The EMBO Journal vol.13 no.20 pp.4765–4775, 1994

Cloning of a murine IL-11 receptor α-chain; requirement for gp130 for high affinity binding and signal transduction

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Communicated by J.Tooze

An adult mouse liver cDNA library was screened with oligonucleotides corresponding to the conserved WSXWS motif of the haemopoietin receptor family. Using this method, cDNA clones encoding a novel receptor were isolated. The new receptor, named NR1, was most similar in sequence and predicted structure to the α-chain of the IL-6 receptor and mRNA was expressed in the 3T3-L1 pre-adipocytic cell line and in a range of primary tissues. Expression of NR1 in the factor-dependent haemopoietic cell line Ba/F3 resulted in the generation of low affinity receptors for IL-11 ($K_d \approx 10$ nM). The capacity to bind IL-11 with high affinity (K_d = 300–800 pM) appeared to require co-expression of both NR1 and gp130, the common subunit of the IL-6, leukaemia inhibitory factor (LIF), oncostatin M (OSM) and ciliary neurotrophic factor (CNTF) receptors. The expression of both NR1 and gp130 was also necessary for Ba/F3 cells to proliferate and M1 cells to undergo macrophage differentiation in response to IL-11.

Key words: cytokine receptor/interleukin-11 receptor

Introduction

Interleukin (IL)-11 is a functionally pleiotropic cytokine (Yang and Yin, 1992; Du and Williams, 1994) that was initially characterized because of its ability to stimulate the proliferation of an IL-6-dependent plasmacytoma cell line, T1165 (Paul *et al.*, 1990). Other biological actions of IL-11 are shared with IL-6, leukaemia inhibitory factor (LIF), ciliary neurotrophic factor (CNTF) and oncostatin M (OSM) (Rose and Bruce, 1991; Hilton, 1992; Kishimoto *et al.*, 1992; Davis and Yancopoulos, 1993; Du and Williams, 1994). These activities include the induction of multipotential haemopoietic progenitor cell proliferation (Musashi *et al.*, 1991; Schibler *et al.*, 1992; Tsuji *et al.*, 1992), enhancement of megakaryocyte and platelet formation (Burstein *et al.*, 1992; Teramura *et al.*, 1992; Yonemura *et al.*, 1992; Hangoc *et al.*, 1993), stimulation of acute phase protein synthesis (Baumann and Schendel, 1991), inhibition of adipocyte lipoprotein lipase activity (Kawashima *et al.*, 1991; Keller *et al.*, 1993) and effects on neurotransmitter phenotype (Fann and Patterson, 1994).

Overlapping biological function has been explained, in part, by the observation that certain cytokines can interact with receptor complexes containing common components (Nicola and Metcalf, 1991; Miyajima *et al.*, 1992; Kishimoto *et al.*, 1994). Three groups of cytokines have been defined in this way. IL-3, IL-5 and GM-CSF bind to specific α-chains with low affinity and interact with a common β-chain to generate a high affinity receptor capable of signal transduction (Nicola and Metcalf, 1991; Miyajima, 1992). Likewise IL-2, IL-4, IL-7, IL-9 and IL-13 are thought to bind both to specific receptor chains and to a common subunit, isolated initially as the γ-chain of the IL-2 receptor (Kishimoto *et al.*, 1994). The third group is even more complicated and includes the receptors for IL-6, IL-11, LIF, OSM and CNTF (Gearing and Ziegler, 1993; Kishimoto *et al.*, 1994). In this group, two subunits, the LIF receptor and gp130, are components of several receptors. IL-6, for example, binds first to its specific low affinity α-chain (Yamasaki *et al.*, 1988) and then to two subunits of gp130 to generate a high affinity complex (Hibi *et al.*, 1990; Murakami *et al.*, 1993). Similarly, CNTF initially binds to a specific α-chain (Davis *et al.*, 1991) and then to the LIF receptor and gp130 (Ip *et al.*, 1992; Davis *et al.*, 1993). Although they may exist, specific receptor α-chains have not yet been described for the LIF and OSM receptors. Rather, LIF binds to the LIF receptor with low affinity and to a complex of the LIF receptor and gp130 with high affinity (Gearing *et al.*, 1991, 1992), while OSM appears to bind to gp130 with low affinity and a complex of gp130 and the LIF receptor with high affinity (Gearing and Bruce, 1992; Gearing *et al.*, 1992).

The structure of the IL-11 receptor is less well defined (Yang and Yin, 1992). Since neutralizing antibodies to gp130 inhibit IL-11-dependent proliferation of TF-1 cells (Yin *et al.*, 1993), it is likely that gp130 also forms a part of the IL-11 receptor. The molecular weight of one component of the IL-11 receptor has been estimated to be 151 000 using cross-linking reagents. This is also consistent with binding to a receptor containing gp130 (Yin *et al.*, 1992). No evidence exists at present, however, for an IL-11 receptor α-chain similar in structure to the IL-6 and CNTF receptor α-chains

The receptor α- and β-chains described above form part of the haemopoietin receptor family (Gearing *et al.*, 1989a; Bazan, 1990; Cosman *et al.*, 1990). The family is

extensive and includes the specific binding subunits of the IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, erythropoietin, thrombopoietin (mpl-ligand), growth hormone, prolactin, CNTF, G-CSF and GM-CSF receptors, the IL-3, IL-5 and GM-CSF common β-chain, the IL-3-specific β-chain, the IL-2 receptor γ-chain, gp130, the LIF receptor and the p40 subunit of IL-12. The defining features of receptors of the haemopoietin family are found primarily in the extracellular domain and include four conserved cysteine residues, series of alternating hydrophobic and polar residues and the five amino acid motif Trp-Ser-Xaa-Trp-Ser (WSXWS).

In this study, degenerate oligonucleotides designed from the sequence encoding the WSXWS motif have been used to clone members of the haemopoietin receptor family without prior knowledge of their ligand. The application of this technique to a liver cDNA library resulted in the cloning of cDNAs for the LIF receptor, gp130, IL-7 receptor and a novel haemopoietin receptor, termed NR1. NR1 is demonstrated to be an α-chain of the IL-11 receptor and the formation of a high affinity IL-11 receptor capable of signal transduction is shown to require co-expression of NR1 and gp130, the common component of the IL-6, LIF, OSM and CNTF receptors.

Results

Cloning cytokine receptors on the basis of sequence similarity

Members of the haemopoietin receptor family exhibit a relatively low level of sequence similarity. One of the defining features of receptors in this family is the five amino acid motif Trp-Ser-Xaa-Trp-Ser (WSXWS; Gearing *et al.*, 1989a; Bazan, 1990; Cosman *et al.*, 1990). In an attempt to clone novel haemopoietin receptors, 10^6 plaques from an adult mouse liver cDNA library were screened with degenerate oligonucleotides corresponding to the WSXWS motif. λ Bacteriophage plaques that appeared positive on the duplicate primary filters were picked, eluted and isolated by two subsequent rounds of plaque enrichment. DNA from pure hybridizing plaques was then sequenced.

The utility of this technique was demonstrated by the identification of cDNAs encoding the murine IL-7 receptor using oligonucleotide HYB1, gp130 and the LIF receptor using oligonucleotide HYB2 and a novel sequence that appeared related to members of the haemopoietin receptor family using oligonucleotide HYB3 (Table I). We called this new receptor NR1. The cDNA (NR1-AZ36) encoding this receptor was sequenced in full and although it contained a polyadenylation signal and an extensive poly(A) tail, it was clearly truncated at the 5′ end (Figure 1).

Isolation of full-length NR1 cDNA and characterization of the novel cytokine receptor

To isolate a full-length NR1 cDNA, the original library and a second adult mouse liver cDNA library were screened with oligonucleotides (26 and 60; Table I) designed from the 5′ end of clone NR1-AZ36. Eight cDNA clones were isolated and four were sequenced completely (Figure 1). Analyses of the cDNA sequences revealed an open reading frame of 1296 bp which is predicted to encode a protein of 432 amino acids in length. The predicted primary sequence included a potential hydrophobic leader sequence (residues 1–23), an extracellular domain with two potential N-linked glycosylation sites (residues 24–367), a transmembrane domain (residues 368–393) and a short cytoplasmic tail (residues 394–432).

