CD8$^-$ subset that produces IL-4 and IL-5 (*15*). The observation that a proportion of alloreactive CD8 cells can be diverted by IL-4 from the normal cytolytic CD8 phenotype suggests a possible therapy in the area of transplant rejection. Potentially graft-destructive CD8 lytic responses may be able to be turned into more tolerable or appropriate lymphokine responses by the administration of IL-4 (*16*).

REFERENCES AND NOTES

1. G. Le Gros, S. Z. Ben-Sasson, R. Seder, F. D. Finkelman, W. E. Paul, *J. Exp. Med.* **172**, 921 (1990); S. L. Swain, A. D. Weinberg, M. English, G. Huston, *J. Immunol.* **145**, 3796 (1990); M. Kopf *et al.*, *Nature* **362**, 245 (1993).
2. F. Erard, M. Nabholz, A. Dupuy-d'Angeac, H. R. MacDonald, *J. Exp. Med.* **162**, 1738 (1985); F. Erard *et al.*, *J. Immunol.* **134**, 1644 (1985).
3. On day 6 of culture, approximately 3×10^5 to 5×10^5 cells/ml were recovered from all culture conditions, which indicates a doubling time of 14 to 15 hours. Purified CD4$^+$ lymph node cells (1000 cells per well) cultured in the presence of PMA, ionomycin, IL-2, and IL-4 achieved concentrations of 3×10^5 to 7×10^5 cells/ml by day 6.
4. Resting CD8$^+$ cells do not express perforin [E. R. Podack, H. Hengartner, M. G. Lichtenheld, *Annu. Rev. Immunol.* **9**, 129 (1991); A. Zlotnik, D. I. Godfrey, M. Fischer, T. Suda, *J. Immunol.* **149**, 1211 (1992)].
5. Cytokine production by CD8 cells from cultures treated for 6 days with PMA and IL-2 or PMA, IL-2, and IL-4 was identical to cytokine production by CD8 cells from cultures treated with PMA, ionomycin, and IL-2.
6. P. A. Geuerne, P. F. Piguet, P. Vassalli, *J. Immunol.* **130**, 2225 (1983); A. Kelso and A. Glasebrook, *ibid.* **132**, 2924 (1984).
7. T. R. Mosmann, H. Cherwinski, M. W. Bond, M. A. Giedlin, R. L. Coffman, *ibid.* **136**, 2348 (1986).
8. T. Owen, *Eur. J. Immunol.* **18**, 395 (1988); R. J. Noelle and E. C. Snow, *Immunol. Today* **11**, 361 (1990).
9. No IgG1 production by B cells was induced by CD8 cells from cultures treated with PMA and IL-2 or PMA, IL-2, and IL-4.
10. J. White *et al.*, *J. Immunol.* **143**, 1822 (1989).
11. Sorted CD8$^-$ cells had no detectable lytic activity (<2%) against allogeneic targets (BW5147 cells) at an effector:target ratio of 12:1, whereas CD8$^+$ cells had easily detectable activity (28%).
12. F. P. Heinzel, M. Sadick, S. S. Mutha, R. Locksley, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 7011 (1991).
13. R. G. Titus *et al.*, *Eur. J. Immunol.* **17**, 1429 (1987); I. Muller, T. H. Pedrazzini, P. Kropf, J. A. Louis, G. Milon, *Int. Immunol.* **3**, 587 (1991).
14. P. Salgame *et al.*, *Science* **254**, 279 (1991).
15. J. B. Margolick *et al.*, *Clin. Immunol. Immunopathol.* **51**, 348 (1989); E. Maggi *et al.*, *Eur. J. Immunol.* **17**, 1685 (1987); G. M. Shearer and M. Clerici, *Prog. Chem. Immunol.* **54**, 21 (1992); A. Sher *et al.*, *Immunol. Rev.* **127**, 183 (1992).
16. V. S. Abraham, D. Sachs, M. Sykes, *J. Immunol.* **148**, 3746 (1992).
17. S. Alonso, A. Minty, Y. Bourlet, M. Buckingham, *J. Mol. Evol.* **23**, 11 (1986).
18. J.-C. Cerottini, H. D. Engers, H. R. MacDonald, K. T. Brunner, *J. Exp. Med.* **140**, 703 (1974).
19. J. A. Garcia-Sanz and E. R. Podack, *Eur. J. Immunol.*, in press.

