Identification of a gp130 Cytokine Receptor Critical Site Involved in Oncostatin M Response*

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Gp130 cytokine receptor is involved in the formation of multimeric functional receptors for interleukin-6 (IL-6), IL-11, leukemia inhibitory factor (LIF), oncostatin M (OSM), ciliary neurotrophic factor, and cardiotrophin-1. Cloning of the epitope recognized by an OSM-neutralizing anti-gp130 monoclonal antibody identified a portion of gp130 receptor localized in the EF loop of the cytokine binding domain. Site-directed mutagenesis of the corresponding region was carried out by alanine substitution of residues 186–198. To generate type 1 or type 2 OSM receptors, gp130 mutants were expressed together with either LIF receptor β or OSM receptor β. When positions Val-189/Tyr-190 and Phe-191/Val-192 were alanine-substituted, Scatchard analyses indicated a complete abrogation of OSM binding to both type receptors. Interestingly, binding of LIF to type 1 receptor was not affected, corroborating the notion that in this case gp130 mostly behaves as a converter protein rather than a binding receptor. The present study demonstrates that positions 189–192 of gp130 cytokine binding domain are essential for OSM binding to both gp130/LIF receptor β and gp130/OSM receptor β heterocomplexes.

gp130 cytokine receptor participates to the formation of high affinity receptor complexes for a family of cytokines involved in the regulation of hematopoiesis (1), immune response (2), inflammation processes (3), bone modeling (4), neural development (5), and mammalian reproduction (6). This family encompasses IL-6 (7), IL-11 (8), leukemia inhibitory factor (LIF) (9), oncostatin M (OSM) (10), ciliary neurotrophic factor (11), and cardiotrophin-1 (12).

Depending on the identity of the activating ligand, gp130 can either homodimerize in the presence of IL-6 (13) and IL-11 (14) or associate with a related type I cytokine receptor, LIFRβ (15), when recruited by LIF, OSM (16), ciliary neurotrophic factor (17), or cardiotrophin-1 (18). Beside the common LIF/OSM receptor complex made of gp130/LIFRβ, OSM is also able to activate a specific heterodimeric receptor, implicating an association of gp130 with OSMRβ (19).

For some of these receptor complexes a third additional α component leading to an increased specificity and affinity to one given ligand is required to generate fully functional receptors. This is the case for ciliary neurotrophic factor Re (20), IL-6Ra (21) and IL-11Ra (22).

Upon cytokine binding, dimerization of large size receptors (gp130, LIFRβ, and OSMRβ) will initiate the onset of the signaling cascade by inducing a phosphorylation and activation of the Jak family of tyrosine kinases (23). Phosphorylation of tyrosine residues located in the intracellular domain of the cytokine receptors will serve as docking sites for adapters and transcription factors. This is the case for the signal transducers and activators of transcription (STAT) family of transcription factors, which bind to the receptors before being activated and migrate into the nucleus, where they initiate the transcription of the IL-6 family of target genes (24, 25).

gp130 belongs to the type 1 cytokine receptor family defined by the presence of a cytokine binding domain (CBD), that is characterized by four conserved cysteine residues and a common WSXWS box found in most of the cytokine receptors (26). The extracellular domain of gp130 also contains an N-terminal immunoglobulin-like domain and, on the C-terminal side of the CBD, three fibronectin type III domains (27). Crystal structures of neighboring hormone and cytokine receptors containing a CBD, the growth hormone receptor (28), the prolactin receptor (29), erythropoietin receptor (30), and gp130 (31) were defined and used as modeling guides for site-directed mutagenesis (32, 33). Mutation studies allowed the identification of binding and activation sites in the external portion of gp130 (32–35). Implication of the immunoglobulin-like domain was shown to be important for IL-6 signaling (34, 35). Substitution of amino acid residues in the CBD of gp130 led to the identification of Val-252, located in the B’C’ loop of the receptor, essential for IL-6 binding and subsequent activation processes (33). A recent study also reported that amino acids Tyr-190 and Phe-191 located in the EF loop are prevalent for the interaction of gp130 with IL-6 and IL-11 when associated with their respective α receptor components (35).

