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 enhancement of osteoclast-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 encodes a transmembrane protein, we have demonstrated that, like IL-6R and CNTF-R [16,17], it is biologically active in a soluble form, where it mediates the association of IL-11 with gp130 [14].

Here we report the molecular cloning and characterization of a second IL-11R gene (hereafter termed *IL-11Rβ*), which exhibits 99 % sequence identity with *IL-11Rα* 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 (129Sv^{Pas}) cells or mouse tissues and was analysed by Southern-blot hybridizations as described previously [12].

RNA isolation and reverse transcription (RT)–PCR analysis

Total RNA from tissues and embryos from 129Sv^{Pas} mice was isolated as described previously [12]. cDNA synthesis and PCRs 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′-TGTGGCTGGGCTGCCACACG-3′ and (II) 5′-CTGCCCCGCCTCTTTCTGCC-3′ were used in a first PCR, and primers (III) 5′-GGATGCTGGCACCTGGAGCG-3′ and (II) 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 *Bam*HI, followed by analysis on 2 % agarose gels. RT-PCR specific for *IL-11Rα* was done with primers (IV) 5′-GGAGGAAGTCTTGGAGGCC-3′ (which binds in exon 1b) and (V) 5′-GGTCTGGCAGACATAAGTGC-3′ (which binds in exon 4), and PCR for *IL-11Rβ* was done with primers (VI) 5′-GAGACATCTGTCCTCAAAGGA-3′ (which binds in exon 1) and (V). As a control, primers for β-actin (5′-TGGAGAAAATCTGGCACCAC-3′ and 5′-AATGGTGATGACCTGGCCGT-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) TGGCACTCAGTCACTGTGA (for *IL-11Rα*) and (VIII) GGCTCTTCAGGAATGAAGCC (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)/1 mM EDTA and digested with *Bam*HI; then they were analysed on 1.5 % agarose gels. Phages that gave rise to a *Bam*HI-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-11Rα* gene

Restriction mapping of genomic phage clones that were isolated with *IL-11Rα* 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-11Rα* and *IL-11Rβ* genes (Figure 1A). All subcloned regions in *IL-11Rα* and *IL-11Rβ* that hybridized to *IL-11Rα* cDNA probes were identified by Southern-blot hybridizations and were sequenced. On the basis of this analysis, *IL-11Rα* 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-11Rα* 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-11Rα* locus encodes both earlier published cDNAs, termed Etl2 and NR1 [12,13], which represent two different naturally occuring transcripts, referred to as *IL-11Rα* 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-11Rα* cDNA sequence showed that the *IL-11Rβ* sequence has 11 nucleotide exchanges that lead to 5 amino acid alterations (Ala-283 → 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-11Rα* protein (Figure 2), suggesting that *IL-11Rα* and *IL-11Rβ* encode very closely related but distinct proteins. A structure-based sequence comparison between the predicted IL-11Rα and IL-11Rβ proteins, using the prolactin receptor as a template, suggested that the amino acid differences

