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Molecular Cloning and Expression of an IL-6 Signal Transducer, gp130

Masahiko Hibi, Masaaki Murakami, Mikiyoshi Saito, Toshio Hirano, Tetsuya Taga, and Tadamitsu Kishimoto
Division of Immunology
Institute for Molecular and Cellular Biology
Osaka University
1-3, Yamada-oka, Suita
Osaka 565
Japan

Summary

Interleukin-6 (IL-6) signal is transduced through a membrane glycoprotein, gp130, which associates with IL-6 receptor (IL-6-R). A cDNA encoding human gp130 has been cloned, revealing that it consists of 918 amino acids with a single transmembrane domain. The extracellular region comprises six units of a fibronectin type III module, and part of this region of ~200 amino acids has features typical of a cytokine receptor family. A cDNA-expressed gp130 showed no binding property to IL-6 or several other cytokines. Although a transfectant with an IL-6-R cDNA expressed mainly low affinity IL-6 binding sites, an increase in high affinity binding sites was observed after cotransfection with a gp130 cDNA. This confirmed that a gp130 is involved in the formation of high affinity IL-6 binding sites. A cloned gp130 could associate with a complex of IL-6 and soluble IL-6-R and transduce the growth signal when expressed in a murine IL-3–dependent cell line.

Introduction

Pleiotropy and redundancy are characteristic features of most cytokines (Paul, 1989). IL-6 is a typical example of such a multifunctional cytokine (Kishimoto, 1989); i.e., it promotes the growth of myeloma/plasmacytoma cells, T cells, and renal mesangial cells (Van Damme et al., 1987; Lotz et al., 1988; Horii et al., 1989); it inhibits the growth of myeloid leukemic cell lines and certain carcinoma cell lines (Miyaura et al., 1988; Chen et al., 1988); and it induces differentiation or tissue-specific gene expression, including neural differentiation of PC12 cells (Satoh et al., 1988), megakaryocyte maturation (Ishibashi et al., 1989), macrophage differentiation of myeloid leukemic cell lines (Miyaura et al., 1988; Shabo et al., 1988), immunoglobulin production in B cells (Hirano et al., 1986), and acute-phase protein synthesis in hepatocytes (Andus et al., 1987; Gauldie et al., 1987).

To clarify the molecular mechanism that allows IL-6 to generate such diverse signals, it is essential to know the molecular structure of the IL-6 receptor (IL-6-R). Recent molecular cloning of most of the cytokine receptors, including IL-2-R (β chain) (Hatakeyama et al., 1989a), IL-3-R (Itoh et al., 1990), IL-4-R (Mosley et al., 1989), IL-6-R (Yamasaki et al., 1988), IL-7-R (Goodwin et al., 1990), erythropoietin (Epo)-R (D'Andrea et al., 1989a), granulocyte–macrophage colony-stimulating factor (GM-CSF)-R (Gearing et al., 1989), and granulocyte colony-stimulating factor (G-CSF)-R (Fukunaga et al., 1990), has revealed that they possess common structural features in their extracellular regions and thus they comprise a cytokine receptor family (Bazan, 1989; Gearing et al., 1989; Goodwin et al., 1990). However, the intracytoplasmic regions of these receptors do not show the presence of any particular consensus elements. The intracytoplasmic regions of IL-6-R and GM-CSF-R are relatively short and seem not to be involved in signal transduction. We have shown that IL-6 triggers the association of IL-6-R and another nonligand-binding membrane glycoprotein, gp130. Moreover, a complex of IL-6 and soluble IL-6-R could interact with cell surface gp130 and generate IL-6 signal, showing that the association of IL-6-R and gp130 takes place extracellularly and gp130 is most likely a signal transducer (Taga et al., 1989).

In several other cytokine receptor systems, only low affinity binding sites were observed when cloned cDNAs for receptors such as IL-3-R, GM-CSF-R, and NGF-R were expressed in COS7 cells or factor-nonresponsive cells, while both high and low affinity sites were detected on factor-responsive cells (Itoh et al., 1990; Gearing et al., 1989; Hempstead et al., 1989). It was also shown that low affinity NGF-R could not transduce the signal (Green et al., 1986). All the results suggest the existence of a receptor-associated signal-transducing molecule like gp130. The second chain may stabilize the binding of the ligand to the receptor, resulting in the formation of high affinity binding sites. If a common or structurally related molecule(s) like gp130 is involved in the signal transduction of various cytokines, it could be responsible for the functional redundancy of cytokines. And the assumption of the presence of a variant of a signal transducer in different tissues could explain the pleiotropy of cytokines.

In this paper, we describe the characterization of the cDNA encoding gp130. The results demonstrate that gp130 is involved in the formation of high affinity IL-6 binding sites and in IL-6 signal transduction, although gp130 has no IL-6 binding property. The extracellular region of gp130, part of which belongs to a cytokine receptor family, comprises six units of a fibronectin type III domain, suggesting its ability to interact with other polypeptide chains.

