Interleukin-11 Regulates the Hepatic Expression of the Same Plasma Protein Genes as Interleukin-6*

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The coordinate stimulation of acute phase plasma protein genes in adult mammalian liver has proven to be a sensitive indicator for the action and specificity of "inflammatory" cytokines (1). By using a variety of tissue culture and in vivo test systems, tumor necrosis factor-α, IL-1, IL-6, LIF, and glucocorticoids have been identified as potential mediators of the hepatic acute phase response (1, 2). Specific combinations of these factors were able to induce the acute phase response in cultured liver cells in a qualitatively and quantitatively reproducible manner (3). The corresponding in vivo functional involvement of these factors also has been inferred from these findings (1, 4). Obviously, the present list of potential acute phase regulating hormones is neither complete nor have all components relevant for the in vivo response been recognized.

During the last few years, a multitude of novel polypeptide factors have been cloned based on seemingly unrelated biological activities. Subsequent analyses have demonstrated, however, multifunctional properties and much broader cell specificities than originally expected. A striking example is presented in this paper which describes a function for IL-11 as a liver-regulating factor similar to IL-6 and LIF.

IL-6 is considered to play a central role in hepatic acute phase regulation probably for all mammals (4). The effect of IL-6 is characterized, for instance, by the pattern of plasma protein gene expression in rat hepatoma H-35 cells (3). IL-6, alone or in conjunction with dexamethasone, stimulates expression of the type 2 acute phase proteins (fibrinogen, haptoglobin, α1-antichymotrypsin, α1-antitrypsin, and α2-macroglobulin) and when combined with IL-1, enhances expression of the type 1 acute phase proteins (α2-acid glycoprotein, complement C3, haptoglobin, hemopexin, and serum amyloid A). Surprisingly, LIF displays the same qualitative regulatory pattern as IL-6 (5). LIF and its plasma membrane receptor are structurally distinct from IL-6 and its receptor, but both systems seem to function, in part, through common signaling pathways (5).

Studies with liver cells have indicated that several cytokines, implicated in growth and differentiation control of hematopoietic cells, can also modulate gene expression in hepatocytes. This is demonstrated anew with IL-11. IL-11 is a product of bone marrow stromal cells and has been defined as a growth factor for IL-6-dependent plasmacytoma cells (6). The cloned cDNA of IL-11 encoded a 180-amino acid polypeptide with a primary structure unrelated to any other cytokine. IL-11 is functionally related to IL-6, substituting for IL-6 as a proliferation-promoting factor in several assays. Here we show that the functional similarity between IL-11 and IL-6 also applies to hepatic cells.

MATERIALS AND METHODS

Factors—Bacterially expressed human recombinant IL-11 was purified to homogeneity and had a specific activity of 1–3 × 10⁶ units/mg in a T185 proliferation assay (6). For treatment of hepatoma cells, a stock solution of 10,000 units of IL-11/ml of serum-free medium was prepared. COS cell-derived human recombinant IL-6 (1 × 10⁶ units/mg) was obtained from Genetics Institute, and bacterially expressed human recombinant IL-19 (2 × 10⁶ units/mg) was from Immunex Corp. Human recombinant LIF was expressed in Chinese hamster ovary cells (Genetics Institute), and 3-day conditioned medium (1 × 10⁶ hepatocyte-stimulating factor-III; units/ml) served as a stock solution. Goat immunoglobulins against human IL-6 (Genetics Institute) and rabbit immunoglobulins against human LIF (anti-hematocyte-stimulating factor-III; 5) were used for activity neutralization.

Cell Treatments and Analysis—The effect of cytokines on plasma protein production was determined on confluent monolayers of HepG2 (7) and H-35 cells (3) in 24-well cluster plates and on primary cultures of adult male Fischer-344 rat hepatocytes in six-well cluster plates as described (8–10). The amounts of secreted plasma proteins were measured by rocket immunoelectrophoresis using one- or two-layered antibody-containing agarose gels (11).