20. We thank M. Wesp and A. Kiefer for flow cytometry, S. Walti for technical assistance, B. Pfeiffer for artwork, Dr. P. Cosson for the CD8α cDNA probe, and F. Ronchese, T. Staehelin, C. Heusser, and R. Jessberger for critical review of the manuscript. The Basel Institute for Immunology was founded and is supported by F. Hoffmann–La Roche Ltd.

1 February 1993; accepted 13 May 1993

LIFRβ and gp130 as Heterodimerizing Signal Transducers of the Tripartite CNTF Receptor

Samuel Davis,* Thomas H. Aldrich, Neil Stahl, Li Pan, Tetsuya Taga, Tadamitsu Kishimoto, Nancy Y. Ip, George D. Yancopoulos*

The ciliary neurotrophic factor (CNTF) receptor complex is shown here to include the CNTF binding protein (CNTFRα) as well as the components of the leukemia inhibitory factor (LIF) receptor, LIFRβ (the LIF binding protein) and gp130 [the signal transducer of interleukin-6 (IL-6)]. Thus, the conversion of a bipartite LIF receptor into a tripartite CNTF receptor apparently occurs by the addition of the specificity-conferring element CNTFRα. Both CNTF and LIF trigger the association of initially separate receptor components, which in turn results in tyrosine phosphorylation of receptor subunits. Unlike the IL-6 receptor complex in which homodimerization of gp130 appears to be critical for signal initiation, signaling by the CNTF and LIF receptor complexes depends on the heterodimerization of gp130 with LIFRβ. Ligand-induced dimerization of signal-transducing receptor components, also seen with receptor tyrosine kinases, may provide a general mechanism for the transmission of a signal across the cell membrane.

Ciliary neurotrophic factor, named for its ability to maintain survival of ciliary neurons, is now known to have a much broader spectrum of activities; almost all of these activities, however, are restricted to cells of the nervous system (*1*). Molecular cloning of a CNTF binding protein (CNTFRα) (*2*) revealed that it was related to one of the two components of the IL-6 receptor (here designated IL-6Rα) (*3, 4*). The finding that CNTF and IL-6 bind related receptor proteins was consistent with the observation that CNTF is distantly related to a family of cytokines that includes IL-6 as well as LIF and Oncostatin M (*5*). It has recently been shown that CNTF and its distant cytokine relatives all share the use of a signal-transducing receptor component, gp130, which was initially identified as the signal transducer for IL-6 (*6–11*). A LIF binding protein (here designated LIFRβ) has been cloned (*12*) that, in combination with gp130, forms high-affinity binding sites for both LIF and Oncostatin M (*9*). Although the role of LIFRβ as a partner of gp130 appears to be similar to that of IL-6Rα and CNTFRα, its structure more closely resembles that of gp130 itself (*12*). Thus, LIFRβ might have a signal-transducing role like that of gp130.

Whereas gp130 is tyrosine-phosphorylated in response to IL-6, both gp130 and another protein the size of LIFRβ are tyrosine-phosphorylated in response to CNTF or LIF (*8*). We have therefore proposed that LIF and CNTF initiate signaling by inducing heterodimerization between gp130 and LIFRβ and, by analogy, that IL-6 acts by inducing homodimerization of gp130 (*8, 11*). We also suggested that the signal transducers gp130 and LIFRβ together could comprise a functional LIF receptor complex (*8, 9*) and that addition of the CNTFRα chain to this complex might be sufficient to convert this bipartite LIF receptor into a tripartite CNTF receptor (*8*). The limited distribution of CNTFRα (predominantly in the nervous system) apparently restricts CNTF to neuronal actions (*13*).

To determine the composition of the CNTF receptor complex, we undertook reconstitution experiments in COS cells in which various combinations of CNTFRα, gp130, or LIFRβ were expressed. The components were expressed as fusion proteins that contained epitope tags to allow for immunoprecipitation and detection. Cells were treated with either LIF or CNTF and then examined for the formation of complexes between the components, as defined by the ability of one component to co-immunoprecipitate with another in a ligand-dependent manner. Functional activation of the complex was assessed by the induction of tyrosine phosphorylation of gp130 and LIFRβ (*14*). In cells transfected with gp130 and LIFRβ, LIF triggered their association and tyrosine phosphorylation, but CNTF did not (Fig. 1). In cells transfected with all three components, CNTF triggered the association of gp130 with both LIFRβ and CNTFRα and induced tyrosine phosphorylation of gp130 and LIFRβ (Fig.