Results

Blocking of the Formation of High Affinity IL-6 Binding Sites by Anti-gp130 MAbs

Monoclonal anti-human gp130 antibodies were obtained by immunizing mice with gp130 purified from U266 cells or placenta. As shown in Figure 1A, anti-gp130 MAbs would reimmunoprecipitate the gp130 molecule that had been copurified with IL-6-R from IL-6–stimulated U266 cells. One of the MAbs, AM64, was shown to recognize the

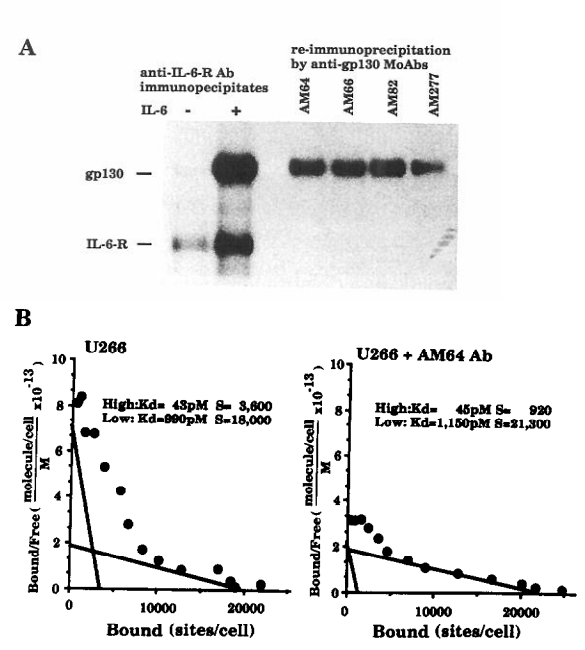

Figure 1. Analysis with Anti-gp130 MAbs

(A) Immunoprecipitation of gp130 by MAbs. Surface-iodinated U266 cells were stimulated with or without IL-6 and solubilized with digitonin-lysis buffer. Immunoprecipitation was performed using anti-IL-6-R MAb MT18 and analyzed by SDS–PAGE (two left lanes). IL-6-R–gp130 complex shown in the second lane was used for reimmunoprecipitation experiments with anti-gp130 MAbs and analyzed by SDS–PAGE (four right lanes).

(B) Blocking of high affinity IL-6 binding sites on U266 cells by MAb AM64. U266 cells were preincubated with or without 40 μg/ml of MAb AM64, and binding assays were carried out in the presence or absence of 20 μg/ml MAb AM64. Scatchard plots are shown.

extracellular region of gp130 by immunostaining of U266 cells and blocked the IL-6–induced association of gp130 and IL-6-R (data not shown). The effect of MAb AM64 on the binding profile of high and low affinity IL-6-R was examined. As shown in Figure 1B, high affinity IL-6 binding sites on U266 cells markedly decreased in the presence of MAb AM64, while low affinity binding sites remained at approximately the same level. The results indicate that gp130 is involved in the formation of high affinity IL-6 binding sites, presumably by stabilizing the binding of IL-6 and IL-6-R.

Cloning and Characterization of a cDNA for gp130

Approximately a half-million plaques from a human placental λgt11 cDNA library were immunoscreened with a mixture of the MAbs, and a clone, λ130-1, was obtained. Using the cDNA insert of this clone as a probe, 70 clones were obtained. λ36 and λ53 were subjected to structural analysis (Figure 2A). A λgt10 cDNA library constructed from mRNA of U266 cells was screened with λ130-1, and then λu-5 and λu-7 were obtained as shown in Figure 2A.

All the clones from placental and U266 libraries above were found to be superimposable or overlapping. The entire sequence has a long open reading frame capable of encoding 918 amino acids (Figure 2B). The nucleotide sequence surrounding the first methionine corresponds well to the consensus sequence for translation initiation sites (Kozak, 1987). A hydropathy plot of the deduced amino acid sequence predicts two major hydrophobic regions. The first spans the initial 22 amino acids and is presumably a signal peptide (von Heijne, 1986). The second region, spanning amino acids 620–641, is presumably a putative transmembrane region. Thus the mature gp130 would consist of an extracellular region of 597 amino acids, a membrane spanning region of 22 amino acids, and a cytoplasmic region of 277 amino acids. There are 14 potential N-linked glycosylation sites. The calculated molecular weight of the mature gp130 of 896 amino acids is 101,000, which agrees well with the M_r (100,000) of glycanase-treated gp130 (Taga et al., 1989).

Six Units of a Fibronectin Type III Module in the Extracellular and GTP Binding Motif–Like Sequences in the Intracytoplasmic Regions

The comparison of the gp130 sequence with the data obtained from the GenBank and National Biomedical Research Foundation data bases showed that the gp130 sequence is unique. However, part of the extracellular region (amino acids 125–320) showed significant homology with the receptors for prolactin (Boutin et al., 1988), growth hormone (Leung et al., 1987), IL-2 (β chain), IL-3, IL-4, IL-6, IL-7, Epo, GM-CSF, and G-CSF, which were shown to be members of a cytokine receptor family (data not shown). This domain in gp130 possesses four conserved cysteins (amino acids 134, 144, 172, and 182) and a WSXWS motif (amino acids 310–314) commonly seen in the family (Gearing et al., 1989), which are indicated by thick bars in Figure 3. The carboxy-terminal part of the extracellular region (amino acids 321–619) has homology with part of contactin (Ranscht and Dours, 1988; data not shown). Within the cytokine receptor family, G-CSF-R is found to have significant homology with gp130 throughout the extracellular region (Fukunaga et al., 1990). Since contactin possesses fibronectin type III modules in the region that has homology with gp130 and G-CSF-R, and since it has also been noted that one fibronectin type III module exists in the carboxy-terminal half of the domain of the cytokine receptor family (Patthy, 1990), alignment of the amino acid sequences of the extracellular regions of gp130 and G-CSF-R with the fibronectin type III module (Skorstengaard et al., 1986) was carried out. As shown in Figure 3, the extracellular regions of gp130 and G-CSF-R contain six units of the fibronectin type III module, including the two in the region homologous to the cytokine receptor family.