Transfection—IL-6-responsive reporter gene constructs included...
RESULTS AND DISCUSSION

Stimulation of Acute Phase Plasma Proteins by IL-11 and H-35 Cells—The potential of IL-11 to regulate the expression of plasma proteins was determined in H-35 cells. The specificity of the IL-11 response was then compared with that of IL-1β, IL-6, and LIF. The most salient features of IL-11 action are illustrated by the representative data in Fig. 1. Here, the regulation of two prominent type 1 (α1-antitripsin and complement component C3) and type 2 acute phase proteins (thiostatin and fibrinogen) is reproduced. The change of haptoglobin, hemopexin, α2-macroglobulin, and α1-antitrypsin had also been measured, but those data are not shown. The results of several independent experiments (see also data included into Figs. 3 and 5A, below) indicated that IL-11 stimulated the production of all those plasma proteins that were affected by IL-6 and LIF and did so in a dose-dependent fashion. IL-11 was as effective as IL-6 and LIF in enhancing fibrinogen, thioseitin, α1-antitrypsin, hemopexin, and haptoglobin. Dexamethasone synergized with IL-11 in stimulating fibrinogen, α2-macroglobulin, and haptoglobin. A synergistic action of dexamethasone and IL-11 was also detectable on the expression of thioseitin but only at submaximal dose of IL-11. No further increase of thioseitin production was achieved at high concentration (≥1,000 units/ml) of IL-11 (see Figs. 1 and 3). The maximal level of all type 2 acute phase proteins attained in the presence of dexamethasone, however, was lower than observed with IL-6 and dexamethasone. This result is similar to that seen with LIF and dexamethasone (5). On the other hand, IL-11 differed from LIF and functioned more like IL-6 when tested in combination with IL-1. An additive effect of IL-11 and IL-1 was recorded for the type 1 acute phase proteins haptoglobin and hemopexin. A strong synergistic action of IL-11 and IL-1 was observed on complement C3 and α1-acid glycoprotein. In agreement with previous studies (3), the regulation of the former protein was independent of dexamethasone whereas that of the latter protein was strictly dependent on the steroid (Fig. 1). IL-1 was inhibitory on basal level expression and also on IL-11 stimulation of the type 2 proteins (e.g. fibrinogen and thiostatin, Fig. 1), a property of IL-1 which had been noted before in context of IL-6 and LIF (2).

IL-11-dependent regulation of acute phase plasma protein was not unique to H-35 cells. Treatment of primary cultures of adult rat hepatocytes with IL-11 yielded a change in plasma protein production which was qualitatively very similar to the one observed in H-35 cells (representative example in Fig. 2). Although primary cultures of hepatocytes showed a substantially higher basal level expression of most acute phase proteins than H-35 cells, their IL-11 response was nevertheless characterized by the same spectrum of stimulated proteins and by the same addition to synergistic action with dexamethasone and IL-1. From this we concluded that the response of H-35 cells was representative for the normal hepatic action of IL-11. The fact that the qualitative response pattern of rat hepatic cells to IL-11 was congruent with that of IL-6 and LIF suggested that all three factors shared functional properties. The quantitative response indicated that the overall efficacy of IL-11 as a regulator of acute phase proteins falls between that of IL-6 and LIF.

IL-11 Interaction with Hepatoma Cells Is Independent of IL-6 and LIF Receptors—Although the primary structure of IL-11 is distinct from that of IL-6 and LIF (6), its function...
Acute phase protein could still be explained by interaction with the IL-6 or LIF receptor. The presence of a separate receptor systems is, however, favored based on the following results. Treatment of H-35 cells with combinations of IL-11 and IL-6 (Fig. 3) or IL-11 and LIF (data not shown) yielded an additive effect on type 2 acute phase proteins. Surprisingly, however, the maximal level of stimulation with any factor never exceeded that achieved by IL-6 and dexamethasone. The independence of IL-11 regulation from the IL-6 and LIF receptor systems was also demonstrated in human hepatoma cells. Type 2 acute phase proteins of HepG2 cells, which in contrast to rat cells also include haptoglobin (8, 14), were strongly stimulated by IL-6 and LIF did not detectably modify its biological activity on H-35 cells, which differ from HepG2 cells, however, by their nonresponsiveness to LIF (8) (data not shown).

