A new hepatocyte stimulating factor: cardiotrophin-1 (CT-1)

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Abstract Recently, a novel cytokine, cardiotrophin-1 (CT-1), was cloned and found to induce cardiac myocyte hypertrophy in vitro. Amino acid sequence similarity showed CT-1 to be a member of the IL-6/LIF/CNTF/OSM/IL-11 cytokine family. Since all known members of the IL-6 cytokine family induce an hepatic acute phase protein (APP) gene expression, we investigated the ability of CT-1 to induce a liver acute phase response. Upon stimulation of rat hepatoma cells, CT-1 and LIF induced the strongest rat fibrinogen mRNA expression, OSM and IL-6 induced a less pronounced response. When human hepatoma cells and primary rat hepatocytes were stimulated with CT-1, the expression of human haptoglobin and rat α_2-macroglobulin mRNA was induced. The induction of the acute phase response was dose- and time-dependent. In this study we demonstrate that CT-1, a novel cytokine belonging to the IL-6 cytokine family, is a hepatocyte stimulating factor.

Key words: Acute-phase response; Cardiotrophin 1; Hepatocyte; Interleukin-6; Interleukin-6-cytokine family

1. Introduction

Inflammation, tissue injuries, neoplastic cell growth, and immunological disorders are characterized by marked alterations in plasma levels of a number of liver-derived proteins called acute phase proteins (APPs). The induction of this hepatocyte response is mediated primarily through soluble cytokines that interact with specific receptors on the surface of hepatocytes to induce the expression of genes for APP [1]. IL-6 seems to be the major inducer of APP response in vivo although it has been shown that other members of this cytokine family exhibit hepatocyte stimulating activities in vitro. Members of the interleukin-6 family are interleukin-6 (IL-6) [2,3], leukemia inhibitory factor (LIF) [4,5], oncostatin M (OSM) [6], interleukin-11 (IL-11) [7], and ciliary neurotrophic factor (CNTF) [8].

This cytokine family acts on target cells via specific cell surface receptors generally consisting of different subunits for ligand binding and signal transduction. The IL-6 receptor family is composed of multisubunit complexes that share a common signaling subunit, gp130 [9,10]. Some members of the IL-6 cytokine family (IL-6 and IL-11) [11,12] induce the homodimerization of gp130, while others (LIF, OSM; and CNTF) induce the heterodimerization of gp130 and of the 190 kDa LIF receptor [13,14]. Following dimerization of the signaling components, these receptors induce a number of intracellular signaling events including activation of the transcription factor NF-IL-6 and activiation of the Jak/STAT signaling pathway [15].

Cardiotrophin-1 is a novel cytokine that induces cardiac myocyte hypertrophy in vitro [16]. It has been demonstrated that CT-1 leads to a heterodimerization of gp130 and the LIF receptor [17]. Based on the fact that CT-1 seems to be a member of the IL-6 cytokine family [16] and acts via the LIF receptor/gp130 heterodimer, we asked the question, whether CT-1 is a hepatocyte stimulating factor. In this study, we show that CT-1 regulates acute phase protein expression in human and rat hepatoma cells as well as in primary rat hepatocytes. The extent of stimulation by CT-1 is comparable to the one obtained with LIF.

2. Materials and methods

2.1. Materials

Human haptoglobin cDNA was a gift of Dr. D. Samols (Cleveland, OH, USA), rat β-fibrinogen cDNA was supplied by Dr. A. Mitchell, Parkville, Australia. Recombinant human (rh) IL-6 was prepared as described [18]. Oncostatin M and leukemia inhibitory factor (LIF) were obtained from Dr. J. Brakenhoff, CLB, Amsterdam, NL. CT-1 was prepared as recently described [16]. Soluble human IL-6 receptor (shIL-6R) was expressed as described [19]. [α-^{32}P]dATP (110 TBq/mmol) was from Amersham International (Amersham, UK).

2.2. Cell culture

Human hepatoma cells (HepG2 cells, HepG2-IL-6 cells [19]), rat hepatoma cells (Fao cells) were grown in DMEM at 5% CO_2 in a water-saturated atmosphere. All cell culture media were supplemented with 10% FCS, 100 mg/l streptomycin, and 60 mg/l penicillin. Primary rat hepatocytes from male Sprague–Dawley rats were isolated as described [20,21] and cultivated for 48 h prior to cytokine stimulation.