In the intracellular region of gp130, the consensus sequence for nucleotide binding, Gly-X-Gly-X-X-Gly-X-Val, which is commonly found in protein kinases (Hanks et al., 1988), is observed at positions 878 through 885, but the motifs in a catalytic domain of protein kinases are absent. Four stretches of amino acid sequences present in the cytoplasmic region of gp130, Gly-Pro-Gly-Thr-Glu-Gly-Gln (residues 878–884), Asp-Ala-Phe-Gly (875–878), Asn-Lys-Arg-Asp (642–645), and Glu-Val-Ser-Ala (870–873) seem partially to fit the consensus elements that were reported

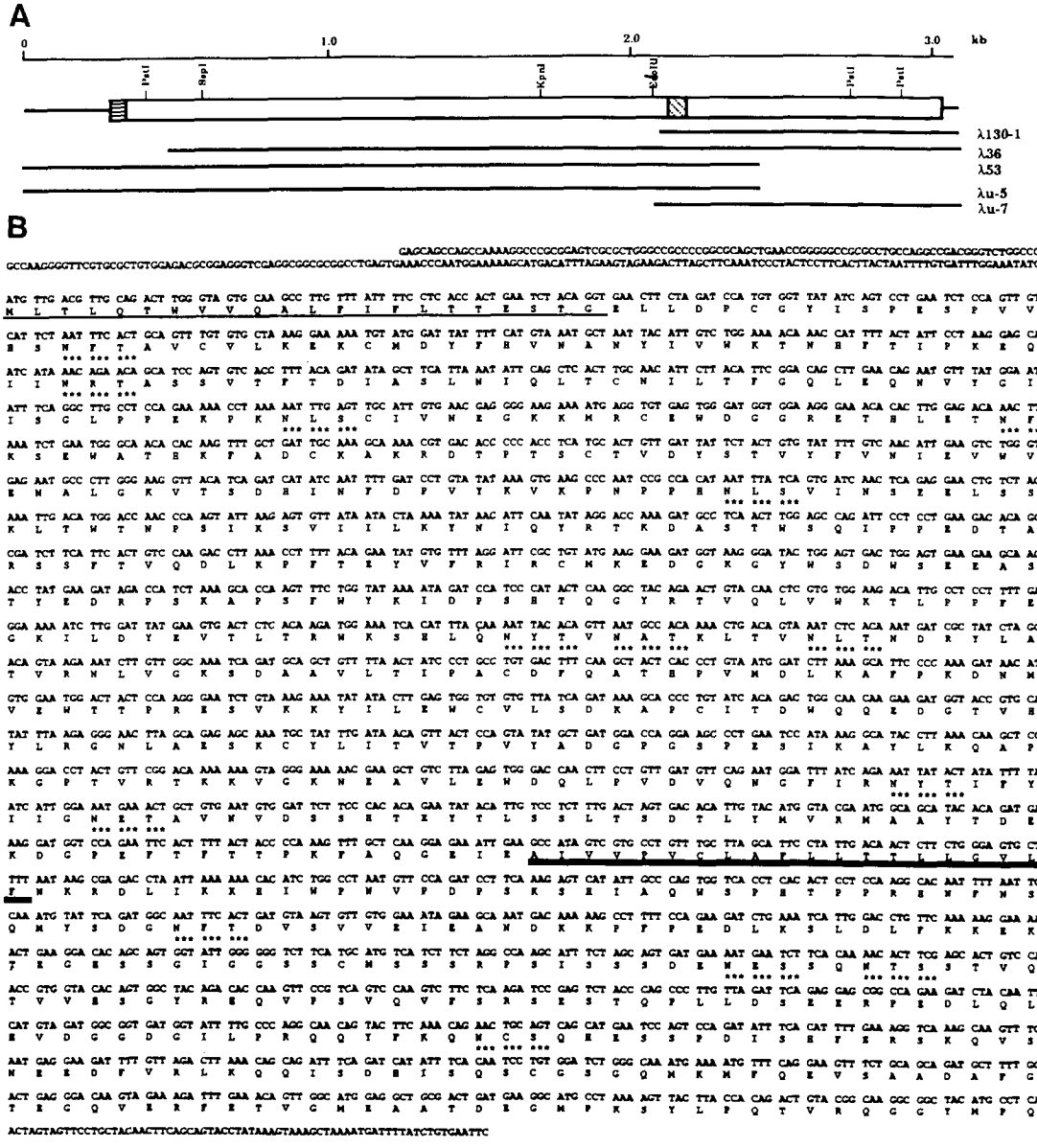

Figure 2. Human gp130 cDNA

(A) Schematic representation and restriction map of independent cDNAs from placental (λ130-1, λ36, λ53) or U266 (λu-5, λu-7) libraries. The coding region is boxed.