In the present study, based on the molecular identification of an EF loop epitope implicated in the binding of a neutralizing OSM anti-gp130 antibody, we have substituted the amino acid residues of this portion with alanines. The results show that positions 189–192 of gp130 cytokine binding domain are essential for OSM binding to both gp130/LIF receptor β and gp130/OSM receptor β heterocomplexes.

**MATERIALS AND METHODS**

Cells, Reagents, and Transfections—The COS-7 cell line was grown in RPMI culture medium supplemented with 10% fetal calf serum.
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COS-7 cells used in this study were particularly chosen for their low endogenous expression level of gp130, LIFRβ, and OSMRβ. Previously described anti-gp130 antibodies were IgG1, with the exception of B-R3, which was an IgG2a (36). AN-E1 (IgG1) and AN-E2 (IgG2) were mAbs recently derived in our laboratory that recognized the human forms of LIFRβ and OSMRβ. Mutations were verified by DNA sequencing using the T7 sequencing kit from Amersham Pharmacia Biotech, bought from Upstate Biotechnology, Inc. (Lake Placid, NY). Goat anti-mouse peroxidase-labeled immunoglobulins were from Dako (Glostrup, Denmark). Purified human recombinant LIF (10^3 units/ml) produced in the Chinese hamster ovary cell line was kindly donated by Dr. K. Turner (Genetics Institute, Boston, MA). OSM (2 x 10^6 units/ml) was purchased from Peprotech (Canton, MA).

The cDNAs encoding LIFRβ and gp130 were introduced into PCMX expression vector. OSMRβ in pDC409 was kindly provided by Dr. B. Mosley (19). COS-7 cells were transfected by using the DEAE-dextran method as described previously (37). For each experiment a control plate was transfected with a PCMX plasmid encoding β-galactosidase. Since expression of large size-signaling cytokine receptors on the membrane is usually weak, experiments were pursued only when more than 80% of the cells were blue-stained 48 h after transfection.

Flow Cytometry Analysis—The immunofluorescence studies were performed using the following protocols, and the cells were then analyzed on a FACSCAN from Becton Dickinson (Moutain View, CA).

Screening of Phages with Phage Display Peptide Library—To identify phage-display peptide phage-antibody interactions, a 16-mer random peptide was kindly provided by G. Smith (Columbia, MO). The library (10^11 individual clones) was made by peptide insertion in the N terminus of the major PVIII coat protein of the nonlytic fd-tet phage (38). Briefly, a DNA encoding a 16-mer random peptide with 2 fixed cysteines at positions 5 and 12 was ligated in HindIII and PstI sites, replacing the 21–27 amino acids in the wild type PVIII protein. For the library screening, 35-mm plastic dishes were coated overnight at 4 °C with 10 μg/ml streptavidin in 100 mM carbonate buffer, pH 8.8. Plates were then saturated with a 5% bovine serum albumin TBS solution (150 mM NaCl, 50 mM Tris HCl) for 1 h at room temperature. After 6 washes, 10 μg of B-S1 or B-N4-biotinylated mAbs in 400 μl of 0.5% Tween 20 TBS were added to the streptavidin coat for a 1-h incubation at room temperature. A 4-h contact between the phage library (10^10 particles) and the antibody was carried out at 4 °C in a Tween 20 TBS solution. After extensive washes, bound phages were eluted by acidic treatment using a 0.1 M HCl glycerine buffer, pH 2.2, containing 1 mg/ml bovine serum albumin. Eluted material was neutralized and then used to infect K91 Kan Escherichia coli cells. Bacteria were amplified overnight at 37 °C in the presence of 40 μg/ml tetracyclin (39). For each screening round, the selection efficiency was determined by the number of cpm per input cpm in the infectious titer of the input samples compared to the dish compared with the output samples eluted from the dish (input/output ratio). The next two enrichment steps were realized in a similar manner, except that the phage/antibody contact was carried out in a similar manner, except that the phage/antibody contact was carried out in 0.1% Tween 20 phosphate-buffered saline before being saturated with 0.1% bovine serum albumin TBS for 2 h at room temperature. Plates were then incubated overnight at 4 °C with increasing concentrations of antibody (Fig. 2). A colorimetric detection of phage-bound antibody indicates a specific recognition of an almost unique motif representing 90% analyzed epitopes using the peptide phage display technology (38–46). In the present work we focused on a family of antibodies (B-N4 and B-S1) able to specifically antagonize the biological function of OSM (36, 42, 47).