2.3. Extraction of total RNA and Northern (RNA) blot analyis

Total RNA was prepared from cells using the phenol extraction method [22]. 10 μg of heat-denatured RNA per sample was fractionated on a 1% agarose gel with 7% formaldehyde. The separated RNA was transferred to GenScreen Plus membranes (Dupont-New England Nuclear, Dreieich, Germany) according to the manufacturers instructions. The filters were prehybridized at 68°C for 2h in 10% dextran sulfate, 1 M NaCl, 1% SDS, and hybridized in the same solution with ^{32}P-labeled cDNA fragments by random priming [23]. The following probes were used: 0.9-kB *Hin*fII restriction fragment of human Haptoglobin cDNA, 0.66-kB *Pst*I fragment of rat α_2-macroglobulin, and a 0.7-kB *Eco*RI fragment of rat β-fibrinogen. Photographs of the ethidium bromide stained gels were used to ensure equal loading of the RNA gels (data not shown).

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Abbreviations: APP, acute phase proteins; α_2M, α-2-macroglobulin; HPT, haptoglobin; IL-6, interleukin-6; IL-6R, interleukin-6 receptor; LIF, leukemia inhibitory factor; OSM, oncostatin M.

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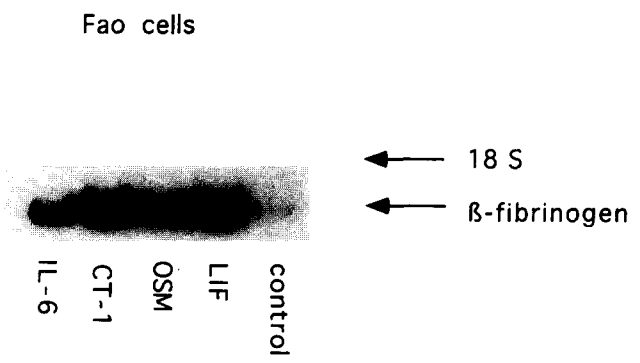

Fig. 1. Effects of CT-1, LIF, OSM, and IL-6 on acute-phase protein expression in Fao cells. Following incubation of the cells with 20 ng/ml CT-1, 50 ng/ml LIF, 50 ng/ml OSM, and 10 ng/ml IL-6 for 18 h, total RNA was prepared and subjected to Northern blot analysis. The filters were hybridized with a ^{32}P-labeled cDNA coding for rat β-fibrinogen.

3. Results and discussion

To examine whether CT-1 exhibits hepatocyte stimulating activity, we incubated HepG2 cells and Fao cells for 18 h in the presence of CT-1. For comparison, cells were incubated with LIF, OSM, and IL-6, which are well-documented hepatocyte-stimulating factors [2–4,24,25].

Fig. 1 shows the stimulation of mRNA expression of the acute phase protein β-fibrinogen in Fao cells: incubation with LIF and CT-1 gave the strongest stimulation of β-fibrinogen mRNA, incubation with OSM and IL-6 led to a less marked induction of acute phase proteins.

Time course experiments were carried out incubating Fao cells with CT-1 and IL-6 (Fig. 2A). Upon stimulation with CT-1, there was a full induction of β-fibrinogen expression after 2 h which was only slightly increased when incubation periods were extended to 18 h. To further confirm this experiment, Fao cells were incubated for 20 min, 40 min, and one hour with the same dose of CT-1. A full induction of β-fibrinogen was seen after 40 min (data not shown). Following stimulation with IL-6, maximal induction of β-fibrinogen was seen after 18h. Figure 2B shows dose–response experiments demonstrating that maximal β-fibrinogen induction after stimulation with CT-1 was seen at a concentration of 20 ng/ml.

Fig. 3A shows the stimulation of mRNA expression of the acute phase protein haptoglobin in HepG2 cells: while IL-6 and OSM treatment led to the strongest stimulation, CT-1 and LIF induced acute phase protein synthesis to a comparable and less pronounced extent. Fig. 3B demonstrates a similar experiment with HepG2-IL-6 cells [19] which are stably transfected with the human IL-6 cDNA, do not express gp80 IL-6 receptors on their surface and have lost their responsiveness to IL-6 but not to other cytokines such as LIF or TGF-β. It can be seen from the figure that incubation of these cells with CT-1 and LIF, but not with IL-6, showed an equally strong induction of haptoglobin mRNA expression. We conclude that the IL-6R is not required for CT-1 activity. The different extent of acute phase protein mRNA induction found in Fao cells and HepG2 cells stimulated with either CT-1 and LIF or with IL-6 and OSM which is demonstrated in Figs. 1 and 3, is most likely due to different relative expression levels of LIF receptor (primary receptor for CT-1 and LIF) or gp130 (primary receptor for OSM, signal transducer for IL-6).