(B) Combined nucleotide and deduced amino acid sequences of gp130. A predicted signal sequence is underlined. The thick underline indicates a presumed transmembrane region. The sets of asterisks identify possible N-glycosylation sites.

to be required for GTP binding in *ras* and *ras*-related proteins: Gly-X-X-X-Gly-Lys, Asp-X-X-Gly, Asn-Lys-X-Asp, and Glu-X-Ser-Ala (Dever et al., 1987; Santos and Nebreda, 1989). However, the order of the four motifs and spacing between them in gp130 are different from those in *ras* and *ras*-related proteins. The gp130 molecule shows no significant similarity with *ras* or *ras*-related proteins except for the GTP binding motifs.

Ubiquitous Expression of gp130 mRNA

Expression of gp130 was analyzed by Northern hybridization using the entire coding region of gp130 cDNA as a probe. As shown in Figure 4 (upper panel), the presence of a 7 kb gp130 mRNA was not restricted to IL-6–responsive cells, but was observed in a variety of cells such as a myeloma cell line U266; an EB virus–transformed B cell line, BMNH; a hepatoma line, HepG2; a glioblastoma line, SK-MG-4; an NK-like cell line, YT; a bladder carcinoma line, T24; T cell lines Jurkat and CEM; a non-T, non-B line, Reh; and a Burkitt's lymphoma line, Daudi. All the cell lines tested were found to express gp130 mRNA. In contrast, IL-6-R mRNA was not detected in all cell lines, although it showed a wide distribution (lower panel).

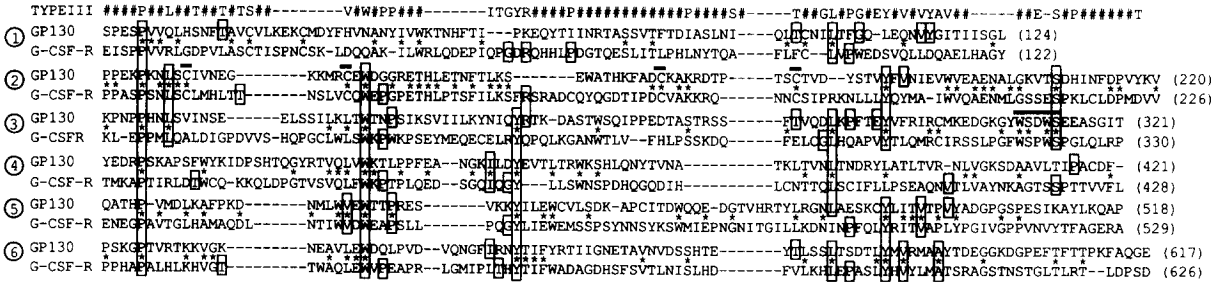

Figure 3. Alignment of gp130 and G-CSF-R with a Consensus Sequence of a Fibronectin type III Module

The consensus sequence is adopted from Skorstengaard et al. (1986) with slight modifications, where amino acid residues observed in at least seven out of 15 type III repeats in fibronectin are indicated. Nonconserved amino acid residues and gaps are indicated by # and –, respectively. The amino acid sequences from 32 to 617 of gp130 and from 30 to 626 of G-CSF-R were aligned (the translation start site of each molecule was defined as 1). The numbers within parentheses are the positions of amino acids. The residues identical with those of the type III module are boxed. Asterisks represent identical amino acids in gp130 and G-CSF-R.

Involvement of gp130 in the Formation of High Affinity IL-6-R

Stable transfectants expressing gp130 were prepared to examine the binding property of the gp130 molecule. The gp130 cDNA spanning the entire coding sequence was inserted into pZipNeoSV(X), carrying the neomycin resistance gene (Cepko et al., 1984), and pZip130 was obtained. The plasmid pZip130, alone or together with the plasmid containing a hygromycin resistance gene (Hatakeyama et al., 1989b), was introduced into a murine IL-3–dependent cell line, BAF-B03 (Hatakeyama et al., 198b), or JIL6R (Taga et al., 1989), which is a neomycin-resistant transfectant of the Jurkat T cell line expressing cDNA-encoded human IL-6-R. To test whether gp130 could bind IL-6 or some other cytokines, a binding assay was performed using a BAF-B03 transfectant, the cell line BAF-130-9. Radioiodinated human IL-6, IL-3, IL-2, and G-CSF, as well as murine IL-3 (as a positive control for the binding assay), were used. As shown in Figure 5A, BAF130-9 binds none of these ligands except murine IL-3, indicating that gp130 has no ability to bind to these cytokines, including IL-6.