Screening of a Peptide Display Phage Library—To identify the nature of the defined epitope(s), a peptide display phage library was screened on the B-S1 and B-N4 antibodies. In the chosen library, a 16-mer random peptide was fused to the N terminus of the PVIII coat protein of the phage. In addition, 2 cysteines were introduced at positions 5 and 12 to induce disulfide links and loop formation in generated peptides.

To select phage-displaying peptides recognized by the anti-gp130 mAbs, biotinylated antibodies immobilized with streptavidin were incubated in the presence of 10^11 phages. Bound phages were eluted, amplified, and submitted to two additional cycles of biopanning. Successive comparative recoveries (selected phages/phage input) showed 10^3-10^5-fold enrichments in agreement with the initial phage concentration used. For each studied antibody, 10–12 independent phages were picked up, and the DNA encoding the peptide region was sequenced (Fig. 1). Phages selected with the B-S1 and B-N4 antibodies (referred to as P8-S1 and P8-N4) led to the identification of an almost unique motif representing 90% analyzed sequences and named peptide #1. Interaction specificity between selected phages (P8-S1, P8-N4) and B-S1 or B-N4 mAbs was studied. Experiments were carried out by coating the phage expressing the peptide #1 to the plastic and adding increasing concentrations of antibody (Fig. 2). A colorimetric detection of phage-bound antibody indicates a specific recognition of the selected phage and anti-gp130 OSM-neutralizing mAbs. Irrelevant phages or antibodies failed to give any detectable signal in this assay.

Epitope Modeling Study—The alignment of the B-S1-recognized peptide with the external portion gp130 cytokine receptor shows a similarity between residues 6–9 from the isolated peptide and residues 185–188 of gp130 (Fig. 3). The Asp-185–

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RESULTS

Functional Properties of OSM Response-neutralizing Antibodies—We previously reported the characterization of monoclonal antibodies directed against the gp130-signaling receptor, which are able to specifically neutralize the biological response to one given cytokine of the IL-6 family (36, 42). We have further pursued this study by an identification of recognized epitopes using the peptide phage display technology (38–46). In the present work we focused on a family of antibodies (B-N4 and B-S1) able to specifically antagonize the biological function of OSM (36, 42, 47).

Analysis of Antigen Peptide Recognition by Enzyme-linked Immunosorbent Assay—Specificity of antibody phage recognition was determined by developing an enzyme-linked immunosorbent assay as described previously (39). Briefly, microtiter wells were coated overnight at 4 °C with 50 μl of the phage suspension and washed three times with 0.1% Tween 20 phosphate-buffered saline before being saturated with 1% bovine serum albumin in TBS for 2 h at room temperature. Plates were then incubated overnight at 4 °C with increasing concentrations of antibody in 0.1% bovine serum albumin, 0.01% Tween 20, TBS. Bound antibodies were detected with goat anti-mouse peroxidase immunoglobulins (1/2,000), and the reaction was visualized at 405 nm by using 2-2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) as peroxidase substrate.

Modeling Study—The atomic coordinates of the cytokine binding domain of gp130 were obtained from the Brookhaven protein data bank (accession number: 1BQU). Twenty alternative conformations of the loop connecting the β-strands E and F of the first FN III domain (residues 183 to 194) were generated with MODELER (Table 4). Simulating (San Diego, CA) by simulated annealing. The final model (“open” loop conformation) was obtained from the four lowest energy conformations to maximize the solvent exposure of the Tyr-186 to Thr-188 fragment.