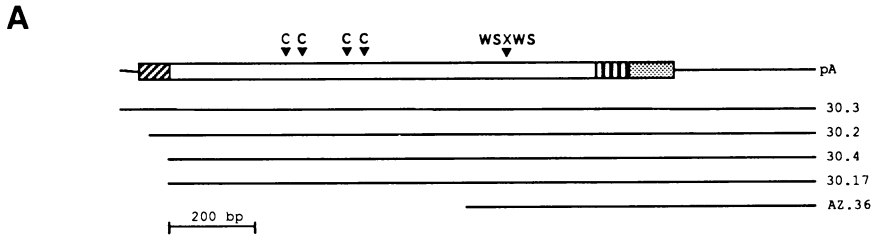
The extracellular domain contained residues characteristic of a classical haemopoietin domain (D200; Bazan, 1990), including proline residues preceding each 100 amino acid sub-domain (SD100), four conserved cysteine residues, a series of polar and hydrophobic residues and a WSXWS motif (Figures 1 and 2). The haemopoietin receptor domain of the new receptor was preceded by an 87 amino acid immunoglobulin-like domain and followed by 37 amino acids before the transmembrane domain. Regarding its overall structure and its primary sequence (Figures 2), the new receptor was most similar to the IL-6 receptor α-chain (24% amino acid identity), the CNTF receptor α-chain (22% amino acid identity) and the p40 subunit of IL-12 (16% amino acid identity).

Expression of NR1 mRNA

The distribution of NR1 mRNA expression was analysed by Northern blot and reverse transcriptase polymerase chain reaction (RT-PCR). Among a survey of polyadenylated RNA from 15 primary tissue samples and 17 cell lines, only RNA from the pre-adipocyte cell line 3T3-L1 yielded a very faint hybridizing band of ~2.0 kb in length

Table I. Sequence of oligonucleotides

Oligonucleotide	Sequence
HYB1	5′ (A/G)CTCCA(C/T)TC(A/G)CTCCA 3′
HYB2	5′ (A/G)CTCCA(A/G)TC(A/G)CTCCA 3′
HYB3	5′ (A/G)CTCCA(N)GC(C/T)CTCCA 3′
26	5′ TGGTCCACGGTGGAGCCCATTGGCT 3′
60	5′ CCACACGCGGTACGAGTCAGTGCCAGGGAC 3′
gt10for	5′ AGCAAGTTCAGCCTGGTTAAG 3′
gt10rev	5′ CTTATGAGTATTTCTTCCAGGGTA 3′
495	5′ CCCTTCATTGACCTCAACTACATG 3′
496	5′ CATGCCAGTGAGCTTCCCGTTCAG 3′
449	5′ GGGTCCTCCAGGGGTCCAGTATGG 3′
285	5′ GGAGGCCTCCAGAGGGT 3′
489	5′ CTCCTGTACTTGGAGTCCAGG 3′
731	5′ GGAAAGCTGTGGCGTGATGGCCGTGGGGCA 3′
30f1	5′ GGGCGGAGGCCGCTGGCGGGCG 3′
30r1	5′ TTATCAGCTGAAGTTCTCTGGGG 3′



B

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- 44                                                    gagagggtgagggcggaggccgctggcggcggctgccgcagaag

  1  ATG AGC AGC AGC TGC TCA GGG CTG ACC AGG GTC CTG GTG GCC GTG GCT ACA GCC CTG GTG
       M  S  S  S  C  S  G  L  T  R  V  L  V  A  V  A  T  A  L  V                  20

 61  TCT TCC TCC TCC CCC TGC CCC CAA GCT TGG GGT CCT CCA GGG GTC CAG TAT GGA CAA CCT
       S  S  S  S  P  C  P  Q  A  W  G  P  P  G  V  Q  Y  G  Q  P                  40

121  GGC AGG CCC GTG ATG CTG TGC TGC CCC GGA GTG AGT GCT GGG ACT CCA GTG TCC TGG TTT
       G  R  P  V  M  L  C  C  P  G  V  S  A  G  T  P  V  S  W  F                  60

181  CGG GAT GGA GAT TCA AGG CTG CTC CAG GGA CCT GAC TCT GGG TTA GGA CAC AGA CTG GTC
       R  D  G  D  S  R  L  L  Q  G  P  D  S  G  L  G  H  R  L  V                  80

241  TTG GCC CAG GTG GAC AGC CCT GAT GAA GGC ACT TAT GTC TGC CAG ACC CTG GAT GGT GTA
       L  A  Q  V  D  S  P  D  E  G  T  Y  V  C  Q  T  L  D  G  V                 100

301  TCA GGG GGC ATG GTG ACC CTG AAG CTG GGC TTT CCC CCA GCA CGT CCT GAA GTC TCC TGC
       S  G  G  M  V  T  L  K  L  G  F  P  P  A  R  P  E  V  S  C                 120

361  CAA GCG GTA GAC TAT GAA AAC TTC TCC TGT ACT TGG AGT CCA GGC CAG GTC AGC GGT TTG
       Q  A  V  D  Y  E  N  F  S  C  T  W  S  P  G  Q  V  S  G  L                 140

421  CCC ACC CGC TAC CTT ACT TCC TAC AGG AAG AAG ACG CTG CCA GGA GCT GAG AGT CAG AGG
       P  T  R  Y  L  T  S  Y  R  K  K  T  L  P  G  A  E  S  Q  R                 160

481  GAA AGT CCA TCC ACC GGG CCT TGG CCG TGT CCA CAG GAC CCT CTG GAG GCC TCC CGA TGT
       E  S  P  S  T  G  P  W  P  C  P  Q  D  P  L  E  A  S  R  C                 180

541  GTG GTC CAT GGG GCA GAG TTC TGG AGT GAG TAC CGG ATC AAT GTG ACC GAG GTG AAC CCA
       V  V  H  G  A  E  F  W  S  E  Y  R  I  N  V  T  E  V  N  P                 200

601  CTG GGT GCC AGC ACG TGC CTA CTG GAT GTG AGA TTA CAG AGC ATC TTG CGT CCT GAT CCA
       L  G  A  S  T  C  L  L  D  V  R  L  Q  S  I  L  R  P  D  P                 220

661  CCC CAA GGA CTG CGG GTG GAA TCC GTA CCT GGT TAC CCG AGA CGC CTG CAT GCC AGC TGG
       P  Q  G  L  R  V  E  S  V  P  G  Y  P  R  R  L  H  A  S  W                 240

721  ACA TAC CCT GCC TCC TGG CGT CGC CAA CCC CAC TTT CTG CTC AAG TTC CGG TTG CAA TAC
       T  Y  P  A  S  W  R  R  Q  P  H  F  L  L  K  F  R  L  Q  Y                 260

781  CGA CCA GCA CAG CAT CCA GCC TGG TCC ACG GTG GAG CCC ATT GGC TTG GAG GAA GTG ATA
       R  P  A  Q  H  P  A  W  S  T  V  E  P  I  G  L  E  E  V  I                 280

841  ACA GAT GCT GTG GCT GGG CTG CCA CAC GCG GTA CGA GTC AGT GCC AGG GAC TTT CTG GAT
       T  D  A  V  A  G  L  P  H  A  V  R  V  S  A  R  D  F  L  D                 300

901  GCT GGC ACC TGG AGC GCC TGG AGC CCA GAG GCC TGG GGT ACT CCT AGC ACT GGT CCC CTG
       A  G  T  W  S  A  W  S  P  E  A  W  G  T  P  S  T  G  P  L                 320

961  CAG GAT GAG ATA CCT GAT TGG AGC CAG GGA CAT GGA CAG CAG CTA GAG GCA GTA GTA GCT
       Q  D  E  I  P  D  W  S  Q  G  H  G  Q  Q  L  E  A  V  V  A                 340

1021 CAG GAG GAC AGC CCG GCT CCT GCA AGG CCT TCC TTG CAG CCG GAC CCA AGG CCA CTT GAT
       Q  E  D  S  P  A  P  A  R  P  S  L  Q  P  D  P  R  P  L  D                 360

1081 CAC AGG GAC CCC TTG GAG CAA GTA GCT GTG TTA GCG TCT CTG GGA ATC TTC TCT TGC CTT
       H  R  D  P  L  E  Q  V  A  V  L  A  S  L  G  I  F  S  C  L                 380

1141 GGC CTG GCT GTT GGA GCT CTG GCA CTG GGG CTC TGG CTG AGG CTG AGA CGG AGT GGG AAG
       G  L  A  V  G  A  L  A  L  G  L  W  L  R  L  R  R  S  G  K                 400