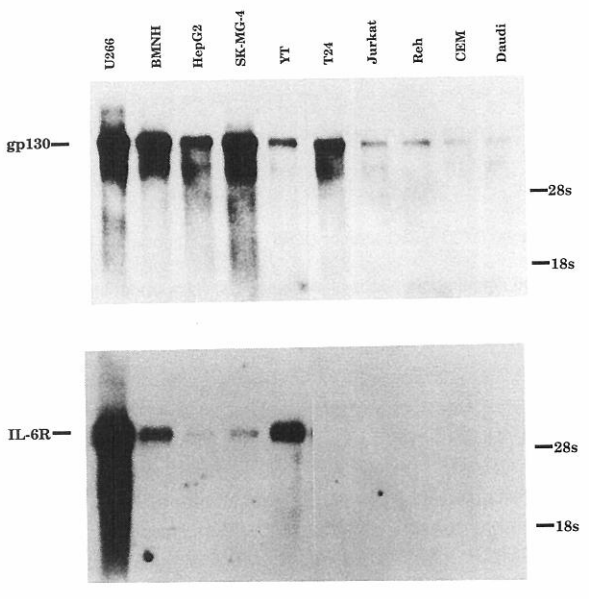

Figure 4. Northern Blot Analysis of gp130 and IL-6-R Transcripts

Two sets of polyadenylated RNA (2 μg) from various cell lines indicated above the figure were electrophoresed through formaldehyde-containing gels and transferred to nylon filter membranes. Filters were hybridized with a gp130 cDNA probe (top) or an IL-6-R cDNA probe (bottom).

Involvement of gp130 in the formation of high affinity IL-6 binding sites was then examined. Although a T cell line, Jurkat, showed no IL-6 binding, its transfectant, JIL6R with IL-6-R cDNA expressed 760 sites/cell of high affinity receptors and 104,000 sites/cell of low affinity receptors (Figure 5B, left panel). After transfection of gp130 cDNA into JIL6R, a resulting transfectant, J130-9, showed an increase of about 5-fold in the number of high affinity binding sites, while the number of low affinity sites remained at approximately the same level (right panel). The small number of high affinity binding sites observed in JIL6R could be due to the low levels of gp130 expression in Jurkat cells as detected by Northern analysis (see Figure 4). When stained with anti-gp130 antibody, AM64, and anti-IL-6R antibody, MT18, mean fluorescence intensities for the respective molecules were 0.14 and 21 in JIL6R and 0.65 and 21 in J130-9 (unstained control values were subtracted; data not shown). This indicates that the levels of gp130 and IL-6-R in J130-9 were not even but existed in a ratio of approximately 1:30, and gp130 expression in J130-9 was only about 5-fold greater than that in JIL6R, which is consistent with the observation of the relatively small increase in high affinity IL-6 binding sites (3,500) over low affinity sites (102,000) after gp130 cDNA transfection into JIL6R. Although several other transfectants were established by introducing gp130 cDNA into JIL6R, all such transfectants also showed relatively low gp130 expression compared with IL-6-R, as is the case of J130-9. J130-9 expressed the highest levels of gp130 among the transfectants, and the result from this transfectant is shown in Figure 5B (right panel). In any of these transfectants, the number of high affinity IL-6 binding sites was correlated with the level of expression of gp130 as measured by cytofluorometry. Anti-gp130 MAb AM64 decreased the number of high affinity IL-6 binding sites in

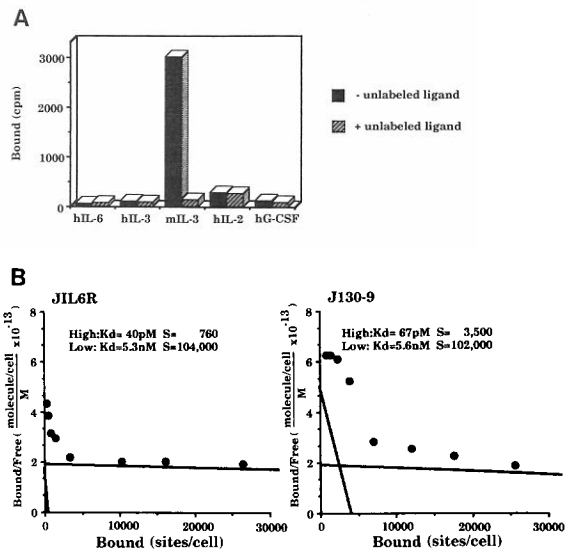

Figure 5. Involvement of gp130 in the Formation of High Affinity IL-6 Binding Sites

(A) Lack of binding properties of gp130 to several cytokines. A BAF-B03 (a murine IL-3–dependent cell line) transfectant, BAF130-9, with gp130 cDNA was used in a binding assay with radioiodinated recombinant human IL-6, human IL-3, murine IL-3, human IL-2, or human G-CSF (2.0×10^4 cpm each in 80 μl of reaction mixture). Data represent average cell-associated counts of duplicates.

(B) Formation of high affinity IL-6 binding sites by transfection with IL-6-R and gp130 cDNAs. JIL6R (a Jurkat transfectant with IL-6-R cDNA) and J130-9 (a Jurkat transfectant with both IL-6-R and gp130 cDNAs) cells were used for Scatchard analysis.

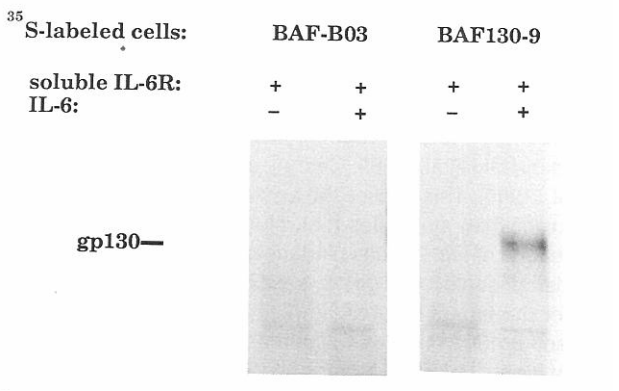

Figure 6. Association of a cDNA-Expressed gp130 with a Complex of Soluble IL-6-R and IL-6

Metabolically labeled BAF-B03 or BAF130-9 cells were incubated with 5 μg/ml of soluble IL-6-R (~100 nM) in the presence or absence of 500 ng/ml of IL-6 (~25 nM), then solubilized with digitonin-lysis buffer. Immunoprecipitation was employed using anti-IL-6-R MAb MT18 and analyzed by SDS–PAGE.

those transfectants in the same manner as shown in Figure 1B, using U266 myeloma cells. All the results indicate that gp130 is involved in the formation of high affinity IL-6 binding sites.

