Mediation of interleukin-11-dependent biological responses by a soluble form of the interleukin-11 receptor

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Interleukin-11 (IL-11) is a polyfunctional cytokine whose biological actions require a specific IL-11 receptor (IL-11R) and the transmembrane transducer gp130. Here we report the production of a soluble form of the murine IL-11R and demonstrate that it interacts with IL-11 ligand with high affinity. The affinity of IL-11 alone for gp130 is below the level of detection, but a complex of IL-11 and soluble IL-11R interacts with gp130 with high affinity. The addition of soluble IL-11R potentiates the effects of exogenous IL-11 in cells that are normally responsive to IL-11. A biological response to IL-11 can be reconstituted in BAF cells transfected with gp130 by addition of IL-11 and soluble IL-11R. These findings show that the cytoplasmic domain of the IL-11R is not required for the biological effects of IL-11 and that a complex of IL-11 and IL-11R mediates signalling by association with gp130.

INTRODUCTION

Interleukin-11 (IL-11) is a polypeptide cytokine that was originally cloned on the basis of its ability to stimulate the proliferation of a murine plasmacytoma cell line T1165 [1] and inhibit adipogenesis in cultured 3T3 L1 fibroblasts [2]. Further biological characterization has revealed that IL-11 has multiple activities *in vitro*, including the ability to stimulate the multiplication of a variety of plasmacytoma cell lines [3,4], induction of acute-phase-response proteins in hepatocytes [5], suppression of lipoprotein lipase activity in adipocytes [6] and induction of differentiation in immortalized hippocampal neurons [7]. In the presence of accessory cytokines, IL-11 has multiple effects on the proliferation and differentiation of a variety of cell types of haemopoetic origin, including megakaryocytes, myeloid progenitors, B-lymphocytes and multipotential progenitor cells (reviewed in [8]). *In vivo* administration of IL-11 results in stimulation of megakaryopoesis and increased platelet counts [9,10], increased levels of circulating acute-phase-response proteins [10], loss of body fat and behavioural changes [11].

The biological effects of IL-11 have been reported to be mediated by association with the transmembrane signal-transducing receptor gp130 [12–16]. This can explain why many of the biological effects of IL-11 are shared with other agents that also interact with gp130 [15]. These include interleukin-6 (IL-6), leukaemia inhibitory factor (LIF), oncostatin M (OSM) and ciliary neurotrophic factor (CNTF). These, and other cytokines such as cardiotrophin-1 [17], form part of a family of agents that employ gp130 as a common signal transducer. There exists a variety of means by which these cytokines elicit activation of gp130-dependent signalling. In the case of IL-6, oligomerization of gp130 is mediated by association of the ligand with a specific IL-6 receptor (IL-6R) [18]. The IL-6R does not require a cytoplasmic domain to participate in the process of signal transduction, since a soluble form of the IL-6R can be shown to mediate gp130-dependent signalling in the presence of IL-6 [19]. Association of IL-6 with the IL-6R promotes gp130 homodimerization and consequential activation of intracellular responses [20,21], most probably by formation of a hexameric complex containing two molecules of IL-6, two molecules of IL-6R and two molecules of gp130 [22,23]. The IL-6R therefore represents an example of a receptor whose primary function is to confer specificity for a particular ligand in triggering a signalling pathway that can be activated by a number of different agents.

Hilton et al. [24] recently reported the cloning of a specific IL-11 receptor (IL-11R) (NR1) that proved to be a transmembrane protein with similarity in sequence to the IL-6R, the p40 IL-12 receptor, the granulocyte–macrophage colony-stimulating-factor receptor and the CNTF receptor. NR1 exhibits almost complete sequence identity with *ETL2*, a gene identified in the vicinity of an enhancer trap integration event [25]. *ETL2* was found to be expressed in the developing nervous system, hair follicles, dermis and sites of skeletogenesis in the developing embryo. We have expressed the predicted extracellular domain of ETL2/NR1 *in vitro* as a soluble protein and find that it not only binds IL-11 with high affinity but is able to confer IL-11-dependent responses on target cell lines that express gp130. These findings demonstrate that ETL2/NR1 is a specific IL-11R whose function is to promote the formation of a high-affinity complex between the IL-11 ligand and the gp130 transducer.

