The Membrane Proximal Cytokine Receptor Domain of the Human Interleukin-6 Receptor Is Sufficient for Ligand Binding but Not for gp130 Association*

(Received for publication, April 14, 1998, and in revised form, May 28, 1998)

Suat Özbek, Joachim Grötzinger‡, Barbara Krebs, Martina Fischer, Axel Wollmer‡, Thomas Jostock, Jürgen Müllberg, and Stefan Rose-John§

From the I. Medizinische Klinik, Abteilung Pathophysiologie, Johannes Gutenberg-Universität Mainz, Obere Zahlbacher Strasse 63, D-55101 Mainz, Germany and the Institute für Biochemie, Klinikum der RWTH Aachen, Pauwelsstrasse 30, D-52057 Aachen, Germany

Interleukin-6 (IL-6) belongs to the family of the “four-helix bundle” cytokines. The extracellular parts of their receptors consist of several Ig- and fibronectin type III-like domains. Characteristic of these receptors is a cytokine-binding module consisting of two such fibronectin domains defined by a set of four conserved cysteines and a tryptophan-serine-X-tryptophan-serine (WSXSWS) sequence motif. On target cells, IL-6 binds to a specific IL-6 receptor (IL-6R), and the complex of IL-6/IL-6R associates with the signal transducing protein gp130. The IL-6R consists of three extracellular domains. The NH₂-terminal Ig-like domain is not needed for ligand binding and signal initiation. Here we have investigated the properties and functional role of the third membrane proximal domain. The protein can be efficiently expressed in bacteria, and the refolded domain is shown to be sufficient for IL-6 binding. When complexed with IL-6, however, it fails to associate with the gp130 protein. Since the second and the third domain together with IL-6 can bind to gp130 and induce signaling, our data demonstrate the ligand binding function of the third domain and point to an important role of the second domain in complex formation with gp130 and signaling.

Interleukin-6-type cytokines (IL-6, IL-11, CT-1, CNTF, LIF, OSM) are cytokines with a characteristic helical fold (1, 2). All these cytokines act via receptor complexes, which contain at least one molecule of gp130, the common signal transducing protein of the IL-6 family of cytokines (2). IL-6 and IL-11 act via a homodimer of gp130, whereas CT-1, CNTF, LIF, and OSM require a heterodimer of gp130 and the related protein LIF-receptor (LIF-R) (2). Recently it has been shown that in the human system OSM additionally binds and acts via a receptor complex consisting of gp130 and the OSM receptor, a protein related to gp130 and LIF-R (3). In the murine system, the gp130 and OSM receptor complex seems to be the only complex that is bound and activated by OSM (4).

Interestingly, the cytokines IL-6, IL-11, CT-1, and CNTF first bind to their specific receptor proteins and induce intracellular signaling by subsequent association of this complex with a gp130/gp130 homodimer or a gp130/LIF-R heterodimer. In contrast, LIF and OSM bind directly to LIF-R and gp130, respectively, leading to the formation of heterodimeric gp130-LIF-R or gp130/OSM-R complexes (2). Thus, LIF and OSM do not require specific cytokine receptor subunits as do IL-6, IL-11, CT-1, and CNTF.

The three-dimensional structures of IL-6 (5), CNTF (6), and LIF (7) have been solved and have been shown to share a common “four-helix bundle” fold. The specific ligand binding receptors for IL-6, IL-11, and CNTF are membrane proteins, the extracellular parts of which consist of three domains. Cytokine binding occurs via the cytokine-binding module (CBM), which consists of the second (D2) and the third (D3) domains. The three-dimensional structure of the CBM domain of gp130 has recently been solved (8). It is structurally highly related to the extracellular parts of the growth hormone receptor (9), the prolactin receptor, and the erythropoietin receptor.