1201 GAT GGA CCG CAA AAA CCT GGG CTC TTG GCA CCC ATG ATC CCG GTG GAA AAG CTT CCA GGA
       D  G  P  Q  K  P  G  L  L  A  P  M  I  P  V  E  K  L  P  G                 420

1261 ATT CCA AAC CTG CAG AGG ACC CCA GAG AAC TTC AGC TGA tttcatctgtaacccggtcagactggg
       I  P  N  L  Q  R  T  P  E  N  F  S  *

1327 ggcagaaagaggcggggcagtggatccctgtggatggaggtctcagctgaaagtctgagctcttttctttgacacctat
1405 actccaaacttgctgccggctgaaggctgtctggacttccgatgtcctgaggtggaagtccacctgaggaatgtgtaca
1483 gaagtctgtgttcctgtgatcgtgtgtgtatgtgagacagggagcaaaagttctctgcatgtgtgtacagatgattgga
1561 gagtgtgtgcggtcttgggcttggcccttctgggaagtgtgaagagttgaaataaaagagacggaagtttttggaaaaa
1639 aaaaaaaaaaaaaaaaaa
```

Fig. 1. Nucleotide sequence, predicted amino acid sequence and cDNA structure of NR1. (**A**) Structure of the NR1 cDNA, showing the 5′ and 3′ untranslated regions (solid line) and the coding region containing the predicted signal sequence (▨), the mature extracellular domain (□), the transmembrane domain (▥) and the cytoplasmic domain (⊡). The approximate position of the cysteines and WSXWS motif conserved among members of the haemopoietin receptor family are shown above. The size and extent of each of the NR1 cDNA clones that were sequenced completely are shown below. (**B**) The nucleotide and predicted amino acid sequence of NR1. The untranslated region is shown in lower case and the coding region in upper case. The conventional one letter code for amino acids is employed throughout. The two potential asparagine-linked glycosylation sites (NXS/T) are shown underlined and the conserved cysteines and WSXWS motif are shown in bold type. The potential signal sequence and the transmembrane domain are highlighted by bars between the nucleotide and amino acid sequence. The 200 amino acid haemopoietin domain (D200) is boxed and a broken line separates the two 100 amino acid sub-domains (SD100) that comprise the D200 domain. A consensus polyadenylation signal in the 3′ untranslated region is shown in bold type.

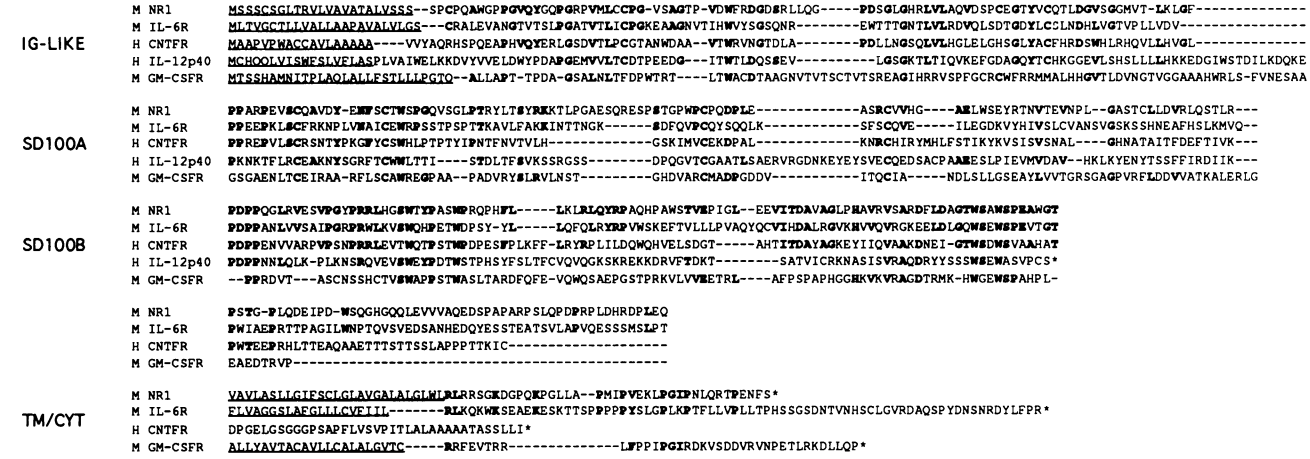

Fig. 2. Comparison of NR1 with other members of the haemopoietin receptor family. Amino acid sequence alignment of murine NR1, the murine IL-6 receptor α-chain, the human CNTF receptor α-chain, the p40 subunit of human IL-12 and the murine GM-CSF receptor. Alignments were carried out by eye and gaps were introduced to increase apparent sequence similarity. The leader sequences and potential transmembrane regions are underlined and residues identical to NR1 are shown in bold type.

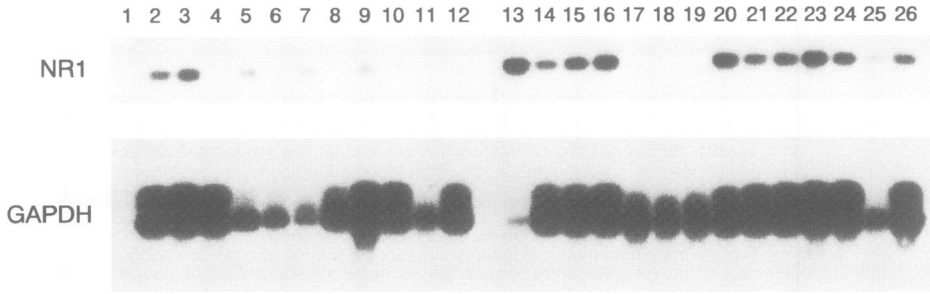

Fig. 3. RT-PCR analyses of NR1 mRNA. Cytoplasmic RNA was prepared from the following sources: lane 2, 3T3-L1 cells; lane 3, BAd cells; lane 4, UMR-106 cells; lane 5, PC13 cells; lane 6, NFS-60 cells; lane 7, FDCP-1 cells; lane 8, 32D cells; lane 9, D35 cells; lane 10, M1 cells; lane 11, J774 cells; lane 12, WEHI-3B D^- cells; lane 13, human bone marrow; lane 14, mouse bone marrow; lane 15, mouse spleen; lane 16, mouse thymus; lane 17, mouse ovary; lane 18, mouse uterus; lane 19, mouse testis; lane 20, mouse epididymus; lane 21, mouse brain; lane 22, mouse heart; lane 23, mouse kidney; lane 24, mouse thigh muscle; lane 25, mouse liver and lane 26, mouse salivary gland. Each RNA sample (1 μg) and a control containing no RNA (lane 1) was subject to reverse transcription, with an identical reaction performed in the absence of reverse transcriptase. Aliquots (5 μl) of the first strand cDNA reaction were subjected to PCR with primers specific for NR1 (upper panel) or the control GAPDH (lower panel). PCR products were resolved on a 1.0% (w/v) agarose gel, transferred to Zetaprobe and hybridized with internal oligonucleotides specific to GAPDH or NR1.

on a Northern blot. This compares with a length of ~1650 bp for the longest NR1 cDNA isolated and suggests that this clone may not be complete at the 5′ end.

The low abundance of the NR1 mRNA suggested from Northern analyses prompted us to use RT-PCR as a more sensitive means of detection. All samples contained GAPDH mRNA as judged by RT-PCR (Figure 3); however, only 3T3-L1 cells, the stromal line BAd, the embryonic carcinoma cell line PC13 and the factor-dependent haemopoietic cell lines FDCP-1 and D35 expressed NR1 mRNA (Figure 3). A wide range of primary tissues were also positive (Figure 3), including the haemopoietic tissues bone marrow, spleen and thymus, as well as the liver, brain, heart, kidney, muscle and salivary gland. In mRNA samples from several cell lines and tissues, transcripts for NR1 could not be detected. Such negative results need to be confirmed using a more quantitative approach to mRNA analysis. In control experiments, PCR was performed on mRNA that had not been subjected to reverse transcription; in none of these samples was a NR1 product detected.

NR1 is a low affinity receptor for IL-11 which interacts with gp130 to generate a high affinity IL-11 receptor

Given its sequence similarity with the IL-6 and CNTF receptor α-chains and the expression of NR1 mRNA by 3T3-L1 cells, we reasoned that NR1 might be a receptor α-chain which interacts with gp130 and/or the LIF receptor to generate a high affinity complex capable of signal transduction. Since no receptor α-chains similar in structure to the IL-6 receptor α-chain have been described for LIF, OSM or IL-11, these cytokines represented attractive candidates for the cognate ligand of NR1.

To test whether LIF, OSM or IL-11 bound to the new receptor, we stably transfected the factor-dependent haemopoietic cell line Ba/F3 and the mouse leukaemic cell line M1 with the vector pEF-BOS containing the cDNA encoding NR1. Parental M1 cells express the LIF receptor and gp130 and therefore bound ^{125}I-labelled LIF and ^{125}I-labelled OSM. Expression of NR1 in M1 cells did not result in altered binding of either ^{125}I-labelled LIF

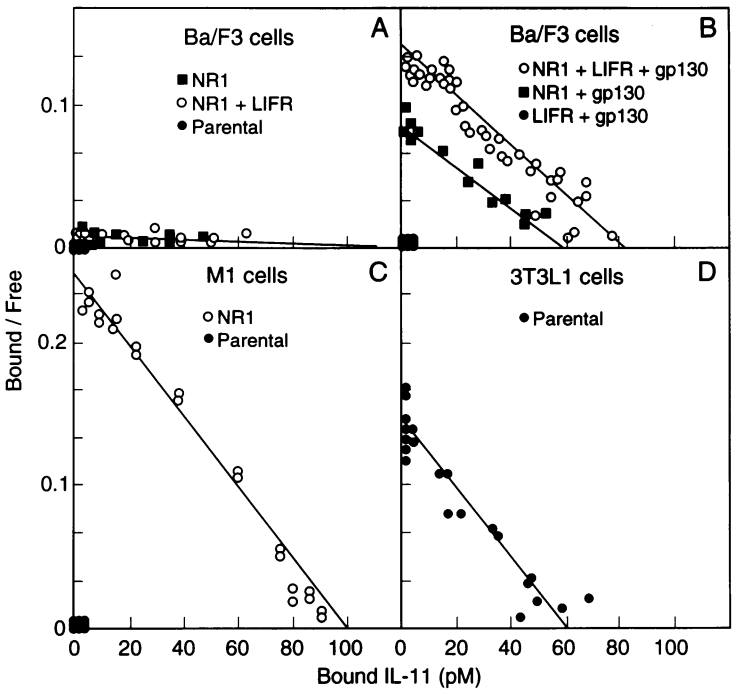