MATERIALS AND METHODS

Expression constructs

pIG-1/IL-11R and pIG-1/gp130 constructs

The basis for all eukaryotic expression constructs was pIG-1, provided by D. L. Simmons (Institute for Molecular Medicine, Oxford, U.K.), which contains the simian virus 40 (SV40) origin

Abbreviations used: IL-11, interleukin-11; bIL-11, biotinylated IL-11; hIL-11, human IL-11; IL-11R, IL-11 receptor; sIL-11R, soluble IL-11R; IL-6, interleukin-6; IL-6R, IL-6 receptor; LIF, leukaemia inhibitory factor; LIF-R, LIF receptor; OSM, oncostatin M; CNTF, ciliary neurotrophic factor; ETL2, enhancer trap locus 2; CMV, cytomegalovirus; HRV, human rhinovirus; FCS, foetal calf serum; DMEM, Dulbecco's modifed Eagle's medium; HRPO, horseradish peroxidase; SV40, simian virus 40; UTR, untranslated region; GST, glutathione S-transferase.

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for replication in mammalian cell lines expressing the SV40 large T antigen, the cytomegalovirus (CMV) enhancer and promoter, a splice acceptor site and the genomic human IgG1-Fc gene (hinge CH2 CH3) followed by an intron and a poly(A)$^+$ site for expression in mammalian cell lines. ETL2 cDNA for IL-11R cloning was obtained as described previously [25]. A fragment of 1104 bp that codes for amino acids 1–363 (i.e. the signal sequence and the extracellular region except for the three membrane-proximal amino acids) was amplified from this cDNA by PCR and cloned into pIG-1 vector cut with *Eco*RI and *Bam*HI. The ETL2 cDNA differs from NR1 cDNA [24] in the 5′ untranslated region (UTR) and, in particular, contains a small open reading frame starting in the untranslated leader sequence that precedes the start codon of IL-11R. A second construct was produced by PCR, in which the region of divergence between ETL2 and NR1 was changed to the reported sequence of NR1, using appropriate PCR primers. Initial studies showed that protein yields from the NR1–IL-11R construct were higher than those obtained with the ETL2–IL-11R construct. A second version of soluble IL-11R (sIL-11R) was obtained by the introduction of a recognition site [26,27] for human rhinovirus (HRV) protease 3C into the gene encoding the hinge region of the IgG1-Fc domain in the vector pIG-1. The recognition site was first introduced into pIG-1. Subsequently, the modified hinge–IgG1-Fc fragment was exchanged for the unmodified fragment in the pIG-1/IL-11R construct. This construct was designated pIG-1/IL-11R/3C and used for the experiments reported in this paper. A 1867 bp fragment encoding the extracellular domain of mouse gp130 [28] was amplified from cDNA by PCR. The blunt-ended fragment was cloned into the pIG vector containing the protease 3C recognition site. The vector was cut with *Hin*dIII/*Bam*HI, treated with alkaline phosphatase and filled in with Klenow fragment. All PCR-cloned inserts were sequenced by the chain-termination method. Reactions were performed using a sequencing kit (Sequenase Quick-Denature Plasmid Sequencing Kit; United States Biochemical), following the protocol provided by the manufacturer.

PCR

PCR was performed using either 5 units of *Taq* polymerase (Perkin Elmer)/100 ng of template/200 ng of primers/50 mM dNTPs (Pharmacia Biotech)/1.5 mM $MgCl_2$/*Taq* buffer (Perkin Elmer) or 5 units of Vent polymerase (BioLabs)/100 ng of template/800 ng of primers/400 mM dNTPs/Vent buffer (BioLabs) in a final volume of two tubes each with 100 ml, covered with light mineral oil. The following cycle conditions were used: cycle 1: 94 °C, 90 s; 52 °C, 75 s; 72 °C, 120 s; cycles 2–22: 94 °C, 25 s; 52 °C, 45 s, 72 °C, 120 s; followed by 72 °C, 360 s. The PCR products were analysed on a Tris/borate/EDTA–agarose (1 %) gel (10 % of PCR product was applied). Primers, nucleotides, salts and *Taq* polymerase were removed by affinity chromatography, using a PCR-product purification kit (Wizard PCR Preps DNA Purification System; Promega), following the manufacturer's protocol.

pGEX-2T/HRV-3C construct

HRV protease 3C was expressed as a glutathione S-transferase (GST) fusion protein [29] in *Escherichia coli* and purified by glutathione affinity chromatography. Following isopropyl β-D-thiogalactoside induction, cells were lysed by sonication. Triton X-100 (Boehringer) was added to 1 %, and the lysate was centrifuged for 15 min at 25000 ***g***. The supernatant was applied to 300 μl of glutathione–Sepharose 4B (Pharmacia Biotech) that had been washed with mouse tonicity PBS and equilibrated with PBS/1 % Triton X-100. The column was washed with mouse tonicity PBS, and HRV-3C–GST was eluted in 0.5 ml fractions with 10 mM reduced glutathione/50 mM Tris. The fractions were analysed by gel electrophoresis. In order to remove glutathione from the eluate, the fractions were dialysed against 20 mM Tris (pH 8.0)/100 mM NaCl/1 mM EDTA for 16 h. Native HRV protease 3C was generated from the GST fusion protein by thrombin cleavage while bound to the glutathione–Sepharose column. Protein concentration was determined by the Coomassie Brilliant Blue protein assay (Bio-Rad), compared with BSA standards of known concentrations.

