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Up-regulation of the interleukin-6-signal transducing protein (gp130) by interleukin-6 and dexamethasone in HepG2 cells

Heidi Schooltink[a], Hildegard Schmitz-Van de Leur[a], Peter C. Heinrich[a] and Stefan Rose-John[a]

[a]*Institut für Biochemie der RWTH Aachen, Pauwelsstr. 30, D-5100 Aachen, Germany*

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The hepatic IL-6-receptor is composed of an 80 kDa IL-6-binding protein and a 130 kDa polypeptide (gp130) believed to be involved in signal transduction. Previous experiments have shown that the 80 kDa IL-6-receptor is up-regulated by glucocorticoids, but not by IL-6. Here we demonstrate that IL-6 together with the synthetic glucocorticoid dexamethasone induces the expression of mRNA for gp130 approximately 5-fold in HepG2 cells. The induction was dose- and time-dependent. Dexamethasone alone, interferon-γ, IL-1α and IL-1β had no effect. A possible role for the regulation of the IL-6-signal transducing protein gp130 in various inflammatory states is proposed.

Hepatic IL-6-receptor; gp130; Interleukin-6; Glucocorticoid; Acute-phase response; Signal transduction

1. INTRODUCTION

IL-6 is a multifunctional cytokine involved in the immune response, in hematopoiesis and in the acute-phase reaction [1–5]. The main activities exerted by IL-6 are induction of cell differentiation or gene activation and induction or inhibition of cell growth [1–5]. IL-6 acts via cell-surface receptors on target cells. The IL-6-receptor (IL-6-R) is composed of an 80 kDa binding protein (gp80) and a 130 kDa glycoprotein involved in signal transduction (gp130). Both IL-6-R subunits have recently been identified by molecular cloning from human [6–8] and rodent sources [9,10]. The two IL-6-R subunits belong to a recently defined receptor superfamily designated as the hematopoietic receptor family [11,12]. The members of the family are characterized by four conserved cysteine residues and a tryptophan-serine-x-tryptophan-serine (WSXWS) motif [13]. IL-6 is a major regulator of acute-phase protein synthesis in liver cells [14–17]. Therefore, the regulation of the hepatic 80 kDa-IL-6-R subunit has been studied. It was found that glucocorticoids, but not IL-6 up-regulated mRNA as well as functional protein expression of gp80 [18,19]. Thus far, nothing is known about the regulation of the second subunit of the hepatic IL-6-R (gp130).

In this study we show for the first time that the gp130-mRNA is slightly up-regulated by IL-6 and to a greater extent by the combination of IL-6 with dexamethasone.

Abbreviations: IL-6, interleukin-6; IL-6-R, interleukin-6-receptor; rh, recombinant human

Correspondence address: P.C. Heinrich, Institut für Biochemie der RWTH Aachen, Pauwelsstraße 30, 5100 Aachen, Germany. Fax: (49) (241) 875992.

2. MATERIALS AND METHODS

2.1. *Chemicals*

Restriction enzymes and the random primed DNA labeling kit were purchased from Boehringer Mannheim (Mannheim, Germany). [α-^{32}P]dCTP (110 TBq/mmol) was obtained from Amersham International (Amersham, UK). DMEM was from Gibco (Eggenstein, Germany). Recombinant human (rh)IL-6 was prepared as described [20]. The specific activity obtained was in the range of 1.5×10^6 B-cell stimulatory factor-2 units/mg protein [21]. RhIL-1α and rhIL-1β with a specific activity of 2×10^7 LAF units/mg protein was a generous gift of Dr. A.R. Shaw (Glaxo Institute for Molecular Biology, Geneva, Switzerland). RhIFNγ was obtained from Dr. E. Bill (Bioferon, Laupheim, Germany).

2.2. *Cell culture*

HepG2 cells were obtained from the American type culture collection (Rockville, MD, USA) and cultured in DMEM/DMEM-F12 medium. Culture medium was supplemented with 10% fetal calf serum, streptomycin (100 mg/l) and penicillin (61 mg/l). Cells were grown at 37°C in a water-saturated atmosphere in the presence of 5% CO_2.

