Six Different Cytokines That Share GP130 as a Receptor Subunit, Induce Serum Amyloid A and Potentiate the Induction of Interleukin-6 and the Activation of the Hypothalamus-Pituitary-Adrenal Axis by Interleukin-1

By Fabio Benigni, Giamilia Fantuzzi, Silvano Sacco, Marina Sironi, Pietro Pozzi, Charles A. Dinarello, Jean D. Sipe, Valeria Poli, Manuela Cappelletti, Giacomo Paonessa, Diane Pennica, Nikos Panayotatos, and Pietro Ghezzi

Ciliary neurotrophic factor (CNTF) and interleukin-6 (IL-6) potentiate the elevation of serum corticosterone induced by suboptimal doses of interleukin-1 (IL-1). CNTF also potentiates IL-1-induced serum IL-6. Here, we report that four other cytokines (leukemia inhibitory factor [LIF], oncostatin M [OSM], interleukin-11 and cardiotrophin-1) also potentiate the elevation of serum corticosterone and IL-6 levels induced by IL-1. Furthermore, all the six cytokines studied induced the acute-phase protein serum amyloid A when administered alone. Because these cytokines differ both in structure and in function, but share gp130 as a subunit of their receptors, these results indicate that signaling through gp130 mediates potentiation of IL-1 activities. The potentiation of IL-1-induced serum corticosterone levels is not a consequence of the increased serum IL-6 levels observed after IL-1 administration. In fact, in IL-6 deficient mice, IL-1 increased serum corticosterone to a level comparable to that observed in wild-type mice. Thus, either endogenous IL-6 does not mediate IL-1-induced corticosterone increase, or its role may be fulfilled by other cytokines. To the extent that gp130-dependent cytokines may serve this role, they may be important feedback regulators of inflammation through the activation of the hypothalamus-pituitary-adrenal axis and the potentiation of acute-phase protein synthesis.

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THE RECEPTORS FOR the cytokines interleukin-6 (IL-6), interleukin-11 (IL-11), leukemia inhibitory factor (LIF), ciliary neurotrophic factor (CNTF), oncostatin M (OSM), and the recently discovered cardiotrophin-1 (CT-1) share the signal transduction protein gp130. As indicated by their names, these cytokines have disparate activities on neurons, leukemia cells, cardiac myocytes, and the hematopoietic system.

The reason for the observed differences in biological activities resides in the fact that, while the gp130 signal transduction protein is ubiquitously expressed, the expression of the specific cytokine-binding receptor subunit(s) is restricted to specific cell types. For example the CNTF-specific receptor subunit (CNTFRα) is expressed mostly on neuronal and muscle cells. On the other hand, hepatocytes are likely to have the receptors required for functional response to all members of this cytokine family, as all of these cytokines directly induce the synthesis of acute-phase proteins by hepatocytes or hepatoma cells in vitro and in vivo (and this report). The induction of acute-phase proteins might be a protective mechanism against infection and inflammation because many of these proteins are antioxidants or proteinase inhibitors.

CNTF and IL-6 are pyrogens. We recently found that CNTF and IL-6 potentiate the increase of serum corticosterone levels induced by suboptimal doses of IL-1 in mice. In the same model, CNTF potentiated the increase of serum IL-6 induced by IL-1 administration. The increase of serum corticosterone levels induced by IL-1 is due to a central action of this cytokine to activate the hypothalamus-pituitary-adrenal axis (HPAA). This activation is thought to be an important negative feedback system because corticosteroids are potent inhibitors of the synthesis of proinflammatory cytokines such as IL-1, IL-8, and tumor necrosis factor. It should be noted that CNTF, while potentiating IL-6 and corticosterone induction by IL-1, had no effect on the endogenous levels of IL-6 and corticosterone when administered alone.

In the present study, we tested six cytokines (IL-6, IL-11, LIF, CNTF, OSM, and CT-1) in the in vivo experimental model used to characterize the activities of CNTF. The various gp130 ligands were administered at doses of 2 to 10 μg/mouse, based on previously published dose-response studies of the in vivo effects of IL-6 or CNTF on the HPAA. These cytokines were administered to mice alone or in combination with a low dose of IL-1, and serum corticosterone and IL-6 levels were measured. The effect of these cytokines on serum amyloid A (SAA) levels was also studied, as administration of CNTF or IL-6 increases the levels of this acute-phase protein.

IL-6 has been the subject of various reports indicating its role in the activation of the HPAA. Because high levels of IL-6 are induced in vivo by administration of IL-1, using IL-6-deficient mice generated by gene targeting, we investigated the possibility that the activation of the HPAA by IL-1 might be mediated by IL-6.

MATERIALS AND METHODS

Materials. rhCNTF, purified from Escherichia coli, was from Regeneron Pharmaceuticals Inc, Tarrytown, NY; rhIL-11 was a kind
Eight- to ten-week-old IL-6−/− and +/+ mice were bred at the Istituto di Ricerche di Biologia Molecolare P. Angeletti, Pomezia, Rome, Italy. The generation and genetic background of these mice has been described previously.2,23 Mice were treated intraperitoneal (IP) with IL-1 (500 ng/mouse) and bled 2 hours later for corticosterone determination.

RESULTS

Effect of gp130-dependent cytokines on IL-1-induced corticosterone and IL-6. Figure 1 shows the effect of gp130-dependent cytokines on the elevation of serum corticosterone levels induced by IL-1. While the administration of each cytokine alone had no significant effect on serum corticosterone levels, all the cytokines tested markedly increased (1.5 to two-fold) the elevation of serum corticosterone induced by IL-1.