S. Davis, T. H. Aldrich, N. Stahl, L. Pan, N. Y. Ip, G. D. Yancopoulos, Regeneron Pharmaceuticals, Inc., 777 Old Saw Mill River Road, Tarrytown, NY 10591.
T. Taga, Institute for Molecular and Cellular Biology, Osaka University, 1-3, Yamada-oka, Suita, Osaka 565, Japan.
T. Kishimoto, Institute for Molecular and Cellular Biology, Osaka University, and Department of Medicine III, Osaka University Medical School, 1-1-50, Fukushima, Fukushima-ku, Osaka 553, Japan.

*To whom correspondence should be addressed.

1). Thus, CNTFRα is necessary for the CNTF-induced association of gp130 with LIFRβ and for the induction of tyrosine phosphorylation. Similar experiments with the soluble form of CNTFRα showed that this form could also trigger CNTF-induced association of gp130 with LIFRβ and tyrosine phosphorylation of those subunits (Fig. 1), which is consistent with observations that the soluble form of CNTFRα can mediate CNTF function in cells normally responsive only to LIF (*15*).

To determine whether LIFRβ was required for complex formation and for tyrosine phosphorylation, we transfected cells with gp130 and CNTFRα in the absence of LIFRβ. In these cells, CNTF triggered the association of gp130 and CNTFRα (Fig. 2A) (*16*). In this respect, CNTFRα resembles the related protein IL-6Rα, which associates with gp130 in response to IL-6 (6). However, although a complex containing IL-6, IL-6Rα, and gp130 is capable of initiating signal transduction (Fig. 2B) (*4, 7*), the combination of CNTF, CNTFRα, and gp130 did not induce tyrosine phosphorylation of gp130 (Fig. 2B). Like LIF, CNTF induced tyrosine phosphorylation of its receptor components only when LIFRβ was included in the receptor complex (Fig. 1B) (*17*). Thus, CNTF, CNTFRα, and gp130 apparently form an intermediate that must engage LIFRβ in order to complete assembly of the receptor complex; this last step apparently initiates signaling (*18*).

These results are consistent with the proposal that IL-6 initiates signal transduction by causing homodimerization of gp130, whereas CNTF and LIF activate signaling pathways by causing heterodimerization of gp130 with LIFRβ; such a model considers gp130 and LIFRβ as essentially equivalent signal transducers. Alternatively, LIFRβ might act as a specificity-conferring element that is necessary for ligand binding, but signal transduction by CNTF and LIF might require homodimerization of gp130 (*19*). The role of homodimerization of gp130 in IL-6 signaling has recently been verified (*20*): disulfide-linked homodimers of gp130 can be recovered after stimulation of cells with IL-6 and only these homodimers are associated with tyrosine kinase activity (*20*). We therefore tested whether CNTF and LIF also induce disulfide-linked homodimerization of gp130 or whether they cause heterodimerization of gp130 with LIFRβ. The Ewing sarcoma cell line (EW-1) was used for these studies because it was particularly easy to detect disulfide-linked dimers in these cells. Treatment of EW-1 cells with the combination of IL-6 and soluble IL-6Rα resulted in the formation of a complex (~290 kD) that contained phosphotyrosine and could be immunoprecipitated with antibodies to gp130 but not by antibodies to LIFRβ (Fig. 3); the ~290-kD complex was not observed if the immunoprecipitates were reduced before electrophoresis. This complex appeared to correspond to a disulfide-linked dimer of gp130 on the basis of its size, its sensitivity to reducing agents, and its characteristics upon immunoprecipitation. In contrast, treatment of EW-1 cells with LIF or CNTF produced a larger complex (~335 kD) that was also sensitive to reduction and that could be immunoprecipitated with antibodies to either gp130 or LIFRβ, which indicates that this complex was a heterodimer of gp130 and LIFRβ. After immunodepletion of the heterodimers with antibody to LIFRβ, homodimers of gp130 could not be detected, which demonstrates that stimulation of cells with CNTF or LIF produces only heterodimers.