The specific receptors for IL-6, IL-11, CT-1, and CNTF cytokines exist in a membrane bound and a soluble form (10–13). In contrast to most soluble receptors for cytokines and growth factors, the soluble receptors of the IL-6 family complex with their ligands can elicit a biological signal on cells that only express the signaling subunits gp130 and LIF-R. This process has recently been called transsignaling (14). Whereas virtually all cells of the body express gp130, the proteins LIF-R, IL-6R, CNTF-R, and IL-11R are only expressed on some cell types (2). Recently, it has been shown that cell types that respond exclusively to IL-6/IL-6R, but not to IL-6 alone, include hematopoietic progenitor cells (15), endothelial cells (16), osteoclasts (17), and neuronal cells (18).

Structure-function analyses of the human IL-6R (19, 20) have shown that the NH₂-terminal Ig-like domain of the receptor protein is not needed for ligand binding and biological activity. So far, the contribution of the single D2 and D3 domains to ligand binding and gp130 activation has not been analyzed. Here we show that the D3 domain alone is sufficient for IL-6 binding but cannot associate with gp130.

MATERIALS AND METHODS

Chemicals—Restriction enzymes were obtained from AGS (Heidelberg, Germany). Vent polymerase was from New England Biolabs (Beverly, MA). Isopropyl-β-D-thiogalactopyranoside was purchased from GEBU (Gaisheim, Germany). Guanidine hydrochloride was from...
Ligand Binding Domain of the Human Interleukin-6 Receptor

21375

Fluka (Buchs, Switzerland). The preparation of the polyclonal mono-
specific antisera against IL-6R (6.2) was described previously (21).

Anti-rabbit IgG POD conjugate was purchased from Sigma (Deisen-
hofen, Germany). Precipitating and soluble BM blue substrates for
detection of peroxidase activity were obtained from Boehringer Mann-
heim (Mannheim, Germany). Skimmed milk powder was from ICN
(McKenheim, Germany).

Construction of a D3 Expression Vector—The D3-encoding region of
the IL-6R-cDNA was amplified by PCR using an IL-6R-cDNA (22) as a
template. Ndel and HindIII sites were introduced in the 5’- and 3’-
primers, respectively, to enable cloning of the amplified DNA in the
Ndel and HindIII sites of the pUC19 expression vector pSET 5b
(23, 24) (sense-primer, 5’ CCG CCG CAT ATG GGA ATC TTG CAG
CCT G 3’; antisense-primer, 5’ GGC CCA AGC TTA AGT AAG
TGC CTT G 3’). PCR was performed in a total volume of 50 µl of PCR
buffer (10 mM KCl, 20 mM Tris-HCl, pH 8.8, 5 mM (NH4)2SO4, 2 mM
MgSO4, 0.1% Triton X-100, 0.2 mM of each dNTP) containing 2.5 ng
of template DNA, 0.5 unit of Vent DNA-polymerase, and 1 µM D3 sense
and antisense primer pairs, respectively. Amplification of D3 DNA by
PCR was performed with 25 cycles of denaturation (45 s, 94 °C), primer
annealing (45 s, 60 °C), and elongation (45 s, 72 °C). PCR products were
purified, subsequently digested with Ndel and HindIII, and ligated into
the pSET 5b vector opened with Ndel and HindIII. The D3 cDNA
construct was verified by restriction analysis and automated DNA
sequencing.

Expression of D3 in Escherichia coli—E. coli BL21 (DE3) cells (23, 24)
were transformed with the D3 expression vector. 200 ml of minimal
medium as has been described (25) were supplemented with ampicillin
(50 µg/ml), glucose (5 g/liter), and trace elements (25), inoculated with
a 2-ml culture of a single clone, and cultured overnight at 37 °C. A
biofermentor (BIOPLO 3000, New Brunswick Scientific, Edison, NJ)
was charged with 3 liters of minimal medium, inoculated with the total
overnight culture, and incubated at 37 °C and a minimum oxygen
saturation of 30%. Expression of the recombinant protein was induced
by adding isopropyl-β-D-thiogalactopyranoside (final concentration: 0.4
mM) at an A600 of about 3 when a specific growth rate of µ = 0.7 was
reached, and the fermentation process was maintained for a further 2 h.
Cells were harvested by centrifugation and resuspended in lysis buffer
(50 mM Tris-HCl, pH 7.5, 1 mM EDTA, 1% Tween 20, 1 mM dithiothre-
itol, 1 mM phenylmethylsulfonyl fluoride). Complete lysis of bacteria
was achieved by three freeze/thaw steps followed by two cycles of
sonication (3 min) and several cycles of washing and centrifugation
resulting in a preparation of purified inclusion bodies. Supernatants
and pellets were analyzed by SDS-PAGE and Western blotting. Inclu-
sion bodies were solubilized in 6 M guanidine HCl, 50 mM Tris-HCl, pH
8.0, 10 mM dithiothreitol.

