Identification of Three Distinct Receptor Binding Sites of Murine Interleukin-11*

Victoria A. Barton‡, Keith R. Hudson§, and John K. Heath¶

From the Cancer Research Campaign Growth Factor Group, Department of Biochemistry, University of Birmingham, Edgbaston, Birmingham, B15 2TT, United Kingdom

Interleukin-11 (IL-11) is a member of the gp130 family of cytokines. These cytokines drive the assembly of multinsubunit receptor complexes, all of which contain at least one molecule of the transmembrane signaling receptor gp130. A complex of IL-11 and the IL-11 receptor (IL-11R) has been shown to interact with gp130, with high affinity, and to induce gp130-dependent signaling. In this study, we have identified residues crucial for the binding of murine IL-11 (mIL-11) to both the IL-11R and gp130 by examining the activities of mIL-11 mutants in receptor binding and cell proliferation assays. The location of these residues, as predicted from structural studies and a model of IL-11, reveals that mIL-11 has three distinct receptor binding sites. These are structurally and functionally analogous to the previously defined receptor binding sites I, II, and III of interleukin-6 (IL-6). This supports the hypothesis that IL-11 signals via the formation of a hexameric receptor complex and indicates that site III is a generic feature of cytokines that signal via association with gp130.

Interleukin-11 (IL-11) is a secreted polypeptide cytokine. It was identified originally from its ability to stimulate the proliferation of a murine plasmacytoma cell line T1165 (1), although it has now been shown that IL-11 is widely expressed and has biological effects on a diverse range of cell types, including hematopoietic cells, hepatocytes, adipocytes, neurons, and osteoblasts (for review, see Ref. 2). In vivo administration of IL-11 results in the stimulation of megakaryopoiesis and increased platelet counts (3), and, in fact, IL-11 has clinical potential for the treatment of thrombocytopenia (4) and oral mucositis (5), both of which can be induced by chemotherapy.

IL-11 mediates its effects by association with the transmembrane signal transducer gp130 (6), explaining why many of the in vitro functions of IL-11 overlap with those of other members of the gp130 family of cytokines, which includes interleukin-6 (IL-6), oncostatin M, leukemia inhibitory factor (LIF), cardiotrophin-1, ciliary neurotrophic factor (CNTF), and an IL-6-like protein encoded by Kaposi’s sarcoma-associated herpesvirus (KSHV-IL-6). All of these cytokines elicit either hetero- or homodimerization of gp130 which activates intracellular signal transduction pathways via protein kinases belonging to the Janus kinase, mitogen-activated protein kinase, and Src families (7–11). Each cytokine drives the assembly of a multiprotein receptor complex that mediates the oligomerization of gp130. In the case of IL-6, association of the ligand with a ligand-specific IL-6 receptor (IL-6R) (12) mediates gp130 homodimerization. It has been shown that a hexameric complex is formed, consisting of two molecules each of IL-6, IL-6R, and gp130 (13, 14). IL-11 has been shown to function in a similar manner, and, as for IL-6, a ligand-specific IL-11 receptor (IL-11R) (15, 16) functions to promote the formation of a high affinity complex between IL-11 and gp130 (17). It has been shown that neither the IL-6R nor the IL-11R requires the cytoplasmic domain to induce gp130-dependent signaling, because soluble forms of each receptor are active (17, 18). There is structural homology between both the ligands and the receptors of the gp130 cytokine family. The extracellular region of all receptors within this superfamily of cytokine receptors contains a cytokine binding homology domain, which is characterized by a WSXWS motif, 4 positionally conserved cysteines, and a proline-rich hinge region (19). The extracellular domains of the IL-6R and the IL-11R share approximately 32% identity in their amino acid sequences.