These results indicate that the CNTFRα chain is sufficient to convert a bipartite LIF receptor into a tripartite CNTF receptor and are consistent with the results of cross-linking analysis (*21*). Both CNTF and LIF induce formation of complexes containing components that are initially physically unassociated. Because we have observed the existence of stable intermediates, we infer that complex formation in response to CNTF occurs by an ordered process in which CNTF first binds to CNTFRα, then recruits a single molecule of gp130, and finally engages LIFRβ as well (Fig. 4). This last step apparently activates signal transduction, which supports the notion that CNTF and its relatives initiate responses through dimerization of signal transducers. In contrast to the action of IL-6, in which the final step in complex formation results in homodimerization of gp130, CNTF and LIF (and presumably Oncostatin M) instead induce heterodimerization of gp130 and LIFRβ (Fig. 4). It is possible that undiscovered members of this family activate signal-

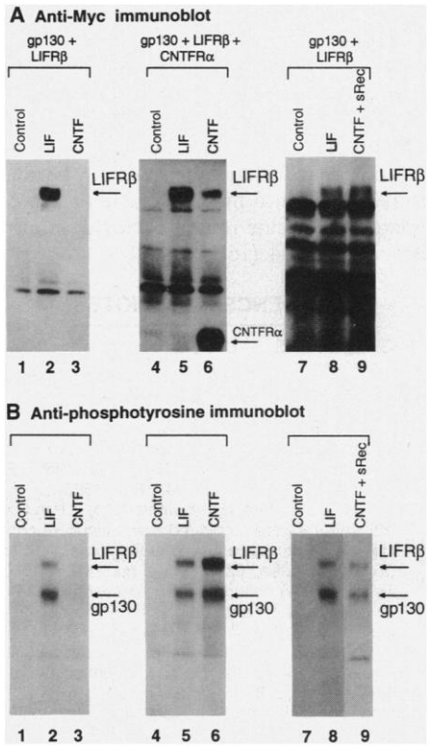

Fig. 1. Complex formation and signal initiation in response to CNTF and LIF. (**A**) Formation of complexes containing gp130, LIFRβ, and CNTFRα. COS cells were transfected with combinations of expression constructs encoding gp130-flag, CNTFRα-Myc, and LIFRβ-Myc as indicated [each suffix refers to a genetically engineered epitope tag (*2, 28*) that was inserted at or near the COOH-terminus of each protein]. Transfected cells were treated with the indicated ligands (50 ng/ml) or with CNTF together with soluble CNTFRα (sRec) (1 μg/ml, lane 9) for 30 min at 37°C. Cells were then solubilized in lysis buffer [50 mM tris (pH 8), 150 mM NaCl, 2.5 mM phenylmethylsulfonyl fluoride, 3 mM sodium orthovanadate, and 1% Brij 96 (Sigma); in some experiments, 1% NP-40 with 0.1% SDS was used in place of Brij 96], and proteins were immunoprecipitated with an antibody to the flag epitope (IBI). Protein immunoblotting was then done with an antibody against the Myc epitope (anti-Myc) to identify components with the Myc epitope that associated with gp130-flag. (**B**) Tyrosine phosphorylation of gp130 and LIFRβ. COS cells transfected with tagged receptor components were treated with the indicated ligands for 5 min at 37°C and then solubilized and immunoprecipitated with a combination of antibodies to the tags. Proteins phosphorylated on tyrosine were detected by protein immunoblotting with antibodies to phosphotyrosine (anti-phosphotyrosine).

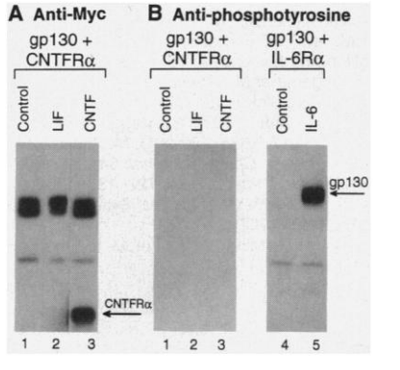

Fig. 2. CNTF-induced formation of complexes between CNTFRα and gp130. COS cells transfected with expression constructs encoding gp130-flag and either CNTFRα-Myc or IL-6Rα were treated with the indicated ligand, solubilized, and analyzed as described (Fig. 1) for complex formation (**A**) or tyrosine phosphorylation (**B**).