Expression of IL-11R constructs

293/tsA1609neo [30] cells were used for transient expression of all IL-11R constructs. They are a transformed human epithelial kidney cell line stably transfected with the temperature-sensitive SV40 large T antigen mutant tsA1609, which produces replication-competent T antigen at 37 °C. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % foetal calf serum (FCS) (Globepharm)/1 mM glutamine/1 mM penicillin/1 mM streptomycin/1 mM pyruvate at 37 °C and 5 % CO_2. Cells were transfected at 50–70 % confluence by calcium phosphate precipitation and cultured for 8–24 h at 37 °C and 5 % CO_2 in DMEM + FCS. The cells were then washed with FCS-free medium, and 30 ml of UltraCho medium (BioWhittaker) was added. Supernatants were harvested after 4–6 days of culture: EDTA and PMSF were added to 1 mM to the supernatants that were subsequently either centrifuged for 15 min at 29000 ***g*** or filtered through a 0.22 μm filter and frozen at −20 °C. Tris (pH 8.0) was added to 100 mM immediately before purification. The concentration of the Fc fusion protein in culture supernatants was determined by ELISA assay, using a goat polyclonal antibody directed against human Fc (gift of Professor R. Jefferis, University of Birmingham, Birmingham, U.K.).

IL-11R/IgG-Fc purification

IL-11R–Fc was purified by affinity chromatography, using Protein A affinity chromatography and gel filtration. A Protein A–Sepharose High Performance column (HiTrap Protein A column; Pharmacia Biotech) was washed with 10 mM Tris (pH 8.0)/100 mM NaCl. Supernatants containing IL-11R–Fc were applied at a flow rate of 0.3 ml/min at 4 °C. The column was washed with at least 10 column vol. of 10 mM Tris (pH 8.0)/100 mM NaCl, and IL-11R–Fc was eluted with 100 mM citric acid (pH 3.0) in 315 μl fractions containing 110 μl of 1.0 M Tris (pH 8.0). The recovery of protein was monitored by gel electrophoresis (Phastgel system; Pharmacia Biotech). Pooled fractions were applied to a PD10 molecular sieve containing Sephadex G-25M (Pharmacia Biotech) after equilibration with 10 mM Tris (pH 8.0)/100 mM NaCl and were eluted with the same buffer in 0.5 ml fractions. The recovery was monitored by gel electrophoresis.

sIL-11R purification

sIL-11R was cleaved from the Fc portion by HRV protease 3C–GST while bound to Protein A–Sepharose. The protease was subsequently moved by binding to glutathione–Sepharose. Protein A–Sepharose fast flow (Pharmacia) was prepared by several washes with 100 mM Tris (pH 8.0). Matrix (100 μl) was added to 50 ml of supernatant and mixed at 4 °C for 90 min. Supernatants were discarded, and the matrix was washed in 50 mM Tris (pH 8.0)/150 mM NaCl/1 mM EDTA. HRV protease 3C–GST

was added in 50 mM Tris (pH 8.0)/150 mM NaCl/1 mM dithiothreitol in a volume equal to the matrix at an estimated m/m ratio of 50:1 (cleavable protein/protease). After a 2 h incubation at 37 °C, cleaved sIL-11R was collected, using wash buffer. Protein recovery was monitored by gel electrophoresis.

Molecular-mass determination

The molecular mass of the constructs was estimated by SDS/PAGE, using 7.5 % or 12.5 % homogeneous PHAST gels (Pharmacia) and protein molecular-mass markers as standards (Pharmacia and Bio-Rad). Either purified protein samples or samples from small-scale Protein A immunoprecipitations were applied. The N-terminal sequence of IL-11R–Fc protein was determined by 10 cycles of automated gas-phase sequencing (Applied Biosystems) after electrophoretic transfer to poly(vinylidene difluoride) filters. Two N-terminal sequences were obtained in approximately equal yields, corresponding to the predicted amino acid sequence of IL-11R beginning at residues 26 and 28.

Purified Fc fusions and cleaved ETL2 were also characterized by immunoblotting, using polyclonal goat anti-(human IgG-Fc) IgG coupled to horseradish peroxidase (HRPO) (Pierce) and polyclonal rabbit anti-(mouse IL-11R) IgG and enhanced chemiluminescence (Amersham) detection.