It has been proposed that IL-11 consists of 4 α-helices (A–D) in an up-up-down-down topology (20). This structure is common to all members of the hematopoietin family of cytokines (21), of which human growth hormone (hGH) is the best characterized (22). hGH homodimerizes two identical receptor subunits, forming a trimeric signaling complex (22, 23). The co-crystal structure of hGH and its receptors (22) together with mutagenesis studies (24–27) have allowed the interaction sites between the units of the signaling complex to be examined. hGH uses two topologically distinct sites (sites I and II) to bind to the two hGH receptors (GHRs). Site I is composed of residues in the carboxyl ends of both the A and D helices and the AB loop, whereas site II is composed of residues in the A and C helices (23–25). It has been shown that IL-6 has two distinct regions that are functionally equivalent to sites I and II on hGH (28). These sites, also termed sites I and II, allow IL-6 to form a trimer with the IL-6R (via site I) and gp130 (via site II), just as hGH forms a trimer with two receptors. The hexameric IL-6 receptor complex described earlier is composed of two of these IL-6-IL-6R-gp130 trimers (13, 14). In addition to sites I and II, a third topologically distinct site has been identified for IL-6 which allows it to bind to a second gp130 molecule (14, 29). This site (site III) contributes to stabilizing the hexameric receptor complex.
In this study we identify residues critical for the binding of mIL-11 to the IL-11R and gp130, which provides evidence that IL-11 has three topologically distinct receptor binding sites structurally and functionally equivalent to sites I, II, and III of IL-6.

**MATERIALS AND METHODS**

**IL-11 Constructs—**Polymerase chain reaction was used to amplify the cDNA sequence (Genetics Institute) encoding the mature form of mIL-11 (amino acids 1–178). The fragment was then cloned into pGEX-2T (Amersham Pharmacia Biotech) using restriction enzymes BamHI and EcoRI. A recognition site for human rhinovirus protease 3C (30) was introduced by the 5′-primer (the sequence of the primers used is available on request). Cleavage of the glutathione S-transferase-mIL-11 fusion protein with 3C protease produces a protein consisting of amino acids 1–178 of mIL-11 with an extra glycine at the NH2 terminus. This was confirmed by NH2-terminal sequencing. All mutant mIL-11 DNA sequences were created by polymerase chain reaction (31) overlap using pGEX-mIL-11 as a template and specific oligonucleotide primers encoding each mutation. The mutant mIL-11 sequences were cloned into pGEX, and the nucleotide sequences of all constructs were confirmed by DNA sequencing using a sequencing reaction kit (Perkin-Elmer).

**Expression and Purification of pIL-11—**mIL-11 and all mutants were expressed as glutathione S-transferase fusion proteins in the Escherichia coli strain ML109. The expression, purification, and cleavage of the fusion proteins were carried out as described previously for human leukemia inhibitory factor (32). The protein concentrations were determined using the Coomassie Plus Protein assay (Pierce). Mutant proteins were also examined by SDS-polyacrylamide gel electrophoresis.

**Monoclonal Antibody Binding Experiments—**The structural integrity of the mIL-11 mutant proteins was assessed by their reactivity with two monoclonal antibodies raised against rhIL-11 (kindly donated by Genetics Institute) in an enzyme-linked immunosorbent assay. A neutralizing monoclonal antibody (11 h3/19.6.1) served as the capture monoclonal antibody (11 h3/19.6.1) served as the capture and a second biotinylated monoclonal antibody (11 h3/15.6.15) was used as the detector. Dot-blot analysis showed that both monoclonal antibodies preferentially recognized native mIL-11 and not denatured mIL-11, indicating that their reactivity with mIL-11 is conformation-dependent.

**IL-11R-Fc and gp130-Fc Expression Constructs—**The construction of the eukaryotic expression plasmids pIG/IL-11R-Fc and pIG/gp130-Fc has been described previously (17).

**Expression and Purification of IL-11R and gp130—**Both the murine IL-11 and murine gp130 were expressed as fusion proteins with the Fc region of human IgG1. The human epithelial kidney 293T cell line (33) was used for the transient expression of the pIG constructs, as described previously (17). Cell lines containing the Fc fusion proteins was harvested after 6 days. Filtered supernatant was then used either directly in ligand binding assays or for the purification of the IL-11R and gp130. Murine IL-11R and gp130-Fc fusion proteins were purified by affinity chromatography using protein A-Sepharose, as described previously (17). Soluble IL-11R and gp130 were cleaved from the Fc portion while bound to the protein A-Sepharose by human rhinovirus 3C protease, as described previously (17). The purified soluble proteins were examined using SDS-polyacrylamide gel electrophoresis.