2.3. *RNA-preparation and Northern-blot analysis*

Total RNA was prepared using the phenol extraction method as described in [22,23]. 5 μg of RNA were heated to 65°C for 10 min in 50% formamide, 20 mM morpholinopropane sulphonic acid (MOPS), 5 mM sodium acetate, 1 mM EDTA, 2.2 M formaldehyde prior to gel electrophoresis in 1% agarose containing 2.2 M formaldehyde, 20 mM MOPS, 5 mM sodium acetate and 1 mM EDTA. Equal loading of the RNA gel was checked by ethidium bromide staining of 18 S and 28 S ribosomal RNA. The separated RNA was transferred to Gene Screen Plus membranes (Dupont-NEN, Dreieich, Germany) according to supplier's instructions. The filters were prehybridized at 68°C for 1 h in 10% dextran sulfate, 1 M sodium chloride, 1% SDS and hybridized in the same solution with a 3 kb *AccII*/*Bam*HI gp 130

cDNA fragment labeled by random priming [24]. After hybridization unspecifically bound radioactivity was removed by washing in 2 × standard saline solution (SSC) at room temperature, followed by two consequent washes in 2 × SSC/1% SDS at 68°C for 30 min each. The filters were then subjected to autoradiography using intensifying screens.

3. RESULTS

Fig. 1 shows the effect of IL-6, dexamethasone, interferon-γ, IL-1α and IL-1β on the expression of gp130-mRNA in HepG2 cells by Northern analysis. It is evident that dexamethasone, interferon-γ, IL-1α and IL-1β do not affect gp130 gene expression. In contrast, IL-6 and in particular IL-6 in combination with dexamethasone led to a marked stimulation of gp130-mRNA synthesis. The dose-dependent stimulation of gp130-mRNA by IL-6 at a constant concentration of 10^{-6} M dexamethasone can be seen in Fig. 2. A steady increase in gp130-mRNA levels was observed at IL-6 concentrations > 10 units/ml. The stimulation of gp130-mRNA synthesis by IL-6 is time-dependent (Fig. 3). A maximum of induction was found between 8 and 18 h. An overall gp130-mRNA induction of about 5-fold was estimated.

4. DISCUSSION

Previous experiments from several laboratories have shown that acute-phase proteins are induced by IL-6 and dexamethasone in vitro and in vivo [14–17,25]. In various inflammatory states elevated glucocorticoid levels are observed. We and others have described that gp80-mRNA as well as the functional protein is induced

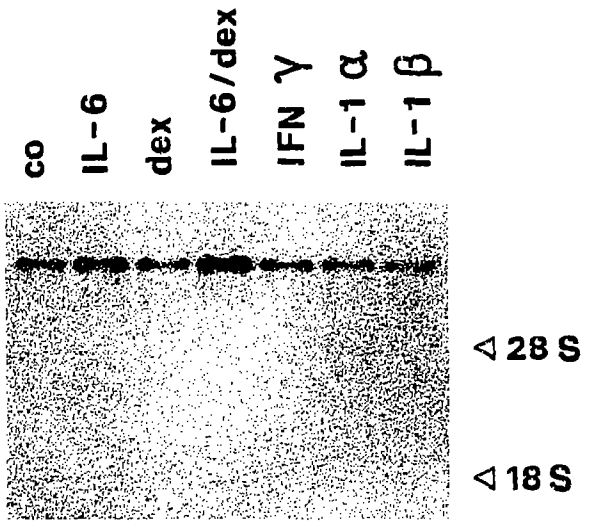

Fig. 1. Gp130 mRNA expression in HepG2 cells treated with various cytokines and dexamethasone. HepG2 cells were treated for 18 h with 100 U/ml rhIL-6, 10^{-6} M dexamethasone, 100 U/ml IL-1α, 100 U/ml IL-1β, or 100 U/ml IFNγ. Total RNA was isolated as described in section 2 and subjected to Northern-blot analysis. RNAs were probed with a gp130-cDNA labelled by random priming.