Antibody neutralization indicated that the structure of active IL-11 was distinct from that of IL-6 and LIF. Treatment of IL-11 with immunoglobulins against human IL-6 and LIF did not detectably modify its biological activity on H-35 cells even though both antibody preparations were highly effective in neutralizing their respective antigens (Fig. 4). We concluded from these experiments (Figs. 3 and 4) that hepatic cells most likely have separate membrane receptors for IL-11, IL-6, and LIF. Our working hypothesis is that the difference in IL-11 responsiveness between H-35 and human hepatoma cells is caused either by different amounts of IL-11 receptor or by different effectiveness of the immediate IL-11 receptor signaling.

IL-11 and IL-6 Utilize Common Elements for Regulating Acute Phase Protein Genes—The similar pattern of regulation of gene expression induced by IL-11, IL-6, and LIF suggests that the intracellular signal transduction initiated at the various receptors will converge before or at the level of the acute phase plasma protein genes. The overlap in transcriptional control between IL-11 and IL-6 was readily apparent in the example of the rat fibrinogen subunit genes (Fig. 5A). The 5'-flanking regions and promoter of each gene, when integrated into a reporter gene construct and transiently introduced into H-35 cells, mediated an IL-11- or IL-6-dependent stimulation of reporter gene expression which closely followed that of the endogenous fibrinogen genes. Moreover, the characteristic synergy of the cytokines with dexamethasone was faithfully reproduced. The fact that the IL-6 regulatory element of the β-fibrinogen gene (9) was equally responsive to IL-11 and IL-6 (lowest panel in Fig. 5A) suggested

![Fig. 3. Additive action of IL-11 and IL-6.](image)

Confluent monolayers of H-35 and HepG2 cells were treated for 24 h with the same medium preparations containing the indicated hormones. The amount of thiostatin (TST) in H-35 cell medium and haptoglobin (HP) in HepG2 cell medium were determined by rocket immunoelectrophoresis. Dex, dexamethasone.

![Fig. 4. Antibody neutralization of cytokines.](image)

Aliquots of IL-11 (500 units), IL-6 (100 units), and LIF (10 units) were brought to 100 μl with serum-free medium. To each sample 5 μl of nonimmune goat immunoglobulin (lanes 1), anti human IL-6 (lanes 2), and anti-human LIF (lanes 3) were added and incubated for 30 min at 37 °C. The samples were diluted with serum-free medium to 1 ml, filtered through 0.22-μm membrane, and added to H-35 cells. Change in fibrinogen (FB) and thiostatin (TST) was measured by rocket immunoelectrophoresis.

![Fig. 5. IL-11 regulation of CAT reporter gene constructs in transiently transfected hepatoma cells.](image)

The indicated CAT reporter gene plasmids were transfected into H-35 (A) and HepG2 (B) cells. The subcultures were treated for 24 h as indicated with serum-free medium alone or containing 1,000 units/ml IL-11, 100 units/ml IL-6, 10 units/ml LIF, or 1 μM dexamethasone. The CAT activity in equal amounts of cell extract was determined. The hormone-mediated change in the expression of the endogenous plasma proteins is illustrated by rocket immunoelectrophoresis of medium from one representative set of cell cultures.
Hepatic Action of IL-11

that at least part of the signal communication by both cytokines involved common components.

Transfection of IL-6-responsive reporter gene constructs into HepG2 cells resulted in a regulation pattern that was comparable to that of the endogenous genes (Fig. 5B). IL-11 elicited a minor but detectable 2-fold activation of the haptoglobin and fibrinogen constructs. In contrast, IL-6 produced a 10–15-fold and LIF a 5–7-fold stimulation.

Taken together, this study documents that IL-11 has the potential to function as a stimulator of hepatocyte gene expression in a manner similar to IL-6 or LIF. However, the data available so far indicate that IL-11 is not simply an IL-6 or LIF substitute. The ability of IL-11 to act additively with IL-6 and LIF might prove to be important for controlling acute phase protein genes in situations in which concentrations of each cytokine are suboptimal. IL-6 has been proposed as being crucial for mediating the systemic acute phase response (4). IL-11 and LIF now must also be considered as candidates for this function in vivo, and the actual contribution of each factor to the homeostatic control of the organism and to the liver activity, in particular, has to be ascertained.

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