As shown in Fig. 4, primary rat hepatocytes were stimulated with CT-1, IL-6, LIF, and OSM. The stimulation of the acute phase protein α_2M was most pronounced upon the incubation with IL-6. Incubation with CT-1, LIF, and OSM led to a very weak response, being only slightly stronger than the control. An explanation for this finding could be that the primary rat hepatocytes used in this experiment expressed much lower levels of LIF receptor than gp130 on their surface. As a consequence, these hepatocytes are more sensitive to IL-6 which only needs gp130 for signaling, compared to CT-1, OSM, and LIF, which require a heterodimer of LIF recpetor and gp130 for signal transduction.

Interestingly, Pennica et al. [16] have shown that CT-1 mRNA was detected in various mouse tissues including liver raising the possibility of an autocrine loop in the regulation of CT-1 expression. However, we failed to demonstrate CT-1 mRNA in unstimulated and CT-1 stimulated HepG2 cells or primary rat hepatocytes (data not shown). These findings may argue against an involvement of an autocrine loop in CT-1 regulation and may also be an indication that CT-1 is expressed in liver cells other than hepatocytes, such as Kupffer cells.

In this study we demonstrate that the newly discovered cytokine CT-1 which seems to belong to the IL-6 cytokine family is a hepatocyte stimulating factor. In vitro experiments have demonstrated that the known members of the IL-6 cytokine family display overlapping activities on target cells. This functional redundancy among members of this family may be

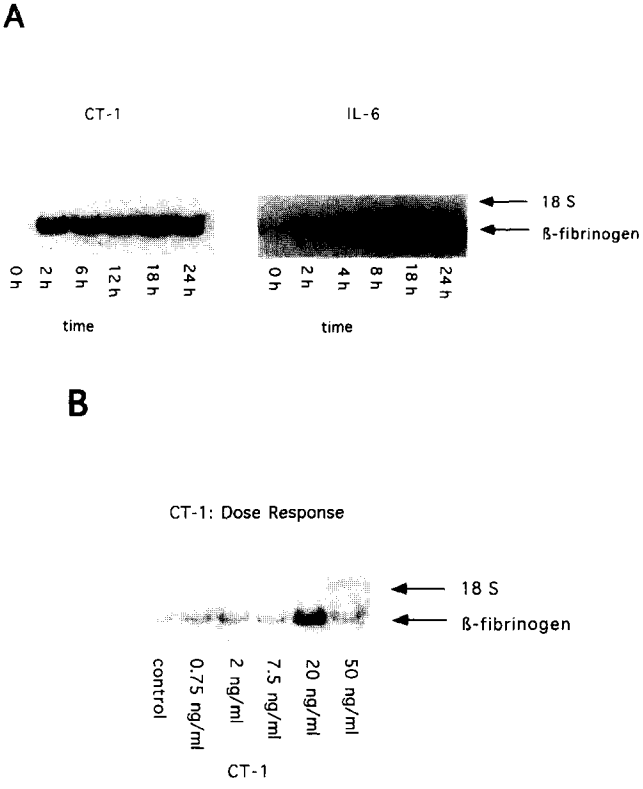

Fig. 2. Time course and dose–response of rat β-fibrinogen induction of Fao cells in response to CT-1. (A) For the time course experiment, cells were stimulated with 20 ng/ml CT-1 and with 10 ng/ml IL-6 for the times indicated. (B) For the dose–response experiments, cells were stimulated for 18 h with various dosages as indicated in the figure. Total RNA was prepared and subjected to Northern blot analysis. The filters were hybridized with a ^{32}P-labeled cDNA coding for rat β-fibrinogen.

caused by interactions of specific binding receptors with the common signal transducing molecule gp130. The importance of this functional redundancy is supported by the fact that IL-6 knock-out mice are still able to elicit an acute phase protein expression upon LPS stimulation [26]. It is still unknown which cytokines besides IL-6 induce the acute phase protein expression in vivo. Given the fact that certain receptor subunits are shared by different cytokines of the IL-6 family, our finding that CT-1 is a hepatocyte stimulating factor highlights the concept of functional redundancy. There might be the possibility that CT-1 represents a candidate which could be a supplement to cytokines like IL-6 in various inflammatory states.