The effect of CNTF, LIF, IL-11, OSM, or CT-1 on the induction of serum IL-6 by a suboptimal dose of IL-1 is shown in Fig 2. All cytokines strongly potentiated (13 to 38-fold) the induction of IL-6 induced by IL-1. In contrast, no serum IL-6 was detectable when each of these cytokines was administered in the absence of IL-1, or in control mice given saline alone (data not shown).

To rule out that the observed increase of serum IL-6 was due to interference of these cytokines with the IL-6 bioassay, we tested the effect of various doses of CNTF, LIF, IL-11, OSM, or CT-1 (0.1, 1, and 10 μg/mL) on the IL-6 standard curve in the 7TD1 proliferation assay. None of the cytokines studied interfered with the assay; also, none of them induced a proliferative response, as previously reported. CNTF was also tested in association with its soluble receptor, sCNTFRα, and no interference in the IL-6 bioassay or IL-6 like activity was observed (data not shown).

Animals and treatments. Male CD-1 mice (25 g body weight, Charles River, Calco, Como, Italy) were used. Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national and international laws and policies (EEC Council Directive 86/609, OF J 358, 1 December 12, 1987; Italian Legislative Decree 116/92, Gazzetta Ufficiale della Repubblica Italiana No. 40, February 18, 1992; NIH Guide for the Care and Use of Laboratory Animals, NIH Publication No. 85-23, 1985). CNTF, LIF, IL-11, IL-6, or OSM were administered as a single dose of 10 μg/mouse, intravenously (IV), in association with IL-1 (100 ng/mouse) or an equal volume of saline; CT-1 was given as a single dose of 2 μg/mouse, IV, due to the limited availability of protein. Blood was taken from the retroorbital plexus 2 hours (for corticosterone and IL-6 determinations) or 8 hours (for SAA determination) later under ether anesthesia and serum prepared. Previous experiments had shown that these times and doses were optimal for the study of the parameters evaluated.

Miscellaneous assays. Corticosterone was measured by radioimmunoassay, using antiserum obtained from Sigma (St Louis, MO) (C-8784) per manufacturer’s instructions. 1H-corticosterone was purchased from Amersham (Amersham, UK). IL-6 was measured as hybridoma growth factor using 7TD1 cells (a kind gift from Dr. J. van Snick) and expressed as costimulatory units/mL using rhIL-6 as a standard. The sensitivity was 50 U/mL. SAA was measured in serum samples using an enzyme-linked immunosorbent assay (ELISA) as previously described.
**Induction of SAA by gp130-dependent cytokines.** Figure 3 reports the effect of gp130-dependent cytokines on SAA levels 8 hours after treatment. All cytokines induced a significant elevation of SAA, in the absence of any other stimulus.

**Corticosterone induction by IL-1 is normal in IL-6-deficient mice.** Figure 4 shows the effect of IL-1 administration on serum corticosterone levels in IL-6+/+ and wild-type (+/+) mice. Basal corticosterone levels were not different between IL-6−/− and IL-6+/+ mice. Following administration of IL-1, a 2.5-fold increase of serum corticosterone was observed in IL-6+/+ mice, as compared with a three-fold increase in IL-6−/− mice. Therefore, IL-6−/− mice displayed a normal increase in serum CS following IL-1 administration.

**DISCUSSION**

The evidence presented in this report indicates that cytokines that share the gp130 signal transducer have overlapping activities on the HPAA and on the liver acute-phase response in terms of SAA induction. Due to limited availability of the various cytokines, we could not obtain dose-responses for each cytokine. Therefore, no conclusions can be drawn in terms of the relative potency of each cytokine. Moreover, the likely differences in pharmacokinetics of the different recombinant cytokines make a quantitative comparison not meaningful in an in vivo system.

It is difficult to identify the pathophysiological significance of the redundancy of action of these cytokines. It is possible that the induced responses are critical for the body, so that it would be detrimental if they were under the control of a single cytokine. The normal HPAA activation by IL-1 (this report) or lipopolysaccharide (LPS) observed in IL-6−/− deficient mice could thus be explained by hypothesizing that other gp130-ligand cytokines act as substitutes for IL-6 in vivo. It is of interest to note that a similar redundancy exists for the hematopoietic actions of cytokines. In particular, different cytokines of the IL-6 family were reported to stimulate thrombopoiesis.

It is also possible that different cytokines are induced under different pathological stimuli. In this case, the same physiological response can be elicited by different stimuli through different cytokines having overlapping activities. In fact, to date only IL-6 and LIF have been detected in the serum of septic patients. Because no data are available on the serum levels of CNTF, IL-11, OSM, or CT-1, it is difficult to make any hypothesis about their role in pathology.

Finally, the possible compartmentalization of these cytokines should be considered. For instance, it is likely that CNTF is induced mainly in the central nervous system and could preferentially act on the HPAA. On the other hand, LIF and IL-6, which are detected in the circulation, might well act on hepatic acute-phase proteins synthesis.

The effectiveness of these cytokines in vivo models indicates that the cell targets responsible for HPAA activation express the relevant receptors. The induction of SAA corroborates the evidence indicating that hepatocytes respond to all of these cytokines in vitro.

In conclusion, we have shown that four other gp130-dependent cytokines besides CNTF and IL-6 potentiate some of the effects of IL-1, namely IL-6 induction and HPAA activation, but have no effect on these parameters when administered alone. The common property of these cytokines responsible for these effects is the gp130 common receptor subunit. Other activities shared by these gp130-dependent cytokines (as well as IL-1 and tumor necrosis factor) include induction of acute-phase proteins synthesis and, as seen for CNTF and IL-6, fever. Further studies will be required to identify the signal transduction mechanism(s) underlying these phenomena.
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