**Biotinylation of IL-11—**The method of mIL-11 biotinylation was adapted from a published procedure (34). After purification of mIL-11, buffer exchange to 100 mM borate (pH 8.2) was carried out using NAP-5 Sephadex columns (Amersham Pharmacia Biotech). Biotin amidoacproate N-hydroxysuccinimide ester (Sigma) was added to give a 10:1 (biotin:mIL-11) molar ratio. After a 4-h incubation at room temperature and then blocked with 1 mM biotin and 1% bovine serum albumin for 1 h, wells were washed with PBS and incubated with 100 μl of IL-11R-Fc-containing supernatant for a minimum of 2 h. After washing the wells with PBS the plates were used for binding assays. The binding of biotinylated ligands to IL-11R-Fc was measured by adding varying concentrations of the biotinylated ligand to each well. Competition assays between biIL-11 and varying concentrations of nonbiotinylated ligand were also carried out. In all cases binding was left for 4 h at room temperature. The wells were washed in PBS and incubated with streptavidin-horseradish peroxidase conjugate (Amersham Pharmacia Biotech) for 1 h. After washing with PBS, bound horseradish peroxidase was visualized using o-phenylenediamine as substrate (Dako), and A490 was determined.

**Complex Formation Studies—**Carried out in a similar manner. As for IL-11R-Fc, gp130-Fc was measured using streptavidin-horseradish peroxidase and o-phenylenediamine substrate, as described above. To test nonspecific binding, the amounts of biotinylated ligand bound in the absence of either gp130-Fc or soluble IL-11R were also measured.

**Cell Culture and Ligand Bioassays—**BaF/3 cells cultured in RPMI (Life Technologies, Inc) supplemented with 10% fetal calf serum, 1 mM glutamine, 1 mM streptomycin, and 1 mM penicillin were transfected with BCansegmp130 (35), which encodes the entire open reading frame for mpg130 (35). The cells were then selected on 600 μg/ml G418. Stable BaF/3-mpg130 transfectants were transfected with pCDNA3/mIL-11R, which encodes the entire open reading frame for mIL-11R (donated by M. Hall) and selected on 100 ng/ml mIL-11. All mutant mIL-11 proteins were tested for biological activity on the BaF3/mpi30G130/mIL-11R cells, using the 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) proliferation assay as described previously (17).

**IL-11 Structure Modeling—**The amino acid sequences of IL-11 and IL-6 were aligned using the progressive pairwise algorithm of Feng and Doolittle (36) implemented in the Pileup program of the GCG package. Predictions of secondary structure of mIL-11 were also made using the GCG package. A structural model of mIL-11 was created, from the IL-6 crystal structure coordinates (37) and the IL-6/mIL-11 sequence alignment (with minor manual editing), using the Modeller 4 computer program (38).

**RESULTS**

**Murine IL-11 Mutagenesis—**The strategy for mIL-11 mutagenesis was influenced by mutagenesis studies for IL-6 (for review, see Ref. 39) and LIF (32). A structural model of IL-11, created using the IL-6 crystal structure coordinates (37) and a sequence alignment of IL-6 and IL-11 combined with predictions of secondary structure, solvent accessibility, and helical wheel projections, was used to identify candidate mIL-11 amino acids for mutagenesis. Multiple alanine substitutions were made to identify those residues important for the binding of mIL-11 to mIL-11R and mpg130.

Amino acids located at the carboxyl terminus (Trp-166, Arg-169, Leu-172, Leu-173, and Thr-176) and within the predicted AB loop (Leu-64 and Leu-67) were selected as potential site I residues. Amino acids predicted to be on the exposed surfaces of helices A (Arg-9, Asp-13, Arg-15, Asp-19, and Val-22) and C (Arg-111, Leu-115, Arg-118, and Leu-121) were selected as potential site II residues, and amino acids predicted to lie within the CD loop or the NH2-terminal end of the D helix (Trp-147 and Arg-151) were selected as potential site III residues. Many of the selected residues within each predicted binding site were found to be in close proximity to one another in the structural model of mIL-11.