Twenty conformations of the CEYSTAGC cyclic fragment of the peptide #1 were generated with MODELER. The final model of the cyclic peptide was chosen to minimize the r.m.s. deviation of the YST peptide fragment with the YST gp130 fragment in the open loop conformation.

The figures were prepared with INSIGHT (MSI). The solvent-accessible surfaces of the D/E/YST fragment in the openloop conformation of gp130 and in the best match of the cyclic peptide were colored as a function of the electrostatic potential. This potential was calculated with DelPhi (MSI), taking into account the charge of the fragment only.
Trp-188 motif is located in the loop joining the \( \beta \)-strands E and F of the first FNIII domain of gp130 (26). The portion corresponding to the EF loop in growth hormone receptor (28), granulocyte colony-stimulating factor receptor (48), IL-5 receptor (49), IL-6 receptor (50), and erythropoietin receptor (51) were reported to be important contact sites with their respective cognate ligands (Fig. 3). The crystal structure of cytokine binding domain of gp130 was recently determined (31), and similarly, the EF loop contains prominent solvent-exposed residues possibly involved in ligand recognition (31).

In the crystal structure of gp130, Tyr-186 is buried within the protein core where it is involved in a network of aromatic residues including Phe-169, His-167, and Trp-164. This orientation should not permit an easy accessibility of tyr-186 to antibodies. Also, we tested the possibility for the existence of an alternative stable loop conformation in which Tyr-186 would be solvent-exposed. An open loop conformation (Fig. 4) was observed on the basis of quality checks and solvent exposure of the DYST motif. The r.m.s. deviation of the C\(_{\alpha}\) traces was 3.0 Å. Larger changes were observed for side chains. The Tyr-186 side chain orientation changed from the \( \gamma_1 \) rotamer in the crystal structure (\( \chi_1 = 54^\circ \)) to the \( \gamma_2 \) rotamer in the model loop (\( \chi_1 = -64^\circ \)), yielding a 10.7-Å shift in the position of the ring barycenter.

Models of the cyclic peptide #1 were also generated (Fig. 5). For 6 out 20 structures, the r.m.s. deviation of the YST heavy atoms with respect to the 186–188 fragment in the open loop conformation was less than 1 Å. The best match, with a r.m.s. deviation of 0.52 Å, is depicted in Fig. 5. It is worth noting the vicinity of the carboxyl groups of glutamic acid located at position 6 in peptide #1 and of gp130 D185, which are separated by 0.73 Å. Due to the backbone constraints of the cyclic peptide, the conservative mutation Asp\(^3\)Glu is required to maintain the similarity of the solvent-accessible area of the D(E)YST fragment.

The best match between YST in the cyclic peptide models and YST in the crystal structure of gp130 had an r.m.s. deviation of 2.82 Å. This corroborates the assumption that peptide #1 mimics an alternative conformation of the EF loop in the crystal structure of gp130.

Site-directed Mutagenesis of the EF Loop of gp130 Cytokine Binding Domain—Since OSM can directly contact gp130 (16), we have undertaken to substitute amino acids 186–198 in the EF loop of gp130 with alanine residues. Single (186–188) or pair mutations (187–198) were introduced in the receptor. Heavy atoms r.m.s. deviation of the mutated proteins was calculated after energy minimization. The mutations did not introduce significant changes in the structure of the cytokine binding domain of gp130 (Table I). And only minor changes

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**Fig. 1.** Screening of phage display library. Cysteine-constrained phage display peptide library was screened with B-S1 and B-N4 anti gp130 antibodies as described under “Materials and Methods.” After three rounds of enrichment, individual phages were isolated, and the DNA peptide-coding region was sequenced.

**Fig. 2.** Specificity of the interaction between phage-expressed peptide and antibody. Isolated (P8-S1, P8-N4) and control phages were coated on 96-well plates. After a saturation step, serial dilutions of anti-gp130 or isotype control mAbs were added to the wells. Detection was carried out by using a goat anti-mouse peroxidase-labeled antibody and 2-2’-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) as substrate.