Protein Purification and Refolding—A Superdex200 (16/60) column
(Amersham Pharmacia Biotech) was equilibrated with refolding buffer
(50 mM sodium phosphate, pH 5, 250 mM NaCl, 1 mM dithiothreitol, 0.5
mM EDTA), loaded with 1.5 ml of solubilized inclusion bodies (200 µg
of protein), and run with a constant flow rate of 1.5 ml/min. Fractions of 2
ml were collected, analyzed by SDS-PAGE, and significant fractions
were pooled and concentrated. By this method 30 mg of purified protein
were obtained from a single fermentation process (3 liters of bacterial
culture).

CD Spectroscopy—CD measurements were carried out on an Aviv
(Aviv Associates, Lakewood, NJ) 62DS CD spectrometer equipped with
a temperature control unit and a Jasco J-600 spectropolarimeter, both
calibrated according to Chen and Yang (26). The spectral bandwidth
was 1.5 nm. The time constant ranged between 1.4 and 4 s and the cell
path length between 0.1 and 10 mm.

Western Blotting—For detection of D3, purified protein or inclusion
bodies from different purification steps were separated on 12.5% SDS-
polyacrylamide gels and electroblotted onto nylon filters (GeneScreen
Plus, NEN Life Science Products). Filters were blocked and incubated
with polyclonal rabbit antibody to IL-6R 1:2000 followed by anti-rabbit
IgG POD conjugate 1:2000. Peroxidase activity was detected using
precipitating BM blue POD substrate.

D3 ELISA—Binding of D3 to IL-6 was analyzed by sandwich ELISA
at 22 °C. Purified recombinant IL-6 was coated on ELISA plate wells
(Microtiter III, Falcon, Oxnard, CA) at 10 µg/ml. After blocking of
unspecific sites the PBS containing 5% skimmed milk was added
D3 was applied at 10 µg/ml. After washing, bound protein was detected
with polyclonal rabbit antibody to IL-6R 1:2000 followed by anti-rabbit
IgG POD conjugate 1:2000. Peroxidase activity was detected with solu-
able BM blue POD. In every ELISA, recombinant soluble IL-6R (con-
sisting of D2 and D3), expressed in Ficha pastoris and purified as
described previously (20), was applied in parallel at 10 µg/ml. For
negative control the equivalent experiment was performed with the
ELISA plate wells coated with PBS containing 5% skimmed milk.

Immunoprecipitation—100 ng of purified D3 protein or sIL-6R were
incubated for 4 h at 4 °C with a 20-fold excess of human IL-6-Fc or a
10-fold excess of human gp130-Fc (alone or in combination with 300 ng
of purified IL-6R) in 50 mM Tris-HCl, pH 7.5, 1 mM dithiothreitol.
All Fe complexes were obtained from supernatants of transiently transfected COS-7 cells and purified via protein A affinity
chromatography. Concentrations were checked using standard protein
assays. Immune complexes were precipitated with protein A-Sepha-
rose, separated on 12.5% SDS-polyacrylamide gels, and blotted as de-
scribed above. Both precipitation experiments and sIL-6R again were
detected using polyclonal rabbit antibody to IL-6R.