**Structural Integrity of the Mutant Proteins—**The abilities of the site I mutants to bind to two conformation-dependent monoclonal antibodies were examined, as described under “Materials and Methods.” All of the site I mutants exhibited binding similar to that of the wild type protein (results not shown), indicating that the mutations had not significantly disturbed the native conformation of the protein. In the case of the sites II and III mutants, the assay of the mutants to bind to IL-11R-Fc served as the control for protein folding and the structural integrity of the proteins. If a mutant has reduced affinity for one receptor and not the other, it suggests that the mutation has caused a local conformational change rather than affecting the global structure of the protein.

**Activity of Site I Mutants—**The activities of the mIL-11 mutants were assessed using two different assays. First, the abil-
Identification of Three Receptor Binding Sites of IL-11

### Table I: Summary of receptor binding and BAF assay data for mIL-11 mutants

| Wild type or mIL-11 mutant | Location of mutation\(\textsuperscript{a}\) | Ratio IL-11R-Fc binding IC\(50\)\textsuperscript{b} mutant/IC\(50\) mIL-11 | Ratio BAF stimulation EC\(50\)\textsuperscript{c} mutant/EC\(50\) mIL-11 |
|---------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| mIL-11                    |                                               | 1.0                                           | 1.0                                           |
| L64A/L67A                 | I                                             | 50.0                                          | 2.9                                           |
| W166A                     | I                                             | 3.0                                           | 1.7                                           |
| R169A                     | I                                             | >1,000                                        | >1,000                                        |
| L172A                     | I                                             | 50.0                                          | 2.1                                           |
| L173A                     | I                                             | 10.0                                          | 1.6                                           |
| T176A                     | I                                             | 0.9                                           | 0.4                                           |
| R9A                       | II                                            | 0.8                                           | 0.6                                           |
| D13A                      | II                                            | 1.3                                           | 4.0                                           |
| R15A/D19A/V22A            | II                                            | 10.0                                          | 14.0                                          |
| R111A/L115A               | II                                            | 0.3                                           | 13.0                                          |
| R115A                     | II                                            | 1.2                                           | 0.4                                           |
| L21A                      | II                                            | 1.1                                           | 1.2                                           |
| W147A                     | III                                           | 0.7                                           | >10,000                                       |
| R151A                     | III                                           | 0.9                                           | 4.8                                           |

\(\textsuperscript{a}\) The predicted binding sites of mIL-11 to which each residue is thought to contribute.

\(\textsuperscript{b}\) IC\(50\) refers to the concentration of mutant or wild type protein required to inhibit 50% of the binding of the biIL-11.

\(\textsuperscript{c}\) EC\(50\) refers to the concentration of mutant or wild type protein to produce 50% maximal stimulation of the BAF cells.

Activity of Site II Mutants—The activities of the site II mutants were assessed in both the IL-11R-Fc binding assay and the Ba/F3-mgp130/mIL-11R proliferation assay (Table I and Figs. 1B and 2B). Most of the site II mutants, including R9A, D13A, R118A, and L121A, showed a significant difference in IL-11R-Fc binding compared with that of the wild type protein (Table I and Fig. 1B). The mutant R111A/L115A showed a 4-fold increase in affinity for the IL-11R-Fc (Table I and Fig. 1B), suggesting that changing these 2 residues in helix C has somehow altered site I such that the ligand binds to IL-11R with a higher affinity.

One of the site II mutants, D13A, which exhibited normal binding to IL-11R-Fc, showed a 4-fold reduction in activity in the cell proliferation assay (Table I and Fig. 1B). The mutant R111A/L115A, which showed an increased binding affinity for the IL-11R, showed more than a 10-fold reduction in biological activity (Table I and Fig. 1B). These results suggest that the residues Asp-13 and either or both of Arg-111 and Leu-115 are involved in the interaction between mIL-11 and gp130, as substitution of these residues reduces the ability of mIL-11 to form a signaling complex with gp130 without reducing the affinity of the ligand for the IL-11R. Other mutants, such as R9A, R115A, and L121A, had activities equivalent to wild type mIL-11 in both assays.