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Amino-terminal Motif

| C | C | V | P | W | Y | Y | WSDWS |
|---|---|---|---|---|---|---|---|

Carboxy-terminal Motif

Phage

GFRLECYSTAGCTIPL

hgp130

TSCTVYSTIV--YFVNIENVVEAE

hEPOR

WCSLPTADTS--SVFPLELVTA

hGHR

SCYPNSSF--T--IWIPYCKLTS

hIL-CSFR

HCCIPRKHLL--LYQNMNWQVAE

hIL-6-Rea

SCQLAVP--EG--DSSFYYSVMCA

hIL-5Re

ACWFPRTFLSKGRD6LVSIVNGS

Fig. 3. Alignment of human gp130 with B-S1-isolated phage-coding sequence and with EF loop residues implicated in ligand binding in other cytokine receptors. The upper part of the figure shows a representation of the extracellular region of human gp130, which contains the CBD. The N-terminal side contains \( \beta \)-strands A through G, and the C-terminal motif contains \( \beta \)-strands A’ through G’. The CBD is further defined by four conserved cysteine position and the presence of the WSXWS box. Additional highly conserved residues were also indicated. The lower part of the figure shows an alignment of the isolated phage with the EF loop of gp130 and the corresponding regions in related cytokine and hormone receptors as described by Bazan (27).

Residues of gp130 that were substituted with alanine in this study are boxed in a gray frame. Residues implicated in ligand binding in the erythropoietin receptor (hEPOR), GH receptor (hGHR), G-CSF receptor (hG-CSFR), IL-6 receptor (hIL-6Re), and IL-5 receptor (hIL-5Re) are in bold.
(deviations <1.4 Å) could be observed in the vicinity of the mutations, indicating that gp130 structure remained unaffected by the modifications.

Mutated proteins expressed in COS-7 fibroblast line were also analyzed by using a panel of anti-gp130 mAbs recognizing conformational epitopes (B-R3, B-S12, B-P8, B-T6, B-T2) (36). No significant variation in flow cytometry analysis could be detected when mutants were compared with the wild type protein (Fig. 6A and data not shown). Interestingly, B-S1 binding was neither affected by amino acid substitutions we made, suggesting that the antibody might recognize a larger conformational epitope on gp130. This notion is further sustained by the fact that linear unconstrained phage display peptide libraries did not allow us to isolate any epitope2 and that B-S1

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antibody entirely failed to recognize a denatured form of gp130 after SDS-polyacrylamide gel electrophoresis.

Amino Acids 189–192 Are Essential for OSM Binding to gp130—Mutated receptors were expressed in COS-7 cells together with LIFRβ chain to generate type 1 high affinity OSM receptors (16). For each experiment, surface expression of gp130 and LIFRβ was monitored by using the B-R3 and AN-H1 mAbs, respectively (in black). An IgG2A antibody (in white, left panel) and an IgG1 antibody (in white, right panel) were used as control isotype mAbs. A, flow cytometry analysis of COS-7 cells co-transfected with expression vectors encoding gp130 or the indicated gp130 mutants and LIFRβ. 48 h after transfection, coexpression of gp130 and LIFRβ was monitored by using the B-R3 and AN-H1 mAbs, respectively (in black). An IgG2A antibody (in white, left panel) and an IgG1 antibody (in white, right panel) were used as control isotype mAbs. B, Scatchard transformation of OSM binding experiments to LIFRβ and gp130 mutant heterocomplexes expressed in COS-7 cells. Binding experiments were performed by incubating the cells in the presence of increasing concentrations of radiolabeled OSM. After a 2-h incubation period at 4 °C, bound and unbound fractions were separated by centrifugation through an oil layer. Specific binding values were obtained by subtracting the bound radioactivity observed in the presence of a 100-fold excess of cold ligand from the total bound radioactivity. The affinity binding components were obtained by Scatchard transformation of the values.