Biotinylation of IL-11

Recombinant human IL-11 (hIL-11) was a gift from N. Serizawa (Sankyo Corporation, Tokyo, Japan). hIL-11 was biotinylated on Lys residues according to a protocol adapted from Harlow and Lane [31]. IL-11 was dialysed against 100 mM borate (pH 8.8) at 4 °C. Biotin amidocaproate-*N*-hydroxysuccinimide ester (Sigma) was added, and the mixture was incubated for 4.5 h at room temperature. NH_4Cl (1 mg per 250 mg of biotin ester) was added and incubated for 10 min at room temperature. IL-11 was dialysed against PBS for 22 h to remove unbound biotin ester.

Binding assay

Immunosorb plates (96 wells; Nunc) were coated with Protein A (2 μg/ml) for 4 h at room temperature. The wells were blocked with PBS/1 % BSA for 1 h. Plates were washed with PBS/0.05 % Tween 20, then IL-11R/IgG-Fc (5 μg/ml in PBS/1 % BSA) was added and incubated for 1 h. After washing with PBS/0.05 % Tween 20, biotinylated IL-11 (bIL-11) and receptors were added at different concentrations diluted in RPMI 1640/20 mM Hepes (pH 7.2)/1 % BSA/1 mM PMSF/1 mM EDTA and incubated for 16 h at 4 °C. The wells were washed with PBS/0.05 % Tween 20, and streptavidin–HRPO (Amersham) diluted 1:1000 in PBS/1 % BSA was added for 1 h. After washing with PBS/0.05 % Tween 20 and PBS, bound HRPO was visualized using *O*-phenylenediamine as substrate, and A_{492} was determined.

Surface plasmon resonance

IL-11R–Fc was immobilized on a CM research-grade sensor chip (Pharmacia Biotech) according to the manufacturer's instructions. Sensograms were obtained using a BIACore 2000 instrument operating at 25 °C and a flow rate of 10 μl/min, using concentrations of IL-11 ranging from 100 nM to 3.25 nM. IL-11R–Fc was regenerated by exposure to 30 μl of 2 mM HCl. Sensogram data were analysed using the BIA Evaluation software package (Pharmacia Biotech).

Cell culture

7TD1

7TD1, a factor-dependent mouse–mouse B-cell hybridoma cell line [32] shown to be IL-6 dependent in its growth [33], was maintained in the presence of IL-6 according to Piquet-Pellorce et al. [34].

BAF-B03-130/190

BAF-B03-130/190 is an interleukin-3- and LIF-dependent murine pro-B-cell line stably transfected with human gp130 and gp190 [LIF receptor (LIF-R)] [35]. BAF cells were cultured in RPMI supplemented with 10 % FCS/1 mM glutamine/1 mM streptomycin/1 mM penicillin/20 ng/ml recombinant human LIF at 37 °C and 5 % CO_2 and were split at a density of $(5–10) \times 10^5$/ml. BAF gp130 cells were derived (H. Vankelecom, personal communication) by transfecting BAF-3 cells with the human pZIPneogp130 construct (gift of Professor T. Kishimoto, Osaka, Japan), described by Saito et al. [28], and selecting for stable transformants in the presence of G418 and IL-6–soluble IL-6R complex. Stable transformants were maintained in the presence of IL-11 and sIL-11R.

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenylterazolium bromide proliferation assays

Cells were extensively washed in cytokine-free medium before being cultured in 96-well flat-bottomed plates in a volume of 110 μl at a density of 5×10^3–2×10^4 (7TD1) or $(1.3–2) \times 10^5$ (BAF) in the presence of various concentrations of cytokines and/or receptors. Assays were performed in triplicate. After 72 or 96 h incubation at 37 °C and 5 % CO_2, 10 μl of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylterazolium bromide [36] was added, and the plates were further incubated for 3–5 h. SDS (100 μl; 10 %)/0.01 M HCl was added subsequently, the stain was allowed to dissolve for 1 h, and A_{570} was determined after incubation for 6 h at 37 °C.

RESULTS

Relationship between ETL2 and NR1

The ETL2 [25] and NR1 [24] cDNAs encode identical proteins but completely diverge in sequence in their 5′ UTR and show minor sequence differences in the 3′ UTR (results not shown). The biological significance of these differences is currently unclear. In initial expression experiments we observed that higher yields of secreted protein were obtained with constructs containing the NR1 5′ UTR than with those containing the ETL2 5′ UTR. This may reflect the existence of a short open reading frame in the 5′ UTR of the ETL2 cDNA. Given the identity of the proteins encoded by ETL2 and NR1, the term IL-11R will be used in the remainder of this paper.