Fig. 4. Scatchard analyses of saturation isotherms of IL-11 binding to various cell lines. (**A**) Parental Ba/F3 cells (●), Ba/F3 cells expressing NR1 (○), Ba/F3 cells expressing NR1 and the LIF receptor (■). (**B**) Ba/F3 cells expressing the LIF receptor and gp130 (●), Ba/F3 cells expressing NR1 and gp130 (■), Ba/F3 cells expressing the NR1, LIF receptor and gp130 (○). (**C**) Parental M1 cells (●), M1 cells expressing NR1 (○) and (**D**) 3T3-L1 cells (●) were incubated with various concentrations of labelled IL-11 in the presence or absence of a 10- to 100-fold excess of unlabelled IL-11. After 18 h incubation on ice, bound and free IL-11 were separated by centrifugation through FCS. Bound and free ^{125}I-labelled IL-11 was quantitated in a γ-counter and the data was depicted as a Scatchard transformation. In each case data were normalized for cell number and shown as binding to 10^6 cells. The amount of non-specific binding varied with cell number and cell type, but was generally between 0.2 and 1% of the total amount of ^{125}I-labelled IL-11 added.

or ^{125}I-labelled OSM. In contrast, Ba/F3 cells expressed neither the LIF receptor nor gp130 and no binding of ^{125}I-labelled LIF and ^{125}I-labelled OSM was observed on either parental Ba/F3 cells or cells expressing NR1.

No binding of ^{125}I-labelled IL-11 could be detected on parental M1 or Ba/F3 cells (Figure 4A and C). Strikingly, however, expression of NR1 in each cell type resulted in the ability to bind ^{125}I-labelled IL-11, which suggested that NR1 might be the α-chain of the IL-11 receptor. Scatchard transformation of the saturation binding isotherms revealed that the affinity of IL-11 for its receptor differed between the two cell types (Figure 4A versus C). Binding of ^{125}I-labelled IL-11 to Ba/F3 cells expressing NR1 was of very low affinity. The apparent equilibrium dissociation constant (K_d) for this interaction was estimated to be ~10 nM and cells expressed an average of between 2000 and 8000 receptors at their surface (Figure 4A). M1 cells transfected with a NR1 cDNA expressed a similar number of IL-11 receptors (Figure 4C) but the affinity of the interaction was higher (K_d = 400–800 pM). The IL-11 receptors expressed on M1 cells transfected with NR1 were similar in affinity to the receptors expressed naturally on 3T3-L1 cells (Figure 4D).

A simple explanation for the generation of low affinity or high affinity receptors according to the cell type in which NR1 is expressed is that NR1 itself has an intrinsically low affinity for IL-11, but M1 cells express an excess of an additional receptor component required for the generation of a high affinity complex. Indirect evidence exists for the role of gp130 in IL-11 receptor signal transduction, since neutralizing antibodies to gp130 inhibited IL-11-induced proliferation of TF-1 cells (Yin *et al.*, 1993). In order to test this proposition directly, we expressed gp130 and/or the LIF receptor in parental Ba/F3 cells or in Ba/F3 cells expressing NR1.

Parental Ba/F3 cells and Ba/F3 cells expressing gp130 and the LIF receptor, alone or in combination did not bind IL-11 (Figure 4A and B). Ba/F3 cells expressing NR1 and the LIF receptor bound IL-11 with a very low affinity that was indistinguishable from cells expressing NR1 alone (Figure 4A). In contrast, when gp130 and NR1 were co-expressed in Ba/F3 cells, high affinity receptors for IL-11 were generated (Figure 4B). The affinity of these receptors was similar to that of receptors expressed by 3T3-L1 cells and M1 cells expressing NR1 (Figure 4B–D). Expression of the LIF receptor with NR1 and gp130 did not increase the affinity of IL-11 binding (Figure 4B).

NR1 appears to be a receptor that is specific for IL-11, since the binding of ^{125}I-labelled IL-11 to Ba/F3 cells expressing NR1 was competed for by unlabelled IL-11, but not IL-6, LIF, OSM or IL-3 (Figure 5). A more complex situation existed in cells in which NR1 was expressed with gp130 and the LIF receptor. The binding of ^{125}I-labelled IL-11 to Ba/F3 cells expressing NR1 and gp130 was competed for by OSM and unlabelled IL-11 (Figure 5), while binding to Ba/F3 cells expressing NR1, gp130 and the LIF receptor was competed for by LIF, as well as OSM and IL-11 (Figure 5).

Co-expression of NR1 and gp130 allows a proliferative and differentiative response to IL-11

Many cytokines affect cell differentiation as well as cell division. In the absence of differentiative stimuli, colonies

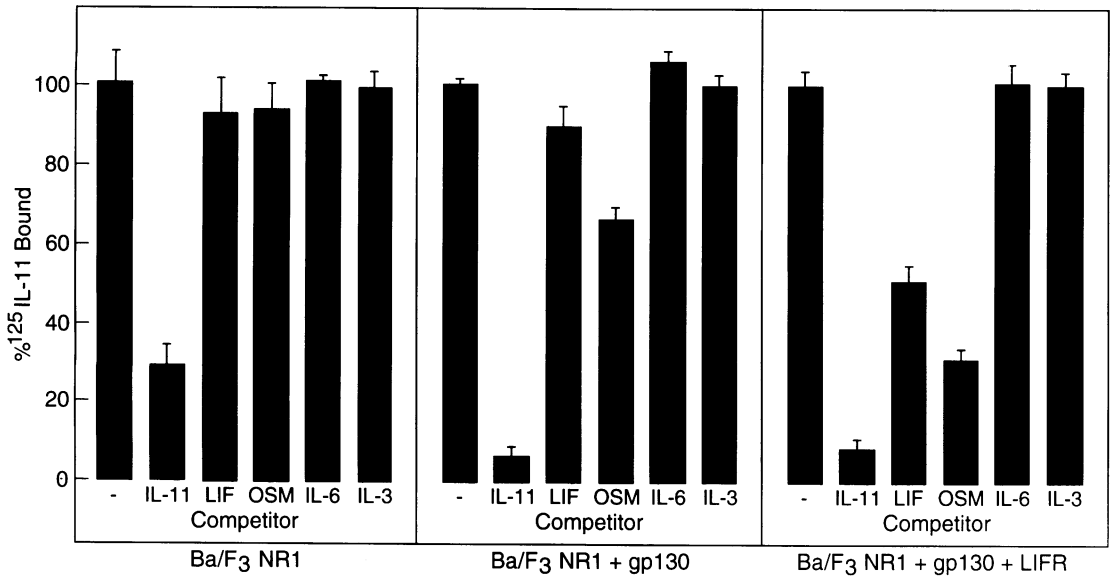

Fig. 5. Molecular specificity of IL-11 binding to various cell lines. Ba/F3 cells expressing the designated receptors were incubated in 100 µl medium containing 60 000 c.p.m. (Ba/F3 NR1) or 6000 c.p.m. ^{125}I-labelled IL-11 (Ba/F3 NR1/gp130 and Ba/F3 NR1/gp130/LIF receptor) in the presence or absence of 20 ng IL-11 or 200 ng IL-6, LIF, OSM or IL-3. After 60 min incubation on ice, bound and free IL-11 were separated by centrifugation through FCS. Bound and free ^{125}I-labelled IL-11 were quantitated in a γ-counter and the amount of binding was expressed as a percentage of that observed in the absence of competitor. Results are shown as the mean and range of duplicate determinations and similar results were obtained from two other independent experiments.

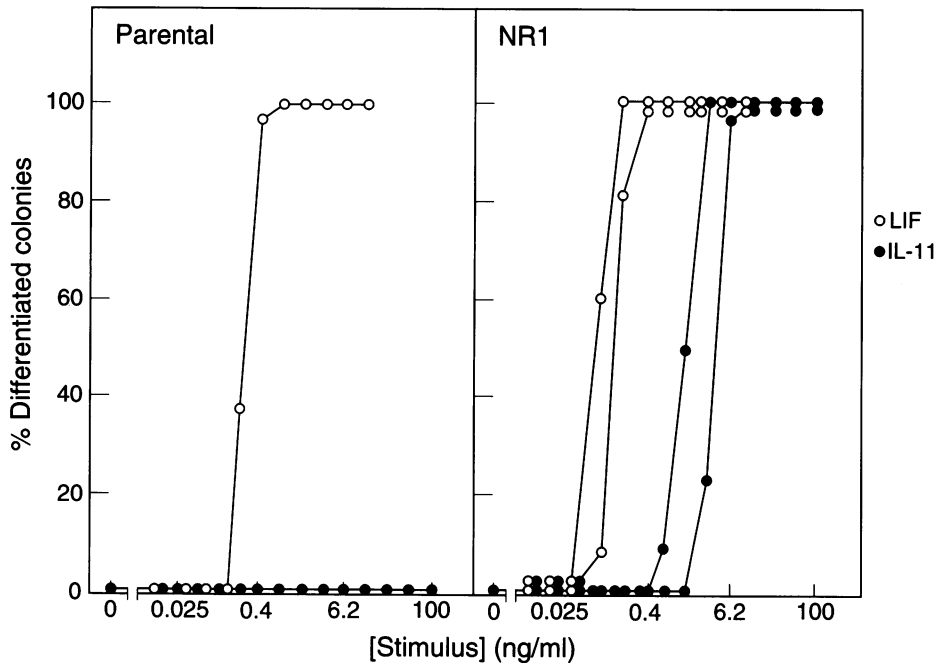

Fig. 6. Differentiation of M1 cells expressing NR1 in response to IL-11. Three hundred parental M1 cells (left panel) or M1 cells expressing NR1 (right panel) were cultured in 1 ml semi-solid agar with the designated concentration of LIF (○) or IL-11 (●). After 7 days, the proportion of colonies containing differentiated cells was determined.

of parental leukaemic M1 cells are tightly packed and are composed of undifferentiated blast cells. In response to LIF, OSM and IL-6, but not IL-11, M1 colonies grown in semi-solid agar become dispersed because of the induction of macrophage differentiation (Figure 6). In addition, LIF, OSM and IL-6 suppress the clonogenicity of M1 cells, resulting in the development of reduced numbers of colonies. M1 cells expressing NR1 exhibited a normal response to LIF, OSM and IL-6, but now differentiated into macrophages when stimulated by IL-11 (Figure 6). As with LIF, IL-6 and OSM, fewer colonies were produced by M1 cells expressing NR1 in the presence of IL-11 than in control cultures and these colonies contained fewer cells.