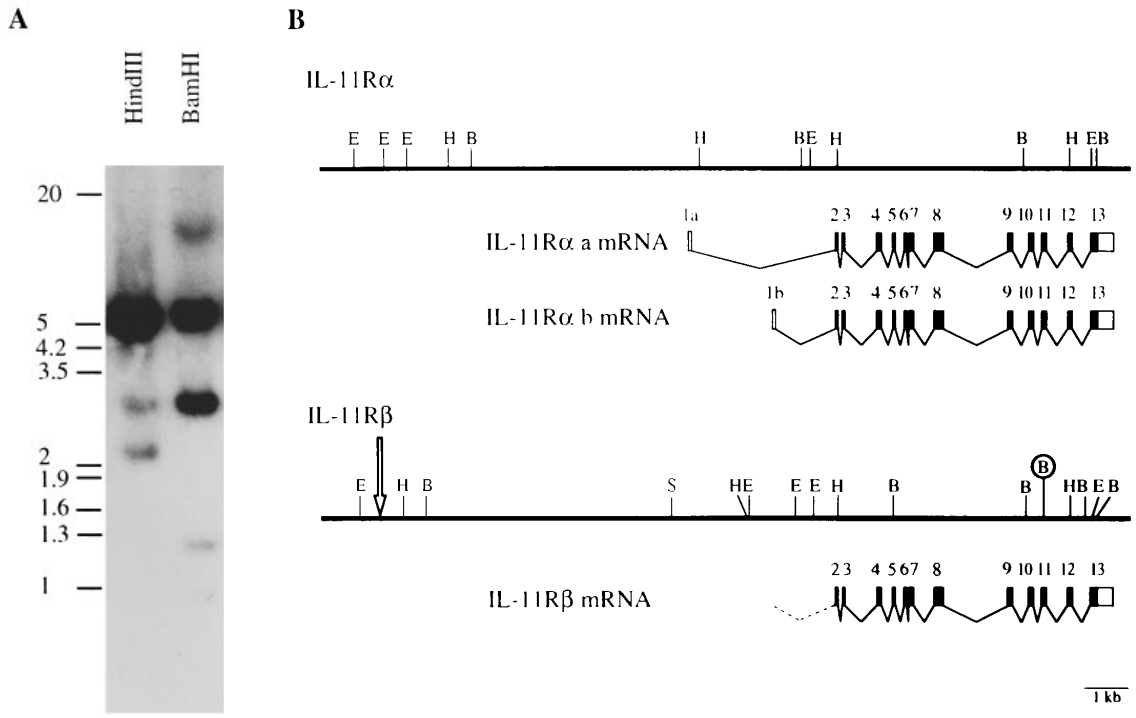

Figure 1 Southern-blot hybridization of genomic DNA with IL-11R cDNA and structures of the *IL-11Rα* and *IL-11Rβ* genomic loci

(**A**) Southern-blot hybridization of genomic 129/Sv DNA with a *IL-11Rα* probe spanning exons 1b–12. The probe detects multiple bands that correspond to and are consistent with DNA fragments of the *IL-11Rα* and *IL-11Rβ* loci. (**B**) Restriction maps and exon/intron structures of the *IL-11Rα* 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 *Bam*HI site used to distinguish between *IL-11Rα* and *IL-11Rβ* transcripts is circled. The first exon of *IL-11Rβ* is not yet localized in the genomic sequence. Abbreviations used: E, *Eco*RI; B, *Bam*HI; H, *Hin*dIII; S, *Sal*I.

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IL-11Rβ   MSSSCSGLTR VLVAVATALV SSSSPCPQAW GPPGVQYGQP GRPVMLCCPG      50
IL-11Rα   MSSSCSGLTR VLVAVATALV SSSSPCPQAW GPPGVQYGQP GRPVMLCCPG      50
                signal sequence

IL-11Rβ   VSAGTPVSWF RDGDSRLLQG PDSGLGHRLV LAQVDSPDEG TYVCQTLDGV      100
IL-11Rα   VSAGTPVSWF RDGDSRLLQG PDSGLGHRLV LAQVDSPDEG TYVCQTLDGV      100

IL-11Rβ   SGGMVTLKLG FPPARPEVSC QAVDYENFSC TWSPGQVSGL PTRYLTSYRK      150
IL-11Rα   SGGMVTLKLG FPPARPEVSC QAVDYENFSC TWSPGQVSGL PTRYLTSYRK      150

IL-11Rβ   KTLPGAESQR ESPSTGPWPC PQDPLEASRC VVHGAEFWSE YRINVTEVNP      200
IL-11Rα   KTLPGAESQR ESPSTGPWPC PQDPLEASRC VVHGAEFWSE YRINVTEVNP      200
                                  domain 1

IL-11Rβ   LGASTCLLDV RLQSILRPDP PQGLRVESVP GYPRRLHASW TYPASWRRQP      250
IL-11Rα   LGASTCLLDV RLQSILRPDP PQGLRVESVP GYPRRLHASW TYPASWRRQP      250

IL-11Rβ   HFLLKFRLQY RPAQHPAWST VEPIGLEEVI TDTVAGLPHA VRVSARDFLD      300
IL-11Rα   HFLLKFRLQY RPAQHPAWST VEPIGLEEVI TDAVAGLPHA VRVSARDFLD      300
                                  domain 2

IL-11Rβ   AGTWSAWSPE AWGTPSTGLL QDEIPDWSQG HGQQLEAVVA QEDSLAPARP      350
IL-11Rα   AGTWSAWSPE AWGTPSTGPL QDEIPDWSQG HGQQLEAVVA QEDSPAPARP      350

IL-11Rβ   SLQPDPRPLD HRDPLEQVAV LASLGIFSCL GLALGALALG LWLRLRRSGK      400
IL-11Rα   SLQPDPRPLD HRDPLEQVAV LASLGIFSCL GLAVGALALG LWLRLRRSGK      400
                              transmembrane domain

IL-11Rβ   EGPQKPGLLA PMIPVEKLPG IPNLQRTPEN FS                        432
IL-11Rα   DGPQKPGLLA PMIPVEKLPG IPNLQRTPEN FS                        432
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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, prolactin domains 1 and 2, and the membrane-spanning domain are indicated by grey shading.