Kinetic Assay—IL-6 was covalently immobilized to a carboxymethyl
dextran matrix (Fisons, Loughborough, UK) at 2.25 µg/ml for 2 min in
10 mM sodium acetate buffer, pH 5.0, as recommended by the manu-
facturer (28). Binding experiments were performed at controlled tem-
perature (25 °C) with 10 different concentrations of purified D3 protein
using the IASYS™ (Fisons) optical biosensor. Association was moni-
tored for at least 2 min, the sample was replaced by PBS/0.05% Tween
20 (PBST), and dissociation was monitored accordingly before the
cuvette was regenerated with 5 mM HCl and equilibrated again in PBST.
Association and dissociation affinigrams were analyzed by nonlinear
regression with the FASTfit (Fisons) software, which uses the Mar-
quardt-Levenburg algorithm for iterative data fitting.

RESULTS

Expression, Purification, and Refolding of D3—For expression
of D3 of IL-6R in E. coli, the respective cDNA fragment
was amplified by PCR and cloned into the expression vector
pSET 5b (23, 24). The expressed protein consisted of D3
together with the epitope recognized by the antibody 6.2 (Fig.
LA) (21). After expression in a biofermenter and lysis of cells,
the protein was exclusively found in inclusion bodies (IB).
No recombinant protein was found in the supernatant of the inclu-
sion bodies (SN IB). The expressed protein was visualized as
a 17-kDa band by SDS-PAGE (Fig. LB) and Western blotting (Fig.
1C). Refolding and purification of the highly enriched protein
was achieved by solubilization of the inclusion bodies in guanidine
hydrochloride and subsequent gel filtration on a Superose200
column equilibrated with sodium phosphate buffer (29). Upon
rechromatography of the refolded protein, a single peak was
observed indicating that no dimers or higher aggregates were
formed during the recovery process of D3 (data not shown).

Structural Characterization of D3—The folding state of the protein
was characterized by CD spectroscopy. Fig. 2A shows the
far-UV CD spectrum of D3. The spectrum was indicative of
a protein in a folded state. The prominent band at 232 nm is the
positive lobe of a couplet, which can be attributed to interacting
tryptophan side chains (30). It had also been observed in the
far-UV CD spectrum of the corresponding domain of the
granulocyte-colony-stimulating factor receptor (31) and the third
extracellular domain of gp130 (29). This band seems to be
characteristic of the WSXWS motif present in the COOH-ter-
minal domain of the CBM in all class I cytokine receptors.
Secondary structure analysis of the CD spectrum showed that
the protein mainly consisted of β-sheet (D3: β-sheet = 56%; α-
helix = 0%), confirming the secondary structure prediction
(32). The folded state of the protein was also evident from the
near-UV CD spectrum (Fig. 3B), which showed several distinct
bands attributable to tyrosine and tryptophan side chains (30).
These data demonstrated that purification and refolding of D3
from solubilized inclusion bodies could be achieved in a one-
step procedure using size-exclusion chromatography.

Thermal Unfolding of D3—The thermal stability of the mol-
ecule at pH 6.8 was also studied by CD spectroscopy. Fig. 3A
shows a series of far-UV CD spectra recorded at different

2T. Jostock, S. Rose-John, and J. Mullberg, manuscript in preparation.
temperatures. With increasing temperature the prominent positive band at 232 nm decreases. The ellipticity at 232 nm was plotted as a function of temperature in Fig. 3. The transition midpoint for D3 at pH 6.8 is 35 °C, indicating a remarkable thermal instability of this domain.

Binding of D3 to IL-6 as Detected by Sandwich ELISA—We next asked whether D3 was able to bind human IL-6. IL-6 was immobilized to a 96-well ELISA dish, and binding was assayed by indirect ELISA as described under "Materials and Methods." D3 was recognized by the antibody 6.2 as efficiently as sIL-6R, as shown by Western blotting (Fig. 1C). In Fig. 4, measurements of D3 and of sIL-6R binding to IL-6 are shown. Binding of IL-6 by D3 and by sIL-6R (consisting of domains D2 + D3) were comparable.