The IL-3-dependent haemopoietic cell line Ba/F3 has been used to study the capacity of a variety of cytokine receptors to transduce a proliferative signal. Ba/F3 cells are absolutely dependent on IL-3 for proliferation, but do not proliferate in response to IL-11, LIF or IL-6. We therefore determined whether expression of NR1, gp130

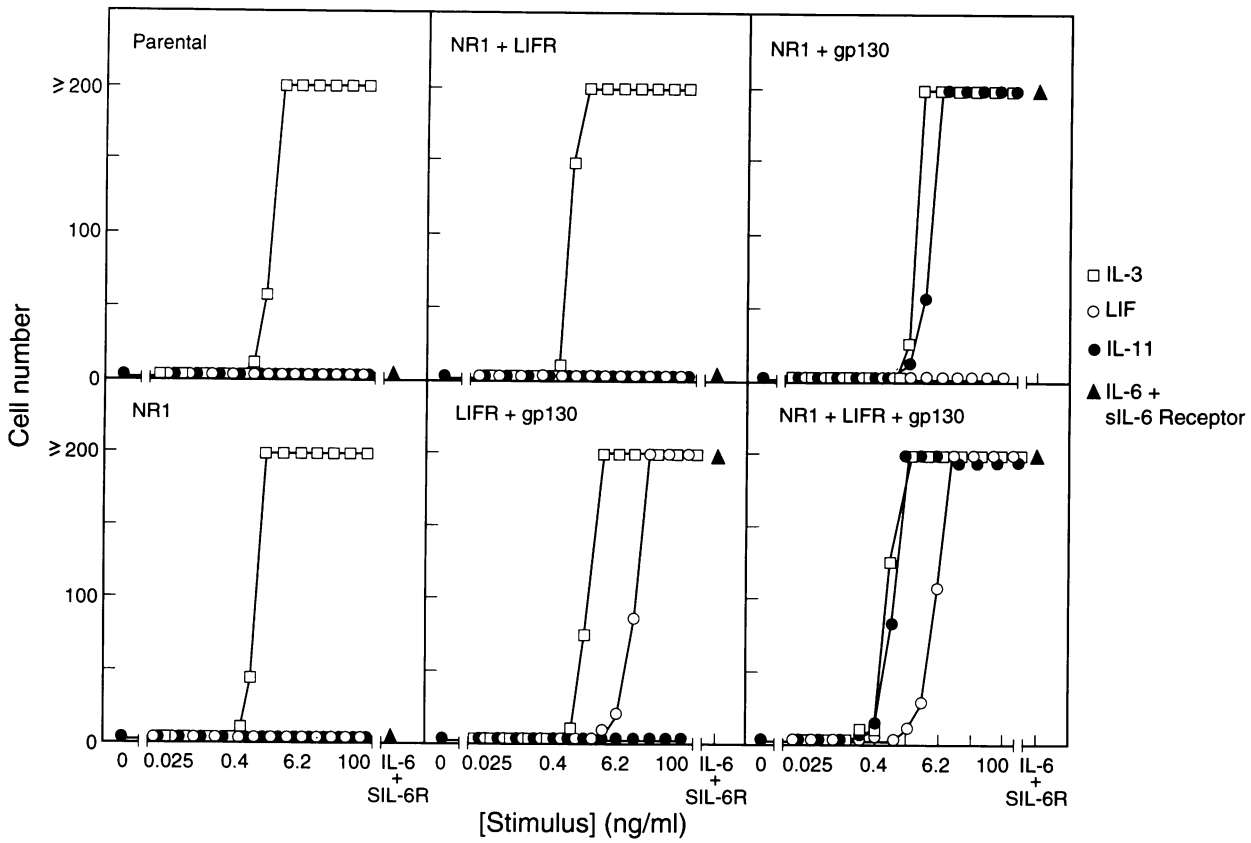

Fig. 7. Factor-dependent proliferation of Ba/F3 cells expressing various combinations of NR1, gp130 and the LIF receptor. Parental Ba/F3 cells, Ba/F3 cells expressing NR1, Ba/F3 cells expressing the NR1 and the LIF receptor, Ba/F3 cells expressing LIF receptor and gp130, Ba/F3 cells expressing NR1 and gp130 and Ba/F3 cells expressing NR1, the LIF receptor and gp130 were incubated at 200 cells/well in a volume of 15 μl with the designated concentrations of IL-11 (●), IL-3 (□) or LIF (○) or with 3 μg/ml IL-6 and 500 ng/ml soluble IL-6 receptor α-chain (▲). After 48 h the numbers of viable cells were counted.

and the LIF receptor broadened the spectrum of cytokines to which these cells could respond. While none of the cell lines examined could proliferate in response to IL-6 alone, each cell line that expressed gp130, irrespective of whether or not other receptors were co-expressed, proliferated in response to a combination of IL-6 and soluble IL-6 receptor α-chain (Figure 7). Proliferation in response to LIF required co-expression of the LIF receptor and gp130 (Figure 7) but these cells were unable to proliferate in response to IL-11. Likewise, Ba/F3 cells expressing NR1 alone or NR1 and the LIF receptor were incapable of responding to IL-11 (Figure 7). Response to IL-11 required co-expression of both NR1 and gp130 (Figure 7). Half-maximal proliferation of these cells occurred at an IL-11 concentration of between 20 and 100 pg/ml. Expression of the LIF receptor, in addition to NR1 and gp130, did not alter this response (Figure 7).

Discussion

Members of the TNF/NGF receptor family (Camerini *et al.*, 1991; Itoh *et al.*, 1991; Armitage *et al.*, 1992; Goodwin *et al.*, 1993; Smith *et al.*, 1993; Suda *et al.*, 1993), the G-protein-coupled receptors (Buck and Axel, 1991) and the receptor kinases (Peles *et al.*, 1992; Lyman *et al.*, 1993) have all been cloned without reference to their physiological ligand. Previously, however, this has proved difficult for members of the haemopoietin receptor family. In this study, a general method for cloning members of the haemopoietin receptor family has been described. The utility of this technique was highlighted by the cloning of cDNAs for the LIF receptor, gp130 and the IL-7 receptor, as well as a novel member of the haemopoietin receptor family, NR1. NR1 contained all of the features of a typical haemopoietin receptor, including four conserved cysteine residues and the five amino acid motif WSXWS and was shown to be the α-chain of the IL-11 receptor.

The IL-11 receptor

The properties of the IL-11 receptor described in this paper agree well with the preliminary characterization made by Yang and colleagues (Yang and Yin, 1992; Yin *et al.*, 1992, 1993). Notably, the affinity (K_d = 300–400 pM) that has been described for the IL-11 receptor on 3T3-L1 cells and other cell lines is consistent with the binding of IL-11 to Ba/F3 cells expressing both the IL-11 receptor α-chain (NR1) and gp130 and to M1 cells expressing the IL-11 receptor α-chain (Figure 4; Yin *et al.*, 1992). Further, the observation that neutralizing antibodies to gp130 inhibit the biological action of IL-11 and the cross-linking of IL-11 to a M_r 151 000 species is consistent with the role of gp130 in IL-11 receptor function demonstrated in this paper (Figures 4–7; Yang and Yin, 1992; Yin *et al.*, 1993).

Parallels between the IL-11, IL-6 and CNTF receptors

The extracellular region of the IL-11 receptor α-chain (NR1) exhibited the greatest sequence similarity to the α-chain of the IL-6 receptor (24% amino acid identity) and the α-chain of the CNTF receptor (22% amino acid identity). In addition to a presumed structural similarity, a number of functional similarities have become evident concerning the receptors for IL-6, CNTF and IL-11. First, the receptor α-chains bind their respective ligands with a low affinity ($K_d \approx 10$ nM for IL-11 and CNTF; $K_d \approx 1$–2 nM for IL-6, Figure 4; Hibi *et al.*, 1990; Gearing *et al.*, 1994). Second, the low affinity α-chains of the IL-6, CNTF and IL-11 receptors, although necessary, are not sufficient for transducing a biological signal (Figure 7; Hibi *et al.*, 1990; Ip *et al.*, 1992; Davis *et al.*, 1993; Gearing *et al.*, 1994). Third, for both IL-6 and IL-11, the generation of high affinity receptors (K_d = 400–800 pM for IL-11, 100–200 pM for CNTF and 40–50 pM for IL-6, Figure 4; Hibi *et al.*, 1990) capable of signal transduction requires co-expression of the receptor α-subunit and gp130 (Figure 7; Hibi *et al.*, 1990). In contrast, the generation of high affinity CNTF receptors requires co-expression of the specific receptor α-subunit, gp130 and the LIF receptor (Davis *et al.*, 1993; Gearing *et al.*, 1994).

It will be interesting to determine whether other parallels between the IL-6 receptor, CNTF receptor and IL-11 receptor also exist. For example, although the IL-6 receptor α-chain is a transmembrane protein, the membrane-spanning and cytoplasmic domains are unnecessary for ligand binding, for IL-6-induced association of the α-chain and gp130 and for signal transduction (Hibi *et al.*, 1990). The α-chain of the CNTF receptor itself lacks a membrane spanning and cytoplasmic domain and is instead tethered in the membrane via a glycosyl-phosphatidylinositol anchor (Davis *et al.*, 1991). The short cytoplasmic domain of the IL-11 receptor α-chain and its sequence similarity to the cytoplasmic domain of the IL-6 receptor α-chain (Figures 1 and 2), suggests that this region may be expendable in terms of generating a biological response. The production of secreted IL-11 receptor α-chain will enable this proposition to be tested.