The mutant R15A/D19A/V22A showed a 10-fold reduction in the IL-11R-Fc binding assay (Table I and Fig. 1B) which was accompanied by a greater than 10-fold reduction in the cell proliferation assay (Table I and Fig. 2B). These data suggest that the alanine substitutions in this mutant have altered the global structure of the protein.

The activities of the site II mutants D13A, L121A, and R111A/L115A were also assessed by direct binding of biotinylated ligands to the receptor. The abilities of these biotinylated ligands to bind to both IL-11R-Fc and gp130-Fc (in the presence of soluble IL-11R) were measured (Table II and Fig. 3) as well as their activities in the Ba/F3-mgp130/mIL-11R proliferation assay. This allowed us to examine the binding of these site II mutants to gp130-Fc (in the presence of soluble IL-11R), which we were unable to do by measuring competitive binding between mIL-11 and the mutants, as the mutant proteins were still able to compete for soluble IL-11R. The biological activities and IL-11R-Fc binding affinities of the biotinylated mutants confirmed the results observed for the nonbiotinylated site II mutants. This served as a control to show that biotinylation had not altered the activity of the mutant proteins. The only biotinylated ligand with activity that differed significantly from that of the wild type was R111A/L115A. This biotinylated mutant bound to IL-11R-Fc with a 5-fold increase in affinity.
compared with the wild type (Table II), as observed for nonbiotinylated R111A/L115A, whereas binding to gp130-Fc (in the presence of IL-11R) was barely detectable (Table II and Fig. 3). These data support the suggestion that either one or both of the residues Arg-111 and Leu-115 are crucial for the binding of mIL-11 to gp130.

Activity of Site III Mutants—The activities of the site III mutants W147A and R151A were assessed in the IL-11R-Fc binding assay and the Ba/F3-mgp130-mIL-11R proliferation assay (Table I and Figs. 1 C and 2 C). Both of these mutants exhibited normal binding to IL-11R-Fc (Table I and Fig. 1 C). The mutant R151A showed a 5-fold reduction in the cell proliferation assay, and W147A produced undetectable stimulation of the Ba/F3-mgp130/mIL-11R cells (Table I and Fig. 1 C). These data suggest that these substitutions reduce the affinity of the ligand for gp130 without affecting the affinity for the IL-11R, and as discussed earlier, this itself provides a control for the structural integrity of the mutant proteins.

The two site III mutants W147A and R151A were also biotinylated, and the binding affinities and biological activities of the biotinylated ligands were examined (Table II and Figs. 3 and 4). The biotinylated mutant R151A was found to behave in a manner similar to that of the biotinylated wild type IL-11. The biotinylated mutant W147A exhibited normal binding to IL-11R-Fc (Table II), as observed for nonbiotinylated W147A, whereas binding to gp130-Fc (in the presence of soluble IL-11R) was barely detectable (Table II and Fig. 3). This was accompanied by undetectable stimulation of the Ba/F3-mgp130/mIL-11R cells (Table II and Fig. 4). These data suggest that the residue Trp-147 is critical for the binding of mIL-11 to gp130 and hence the formation of a signaling complex.

DISCUSSION

Cytokines mediate biological functions through the formation of multichain receptor signaling complexes. Each cytokine drives the assembly of a receptor complex that is stabilized by multiple protein-protein interactions between the various components. The simplest and best characterized example is hGH, which homodimerizes two identical receptor subunits (22). The gp130 family of cytokines, of which IL-11 is a member, share...
the common signal transducer, gp130. In the case of IL-11, gp130-dependent signaling is activated by the homodimerization of gp130. A complex of IL-11 and the IL-11R interacts with gp130 to induce this homodimerization.

Here we have examined the activities of mIL-11 mutants in receptor binding assays and a cell proliferation assay. We have identified residues crucial for the binding of mIL-11 to both IL-11R and gp130. The location of these residues, as predicted from structural studies and a model of IL-11, provides evidence that mIL-11 has three topologically distinct receptor binding sites, which are both structurally and functionally equivalent to the receptor binding sites I, II, and III of IL-6. This supports the suggestion that, in a manner similar to that of IL-6, IL-11 forms hexameric signaling complexes.

