequenced. On the basis of this analysis, IL-11Rz and IL-11Rβ represent two genes with a highly similar exon/intron structure (Figure 1B), identical intron/exon boundaries, and almost identical nucleotide sequence in exons 2–13. The IL-11Rz and IL-11Rβ loci correspond to locus 1 and 2, respectively, in the study by Robb et al. [19], and IL-11Rβ corresponds to the Etl2 genomic locus in the report by Neuhaus et al. [12]. Sequence analysis indicated that the IL-11Rz locus encodes both earlier published cDNAs, termed Etl2 and NR1 [12,13], which represent two different naturally occurring transcripts, referred to as IL-11Rz a (former NR1) and b (former Etl2) transcripts, generated by differential usage of exons containing only 5′ untranslated sequence ([19]; P. Bilinski and A. Gossler, unpublished work).

Comparison of the exon 2–13 sequence with the IL-11Rz cDNA sequence showed that the IL-11Rβ sequence has 11 nucleotide exchanges that lead to 5 amino acid alterations (Ala–Thr; Pro–319 → Leu; Pro–345 → Leu; Val–384 → Leu; and Asp–401 → Glu) in the deduced IL-11Rβ-derived protein compared with the IL-11Rz protein (Figure 2), suggesting that IL-11Rz and IL-11Rβ encode very closely related but distinct proteins. A structure-based sequence comparison between the predicted IL-11Rz and IL-11Rβ proteins, using the prolactin receptor as a template, suggested that the amino acid differences

![Southern-blot hybridization of genomic DNA with IL-11R cDNA and structures of the IL-11Rz and IL-11Rβ genomic loci](image-url)

**Figure 1** Southern-blot hybridization of genomic DNA with IL-11R cDNA and structures of the IL-11Rz and IL-11Rβ genomic loci

(A) Southern-blot hybridization of genomic 129/SvDNA with a IL-11Rz probe spanning exons 1b–12. The probe detects multiple bands that correspond to and are consistent with DNA fragments of the IL-11Rz and IL-11Rβ loci. (B) Restriction maps and exon/intron structures of the IL-11Rz and IL-11Rβ genes. The black horizontal lines represent genomic DNA; exons are indicated by boxes below the genomic DNA. The coding sequence is in black; untranslated regions are in white. The insertion site of an enhancer trap construct in IL-11Rβ (former Etl2 locus described in [12]) is indicated by an arrow. The polymorphic BamHI site used to distinguish between IL-11Rz and IL-11Rβ transcripts is circled. The first exon of IL-11Rβ is not yet localized in the genomic sequence. Abbreviations used: E, EcoRI; B, BamHI; H, HindIII; S, SalI.
Two mouse interleukin-11 receptors

Table 1

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Table 2

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Figure 2 Alignment of the deduced IL-11Rα and IL-11Rβ amino acid sequences

The five amino acids changed in IL-11Rβ compared with IL-11Rα are indicated by white letters. The signal sequence, prolinin domains 1 and 2, and the membrane-spanning domain are indicated by grey shading.

Figure 3 Comparative expression analysis of IL-11Rα and IL-11Rβ

Southern-blot analyses of RT-PCR products obtained with RNA from various embryonic stages and adult tissues with specific primers for IL-11Rα and IL-11Rβ. (A) Localization of oligonucleotides used for PCR and hybridization. The roman numerals refer to the sequences given in the Materials and methods section. (B, C) Expression of IL-11Rα (B) and IL-11Rβ (C). The embryonic stages and adult tissues used as a source for RNA are indicated above each lane. No IL-11Rβ-specific product was detected in skeletal muscle, even after prolonged exposure.