Cloned gp130 Interacts with a Complex of IL-6 and Soluble IL-6-R and Transduces the Signal

Murine IL-3–dependent BAF-B03 cells or transfectant BAF130-9 cells expressing human gp130 were metabolically labeled and incubated with soluble IL-6-R (5 μg/ml) in the presence or absence of IL-6 (500 ng/ml). Cells were lysed with digitonin and immunoprecipitated with the anti-IL-6-R MAb MT18. As shown in Figure 6, a protein with a M_r of 130,000 was detected by SDS–PAGE from BAF-130-9 cells incubated with soluble IL-6-R plus IL-6, demonstrating that cDNA-expressed gp130 could associate with soluble IL-6-R in the presence of IL-6. Considering the concentrations of soluble IL-6-R (~100 nM, on the basis of its M_r of ~50,000; Yasukawa et al., 1990) and IL-6 (~25 nM), the association constant of the complex of soluble IL-6-R and IL-6 for gp130 is presumably greater than 10^7 M^{-1}. However, further studies are required to determine the precise association constant. The association was not observed in the absence of IL-6 nor in parental BAF-B03 cells (Figure 6).

BAF-B03 and BAF130-9 cells were incubated with varying concentrations of IL-6 in the presence or absence of soluble IL-6-R, and the incorporation of [^{3}H]thymidine was measured. As shown in Figure 7, BAF130-9 cells proliferated when cultured with IL-6 plus soluble IL-6-R. The parental BAF-B03 cells did not proliferate under the same conditions. No significant proliferation of BAF130-9 was observed with IL-6 alone. A small amount of the [^{3}H]thymidine that was incorporated by BAF130-9 cells cultured with soluble IL-6-R alone might be due to the presence of IL-6 in the fetal calf serum. All the other BAF-B03 transfectants with a gp130 cDNA also proliferated when cultured with soluble IL-6-R plus IL-6, but neomycin-resistant BAF-B03 transfectants without gp130 expression did not proliferate under the same conditions. The results demonstrated that cDNA-expressed gp130 in IL-3–dependent cells could transduce the proliferative signal after interacting with a complex of soluble IL-6-R and IL-6.

Discussion

Transfection of the cDNAs for cytokine or growth factor

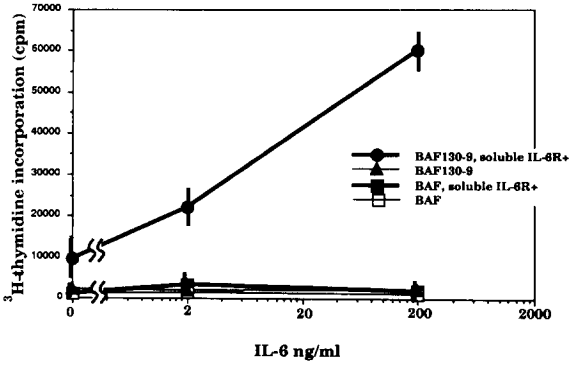

Figure 7. IL-6–Mediated Growth Signal through a cDNA-Expressed gp130

BAF-B03 and BAF130-9 cells were incubated with varying concentrations of IL-6 in the presence or absence of soluble IL-6-R (5 μg/ml) for 40 hr and pulse-labeled with [^{3}H]thymidine for 8 hr. Incorporated radioactivities are shown. Vertical bars represent the SD of triplicate measurements.

receptors such as IL-3-R, IL-6-R, GM-CSF-R, and NGF-R has shown that these cDNAs can encode only low affinity binding proteins in COS7 cells or fibroblast cells, although both high and low affinity binding sites for these cytokines are known to be present in native cells (Itoh et al., 1990; Yamasaki et al., 1988; Gearing et al., 1989; Hempstead et al., 1989). This implies the existence of possible receptor-associated molecules that might be required for the formation of high affinity binding sites. In this study we have demonstrated that gp130 is such a molecule, responsible for the formation of high affinity IL-6 binding sites, since anti-gp130 MAb AM64 remarkably decreased the number of high affinity IL-6 binding sites, and, after introduction of gp130 cDNA into the Jurkat transfectant (JIL6R) possessing IL-6-R cDNA, the resulting transfectant, J130-9, showed an increase in high affinity binding sites that was inhibited by the AM64 MAb. Although the increase was relatively small (5-fold), this could be explained by the results of the flow cytometric analysis of J130-9 and JIL6R, i.e., the staining levels of gp130 and IL-6-R in J130-9 occurred in a ratio of approximately 1:30 and the level of gp130 expression in J130-9 was about 5-fold greater than that in JIL6R, which is consistent with the results of the binding assay. Although several other transfectants obtained by introducing gp130 cDNA into JIL6R cells also expressed relatively low levels of gp130 as J130-9 did, such transfectants (as well as J130-9) showed correlations between the number of high affinity sites and levels of gp130 expression. It is postulated that after association of gp130 with the IL-6-IL-6-R complex, the binding of IL-6 to the receptor might be stabilized, resulting in the appearance of the high affinity IL-6 binding sites. This model might be applied to other receptor systems, like IL-3-R, GM-CSF-R, and NGF-R, in which both high and low affinity sites exist.