Expression of the ectodomain of IL-11R

A region of IL-11R cDNA corresponding to the predicted ectodomain of the protein was cloned into a modified version of the vector pIG. This produces a dimeric soluble fusion protein between the ectodomain of IL-11R and human Fc (IL-11R–Fc). The inclusion of a protease cleavage site in the junction between the IL-11R and the Fc domain permitted cleavage with HRV protease 3C [26] and release of monomeric sIL-11R. The construct was transfected into 293/tsA1609neo cells [30], and, after 4 days of culture, the culture supernatant was subjected to

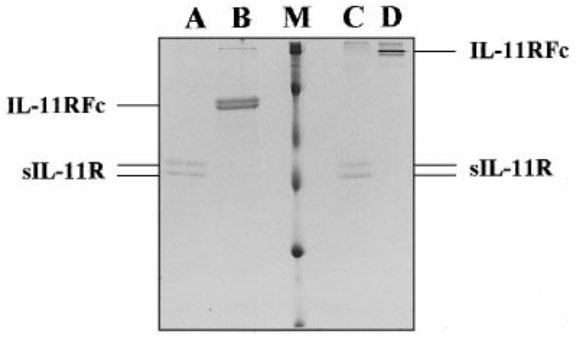

Figure 1 SDS/PAGE analysis of sIL-11R

Lanes A and B show sIL-11R derived by proteolytic cleavage of IL-11R–Fc (lane A) and affinity-purified IL-11R–Fc (lane B) in reducing conditions. Lanes C and D show sIL-11R derived by proteolytic cleavage of IL-11R–Fc (lane C) and affinity-purified IL-11R–Fc (lane D) in non-reducing conditions. Molecular-mass markers (lane M) are myosin (200 kDa), phosphorylase B (97.4 kDa), BSA (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (31 kDa) and lysozyme (21.5 kDa).

Protein A affinity chromatography to recover the IL-11R–Fc fusion protein. SDS/PAGE analysis of the affinity-purified protein under reducing conditions showed a doublet of apparent molecular mass 75000 Da (Figure 1) corresponding to the IL-11R–Fc fusion. This was confirmed by Western blot and ELISA analysis using antibodies directed against human Fc and IL-11R (results not shown). The affinity-purified IL-11R–Fc protein, bound to Protein A beads, was subjected to proteolytic cleavage by protease 3C, and the soluble material was analysed by reducing SDS/PAGE. This reveals a doublet of apparent molecular masses 50 and 52 kDa (Figure 1). Cleaved IL-11R was subjected to amino acid sequence analysis. This revealed two N-terminal sequences (see the Materials and methods section) in approximately equimolar amounts. The sequences confirm the identity of the purified protein as IL-11R and show that the signal peptide is cleaved at two alternate sites during synthesis and secretion of the protein. It is probable that the characteristic doublet bands observed upon SDS/PAGE of sIL-11R correspond to alternate glycosylated forms rather than the two alternately cleaved sIL-11R products (J. Karow, K. R. Hudson and J. K. Heath, unpublished work).

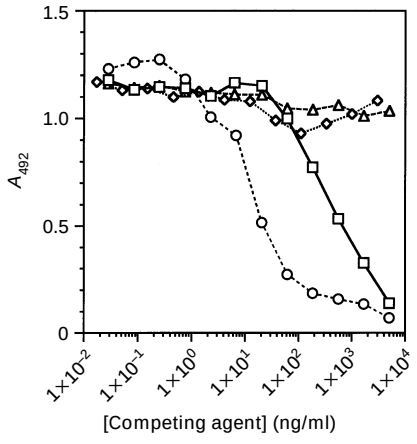

Figure 2 Interaction of bIL-11 with IL-11R–Fc and inhibition by IL-11 and soluble receptors

IL-11R–Fc was immobilized on Protein A-coated plates and incubated with 1.7 nM bIL-11 in the presence or absence of various concentrations of competing agents: IL-11R–Fc (□), LIF-R–Fc (◇), unlabelled IL-11 (○) and parallel wells with no additions (△). Data points are the means of triplicate samples. The S.E.M. was less than 10%.