It is noteworthy that the time course experiment of the induction of β-fibrinogen expression by CT-1 is different from the one obtained with IL-6. We currently have no explanation for these discrepancies but it will be interesting to compare primary receptor events between the two cytokines.

Although CT-1 was isolated based on its ability to induce cardiac myocyte hypertrophy [16,17], it has a much wider spectrum of activities as found for all other members of the IL-6 family [1]. Future studies will be needed to define the specific role of CT-1 within the family of IL-6-type cytokines.

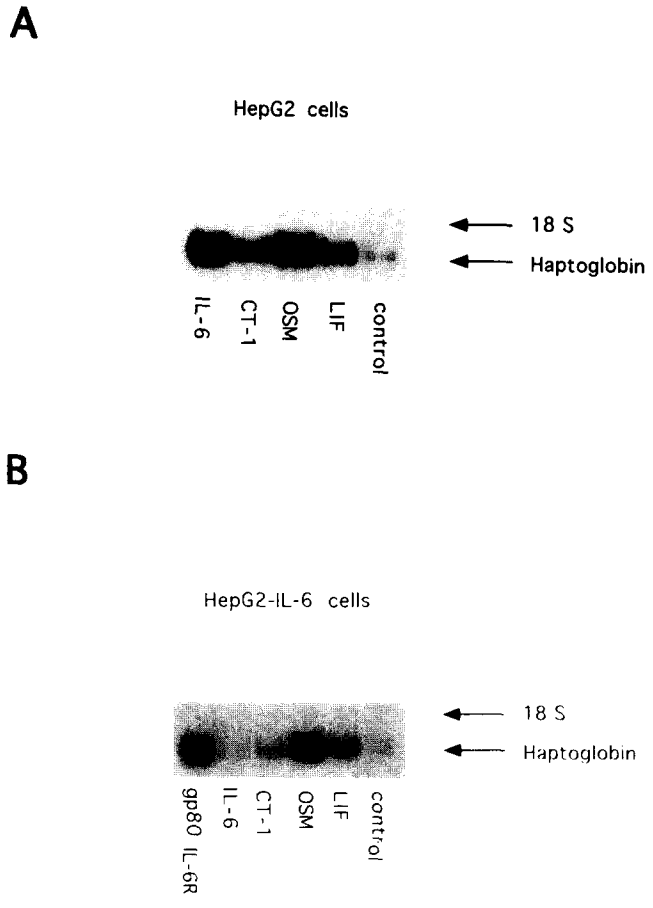

Fig. 3. Effects of CT-1, LIF, OSM, and IL-6 on acute-phase protein expression in (A) human hepatoma HepG2 cells and (B) HepG2-IL-6 cells. Following incubation of cells with 20 ng/ml CT-1, 50 ng/ml LIF, 50 ng/ml OSM, 10 ng/ml IL-6, and 100 ng/ml gp80/IL-6R for 18 h, total RNA was prepared und subjected to Northern blot analysis. The filters were hybridized with a ^{32}P-labeled cDNA coding for human haptoglobin. gp80/IL-6R, soluble form of the ligand binding subunit of the IL-6 receptor complex.

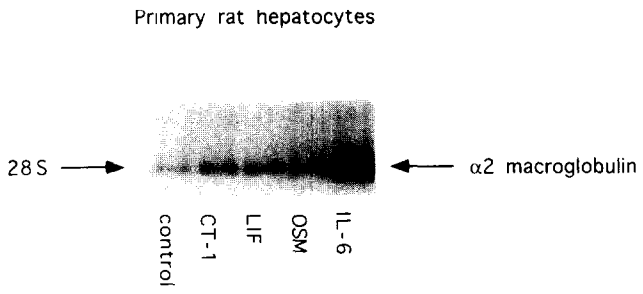

Fig. 4. Effects of CT-1, LIF, OSM, and IL-6 on acute-phase protein expression in primary rat hepatocytes cells. Following incubation of cells with 20 ng/ml CT-1, 10 ng/ml LIF, 20 ng/ml OSM, and 10 ng/ml IL-6 for 18 h, total RNA was prepared und subjected to Northern blot analysis. The filters were hybridized with a ^{32}P-labeled cDNA coding for rat α_2-macroglobulin.

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