A number of implications arise from the observation that IL-6, IL-11, LIF, OSM and CNTF utilize common components in the generation of high affinity receptors (Figures 4–7; Hibi *et al.*, 1990; Gearing and Bruce, 1992; Gearing *et al.*, 1992, 1994; Davis *et al.*, 1993;Yin *et al.*, 1993). If, as has been suggested, the α-chains of the IL-6 and IL-11 receptors play little direct role in signal transduction, then in any cell expressing both the IL-6 and IL-11 receptor α-chains, as well as gp130, the biological response to the two cytokines should be indistinguishable. The same would not be true of a response to CNTF, LIF or OSM, since these cytokines require both the LIF receptor and gp130 for signal transduction.

Further, it would be predicted that a complex relationship exists between the binding of different cytokines to cells expressing various receptor α-chains, in addition to the LIF receptor and gp130. For example, in cells expressing LIF receptors, IL-11 receptor α-chains and limiting numbers of gp130, exposure to LIF or OSM may result in the sequestration of gp130 in high affinity complexes and hence the inability to generate high affinity IL-11 receptors. Indeed, competition for IL-11 receptors by OSM and LIF could be demonstrated using Ba/F3 cells expressing the IL-11 receptor α-chain (NR1), gp130 and the LIF receptor, but not on Ba/F3 cells expressing the IL-11 receptor α-chain (NR1) alone (Figure 5). Whether this reduction in IL-11 binding observed in the presence of LIF and OSM was due to a reduction in the affinity of the IL-11 receptor remains to be determined; however, this type of receptor 'cross-talk' clearly has biological implications. Since, for example, OSM can bind directly to gp130 without eliciting a biological response, it might be anticipated that high concentrations of OSM would antagonize the action of IL-11 and IL-6 in cells that do not also express the LIF receptor. The generation of Ba/F3 cells expressing various combinations of the IL-11 receptor, LIF receptor and gp130 (Figure 7) should allow these possibilities to be explored further.

Implications of a general method for cloning cytokine receptors in the absence of information about their ligand

Although 20 or more members of the haemopoietin receptor family have been described, almost all have been cloned based on their ability to bind to previously discovered cytokines. In turn, most cytokines have been identified because of their biological action on lymphoid cells, committed haemopoietic progenitors and haemopoietic cell lines. The proliferation and differentiation of other cell types, such as haemopoietic stem cells, gastrointestinal tract epithelium and many neuronal populations, have proved more difficult to study *in vitro*. As a consequence, the molecular bases of their regulation are less well defined. The capacity to clone 'orphan' receptors from these tissues and subsequently identify their corresponding ligand is one mechanism by which the difficulties in culturing certain cells may be bypassed. The difficulty in isolating haemopoietin receptors stems from the low degree of sequence similarity exhibited by members of this family (Gearing *et al.*, 1989a; Bazan, 1990; Cosman *et al.*, 1990). The motifs that define the haemopoietin receptor family are present in the extracellular domain and include four conserved cysteine residues, a series of scattered proline and tryptophan residues and the five amino acid motif Trp-Ser-Xaa-Trp-Ser (WSXWS). In this paper a method has been described for the cloning of cDNAs encoding haemopoietin receptors on the basis of their ability to hybridize to oligonucleotides designed against the WSXWS motif. This method should allow the cloning of related receptors, and by extension their cognate ligands, expressed by a wide variety of adult and embryonic tissues.

Materials and methods

Library screening

Commercial adult mouse liver cDNA libraries cloned into λgt10 (Clonetech, CA) and λZAP (Stratagene, La Jolla, CA, USA) were used to infect *Escherichia coli* of the strain LE392. Infected bacteria were grown on 20 150 mm agar plates, to give ~50 000 plaques per plate. Plaques were then transferred to duplicate 150 mm diameter nylon membranes (Colony/Plaque Screen™; NEN Research Products, Boston, MA, USA), bacteria were lysed and the DNA was denatured-fixed by autoclaving at 100°C for 1 min with dry exhaust. The filters were rinsed twice in 0.1% (w/v) SDS, 0.1× SSC (150 mM sodium chloride,

15 mM sodium citrate dihydrate) at room temperature and pre-hybridized overnight at 37°C in 6× SSC containing 2 mg/ml bovine serum albumin, 2 mg/ml Ficoll, 2 mg/ml polyvinylpyrrolidone, 100 μM ATP, 10 μg/ml tRNA, 2 mM sodium pyrophosphate, 2 mg/ml salmon sperm DNA, 0.1% NP-40 and 200 mg/ml sodium azide. The pre-hybridization buffer was removed. Degenerate oligonucleotides (1.2 μg) for hybridization (HYB1, HYB2 and HYB3, Table I) were phosphorylated with T4 polynucleotide kinase using 960 μCi [γ-^{32}P]ATP (Bresatec, Adelaide, S.A., Australia). Unincorporated ATP was separated from the labelled oligonucleotide using a pre-packed gel filtration column (NAP-5; Pharmacia, Uppsala, Sweden). Filters were hybridized overnight at 37°C in 80 ml prehybridization buffer containing 0.1% (w/v) SDS, rather than NP40, and 10^6-10^7 c.p.m./ml labelled oligonucleotide. Filters were briefly rinsed twice at room temperature in 6× SSC, 0.1% (v/v) SDS, twice for 30 min at 45°C in a shaking water bath containing 1.5 l of the same buffer and then briefly in 6× SSC at room temperature. Filters were then blotted dry and exposed to autoradiographic film at −70°C using intensifying screens, for 7–14 days prior to development.

Plaques that appeared positive on orientated duplicate filters were picked, eluted in 1 ml 100 mM NaCl, 10 mM $MgCl_2$, 10 mM Tris−HCl, pH 7.4, containing 0.5% (w/v) gelatin and 0.5% (v/v) chloroform and stored at 4°C. After 2 days LE392 cells were infected with the eluate from the primary plugs and replated for the secondary screen. This process was repeated until hybridizing plaques were pure.