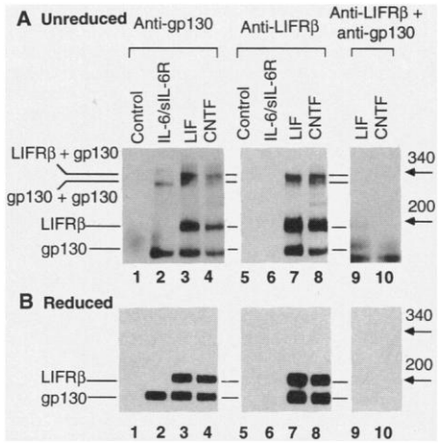

Fig. 3. Heterodimerization of gp130 with LIFRβ in the LIF and CNTF receptor complexes, in contrast to homodimerization of gp130 in the IL-6 receptor complex. EW-1 cells were either untreated (control) or treated with LIF, CNTF, or a combination of IL-6 and soluble IL-6Rα (sIL-6R). After cell lysis, proteins were immunoprecipitated with antibodies to either gp130 (anti-gp130) or LIFRβ (anti-LIFRβ) as indicated; the lysates immunodepleted by antibodies to LIFRβ were then subjected to a second immunoprecipitation with antibodies to gp130 (lanes 9 and 10). Immunoprecipitated proteins were electrophoresed on gradient polyacrylamide gels (3 to 27%), transferred to Immobilon membranes, and probed with antibodies to phosphotyrosine. All samples for electrophoresis were prepared under nonreducing conditions (**A**) or treated with 2-mercaptoethanol (**B**). The approximate molecular sizes of gp130 and LIFRβ are 145 and 190 kD, respectively. We generated polyclonal antisera to gp130 or LIFRβ by immunizing rabbits with synthetic peptides corresponding to sequences derived from the COOH-terminal regions of gp130 and LIFRβ, respectively. Molecular size markers are on the right in kilodaltons.

ing by inducing LIFRβ homodimerization.

Ligand-induced dimerization provides a plausible mechanism for the transmission of a signal across the membrane, reminiscent of the mechanism thought to be responsible for the activation of tyrosine kinase receptors (*22*). Evidence is accumulating that other classes of cytokines may also induce dimerization of signal-transducing receptor components (*23, 24*). Although cytokine receptor components lack tyrosine kinase activity, it is possible that juxtaposition of the signal transducer intracellular domains allows associated tyrosine kinases to be activated. In fact, tyrosine kinase activity is associated with the IL-6 receptor complex (*20*) and with the CNTF and LIF receptor complexes (*25*). The signaling pathways remain to be understood in detail, but the fact that tyrosine kinase inhibitors that block phosphorylation of the signal transducers completely ablate subsequent responses (*8*) is consistent with the notion that tyrosine kinase activation leads to all downstream events.

Receptor activation by means of signal transducer dimerization can also explain the ability of CNTFRα and IL-6Rα to function even in soluble form; the α components need not have intracellular domains in order to bring together the signal transducers. In contrast, truncated forms of the signal transducers should block ligand-induced activation in a manner analogous to that of truncated forms of receptor tyrosine kinases (*22*).

The CNTF receptor complex is unusual in that it requires three separate receptor components for function. Although the IL-2 receptor complex also has three components, its α component (IL-2Rα) increases the affinity of the receptor complex for IL-2 but is dispensable for function (*26*). CNTFRα does not change the affinity of the LIF receptor complex for LIF but rather confers CNTF specificity. CNTF and its cytokine relatives are also unusual in the manner in which they share receptor components. IL-3, IL-5, and granulocyte-macrophage colony-stimulating factor offer a somewhat similar example, in which a shared transducer subunit is responsible for signal initiation (perhaps by also undergoing ligand-induced dimerization), whereas distinct subunits confer specificity for the individual factors (*27*). With CNTF and related receptors, however, signal-transducing subunits can either homo- or heterodimerize and they also may assume specificity-conferring functions (LIF binds directly to LIFRβ and then induces heterodimerization with gp130, apparently in the absence of a subunit similar to CNTFRα and IL-6Rα). Although CNTF, LIF, and IL-6 use different combinations of four distinct receptor components, they may trigger the formation of receptor complexes that are structurally homomorphic and that initiate signaling by a common mechanism requiring signal transducer dimerization.