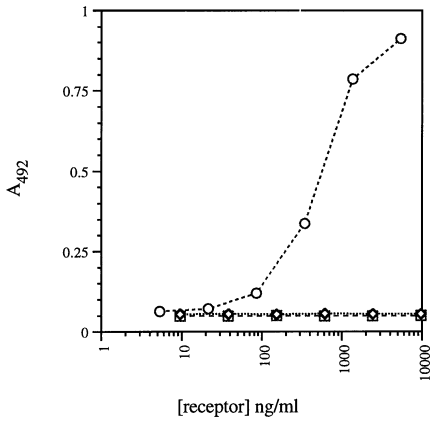

Figure 3 Interaction of bIL-11 with gp130

Gp130–Fc, or control LIF-R–Fc, was immobilized on Protein A-coated plates and incubated with 2.6 nM bIL-11 in the presence of various concentrations of receptors: LIF-R–Fc in the presence of immobilized gp130–Fc (◇), sIL-11R in the presence of immobilized gp130–Fc (○) and sIL-11R in the presence of immobilized LIF-R–Fc (□). Data points are the means of triplicate samples. The S.E.M. was less than 10%.

sIL-11R binds IL-11 with high affinity

The ability of sIL-11R to bind IL-11 (and other cytokines) was examined using a modified ELISA assay. Recombinant hIL-11 was biotinylated (bIL-11) and tested for its ability to bind IL-11R–Fc immobilized on Protein A-coated multiwell plates. The results of the experiments (Figure 2) show that bIL-11 specifically bound to IL-11R–Fc. The interaction of bIL-11 with IL-11R–Fc could be specifically inhibited by co-incubation with unlabelled hIL-11, sIL-11R and IL-11R–Fc (Figure 2) but not by LIF-R–Fc.

The affinity of hIL-11 for the IL-11R was probed in more detail using surface-plasmon-resonance techniques [37]. IL-11R–Fc was immobilized on the sensor chip of a BIAcore instrument, and sensograms were obtained at different concentrations of IL-11. Analysis of these binding data revealed an affinity of IL-11 for IL-11R–Fc of 2.0 ± 0.6 nM (mean of three determinations). Together these findings demonstrate that hIL-11 interacts with a soluble domain of the IL-11R with an affinity consistent with its biological potency *in vitro*.

Interaction of IL-11 with gp130

The ectodomain of gp130 was expressed as an Fc fusion protein, immobilized on Protein A-coated plates and tested for its ability to interact with bIL-11 in the presence of recombinant receptor ectodomains (Figure 3). IL-11 alone, or in the presence of control LIF-R–Fc, failed to bind gp130–Fc with detectable affinity. Significant binding of IL-11 to gp130–Fc, but not control LIF-R–Fc, was, however, observed in the presence of sIL-11R. These findings demonstrate that a complex of IL-11 with sIL-11R is able to interact with gp130.