Kinetics of Binding of D3 to IL-6—When human IL-6 was immobilized to an IAsys™ cuvette (see "Materials and Methods") real-time interaction between IL-6 and D3 could be analyzed. In Fig. 5A association curves recorded for 10 concentrations of D3 are shown. For clarity, dissociation, although measured for all concentrations, is shown only for the highest concentration. The obtained data were used to calculate an association rate, \( k_a = 2.72 \times 10^4 \pm 1467.61 \text{ M}^{-1} \text{s}^{-1} \), and a dissociation rate, \( k_d = 1.04 \times 10^{-2} \pm 0.0009 \text{ s}^{-1} \), between IL-6 and D3. From these values an affinity of \( K_D = 385 \text{ nM} \) can be derived (Fig. 5B). For comparison, reported affinities for the interaction of immobilized IL-6 and entire soluble IL-6R (33) or the immobilized entire sIL-6R and IL-6 (34), which also were measured at controlled temperature (25 °C) (33, 34), were about 10-fold higher.

Immunoprecipitation of D3 with IL-6-Fc—We next asked whether binding of D3 to IL-6 could be detected in solution and whether a D3/IL-6-complex was able to associate with the signal transducing protein gp130. To answer these questions we incubated purified D3 protein with Fc fusion proteins of IL-6 and gp130 in the presence or absence of IL-6. We have recently constructed a fusion protein consisting of human IL-6...
and human soluble IL-6R in which both proteins were covalently linked by a flexible polypeptide linker (27). This fusion protein was called Hyper-IL-6 (H-IL-6). This IL-6/sIL-6R fusion protein was expressed as an Fc fusion protein (H-IL-6-Fc). Since we have shown that in the H-IL-6 fusion protein, the IL-6R binding site of IL-6 is not accessible for free IL-6R (27), this H-IL-6-Fc protein was used as a negative control. Bound protein was immunoprecipitated and detected by Western blotting. As shown in Fig. 6A, D3 could be precipitated with IL-6-Fc (left lane), but not with the combination of IL-6 and gp130-Fc. There was no binding of D3 to gp130-Fc alone or to H-IL-6-Fc. In Fig. 6B the identical experiment is shown using the sIL-6R consisting of D2 and D3, which could be precipitated with both, IL-6-Fc, and the combination of IL-6 and gp130-Fc. Interestingly, the sIL-6R was almost quantitatively precipitated by IL-6-Fc, whereas only 5–10% of the D3 protein was captured by IL-6-Fc, possibly reflecting the lower affinity of D3 for IL-6 (see above). These results clearly show that D3 of sIL-6R, although being able to bind IL-6, could not mediate an association of the complex with gp130.

**DISCUSSION**

Four conclusions can be drawn from our study: (i) the third domain of the human IL-6R can be expressed on its own and correctly refolds in vitro. (ii) The protein is remarkably unstable to thermal perturbation. (iii) D3 on its own binds the ligand IL-6, albeit with a lower affinity than the complete human IL-6R. (iv) Although D3 is sufficient for IL-6 binding, the complex of D3 and IL-6 fails to associate with gp130.

Receptors for the four-helix cytokines have been shown to be composed of Ig and fibronectin type III-like domains (35). Specific ligand recognition has been ascribed to the CBM, which consists of two of such fibronectin type III-like domains defined by a set of four conserved cysteines and a tryptophan-serine-X-tryptophan-serine (WSXWS) sequence motif (32). In the case of the growth hormone-growth hormone receptor complex, it has been demonstrated that both domains of the CBM are involved in ligand binding (9). The extracellular portion of human IL-6R consists altogether of three domains (22). The NH₂-terminal domain has been shown to be dispensable for ligand recognition and signal initiation (19, 20). Recent experiments might point to a role of the NH₂-terminal domain of the IL-6R for intracellular processing and protein stability.³ Ligand recognition by the CBM of human IL-6R is believed to occur via the loops which connect the β-strands within the two fibronectin type III-like domains (36). In analogy to the growth hormone-growth hormone receptor complex, the third domain of IL-6R is predicted to establish a contact with the respective domain of the CBM of gp130 (36). In fact, mutations in a membrane proximal loop region of the IL-6R, which was predicted to be involved in such a contact, turned out to prevent association of the IL-6IL-6R complex with gp130 (37). Such IL-6R muteins turned out to be weak antagonists of IL-6 (37).