IL-6 has been shown to interact with the IL-6R through a region known as site I, which is formed by residues in the COOH-terminal end of helix D and the AB loop (41–43). Our data indicate that the region of mIL-11 responsible for binding to the IL-11R is topologically very similar to that of IL-6 (Fig. 3). The residues Arg-169, Leu-172, and Leu-173, which are predicted to lie within the COOH-terminal end of helix D, are very important for the binding of mIL-11 to the IL-11R. In fact, Arg-169 was found to be crucial for the binding of mIL-11 to the IL-11R and hence its biological activity in the cell proliferation assay. Sequence alignments reveal that the equivalent residues in all known IL-6 sequences and KSHV-IL-6 are also arginines. Mutagenesis studies have shown that this arginine of hIL-6 (Arg-179) is an important site I residue, and in fact, a positive charge in position 179 is an absolute requirement for the interaction between hIL-6 and the IL-6R (41). The mIL-11 mutant L64A/L67A also showed reduced binding to the IL-11R, indicating that either one or both of these residues, predicted to lie within the AB loop, also contribute to the binding of mIL-11 to the IL-11R. On examining the location of these five site I residues, within our structural model of IL-11, it was found that they collectively form a distinct region at one end of the four-helix bundle structure, as observed for other members of this cytokine family such as hGH (23, 26), LIF (32), and IL-6 (42). The residues appear to be clustered around a central

| Biotinylated ligand | Location of mutation | Ratio IL-11R Fc binding | Ratio complex formation | Ratio BAF stimulation |
|---------------------|----------------------|-------------------------|------------------------|----------------------|
| mIL-11              |                     | 1.0                     | 1.0                    | 1.0                  |
| D13A                | II                   | 2.5                     | 2.4                    | 2.8                  |
| R111A/L115A         | II                   | 0.2                     | >100                   | 6.0                  |
| L121A               | II                   | 0.6                     | 0.9                    | 1.1                  |
| W147A               | III                  | 0.8                     | >100                   | >1,000               |
| R151A               | III                  | 1.2                     | 1.4                    | 2.2                  |

* The binding of biotinylated ligands to gp130-Fc in the presence of 1 μg/ml soluble IL-11R.
Arg-169, which proved to be the most important residue for binding to the IL-11R. The configuration of a binding site as a few critical residues surrounded by ones of lesser importance has also been reported for both hGH (26) and LIF (32).

Site II of IL-6 may be defined as the region that interacts with gp130 in a manner similar to that of site II in hGH. It is formed by exposed residues on helices A and C (44). Residues of mIL-11 predicted to be found within this region of the protein were selected for alanine substitution. Binding data indicate that either one or both of the residues Arg-111 and Leu-115 are extremely important for the binding of mIL-11 to gp130. The mutant R111A/L115A had an unexpectedly high activity in the cell proliferation assay, considering the importance of these residues for interaction with gp130 as shown by binding studies. However, the affinity of this mutant for the IL-11R was approximately 4-fold greater than that of the wild type, therefore reducing the impact of the mutation on the biological activity of the ligand; and, as described earlier for CNTF (40), membrane anchoring of the IL-11R is likely to render the IL-11 receptor complex less sensitive to changes in the affinity of the ligand for either of the receptor subunits. The reason for this is not clear. The residues Arg-111 and Leu-115 are found within the predicted C helix. The data also indicate that Asp-13, which is found within the predicted A helix, may play some role in the interaction between mIL-11 and gp130. These residues form a distinct patch on the surface of the IL-11 structural model, well separated from site I (Fig. 5). Further mutagenesis is required to examine this binding region in more detail, although often it is only a few key residues that contribute to the energy of binding. Site II of IL-6 is thought to be composed of residues Tyr-31 (A helix), Ser-118, and Val-121 (C helix) (37, 44, 45). It was noted for hGH that only a few of the residues involved in contacts between the ligand and receptor in the crystal structure actually contributed to the energy of binding (26); that is, the functional epitope is much smaller than the structural epitope.