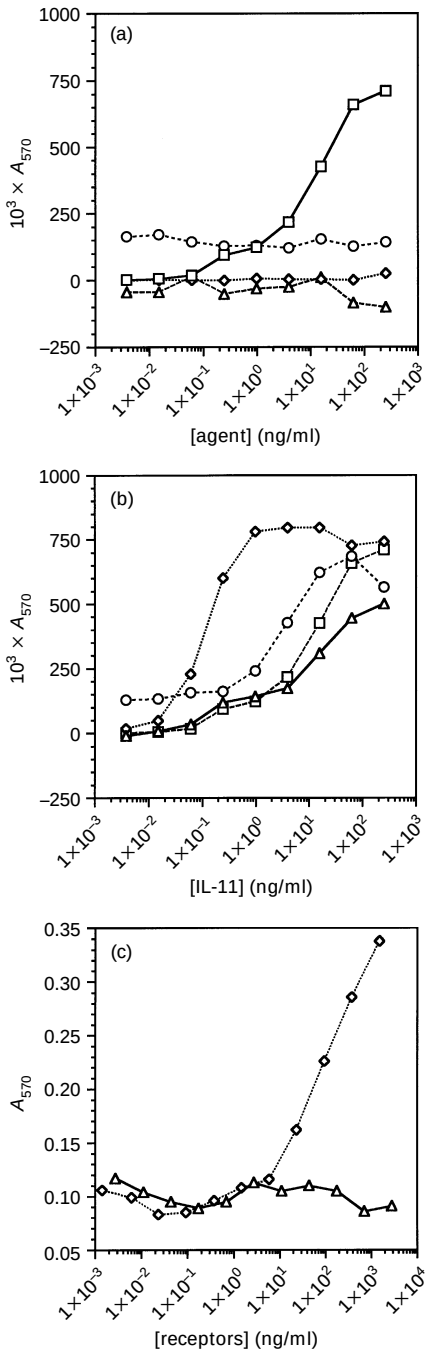

Figure 4 Effect on 7TD1 cells of IL-11 and receptors

(**a**) Biological response of 7TD1 cells to exogenous IL-11 (□), sIL-11R (◇), IL-11R–Fc (○) and LIF-R–Fc (△). Data points are the means of triplicate samples. The S.E.M. was less than 10%. (**b**) Enhancement of IL-11 bioactivity in the presence of sIL-11Rs. 7TD1 cells were exposed to various concentrations of IL-11 in the presence of soluble receptors: 2 μg/ml LIF-R–Fc (△), 2 μg/ml sIL-11R (◇), 2.8 μg/ml IL-11R–Fc (○) and no addition (□). Data points are the means of triplicate samples. The S.E.M. was less than 10%. (**c**) Concentration-dependent stimulation of 7TD1 cells in the presence of 2 ng/ml IL-11 by exogenous IL-11Rs: IL-11R–Fc (△) and sIL-11R (◇). Data points are the means of triplicate samples. The S.E.M. was less than 10%.

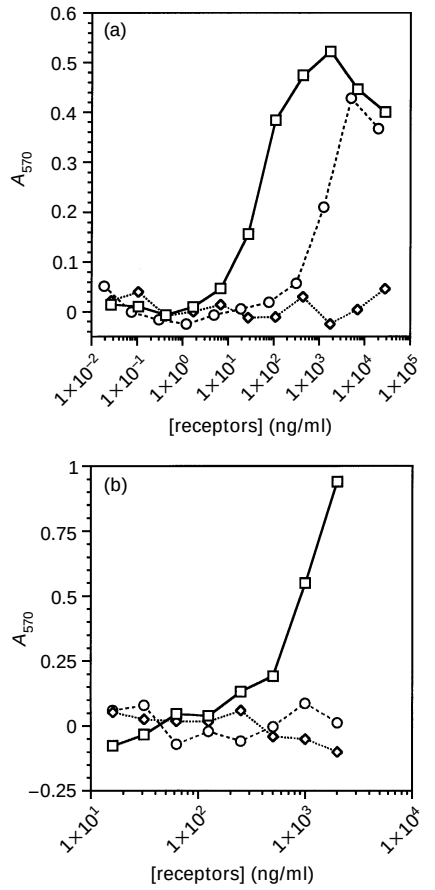

Figure 5 Stimulation of BAF cells

(**a**) Stimulation of BAF cells transfected with gp130 and LIF-R by exogenous receptors. BAF-130/190 cells were exposed to 8.3 nM IL-11 in the presence or absence of various amounts of soluble receptors: IL-11R–Fc (□), parallel wells with IL-11 alone (◇) and sIL-11R (○). Data points are the means of triplicate samples. The S.E.M. was less than 10%. (**b**) Stimulation of BAF cells transfected with gp130 by exogenous receptors. BAF-gp130 cells were exposed to 8.3 nM IL-11 in the presence or absence of various amounts of soluble receptors: IL-11R–Fc (□), parallel wells with no additions (◇) and IL-11R–Fc alone (○).

Biological functions of sIL-11R

IL-11R is predicted to be a transmembrane protein [25]. Having established that sIL-11R bound IL-11, we investigated whether the cytoplasmic domain of the IL-11R is required for IL-11-mediated signal transduction or whether IL-11-dependent responses can be elicited from appropriate target cells when the IL-11R is presented in a soluble form. In view of the reported requirement for gp130 in IL-11-mediated signal transduction [12–16] and the results presented in Figure 3, we selected for these experiments target cell types that we have previously demonstrated to be responsive to ligands whose biological effects are mediated by association with gp130.

7TD1 is a line of B-cell origin that has been shown to exhibit a growth dependency upon IL-6 [32,33] (which requires gp130 and IL-6R for signalling) but not upon LIF, OSM or CNTF [34] (which require gp130 and LIF-R for signalling). Initial experiments (Figure 4a) revealed that 7TD1 cells were responsive to exogenous IL-11 in a dose-dependent fashion, indicating that 7TD1 cells express appropriate transducing components required for response to IL-11. The concentration-dependent response to IL-11 in this assay was markedly potentiated by addition of sIL-11R–Fc (Figure 4b). Furthermore, the activity of limiting concentrations of IL-11 in this assay was significantly potentiated, in a concentration-dependent manner, by addition of sIL-11R

(Figure 4c). It was significant that the monomeric sIL-11R exhibited variable, and invariably stronger, activity in both assays than the dimeric sIL-11R–Fc form.