Our results demonstrate that D3 of human IL-6R on its own is sufficient for ligand binding. The affinity we measured is about 1 order of magnitude lower than the one determined for the entire sIL-6R (33, 34). As suggested by our model of the IL-6-IL-6R complex (36), there are two contact regions between IL-6 and IL-6R. The D2 portion of IL-6R is in contact with the 2c region (loop between the helix A and the helix B) (38). The D3 portion of the IL-6R touches the COOH-terminal part of helix D, which also has been implicated in receptor binding (39).

³ P. Vollmer, B. Oppmann, N. Voltz, and S. Rose-John, submitted for publication.
A possible explanation for the low affinity between D3 and IL-6, which we observed in this study, is that loop residues of D2 contribute to ligand binding and thereby to the affinity of the IL-6R (36). As discussed above, D3 was shown to be essential for the contact between IL-6R and gp130. Our data demonstrate that this contact is not sufficient for association of the D3zIL-6 complex with gp130. This implies that D2 is needed for the formation of the complex between IL-6, IL-6R, and gp130.

We have shown that D3 of the human IL-6R has a remarkably low thermal stability. Unfolding starts already at 28 °C. For comparison, the analogous domain of gp130 is stable up to 34 °C. One consequence of this instability of D3 is that this protein does not exhibit antagonistic activity on cells stimulated with human IL-6 (data not shown). Such assays have to be performed at 37 °C a temperature at which D3 is already unfolded. Since a recombinant version of sIL-6R consisting of D2 and D3 is stable at 37 °C (20), it can be concluded that in complex D2 and D3 must stabilize each other.

It has been suggested recently that the complex of IL-6, IL-6R, and gp130 has a stoichiometry of two molecules each of IL-6, IL-6R, and gp130 (41–43). Steric considerations led us to suggest a tetrameric model of the receptor complex containing one molecule each of IL-6 and IL-6R associated with two molecules of gp130 (36). In this respect it is worth mentioning that mutation of valine 190 in the human IL-6R resulted in loss of complex formation between IL-6zIL-6R and gp130 (19), whereas IL-6 binding to IL-6R was unchanged (19). Valine 190 resides within D2 (22). A peptide corresponding to a part of D3 in the human IL-6R was shown to inhibit the biological activity of IL-6 without disturbing binding of IL-6 to IL-6R (44). From these data it can be concluded that both D2 and D3 seem to be involved in complex formation between IL-6zIL-6R and gp130.

Our data add to the understanding of the molecular mechanism of the association of the components IL-6, IL-6R, and gp130 in the receptor complex. Furthermore no experimental structural information is available on human IL-6R, the reported strategy of bacterial expression and refolding of the IL-6R domain, which is important for ligand binding, might be

---

**Fig. 5. Kinetics of binding of D3 to immobilized IL-6.** A, association of D3 to immobilized IL-6 led to the increase of the resonance angle as a function of time. The association phase was recorded for different nanomolar D3 concentrations, as indicated. The decrease of the resonance angle (shown for the highest concentration only) indicates the dissociation phase resulting from replacement of the D3 solution from the solid phase with triplicate washing with PBS containing 0.05% Tween 20. B, calculation of the obtained data from the curves allowed the determination of the dissociation rate constant from the ordinate intercept and the association rate constant from the slope of the graph. All kinetic experiments were performed at controlled temperature (25 °C).

**Fig. 6. Association of D3 with IL-6, IL-6R, and gp130.** A, 100 ng of D3 were incubated with 2 μg of IL-6-Fc (left), 1 μg of gp130-Fc, 1 μg of gp130-Fc in combination with 300 ng of IL-6, and 1 μg of H-IL-6-Fc. All Fc proteins were precipitated with protein A-Sepharose, and after Western blotting, precipitated D3 was detected with anti-IL-6R antibody. The right lane was loaded with 100 ng of purified D3. Numbers indicate the molecular masses of marker proteins. B, the same experiment performed with 100 ng recombinant sIL-6R consisting of D2 and D3. Note that the 55-kDa band protein, which is recognized by the anti-IL-6R antibody, has been identified as protein A released from the protein A-Sepharose during purification (data not shown).
the basis for experiments aiming at structure elucidation by NMR spectroscopy or x-ray crystallography.