Analyses of positive plaques

DNA was prepared from positive plaques using Promega Magic Lambda DNA columns (Promega Corporation, WI) according to the manufacturer's instructions. DNA (100 ng) from each positive bacteriophage was sequenced using a fmol sequencing kit (Promega Corporation, Madison, WI) with the ^{33}P-labelled oligonucleotide primers gt10for, gt10rev and either HYB1, HYB2 or HYB3. The products were resolved on a 6% polyacrylamide gel and the sequence of each clone was analysed using the Blast database comparison programs and the translation function of the Wisconsin suite of programs.

The sequence of one clone (NR1-AZ-36) contained motifs characteristic of the haemopoietin receptor family. Two oligonucleotides, 26 and 60 (nucleotides 946–970 and 1005–1034, Figure 1 and Table I), were designed from this sequence and used to re-screen the primary filters from the original liver library and two other adult liver cDNA libraries. The initially isolated cDNA clone, NR1-AZ36, and four other cDNA clones (NR1-30.2, 30.3, 30.4 and 30.17) were sequenced completely, on both strands, using the dideoxy method (Sanger *et al.*, 1977) with the Pharmacia T7 polymerase sequencing kit (Pharmacia, Uppsala, Sweden). The sequence of the new receptor was compared with the EMBL and GenBank database using the FASTA program. Alignments with known cytokine receptors were carried out by eye.

Northern blots

Cytoplasmic polyadenylated RNA was prepared from cell lines using an NP-40 lysis method (Gough, 1988) and from tissues using a proteinase K method (Gonda *et al.*, 1982) or a guanidium thiocyanate/phenol method (Chomczynski and Sacchi, 1987). Cytoplasmic polyadenylated RNA was purified by oligo(dT)−cellulose chromatography (Boehringer Mannheim GmbH, Mannheim, Germany) and fractionated on a 1% (w/v) agarose gel containing 20 mM morpholinopropane sulfonic acid (MOPS), 5 mM sodium acetate, 1 mM EDTA and 6% (v/v) formaldehyde and transferred overnight to nitrocellulose with 20× SSC. After transfer the membrane was baked in a vacuum oven for 2 h at 80°C and prehybridized in 2× SSC containing 2 mg/ml bovine serum albumin, 2 mg/ml Ficoll, 2 mg/ml polyvinylpyrrolidone, 100 μM ATP, 10 μg/ml tRNA, 2 mM sodium pyrophosphate, 2 mg/ml salmon sperm DNA and 200 μg/ml sodium azide for 2 h at 60°C. The NR1 probe was prepared using a random decanucleotide labelling kit (Bresatec, Adelaide, S.A., Australia) from the 5′ *Eco*RI fragment of the clone NR1-AZ36. Incorprated [^{32}P]ATP was separated from unincorporated label using a NICK column (Pharmacia, Uppsala, Sweden). The membrane was then hybridized in pre-hybridization buffer containing the probe and 0.1% (w/v) SDS at 60°C for 16 h. The membrane was washed at 65°C in 2× SSC containing 0.1% (w/v) SDS for 2 h, at 65°C in 0.2× SSC containing 0.1% (w/v) SDS for 2 h, rinsed in 2× SSC and exposed to autoradiographic film at −70°C for 12 days.

Reverse transcriptase polymerase chain reaction

First strand cDNA synthesis was performed on 1 μg poly(A)$^+$ cytoplasmic RNA. Reverse transcription was carried out at 42°C for 60 min in 20 μl 50 mM Tris−HCl, pH 8.3, 20 mM KCl, 10 mM $MgCl_2$, 5 mM dithiothreitol, 1 mM each dNTP, 20 μg/ml oligo(dT)$_{15}$ and 12.5 units AMV reverse transcriptase (Boehringer Mannheim GmbH, Mannheim, Germany). Control reactions were performed for each RNA sample under identical conditions, except that reverse transcriptase was omitted from the reaction. The reverse transcription reaction mixture was diluted to 100 μl with water and 5 μl was used for each PCR. PCRs were carried out in 50 μl reaction buffer (Boehringer Mannheim GmbH, Mannheim, Germany) containing 200 μM each dNTP, 1 μM each primer and 2.5 U *Taq* polymerase (Boehringer Mannheim GmbH, Mannheim, Germany). The primers used for amplification of IL-11 receptor α-chain (NR1) cDNA were, from homology with other members of the haemopoietin receptor family, predicted to span at least one intron. These oligonucleotides were 449 and 285 (nucleotides 133–156 and 677–661, Figure 1 and Table I), while for amplification of GAPDH cDNA, primers 495 and 496 were used (Table I). PCR was performed for 30 cycles at 94°C for 2 min, at 60°C for 2 min and at 72°C for 3 min in a Perkin Elmer Cetus Thermal cycler (Perkin Elmer Cetus, Foster City, CA, USA). An aliquot of the reaction mixture was electrophoresed on a 1.0% (w/v) agarose gel and DNA was transferred to a Zetaprobe membrane. Southern blots were performed as described by Reed and Mann (1987). Hybridization was carried out with end-labelled oligonucleotides (489 for the IL-11 receptor α-chain and 741 for GAPDH, Table I).

Tissue culture

Ba/F3 cells (Palacios and Steinmetz, 1985) were grown in RPMI-1640 medium containing 10% (v/v) fetal calf serum (FCS) and 10% (v/v) WEHI-3B D$^-$-conditioned medium as a source of IL-3 (Metcalf, 1984). M1 cells (Ichikawa, 1969) were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% (v/v) FCS. Cells were maintained by twice weekly passage and were used in exponential growth phase.

Expression constructs

NR1-30.3 was used in a PCR with primers 30f1 and 30r1 (Table I) to generate a cDNA that contained little 5′ or 3′ untranslated region. The PCR product was cloned into the *Bst*XI site of pEF-BOS (Mizushima and Nagata, 1990) using *Bst*XI adaptors (Invitrogen, San Diego, CA, USA). The cDNA insert was sequenced on both strands. cDNAs encoding the human LIF receptor (the gift of Dr P.Lock) and mouse gp130 (the gift of Dr T.Taga) were also subcloned into pEF-BOS. Receptor cDNAs in pEF-BOS were linearized with *Aat*II prior to transfection. pBluescript derivatives containing cDNAs encoding the selectable markers puromycin transferase (pPGKpuropA; the gift of Dr S.Cory) and neomycin transferase (pPGKneopA; the gift of Dr P.Lock) transcribed from a PGK promoter and with the β-globin 3′-untranslated region were linearized with *Sca*I.