Northern analysis showed that gp130 mRNA is present ubiquitously. This is in contrast to IL-6-R expression: although IL-6-R shows a broad distribution, some cell lines lack this particular receptor. Since gp130 mRNA is present in all cell lines tested, the introduction of IL-6-R into any cell type should theoretically yield high affinity IL-6-R. In fact, when transfected with IL-6-R cDNA, human Jurkat T cells and mouse L fibroblast cells expressed both high and low affinity IL-6 binding sites, although the number of high affinity binding sites was low. However, this may not necessarily mean that any cell type acquires functional IL-6 signaling capability after introduction of IL-6-R cDNA, since not all elements of the signal transduction machinery working downstream of gp130 exist in every cell. Ubiquitous expression of gp130 suggests the possibility that gp130 might work as a signal transducer for other cytokine receptors. Although gp130 possesses no binding property, at least to human IL-2, IL-3, IL-6, or G-CSF (see Figure 5A), our preliminary study implies that the transfection of a gp130 cDNA into IL-3–dependent cells augmented the sensitivity of the cells to IL-3. If this could be confirmed, it would explain the functional redundancy of cytokines by a mechanism utilizing the same or a similar signal transducer.

It was known that the primary structure, as well as the gene organization, of IL-6 has significant similarity with that of G-CSF (Hirano et al., 1986; Yasukawa et al., 1987). It is notable that the signal–transducing gp130 but not the ligand-binding IL-6-R has an overall structural similarity to G-CSF-R in the extracellular region (see Figure 3). The possibility that gp130 might bind to G-CSF was excluded, since no specific binding of ^{125}I-G-CSF was observed, using gp130-expressing BAF130-9 cells. The finding of six units of a fibronectin type III module in the extracellular region of gp130 and G-CSF-R suggests that these chains might interact with the other cell surface molecules. In fact, gp130 can associate with IL-6-R, in which two units of the fibronectin type III module exist (Patthy, 1990, and unpublished data). Since fibronectin type III modules appear in several adhesion molecules, such as L1 (Moos et al., 1988) and cytotactin (Jones et al., 1988), gp130 might have evolved from cell surface molecules that could have been involved in cell-to-cell communication, or in the recognition of cell membrane–bound ligands. It is interesting to note that there exists a membrane-bound form of IL-1 and M-CSF (Beuscher and Colten, 1988; Rettenmier et al., 1987), but receptors for these ligands (Sims et al., 1988; Sherr et al., 1985) are not the members of the cytokine receptor family that contain fibronectin type III modules. The members of the cytokine receptor family or signal transducers such as gp130 might function as cell adhesion molecules so that they could interact with cells secreting cytokines and receive their signals efficiently.

Very little is known about intracytoplasmic signaling mechanisms of cytokines such as IL-6. The pleiotropy of IL-6 in different tissues might be generated if gp130 variants exist that possess the intracytoplasmic region with a different structure. However, this is unlikely, since gp130 cDNAs cloned from placental and U266 libraries showed identical amino acid sequence throughout the molecule. Thus, the pleiotropy of IL-6 could be generated by a molecule(s) present downstream of gp130 in the IL-6 signaling pathway that might vary in different types of cells. Although particular consensus sequences were not observed among the cytoplasmic regions of the members of the cytokine receptor family, some similarities in the cytoplasmic regions were reported between IL-2-R β chain and Epo-R (D'Andrea et al., 1989b), G-CSF-R and IL-4-R (Fukunaga et al., 1990), and IL-3-R and IL-2-R β chain (Itoh et al., 1990). One part of this region was mapped as a functional signal transduction domain in IL-2-R β chain (Hatakeyama et al., 1989b). We observed weak similarity among the cytoplasmic regions of gp130, G-CSF-R, Epo-R, and IL-2-R β chain. We also noticed a serine-rich region in the middle of the cytoplasmic domain of gp130 (data not shown), as found in IL-2-R β chain, IL-4-R, and G-CSF-R. However, it remains to be resolved whether these regions have any significance in the signal transduction of these cytokines. The finding of GTP binding motif–like sequences in the intracytoplasmic region of gp130 suggests a possible relationship between the *ras* signaling pathway and IL-6 signal transduction, but future studies will be required to examine this possibility, including that of a GTP binding activity in this molecule. A nuclear factor, NF-IL6, which takes part in IL-6 expression, has been shown to be involved also in the expression of acute-phase protein genes

in IL-6–stimulated hepatocytes (Akira et al., 1990). Since NF-IL6 is considered to be a nuclear factor mediating IL-6 function in hepatocytes, filling the blanks between gp130 and NF-IL6 will help to reveal the cytokine signaling mechanisms from membrane to nucleus.