BAF-3 is a cell line of pro-B-cell derivation that has been widely employed in analysis of receptor-mediated signal transduction [38]. In particular, BAF-3 cells are unresponsive to IL-6 but become dependent upon IL-6 and soluble IL-6R after transfection by constructs that express gp130 [39]. We have also shown that BAF-3 cells co-transfected with human gp130 and LIF-R become responsive to exogenous LIF and OSM [35]. It has also been demonstrated that BAF-3 cells transfected with gp130 and LIF-R can respond to IL-11 when transfected with full-length IL-11R cDNAs [24]. BAF-3 cells transfected with gp130 and LIF-R are unresponsive to exogenous IL-11. In the presence of sIL-11R, and IL-11R–Fc, the transfected BAF-3 cells responded to IL-11. This was analysed by titrating the concentration of the soluble receptors in the presence of fixed concentrations of IL-11 (Figure 5a). Similar results were obtained using BAF-3 cells transfected with gp130 alone (Figure 5b), revealing that the presence of IL-11R and gp130 is sufficient to induce a biological response to IL-11, and that BAF-3 cells that express gp130 can respond to IL-11 in the presence of a soluble form of IL-11R. Taken together with the parallel studies of 7TD1 cells, these results demonstrate that soluble forms of the high-affinity IL-11R can confer IL-11-dependent responses in the presence of gp130. IL-11R is therefore required for IL-11 signalling, not as a direct participant in intracellular signal transduction, but as a mediator of gp130 oligomerization.

DISCUSSION

The encoded protein product of the ETL2/NR1 gene is a high-affinity ligand-binding subunit of the functional receptor complex for IL-11. Since many receptors of the cytokine family interact with multiple ligands, it is possible that other ligands that interact with IL-11R remain to be identified. Equally the existence of additional genes that encode additional IL-11Rs cannot, on the present evidence, be eliminated.

We report here that a soluble form of IL-11R is able to mediate IL-11-dependent responses in cell lines that express the transmembrane transducing receptor gp130. We have, in particular, been able to show that a biological response to IL-11 can be reconstituted by addition of soluble receptor to cells transfected with gp130. IL-11R is therefore similar in its mode of action to IL-6R in that the cytoplasmic domain of the protein does not appear to be required for mediating the biological function of the ligand. This suggests that the primary function of the receptor is to confer specificity for ligand in the formation of an oligomeric complex with gp130 [19,22,23]. Recent studies on IL-6 [22,23] suggest that the functional signalling complex contains two molecules of ligand, two molecules of IL-6R and two molecules of gp130: it will be important to determine whether a similar stoichiometry pertains in the case of IL-11. Our analysis of the interaction of IL-11 with gp130 indicates that the affinity of this interaction is rather weak, certainly below the level of detection in our experiments. By contrast a complex of IL-11 and sIL-11R is able to interact with gp130 with high affinity. The high-affinity interaction with IL-11R is therefore the principal determinant of ligand specificity in the formation of the active signalling complex. This also suggests that the formation of a high-affinity complex involves IL-11R-mediated conformational changes in the IL-11 ligand and/or direct interaction between binding sites on gp130 and the IL-11R.

A feature of our studies was the finding that the dimeric form of IL-11R expressed as a fusion with human Fc was significantly less active on 7TD1 cells, which express endogenous IL-11Rs, than on BAF-3 cells transfected with gp130 that have no endogenous receptors. This suggests that the conformation of gp130 may be regulated by the presence of endogenous IL-11Rs in the absence of ligand. This is manifest in a restriction of the ability of gp130 to form a ligand-mediated signalling complex with dimeric, but not monomeric, forms of the IL-11R. Nevertheless the use of cytokine receptor–Fc fusions of the type employed here may be valuable reagents in the analysis of other cytokines, and, in particular, we would predict that dimeric cytokine receptors required for formation of a high-affinity complex rather than signalling would exhibit ligand-dependent biological activity in heterologous systems.

The findings here, along with previous studies [12–16,24], provide an explanation for the overlapping biological actions of IL-6 and IL-11 (reviewed in [8]), in that both agents activate gp130-dependent signalling. Although it has not yet been established whether IL-11R is expressed in a soluble form in any natural source, soluble forms of the IL-6R have been found in a variety of different tissues and cell lines (see, e.g., [40,41]) resulting from either specific proteolysis [42] or use of alternate transcripts [43]. The evidence therefore indicates that the characteristic biological effects of IL-11 are controlled *in vivo* by the tissue-specific expression and bioavailability of the cognate receptors. IL-11R transcripts are expressed at multiple sites in the developing mouse embryo [25], including sites of osteogenesis, the skin, hair follicles and the nervous system. These findings suggest that IL-11 may have actions in the developing embryo in addition to those already reported in the adult animal [9,10] and in particular highlight osteogenesis, hair growth, and differentiation and neuronal function as possible targets for IL-11 action. The significance of these potential tissue targets may be clarified by the construction of gene-knockout models of IL-11R action.

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