Cell transfection

Cells were stably transfected by electroporation. Briefly, cells were washed twice in ice-cold PBS and resuspended in PBS at 5×10^6 cells/ml. Cells (4×10^6) were aliquoted into 0.4 mm electroporation cuvettes with 20 μg pEF-BOS with or without NR1, gp130 or the LIF receptor cloned into the *Bst*XI site and 2 μg selectable markers pPGKpuro or pPGKneo. DNA and cells were incubated for 10 min on ice and electroporated at 270 V and 960 mF in a Bio-Rad Gene-Pulser (Bio-Rad Laboratories, Hercules, CA, USA). The cells were mixed with 1 ml culture medium, centrifuged through 3 ml FCS and resuspended in 100 ml culture medium. Cells were then aliquoted into four 24-well dishes. After 2 days, selection was commenced by the addition of geneticin to a concentration of 1.2 mg/ml or puromycin to a concentration of 40 μg/ml for M1 cells and 5 μg/ml for Ba/F3 cells. After 10–14 days, clones of proliferating cells were transferred to flasks and, after expansion, tested for receptor expression.

Cytokines

Murine IL-3 and IL-11 were purchased from PeproTech (Rocky Hill, NJ, USA), human LIF and human OSM were produced using the pGEX system, essentially as described (Gearing *et al.*, 1989b) and were the gift of Dr C.Owczarek and Ms M.Layton and human IL-6 and the soluble IL-6 receptor α-chain were the gift of Dr R.Simpson.

Biological assays

The proliferation of Ba/F3 cells in response to cytokines was measured in a Lux 60 microwell HL-A plate (Nunc Inc., Roskilde, Denmark). Cells were washed three times in DMEM containing 20% (v/v) new born calf serum and resuspended at a concentration of 2×10^4 cells/ml in the same medium. Aliqouts of 10 μl cell suspension were placed in

the culture wells with 5 µl of various concentrations of purified recombinant IL-3, IL-11 or LIF or with IL-6 at 3 µg/ml and soluble IL-6 receptor α-chain at 500 ng/ml. After 2 days of incubation at 37°C in a fully humidified incubator containing 10% CO_2 in air, viable cells were counted using an inverted microscope.

In order to assay the differentiation of M1 cells in response to cytokines, 300 cells were cultured in 35 mm Petri dishes containing 1 ml DMEM supplemented with 20% (v/v) FCS, 0.3% (w/v) agar and 0.1 ml of serial dilutions of IL-6, IL-11, LIF and OSM. After 7 days culture at 37°C in a fully humidified atmosphere containing 10% CO_2 in air, colonies of M1 cells were counted and classified as differentiated if they were composed of dispersed cells or had a corona of dispersed cells around a tightly packed centre.

Binding studies with IL-11

IL-11 was dissolved at a concentration of 100 µg/ml in 50 mM sodium phosphate, 150 mM NaCl (PBS), 0.02% (v/v) Tween 20 and 0.02% (w/v) sodium azide, pH 7.4. IL-11 was radioiodinated according to the method of Bolton and Hunter (1973). Briefly, 2 µg IL-11 were incubated with 2 mCi monoiodinated Bolton–Hunter reagent (New England Nuclear, North Ryde, NSW, Australia) at room temperature in 20 µl 150 mM sodium borate, pH 8.5. After 2 h the reaction was quenched with 100 µl 1 M glycine in the same buffer and the labelled protein was separated from unincorporated Bolton–Hunter reagent using a pre-packed Sephadex G-25 column (PD-10; Pharmacia, Uppsala, Sweden) equilibrated in PBS containing 0.02% (v/v) Tween 20 and 0.02% (w/v) sodium azide. Prior to use, the ^{125}I-labelled IL-11 was diluted 10-fold with 50 mM Tris–HCl, pH 7.5 containing 0.02% (v/v) Tween 20 and 0.02% (w/v) sodium azide and applied to a 250 µl column of CM-Sepharose CL-4B (Pharmacia, Uppsala, Sweden) equilibrated in the same buffer. The column was washed with 5 ml equilibration buffer and eluted with sequential 5 ml aliquots of DMEM containing 10% (v/v) FCS. At this stage the ^{125}I was >95% precipitable with cold trichloroacetic acid. The bindability of the ^{125}I-labelled IL-11 preparation was assessed as described previously (Hilton *et al.*, 1988) and was ~80%. The specific radioactivity of the ^{125}I-labelled IL-11 was ~130 000 c.p.m./ng and was determined by self-displacement analysis (Calvo *et al.*, 1983).

Binding studies were performed essentially as described (Hilton and Nicola, 1992). Briefly, 5×10^5–1.5×10^7 cells in 40 µl RPMI-1640 medium containing 20 mM HEPES, pH 7.4, and 10% (v/v) FCS (RHF) were incubated overnight on ice with between 5×10^3 and 2×10^6 c.p.m. ^{125}I-labelled IL-11, with or without a 100-fold excess of unlabelled IL-11. In other experiments receptors were saturated with a constant amount of ^{125}I-labelled IL-11 and increasing amounts of unlabelled IL-11. Binding experiments to analyse receptor specificity were carried out by incubating cells with unlabelled IL-11, IL-6, IL-3, LIF or OSM for 20 min on ice and then with ^{125}I-labelled IL-11 overnight. In each type of experiment, cell associated and free ^{125}I-labelled IL-11 were separated by rapid centrifugation through 180 µl FCS and quantitated in a γ-counter. Genbank accession number V14412.

Acknowledgements

The authors gratefully acknowledge Drs Beth Simpson, Simon Foote, Andrew Zinn and Douglas Volrath for their generous advice. Bronwyn Roberts, Dale Cary, Ladina Di Rago, Sandra Mifsud and Jan Boyd are thanked for their excellent technical assistance. The following people are thanked for generously providing reagents: Dr S.Cory (pPGKpuropA), Dr P.Lock (pPGKneopA and pEF-BOS/hLIFR), Dr R.Simpson (IL-6 and soluble IL-6 receptor), Dr C.Owczarek, Ms M.Layton (LIF and OSM) and Dr T.Taga (murine gp130 cDNA). D.J.H. was supported by a Queen Elizabeth II Postdoctoral Fellowship from the Australian Research Council. This work was supported by the Anti-Cancer Council of Victoria, Melbourne, Australia, AMRAD Operations Pty Ltd, Melbourne, Australia, The National Health and Medical Research Council, Canberra , Australia, The J.D. and L.Harris Trust, The National Institutes of Health, Bethesda, MD (grant CA-22556) and the Australian Federal Government Cooperative Research Centres Program.

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Received on June 6, 1994; revised on July 19, 1994