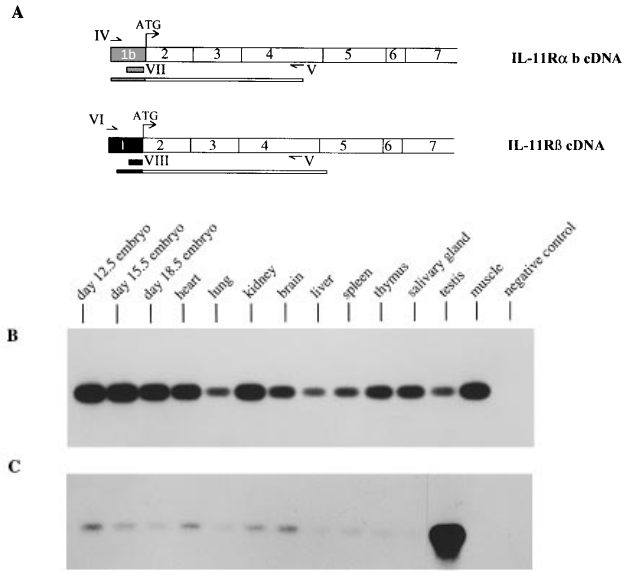

Figure 3 Comparative expression analysis of *IL-11Rα* and *IL-11Rβ*

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.

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.

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 *Bam*HI 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 *Bam*HI site. RT-PCR products from *IL-11Rα*, which do not contain a *Bam*HI site, should result in a fragment that cannot be cleaved with *Bam*HI. In contrast, digestion of the RT-PCR products from *IL-11Rβ* with *Bam*HI 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 *Bam*HI 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 *Bam*HI 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

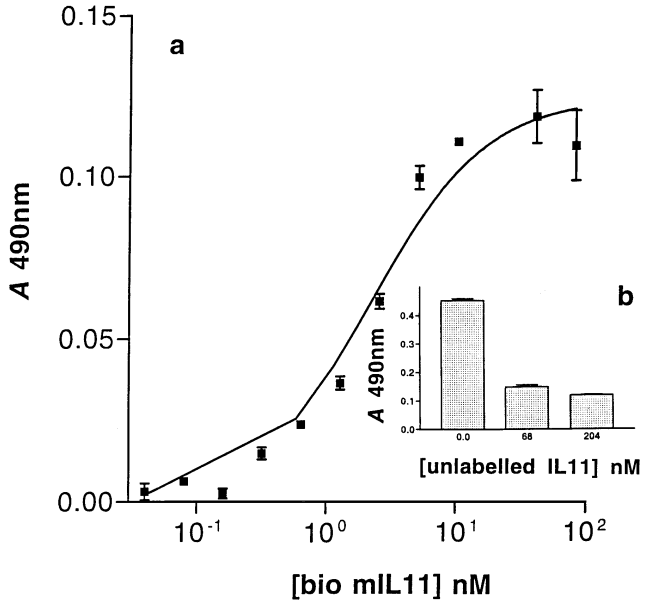

Figure 4 Interaction of IL-11Rβ–Fc with biotinylated murine IL-11 (bio mIL-11)

(**a**) IL-11Rβ–Fc was immobilized on Protein A-coated plates and was incubated with a series of concentrations of biotinylated murine IL-11. (**b**) Binding competition of biotinylated murine IL-11 with unlabelled IL-11. Data points are the results of three separate experiments, each performed in duplicate. The relative concentration of biotinylated IL-11 bound at equilibrium was determined by addition of streptavidin–horseradish peroxidase and colour development with *o*-phenyldiamine. Specific binding was determined by subtraction of values for background binding to parallel control (no IL-11Rβ–Fc) wells.

showed that the *IL-11R*α and *IL-11R*β cDNAs have completely different 5′ untranslated sequences. Expression of *IL-11R*β was re-analysed with mRNA from various embryonic stages and adult tissues by RT-PCR with primers specific for *IL-11R*β and by Southern-blot analysis of the PCR products (Figure 3). The highest levels of *IL-11R*β transcripts were detected in adult testis. Low levels of *IL-11R*β transcripts were detected in embryos and all analysed adult tissues, with the exception of skeletal muscle. In contrast, the highest levels of *IL-11R*α were detected in embryos, and in adult heart, kidney and skeletal muscle, whereas low levels of *IL-11R*α transcripts were detected in testes (Figure 3). On this basis we conclude that *IL-11R*α and *IL-11R*β differ in their tissue-specific expression.

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

Since the extracellular portion of IL-11Rα is sufficient for IL-11 binding [14], the extracellular region encoded by a *IL-11R*β cDNA clone, which contained the complete open reading frame of the IL-11β protein, was introduced into a modified pIG 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 affinity 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-11Rα [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-11R*α gene that encodes the previously cloned IL-11R cDNAs [12,13], and the overall exon/intron structure is well preserved. The *IL-11R*β gene is expressed, indicating that *IL-11R*β does not represent a transcriptionally silent pseudogene, as suggested previously [19]. *IL-11R*α and *IL-11R*β transcripts were detected in all analysed embryonic stages and in all analysed adult tissues except skeletal muscle, which solely expressed *IL-11R*α. By contrast, *IL-11R*β appeared to be more highly expressed in testes than *IL-11R*α. Thus the published RNA *in situ* hybridization results with *IL-11R*α 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-11Rα prototype. The signalling properties of IL-11Rβ, and its association with gp130, are currently under investigation. The amino acid differences between IL-11Rα 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-11Rα and *IL-11R*β 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-11R*α and *IL-11R*β 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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