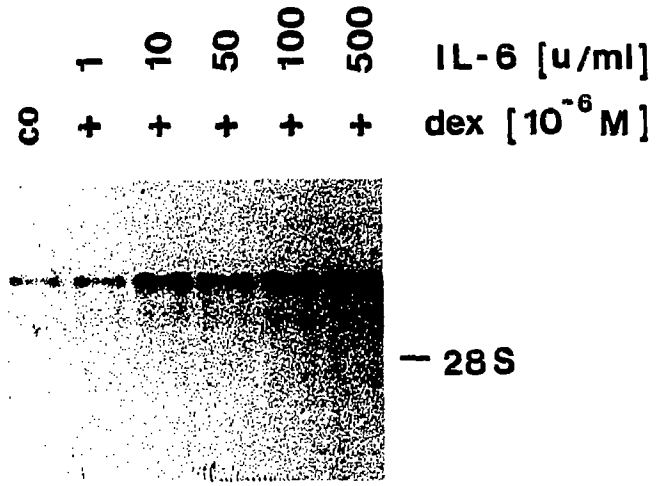

Fig. 2. Dose dependence of gp 130 mRNA expression in HepG2 cells treated with IL-6 and dexamethasone. HepG2 cells were treated for 18 h with 10^{-6} M dexamethasone and various amounts of IL-6 as indicated in the figure. Northern-blot analysis was carried out as described in the legend to Fig. 1.

by glucocorticoids and not by IL-6 [18,19]. In the present study, evidence is presented that IL-6 and in particular the combination of IL-6 and dexamethasone stimulates gp130-mRNA expression in HepG2 cells. This clearly indicates that the expression of the two IL-6-R subunits are regulated by different mechanisms. It has been published by Hibi et al. [8] that in HepG2 cells gp130-mRNA levels are at least 5 times higher than those of gp80. Recent experiments from our laboratory have shown that the exposure of HepG2 cells to high doses of IL-6 leads to a rapid down-regulation of IL-6 binding sites (Zohlnhöfer et al., submitted). Having down-regulated their receptors, HepG2 cells became non-responsive to IL-6 in regard to acute-phase protein induction. However, responsiveness could be reconstituted with the soluble form of the 80 kDa-binding protein/IL-6 complex (Mackiewicz et al., submitted), i.e. the soluble IL-6-R/IL-6 complex acts as an agonist

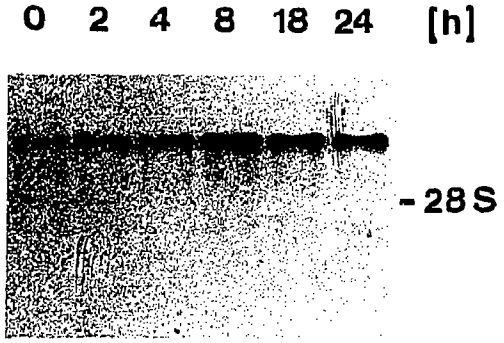

Fig. 3. Time dependence of gp130 mRNA expression in HepG2 cells treated with IL-6 and dexamethasone. HepG2 cells were treated with 100 U/ml IL-6 and 10^{-6} M dexamethasone for the times indicated in the figure. Northern-blot analysis was done as described in the legend to Fig. 1.

on liver cells depleted of membrane-bound gp80 by triggering the aggregation of the soluble gp80 with gp130 leading to signal transduction [26]. The amplitude of the IL-6 response seemed to be determined by the ratio of the two subunits of the IL-6-R on the cell surface; if the amount of gp80 is low, the cell loses responsiveness after an exposure to low levels of IL-6. However, such a refractory state could be overcome by the presence of the shIL-6-R. Finally, the amount of gp130 determines how many complexes of IL-6 bound to the membrane or soluble form of gp80 can induce transduction of a specific signal. Accordingly, it will be of importance to analyze in detail the amounts and ratio of the two IL-6-R subunits in soluble form or on the surface of IL-6 responsive cells. Interestingly, we found high levels of soluble IL-6-R in patient sera with chronic inflammatory diseases like systemic lupus erythematodes and rheumatoid arthritis (H. Schooltink, unpublished results).

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