OSM responses can also be mediated through a second specific heterodimeric receptor or type 2 receptor complex. The type 2 receptor complex involves gp130, which dimerizes to a gp180 protein named OSMRβ (19, 53). The possibility for VY 189/190 and FV 191/192 mutants to fail in OSM binding when associated to OSMRβ was analyzed. Fig. 7 shows that a decrease in OSM affinity for type 2 receptor complex was already observed for position 188. No specific binding could be observed when VY 189/190 and FV 191/192 mutants were coexpressed

binding to the heterodimeric receptor. In contrast, OSM binding was entirely abrogated, and the affinity could not be calculated when amino acids 189–192 were alanine-mutated. A restoration of OSM binding was observed for alanine substitution of positions 193–198.

Fig. 6. Alanine substitution of residues 189/190 and 191/192 abolishes OSM binding to gp130/LIFRβ heterocomplex. A, flow cytometry analysis of COS-7 cells co-transfected with expression vectors encoding gp130 or the indicated gp130 mutants and LIFRβ. 48 h after transfection, coexpression of gp130 and LIFRβ was monitored by using the B-R3 and AN-H1 mAbs, respectively (in black). An IgG2A antibody (in white, left panel) and an IgG1 antibody (in white, right panel) were used as control isotype mAbs. B, Scatchard transformation of OSM binding experiments to LIFRβ and gp130 mutant heterocomplexes expressed in COS-7 cells. Binding experiments were performed by incubating the cells in the presence of increasing concentrations of radiolabeled OSM. After a 2-h incubation period at 4 °C, bound and unbound fractions were separated by centrifugation through an oil layer. Specific binding values were obtained by subtracting the bound radioactivity observed in the presence of a 100-fold excess of cold ligand from the total bound radioactivity. The affinity binding components were obtained by Scatchard transformation of the values.
These results indicate that positions 189/190 and 191/192 in the gp130 CBD EF loop are crucial for OSM binding and subsequent recruitment of LIFRβ or OSMRβ converter proteins.

Alanine Substitution of gp130 Residues 189–192 Did Not Affect LIF Binding to gp130/LIFRβ Heterodimer—Gp130/LIFR complexes is also a functional heterodimeric receptor for LIF (16). In this case a preferential association between LIF and LIFRβ was observed, gp130 playing the role of converter protein for receptor dimerization and subsequent signaling events. T188A gp130 mutant and VY 189/190 AA- and FV 191/192 AA-mutated receptors, which show a decrease or lost their apparent capacity to bind OSM when transfected together with LIFRβ chain in COS-7 cells, Binding experiments were performed by incubating the cells in the presence of increasing concentrations of radiolabeled OSM. After a 2-h incubation period at 4 °C, bound and unbound fractions were separated by centrifugation through an oil layer. Specific binding values were obtained by subtracting the bound radioactivity observed in the presence of a 100-fold excess of cold ligand from the total bound radioactivity. The affinity binding components were obtained by Scatchard transformation of the values.

DISCUSSION

B-S1 and B-N4 antibodies belong to group of anti-gp130 mAbs that are able to neutralize OSM binding to type 1 and type 2 OSM receptors, as well the subsequent signaling events and functional responses (36, 42, 47). In contrast, binding of B-S1 to gp130 did not affect binding and biological responses to LIF or cardiotrophin-1. This result suggested that B-S1 antibody might recognize a critical site on gp130 required for recruitment of gp130 into a productive signaling complex. That B-S1 failed to recognize gp130 when the protein was denatured suggested the epitope was likely of conformational nature. For this reason epitope cloning was carried out starting from a constrained peptide display phage library containing two fixed cysteines in the random peptide sequence. Comparison of the isolated peptide with gp130 led to the identification of a single homology region located in the EF loop of the gp130 CBD. To rule out any possible indirect effect of the antibody on OSM binding and receptor activation, alanine-scanning mutagenesis was conducted on the EF loop.