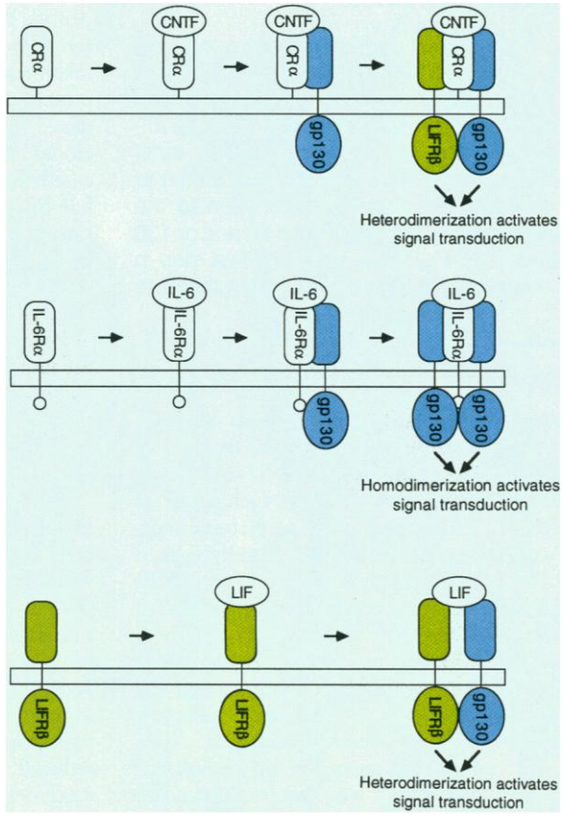

Fig. 4. Model of sequential formation and activation of the CNTF, IL-6, and LIF receptor complexes. The schematic diagram illustrates possible scenarios for the events leading to ligand-induced signaling (the cell membrane is represented by a bar, with the extracellular region depicted above the membrane and the intracellular region below). (**Top**) CNTF binds to CNTFRα (CRα); this complex then binds gp130 and forms a complex that is inactive. Finally, LIFRβ is added to the complex, resulting in heterodimerization of the signal transducers and initiation of signaling. (**Middle**) For IL-6, a similar sequence of events may lead to homodimerization of gp130 and signaling (the intermediate complex containing one gp130 subunit has not yet been isolated). (**Bottom**) LIF binds to LIFRβ and then gp130 is bound, resulting in signaling through heterodimerization of gp130 with LIFRβ. By analogy with the related growth hormone receptor (*21*), we assume a stoichiometry of one ligand molecule per receptor complex; the stoichiometry of the α subunits has not been determined.

REFERENCES AND NOTES

1. N. Y. Ip and G. D. Yancopoulos, *Prog. Growth Factor Res.* **4**, 139 (1992).
2. S. Davis *et al.*, *Science* **253**, 59 (1991).
3. K. Yamasaki *et al.*, *ibid.* **241**, 825 (1988).
4. T. Kishimoto, S. Akira, T. Taga, *ibid.* **258**, 593 (1992).
5. J. F. Bazan, *Neuron* **7**, 197 (1991); T. M. Rose and G. Bruce, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 8641 (1991).
6. T. Taga *et al.*, *Cell* **58**, 573 (1989).
7. M. Hibi *et al.*, *ibid.* **63**, 1149 (1990).
8. N. Y. Ip *et al.*, *ibid.* **69**, 1121 (1992).
9. D. P. Gearing *et al.*, *Science* **255**, 1434 (1992).
10. T. Taga *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 10998 (1992).
11. S. Davis and G. D. Yancopoulos, *Curr. Opin. Neurobiol.* **3**, 20 (1993).
12. D. P. Gearing *et al.*, *EMBO J.* **10**, 2839 (1991).