Cloning of the IL11Rβ cDNA and expression analysis of IL-11Rβ

Sequence and restriction analysis of the IL-11Rα and IL-11Rβ genes revealed a polymorphic BamHI site present in exon 11 of IL-11Rβ (Figure 1B). This polymorphism is present in C57BL/6J, 129/SvPas and CD1 DNA (results not shown). To identify potential transcripts of the IL-11Rβ locus and to distinguish them from IL-11Rα transcripts, we analysed 129/Sv mRNA from various embryonic stages and adult tissues by RT-PCR with primers that amplify the region containing the polymorphic BamHI site. RT-PCR products from IL-11Rα, which do not contain a BamHI site, should result in a fragment that cannot be cleaved with BamHI. In contrast, digestion of the RT-PCR products from IL-11Rβ with BamHI should result in two smaller fragments. Only RT-PCR with RNA from adult thymus and testis gave rise to a DNA fragment that could in part be cleaved with BamHI to the expected fragments (results not shown). A testis cDNA library was screened with a probe containing 1.2 kb of IL-11Rα coding sequence. IL-11Rβ-specific clones were identified with a PCR assay, using the polymorphic BamHI site as described above. Three from 13 positive clones represented IL-11Rβ transcripts and were analysed further. Sequence analysis of these cDNA clones (EMBL Database accession number X98519) confirmed their identity with the deduced nucleotide sequence obtained from genomic sequences of the IL-11Rβ locus and between IL-11Rα and IL-11Rβ do not reside in regions of the protein likely to be involved in ligand recognition, which suggested that the IL-11Rβ protein, if expressed, might have the ability to interact with IL-11.
showed that the IL-11Rα and IL-11Rβ cDNAs have completely different 5′ untranslated sequences. Expression of IL-11Rβ was re-analysed with mRNA from various embryonic stages and adult tissues by RT-PCR with primers specific for IL-11Rβ and by Southern-blot analysis of the PCR products (Figure 3). The highest levels of IL-11Rβ transcripts were detected in adult testis. Low levels of IL-11Rβ transcripts were detected in embryos and all analysed adult tissues, with the exception of skeletal muscle. In contrast, the highest levels of IL-11Rα were detected in embryos, and in adult heart, kidney and skeletal muscle, whereas low levels of IL-11Rα transcripts were detected in testes (Figure 3). On this basis we conclude that IL-11Rα and IL-11Rβ differ in their tissue-specific expression.

**IL-11Rβ protein binds IL-11 with high affinity**

Since the extracellular portion of IL-11Rz is sufficient for IL-11 binding [14], the extracellular region encoded by a IL-11Rβ cDNA clone, which contained the complete open reading frame of the IL-11β protein, was introduced into a modified pIgβ eukaryotic expression vector [14], and the resulting construct was transfected into 293T cells. IL-11Rβ–Fc fusion protein was purified from culture supernatants by Protein A–Sepharose chromatography. Analysis by SDS/PAGE (results not shown) revealed a purified fusion protein of the predicted mass (75 kDa under reducing conditions).

The IL-11Rβ–Fc fusion protein was immobilized on Protein A-coated plates and tested for its ability to bind biotinylated murine IL-11 (Figure 4). IL-11 bound the protein in a saturable manner with an ED_{50} of 9.6 nM. This value is similar to our analysis of IL-11 binding to IL-11Rz [14]. We conclude that the IL-11Rβ gene encodes a specific high-affinity receptor for IL-11.

**DISCUSSION**

IL-11Rβ, the gene for a second high-affinity IL-11 receptor, was cloned and analysed. The gene is 99% identical with the IL-11Rα gene that encodes the previously cloned IL-11R cDNAs [12,13], and the overall exon/intron structure is well preserved. The IL-11Rβ gene is expressed, indicating that IL-11Rβ does not represent a transcriptionally silent pseudogene, as suggested previously [19]. IL-11Rα and IL-11Rβ transcripts were detected in all analysed embryonic stages and in all analysed adult tissues except skeletal muscle, which solely expressed IL-11Rz. By contrast, IL-11Rβ appeared to be more highly expressed in testes than IL-11Rα. Thus the published RNA in situ hybridization results with IL-11Rz cDNA probes [12] probably represent a composite expression pattern of both IL-11R genes at these embryonic stages, which could not be distinguished by Northern blot or RNA in situ hybridization with the probes used in these experiments.

The expressed IL-11Rβ protein binds IL-11 with similar properties to those of the IL-11Rz prototype. The signalling properties of IL-11Rβ, and its association with gp130, are currently under investigation. The amino acid differences between IL-11Rz and IL-11Rβ appear, as predicted, to have little effect on the interaction with the IL-11 ligand. It is unclear at present whether IL-11Rz and IL-11Rβ are functionally interchangeable or have distinct properties and biochemical functions. Distinct properties and functions of the IL-11Rα and IL-11Rβ proteins might be supported by the co-expression of the IL-11Rα and IL-11Rβ genes. However, since the expression of both transcripts on the cellular level is not known, these genes could be expressed in different cells of these tissues and serve very similar or identical functions. Further biochemical and genetic analysis will be required to address these questions.

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