Experimental Procedures

Anti-gp130 MAbs and Immunoprecipitation

U266 cells (8×10^9) were incubated in 60 ml of cell culture medium (RPMI 1640 + 10% FCS) containing 1.5 μg/ml of IL-6 at 37°C for 30 min and solubilized with 60 ml of ice-cold digitonin-lysis buffer (Taga et al., 1989). gp130 was copurified with IL-6-R by the anti-IL-6-R MAb MT18 (Hirata et al., 1989), and the complex of gp130 and IL-6-R, together with MT18-conjugated Sepharose 4B (Pharmacia), was used for immunization. BALB/c mice were immunized six times with 2×10^9 cell-derived gp130–IL-6-R complex, and hybridomas were obtained by a conventional HAT selection. Supernatants from growth-positive cells were harvested and tested for their ability to bind gp130 as follows. U266 cells (5×10^7) were metabolically labeled with [^{35}S]methionine (2.5 mCi) and stimulated with 1.5 μg/ml of IL-6 and solubilized in digitonin-lysis buffer (1 ml). Clear lysate was mixed with a 0.04 ml packed volume of MT18-conjugated Sepharose 4B beads. The beads were washed in digitonin-lysis buffer six times, and ^{35}S-labeled gp130 and IL-6-R were dissociated and eluted in 0.25 ml of digitonin-lysis buffer (pH 3.4) and neutralized by adding 0.025 ml of 1 M Tris (pH 7.4). Hybridoma supernatant was incubated with protein G–Sepharose (Pharmacia), washed, and incubated with an aliquot of ^{35}S-labeled mixture of gp130 and IL-6-R. The immunoprecipitated materials were analyzed by SDS–PAGE to detect gp130-reactive clones, and AM277 was obtained. AM64, AM66, and AM82 were obtained by immunizing mice with U266- or human placental membrane–derived gp130 using a column of AM277-conjugated Sepharose 4B. Placental membrane was prepared as described (Fujita-Yamaguchi et al., 1983), and processed with digitonin-lysis buffer as mentioned above. Surface iodination and immunoprecipitation using soluble IL-6-R and IL-6 were described previously (Taga et al., 1989). Soluble IL-6-R was kindly provided by Dr. Yasukawa (Tosoh Corporation, Kanagawa; Yasukawa et al., 1990).

cDNA Cloning and Nucleotide Sequence Analysis

A λgt11 cDNA library from human placenta (Clontech) was used. Approximately $5–50 \times 10^3$ plaques per 15 cm dish were overlaid with nylon filters (Schleicher and Schuell) and screened with a mixture of anti-gp130 MAbs according to Sambrook et al. (1989). A λgt10 cDNA library from poly(A)$^+$ U266-derived RNA was prepared using a cDNA Synthesis System Plus and a cDNA Cloning System λgt10 (Amersham). Plaque hybridization was carried out as described (Sambrook et al., 1989). DNA sequencing was performed by the dideoxynucleotide chain termination method using Taq polymerase (Promega) and Sequenase (Stratagene) according to the manufacturers' procedures.

Northern Hybridization

Isolation of RNA and Northern blot analysis were performed as described (Sambrook et al., 1989; Sugita et al., 1990). Filters were probed with a 2.8 kb AccII–SpeI fragment of gp130 cDNA (position −6–2761) containing the entire coding region or an FspI–EspI fragment of IL-6-R cDNA (Yamasaki et al., 1988).

Transfectants

JIL6R is a Jurkat (human T cell line) transfectant with a human IL-6-R cDNA and a neomycin resistance gene (Taga et al., 1989). A 2.8 kb AccII–SpeI fragment of gp130 cDNA (position −6–2761) containing the entire coding region was inserted in pZipNeoSV(X)I (Cepko et al., 1984), and pZip130 was obtained. J130-9 was established by transfecting JIL6R with pZip130 and pHygro (obtained from Dr. Hatakeyama; Hatakeyama et al., 1989a) and selected with hygromycin. BAF130-9 was obtained by transfecting BAF-B03 (kindly provided by Dr. Hatakeyama; Hatakeyama et al., 1989b) with pZip130 and selected with neomycin.

Recombinant Cytokines and Binding Assay

Recombinant human IL-6 and IL-2 were provided by Ajinomoto Co. Ltd. (Tokyo). Human and mouse IL-3 were from Kirin Brewery Co. Ltd. (Tokyo) and Dr. Miyajima (DNAX), respectively. Human G-CSF was from Chugai Pharmaceutical Co. Ltd. (Tokyo). Radioiodination was performed as described previously (IL-6, Taga et al., 1987; IL-2, IL-3, and G-CSF, Schreurs et al., 1989). Specific activities of human IL-2, IL-3, IL-6, G-CSF, and mouse IL-3 were 3.3×10^{17}, 4.2×10^{17}, 1.0×10^{18}, 1.3×10^{18}, and 4.1×10^{17} cpm/mol, respectively. Binding assays and Scatchard analysis were processed as described elsewhere (Taga et al., 1987).

Cell Growth Assay

BAF-B03 and BAF130-9 cells were cultured with or without IL-6 in the presence or absence of soluble IL-6-R for 40 hr in microculture plates (1×10^4 cells per well, 0.15 ml per well) and pulse-labeled with [^{3}H]thymidine (1 μCi per well) for 8 hr. Cells were harvested on glass filters, and cell-associated radioactivity was measured.

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