We determined that VY189/190AA and FV191/192AA mutations resulted in a dramatic reduction of OSM binding to gp130/LIFRβ complex receptor as well as the subsequent phosphorylations and signaling events. These mutations apparently
did not affect the protein folding in COS-7 cells, since the binding of a series of anti-gp130 mAb-recognizing conformational epitopes was unaffected by the presence of the mutations. Interestingly, introduced EF mutations did not modify gp130 recognition by the B-S1 antibody. This might be linked to the implication of multipoint noncontiguous stretches in the formation of the conformational epitope recognized by the antibody. Similarly, a neutralizing anti-IL-2 receptor \( \gamma \) chain antibody was shown to bind to the protein through four discontinuous contact sites (54). Moreover, a modeling study of the introduced mutations indicated the absence of significant change in the structure of the CBD of gp130.

VY189/190AA and FV191/192AA mutants were found to also lose their capacity to significantly bind OSM when expressed together with OSMR\( \beta \). This indicates that gp130 is likely activated in a similar fashion in both OSM receptor complexes.

In contrast, mutations at positions 189/190 or 191/192 did not affect the high affinity binding of LIF to the gp130/LIFR\( \beta \) heterodimeric receptor. This observation is in line with a preferential binding of LIF to LIFR\( \beta \), gp130 playing in this case a role of converting protein (16). Moreover when gp130 was expressed alone in mammalian cells, LIF failed to compete with OSM for receptor binding (16, 52). This indicated that different sites are likely required when gp130 behaves as a receptor converter or as a binding subunit. Similarly, a specific site inactivation for GM-CSF and IL-5 high affinity binding, which only weakly affected IL-3 binding, was previously reported in the \( B_9C_9 \) loop of the common \( \beta \) chain for this cytokine family (55). Additionally, introduction of mutations in the EF loop of the common \( \beta \) subunit for GM-CSF, IL-3, and IL-5 failed to affect ligand binding, similar to that observed in the present work for LIF (56). This indicates that cytokine binding to the EF loop of receptors is not an absolute requirement to get functional responses. It will be of interest to analyze the ciliary neurotrophic factor and cardiotrophin-1 behaviors toward gp130 EF loop.

Topological similarities between type I cytokine receptors led to the identification of three loops linking \( \beta \) strand elements important for ligand binding. The BC’ loop located in the second part of the CBD was reported to play a prominent role for binding and activation of growth hormone receptor (57), G-CSF receptor (48), the \( \beta \) components for IL-2 receptor (58).
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and IL-5/GM-CSF receptor (55) complexes, and IL-6 receptor α component (50). Molecular modeling-guided mutagenesis also allowed identification of Val-252 in the corresponding portion of gp130-signaling receptor to be essential for IL-6 binding and subsequent activation (33).

Another potential binding site located in the F’G’ loop of the CDB for G-CSF receptor (48, 59), IL-6 receptor α chain (50), mouse IL-3 receptor, and GM-CSF receptor α chain (51) also defined an important contact site with their respective cognate ligands. A similar observation was reported for implication of Gly-306 and Lys-307 residues in the binding of IL-6 to the F’G’ loop of gp130 (33).

The third identified site is the EF loop in the first subdomain of the CBD, which often contains a prominent aromatic residue involved in ligand binding. This is the case for GH receptor (18, 29, 30, 99) and IL-6 receptor (31, 32), as well as in gp130 B (33). Molecular modeling-guided mutagenesis also pointed out the implication of the Ig-like domain of gp130 and FOSM receptors. In contrast, the EF loop does not appear to be essential for LIF binding.

In the present work we show that similar residues and possibly some of the surrounding amino acids were also important for OSM binding to gp130 and functional recruitment of type 1 and 2 OSM receptors. In contrast, the EF loop does not appear to be essential for LIF binding.

Analysis of naturally occurring mutations in IL-2 receptor γ chain revealed single residue substitutions at positions 131 or 134 in the EF loop were responsible for a decrease in IL-2 binding affinity and part of the X-linked severe congenital immunodeficiency syndromes (63, 64). Similar analyses carried out in gp130 EF loop, as well as in gp130 B’C’ and F’G’ loops, should bring important information in the understanding of cytokine-associated diseases.

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