REFERENCES

1. Bazan, J. F. (1990) Immunol. Today 11, 350–354
2. Taka, T. and Kishimoto, T. (1997) Annu. Rev. Immunol. 15, 797–819
3. Mosley, B., De Imus, C., Friend, D., Boiani, N., Thoma, B., Park, L. S., and Cosman, D. (1996) J. Biol. Chem. 271, 32635–32643
4. Ichihara, M., Hara, T., Kim, H., Murate, T., and Miyajima, A. (1997) Blood 90, 165–173
5. Somers, W., Stahl, M., and Seehra, J. S. (1997) EMBO J. 16, 988–997
6. McDonald, N. Q., Panayotatos, N., and Hendrickson, W. A. (1995) EMBO J. 14, 2689–2699
7. Robinson, R. C, Grey, L. M., Staunton, D., Vankelecom, H., Vernallis, A. B., Moreau, J. F., Stuart, D. I., Heath, J. K., and Jones, E. Y. (1994) Cell 77, 1101–1116
8. Bravo, J., Staunton, D., Heath, J. K., and Jones, E. Y. (1998) EMBO J. 17, 1665–1674
9. De Vos, A. M., Uitgeijzen, M., and Kossiakoff, A. A. (1992) Science 255, 306–312
10. Davis, S., Aldrich, T. H., Ip, N. Y., Stahl, N., Scherer, S., Farruggella, T., Di Stefano, P. S., Curtis, R., Panayotatos, N., Gascan, H., Chevalier, S., and Yancopoulos, G. D. (1993) Science 259, 1736–1739
11. Mullberg, J., Schooltink, H., Stoyan, T., Gunther, M., Graeve, L., Buse, G., Mackiewicz, A., Heinrich, P. C., and Rose-John, S. (1993) Eur. J. Immunol. 23, 473–480
12. Baumann, H., Wang, Y., Morella, K. K., La, C. F., Dama, H., Hilton, D. J., Hawley, R. G., and Mackiewicz, A. (1996) J. Immunol. 157, 284–290
13. Pennica, D., Arce, V., Swanson, T. A., Vejsada, R., Pollock, R. A., Armanini, M., Dudley, K., Phillips, H. S., Rosenthal, A., Kato, A. C., and Henderson, C. E. (1996) Neuron 17, 63–74
14. Rose-John, S., and Heinrich, P. C. (1994) Biochem. J. 300, 281–290
15. Peters, M., Schirmacher, P., Goldschmidt, J., Odenthal, M., Peschel, C., Di Nee, H. P., Fattori, E., Ciliberto, G., Meyer zum Buschenfelde, K. H., and Rose-John, S. (1997) J. Exp. Med. 185, 755–766
16. Romano, M., Sironi, M., Toniatti, C., Polentarutti, N., Fruscella, P., Ghezzi, P., Faggioni, R., Luini, W., van Hinsbergh, V., Sezzani, S., Bussolino, F., Poli, P., Ciliberto, G., and Mantovani, A. (1997) Immunity 6, 315–325
17. Udagawa, N., Takahashi, N., Katagiri, T., Tamura, T., Wada, S., Findlay, D. M., Martin, T. J., Hirotta, H., Taga, T., Kishimoto, T., and Suda, T. (1995) EMBO J. 14, 461–468
18. Marx, P., Cheng, J.-C., Gadient, R. A., Patterson, P., Stoyan, T., Otten, U., and Rose-John, S. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 3251–3256
19. Yawata, H., Yasukawa, K., Natsuka, S., Murakami, M., Yasukawa, K., Hihi, M., Taga, T., and Kishimoto, T. (1996) EMBO J. 12, 1705–1712
20. Vollmer, P., Peters, M., Ehlers, M., Yagame, H., Matsuba, T., Kondo, M., Yasukawa, K., Buschenfelde, K. H., and Rose-John, S. (1996) J. Immunol. Methods 199, 47–54