The epitopes identified in this study which allow mIL-11 to bind to the IL-11R and one gp130 molecule are functionally equivalent to sites I and II, originally identified on hGH for binding to the two GHR molecules. This illustrates how members of the hematopoietin family of cytokines use topologically conserved epitopes to bind to cytokine receptors. More recently a third site has been identified for some members of this family. Site III enables IL-6 to bind to a second gp130 molecule and form hexameric complexes (29), and site III of LIF enables it to bind to the LIF receptor (32). Our data provide evidence that mIL-11 also has a third receptor binding site. Site II of IL-6 may be defined as the region that interacts with gp130 family members, including IL-6, IL-11, LIF, oncostatin M, and CNTF. A tryptophan residue (Tyr-147) found within this region of IL-6, is conserved in nearly all IL-6 sequences of different species, and it has been reported to be one of the most important site III residues (29). When the amino acid sequences of different gp130 family members are compared, this residue aligns not only with Tyr-147 of IL-11 (identified in this study as a site III residue) and a tryptophan in KSHV-IL-6 (which is known to interact with gp130) but with a conserved phenylala-

References—We are grateful to M. Hall for the pCDNA3/mIL-11R construct, Will Somers for the IL-6 crystal coordinate, and Genetics Institute for monoclonal antibodies against rhIL-11.

Identification of Three Receptor Binding Sites of IL-11

References
Identification of Three Receptor Binding Sites of IL-11

5761

Porter, A. G. (1994) Bio/Technology 12, 601–605
31. Ho, S. N., Hunt, H. D., Horton, R. M., Pullen, J. K., and Pease, L. R. (1989) Gene (Amst.) 77, 51–59
32. Hudson, K. R., Vernallis, A. B., and Heath, J. K. (1996) J. Biol. Chem. 271, 11971–11978
33. Dulbridge, R. B., Tung, P., Hsaia, H. C., Leong, P. M., Miller, J. H., and Calos, M. P. (1997) Mol. Cell. Biol. 7, 379–387
34. Harlow, E., and Lane, D. (1988) Antibodies: A Laboratory Manual, p. 341, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
35. Saito, M., Yoshida, K., Hibi, M., Taga, T., and Kishimoto, T. (1992) J. Immunol. 148, 4066–4071
36. Feng, D. F., and Doolittle, R. F. (1987) J. Mol. Evol. 25, 351–360
37. Somers, W., Stahl, M., and Sechra, J. S. (1997) EMBO J. 16, 989–997
38. Sali, A., and Blundell, T. L. (1993) J. Mol. Biol. 234, 779–815
39. Simpson, R. J., Hammacher, A., Smith, D. K., Matthews, J. M., and Ward, L. D. (1997) Protein Sci. 6, 929–955
40. Di Marco, A., Gloaguen, I., Demartis, A., Saggio, I., Graziani, R., Paonessa, G., and Lauffer, R. (1997) J. Biol. Chem. 272, 23069–23075
41. Fontaine, V., Savino, R., Arcene, R., de Wit, L., Brakenhoff, J. P., Content, J., and Ciliberto, G. (1993) Eur. J. Biochem. 211, 749–755
42. Savino, R., Lahm, A., Giorgio, M., Cabilio, A., Tramontano, A., and Ciliberto, G. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 4067–4071
43. Ehlers, M., Grotzinger, J., deHon, F. D., Mullberg, J., Brakenhoff, J. P., Liu, J., Wolffner, A., and Rose-John, S. (1994) J. Immunol. 153, 1744–1753
44. Savino, R., Lahm, A., Salvati, A. L., Ciapponi, L., Sporeno, E., Altamura, S., Paonessa, G., Toniatti, C., and Ciliberto, G. (1994) EMBO J. 13, 1357–1367
45. Savino, R., Ciapponi, L., Lahm, A., Demartis, A., Cabilio, A., Toniatti, C., Delmastro, P., Altamura, S., and Ciliberto, G. (1994) EMBO J. 13, 5863–5870
46. Kraulis, P. J. (1991) J. Appl. Crystallogr. 24, 946–950