13. N. Y. Ip *et al.*, *Neuron* **10**, 89 (1993).
14. Although the function of receptor tyrosine phosphorylation in subsequent events has not been established, its occurrence is a marker of the activation of receptor-mediated intracellular signaling. The induced tyrosine phosphorylations of gp130 and LIFRβ observed in COS cells were qualitatively similar to those previously observed in neuronal cell lines (*8*)—that is, they displayed the same pattern of inhibition by kinase inhibitors (they are inhibited by staurosporine but not by H7) and they were accompanied by the tyrosine phosphorylation of the same set of additional, intracytoplasmic proteins.
15. S. Davis *et al.*, *Science* **259**, 1736 (1993).
16. Although the extent and possibly the stability of this association was variable in different experiments, two other lines of independent evidence support the existence of an intermediate complex containing gp130 and CNTFRα. (i) In the presence of soluble CNTFRα (but not in its absence), we could detect the binding of CNTF to COS cells that overexpressed gp130 alone (S. Davis, unpublished results). (ii) Although CNTF could be cross-linked to all three components in COS cells that overexpressed all three components, cross-linking to only gp130 and CNTFRα was observed either in COS cells that overexpressed only these two components (*20*) or in naturally occurring cell lines that expressed gp130 and CNTFRα but not LIFRβ (*25*).
17. These experiments are consistent with previous observations that cells such as BAFh130 (*10*) or B9 (*15*), which express gp130 but not LIFRβ, can transduce signals initiated by treatment with IL-6 but not those initiated by treatment with CNTF or LIF.
18. In the absence of gp130, we did not detect the CNTF-induced association of CNTFRα and LIFRβ or the tyrosine phosphorylation of LIFRβ alone.
19. J.-L. Boulay and W. E. Paul, *J. Biol. Chem.* **267**, 20525 (1992).
20. M. Murakami *et al.*, unpublished data.
21. N. Stahl *et al.*, *J. Biol. Chem.* **268**, 7628 (1993).
22. J. Schlessinger and A. Ullrich, *Neuron* **9**, 383 (1992); A. Ullrich and J. Schlessinger, *Cell* **61**, 203 (1990).
23. S. S. Watowich *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 2140 (1992); R. Fukunaga, E. Ishizuka-Ikeda, Y. Seto, S. Nagata, *Cell* **61**, 341 (1990).
24. B. C. Cunningham *et al.*, *Science* **254**, 821 (1991); A. M. de Vos, M. Ultsch, A. A. Kossiakoff, *ibid.* **255**, 306 (1992).
25. N. Stahl, unpublished results.
26. T. Takeshita *et al.*, *Science* **257**, 379 (1992); M. Hatakeyama, H. Mori, T. Doi, T. Taniguchi, *Cell* **59**, 837 (1989); M. Hatakeyama *et al.*, *Science* **244**, 551 (1989).
27. A. Miyajima, T. Kitamura, N. Harada, T. Yokota, K. Arai, *Annu. Rev. Immunol.* **10**, 295 (1992); N. A. Nicola and D. Metcalf, *Cell* **67**, 1 (1991).
28. T. P. Hopp *et al.*, *Biotechnology* **6**, 1204 (1988).
29. We thank L. Schliefer and the Discovery group at Regeneron for their enthusiasm and support; L. Defeo, C. New, and Y. Li for technical assistance; and M. J. Macchi for tissue culture expertise.

25 February 1993; accepted 9 April 1993

IL-6–Induced Homodimerization of gp130 and Associated Activation of a Tyrosine Kinase

Masaaki Murakami, Masahiko Hibi, Naoko Nakagawa, Toshimasa Nakagawa, Kiyoshi Yasukawa, Koichi Yamanishi, Tetsuya Taga, Tadamitsu Kishimoto

The biological functions of interleukin-6 (IL-6) are mediated through a signal-transducing component of the IL-6 receptor, gp130, which is associated with the ligand-occupied IL-6 receptor (IL-6R) protein. Binding of IL-6 to IL-6R induced disulfide-linked homodimerization of gp130. Tyrosine kinase activity was associated with dimerized but not monomeric gp130 protein. Substitution of serine for proline residues 656 and 658 in the cytoplasmic motif abolished tyrosine kinase activation and cellular responses but not homodimerization of gp130. The IL-6–induced gp130 homodimer appears to be similar in function to the heterodimer formed between the leukemia inhibitory factor (LIF) receptor (LIFR) and gp130 in response to the LIF or ciliary neurotrophic factor (CNTF). Thus, a general first step in IL-6–related cytokine signaling may be the dimerization of signal-transducing molecules and activation of associated tyrosine kinases.