21. Stoyan, T., Michaelis, U., Schooltink, H., Van Dam, M., Rudolph, B., Heinrich, P. C., and Rose-John, S. (1993) Eur. J. Biochem. 216, 239–245
22. Yasukawa, K., Taga, T., Hirotta, Y., Yawata, H., Kawanishi, Y., Seed, B., Taniguchi, T., Hirano, T., and Kishimoto, T. (1988) Science 241, 825–828
23. Studier, F. W., Rosenberg, A. H., Dunn, J. J., and Dubendorff, J. W. (1990) Methods Enzymol. 185, 60–89
24. van Dam, M., Mullberg, J., Schooltink, H., Stoyan, T., Braekenhoff, J. P., Graeve, L., Heinrich, P. C., and Rose-John, S. (1993) J. Biol. Chem. 268, 15285–15290
25. Neunhoeffer, F., Haggstrom, L., and Enfors, S.-O. (1995) Biotechnol. Bioeng. 47, 139–146
26. Chen, G. C., and Yang, J. T. (1977) Anal. Lett. 10, 1195–1207
27. Fischer, M., Goldschmidt, J., Peschel, C., Kallen, K. J., Braekenhoff, J. P. J., Wollner, A., Grotzinger, J., and Rose-John, S. (1997) Nat. Biotechnol. 15, 142–145
28. Krebs, B., Griffin, H., Winter, G., and Rose-John, S. (1998) J. Biol. Chem. 273, 2868–2865
29. Muller-Newen, G., Pflanz, S., Hesse, U., Rohl, J. W., Wollner, A., Heinrich, P. C., and Grotzinger, J. (1997) Eur. J. Biochem. 247, 425–431
30. Grishina, I. B., and Woody, R. W. (1994) Faraday Discuss. 104, 245–262
31. Anaguchi, H., Hiraoka, O., Yasukawa, K., Naito, S., and Ota, Y. (1995) J. Biol. Chem. 270, 27845–27851
32. Bazan, J. F. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 6934–6938
33. Brakenhoff, J. P., Rosenberg, A. H., Dunn, J. J., and Dubendorff, J. W. (1990) EMBO J. 15, 2726–2737
34. Sprang, S. R., and Bazan, J. F. (1995) Curr. Opin. Struct. Biol. 3, 185–187
35. Grotzinger, J., Kurapkat, G., Wollner, A., KLau, A., and Rose-John, S. (1997) Proteins Struct. Funct. Genet. 27, 86–109
36. Salvati, A. L., Lahm, A., Panness, G., Ciliberto, G., and Toniatti, C. (1995) J. Biol. Chem. 270, 12242–12249
37. Ehlers, M., Grozinger, J., deHoff, D. F., Mullberg, J., Braekenhoff, J. P., Liu, J., Wollmer, A., and Rose-John, S. (1994) J. Immunol. 153, 1744–1753
38. Krutts, A., Rose-John, S., Muller, A., Wollmer, A., Mullberg, J., Hirano, T., Kishimoto, T., and Heinrich, P. C. (1990) FERS Lett. 262, 323–326
39. Leebek, F. W., Kundi, J., Schwabe, M., and Fowlkes, D. M. (1992) J. Biol. Chem. 267, 14832–14838
40. Ward, L. D., Tard, G., Jompol, G., Yasukawa, K., Hamburger, A., Meritz, R. L., and Simpson, R. J. (1994) J. Biol. Chem. 269, 23286–23289
41. Panness, A., Graziani, R., De Serio, A., Savino, R., Cipponi, L., Lahm, A., Salvati, A. L., Toniatti, C., and Ciliberto, G. (1995) EMBO J. 14, 1942–1951
42. Simpson, R. J., Hamburger, A., Smith, D. K., Matthews, J. M., and Ward, L. D. (1997) Protein Sci. 6, 929–935
43. Grube, B. J., and Cochrane, C. G. (1994) J. Biol. Chem. 269, 20791–20797

The Ligand Binding Domain of the Human Interleukin-6 Receptor

21379