When bound to IL-6, the IL-6R [referred to by Davis *et al.* as IL-6Rα (*1*)] associates with gp130 to initiate cytoplasmic signaling processes (*2*, *3*). The binding of IL-6 to its receptor results in the rapid tyrosine phosphorylation of gp130, but this has not been shown to cause its association with other signal-transducing molecules (*2*–*6*). Other cytokines related to IL-6 (*7*), such as LIF or CNTF, cause formation of heterodimers between gp130 and the structurally related LIFR [referred to by Davis *et al.* as LIFRβ (*1*)] and induce tyrosine phosphorylation of both molecules (*1*, *5*, *6*, *8*, *9*).

To examine more closely the potential induction of biochemical changes in gp130 by IL-6, we transfected cells with an expression vector that overexpresses gp130. The entire coding region of human cDNA encoding gp130 (*3*) was inserted into the genome of the vaccinia virus strain LC16mO under the control of a strong promoter, which normally expresses the 7.5-kD viral polypeptide (*10*), and a recombinant virus Vac130 was obtained. Hep 3B cells infected with Vac130 were cultured with [^{35}S]methionine and stimulated with a complex of IL-6 and soluble IL-6R (sIL-6R), which associates with and stimulates gp130 (*2*, *11*). Cell lysates were subjected to immunoprecipitation with monoclonal antibody (mAb) to human gp130 (anti-gp130). As analyzed by SDS–polyacrylamide gel electrophoresis (PAGE) under nonreducing conditions, gp130 was the major protein precipitated from the unstimulated cells (Fig. 1), whereas a protein of 260 kD was detected with gp130 from the cells stimulated with the IL-6–sIL-6R complex. When the same set of immunoprecipitates were treated with a reducing reagent, 2-mercaptoethanol (2-ME; 5%), only the gp130 protein was detected in either unstimulated or stimulated cells. The fact that the 260-kD protein was not present and no major proteins other than gp130 were detected under reducing conditions implied that the 260-kD protein might be a disulfide-linked homodimer of gp130.

To examine whether the 260-kD protein was composed solely of gp130, we performed peptide mapping analysis. The gp130 protein from unstimulated and stimulated cells and the 260-kD protein from stimulated cells were individually subjected to proteolysis with *Staphylococcus aureus* V8 protease. The peptide maps obtained with these three materials were identical (Fig. 1B). This result, taken together with the molecular size of the protein (260 kD, nonreduced; 130 kD, reduced) and its immunoreactivity with polyclonal antibody to human gp130 (*12*), indicated that the 260-kD protein was a disulfide-linked gp130 homodimer. The formation of gp130 homodimers after stimulation by IL-6 alone was also observed in [^{35}S]methionine-labeled IL-6–responsive cells not infected with Vac130, but the amounts observed were very small (*13*), presumably because of the very small amounts of both IL-6R and gp130 protein present in these cells. Thus, gp130 homodimerization may also occur upon binding of IL-6 to membrane-anchored IL-6R as well.

We examined the phosphorylation state of the dimerized gp130 protein. Hep 3B cells infected with Vac130 were incubated with [^{32}P]orthophosphate and stimulated with IL-6 and sIL-6R. Analysis by SDS-PAGE of the anti-gp130 immunoprecipitates under nonreducing conditions revealed that both dimerized and monomeric forms of gp130 protein were phosphorylated in response to stimulation (Fig. 2A). When reduced, the phosphorylated gp130 homodimers dissociated into monomers.

M. Murakami, M. Hibi, T. Taga, Institute for Molecular and Cellular Biology, Osaka University, 1-3 Yamadaoka, Suita, Osaka 565, Japan.
N. Nakagawa, T. Nakagawa, K. Yamanishi, Research Institute for Microbial Diseases, Osaka University, 3-1 Yamada-oka, Suita, Osaka 565, Japan.
K. Yasukawa, Biotechnology Research Laboratory, Tosoh Corporation, 2743-1 Hayakawa, Ayase, Kanagawa 252, Japan.
T. Kishimoto, Department of Medicine III, Osaka University Medical School, 1-1-50 Fukushima, Fukushima-ku, Osaka 553, Japan.