Endocytosis of Interleukin-6—Soluble Interleukin-6 Receptor Complex and Its Intralysosomal Degradation

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 124, No. 11, pp. 527-529, November, 1997
Original article submitted July 30, 1996

Binding and internalization of interleukin-6—soluble interleukin-6 receptor complex by MDCK and MDCK-gp130 (transfected with gp130 signal transductor) cells are studied. Binding of labeled complex depends on the concentration of interleukin-6; an effective internalization of the complex is shown. Binding and endocytosis of the complex are demonstrated in human hepatoma cells expressing interleukin-6 receptor and gp130. These processes depend on the concentration of interleukin-6. The inhibitors of lysosomal functions ammonium chloride, monensin, and leupeptin suppress intralysosomal degradation of the complex, which confirms the important role of intralysosomal cleavage of the complex.

Key Words: *receptor-mediated endocytosis; interleukin-6; soluble interleukin-6 receptor; lysosomes*

Interleukin-6 (IL-6) is a pleiotropic cytokine regulating growth and differentiation of various cells and involved into immunological reactions and production of acute phase proteins [7-10]. Multiple effects of IL-6 are mediated through a receptor complex consisting of two subunits, IL-6 receptor and gp130 signal transductor [8,10]. The binding of IL-6 to the receptor complex induces dimerization of gp130 and is accompanied by rapid phosphorylation of gp130, JAK-kinases, and acute phase protein transcription factor (STAT) [4,8].

The binding of IL-6 to specific cell receptor is followed by its internalization and degradation [11,12]; however, precise localization of this process is still unknown. The fate of soluble IL-6 receptor (IL-6R) also remains unclear.

Since the IL-6—IL-6R complex acts as an agonist on cells expressing gp130 on their surface, a question arises: whether internalization and intracellular degradation of this complex, and especially of IL-6R, occur in these cells.

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MATERIALS AND METHODS

Recombinant human IL-6 was tested for its biological activity [2]. The following reagents were used: fetal bovine serum (Seromed), DMEM and DMEM/F-12 media (Gibco), leupeptin (Boehringer Mannheim), monensin (Calbiochem), and Bolton—Hunter reagent (specific activity 74 TBq/mmol, Amersham). ^{125}I-IL-6R was stored at -20°C; under these conditions its biological activity was preserved [5,11].

MDCK cells and gp130-positive MDCK-gp130 cells [6] were cultured in DMEM supplemented with 10% fetal bovine serum, 60 mg/ml penicillin, and 100 mg/liter streptomycin. Human hepatoma cells (HepG2) were cultured in DMEM/F-12 with the same supplements. For investigation of the binding and internalization of the IL-6—IL-6R complex the cells were grown in 24-well plates [5].

IL-6R (10 ng/ml) were incubated with IL-6 in a binding medium at 4°C for 12 h. Labeled ligand was then added to the cells (200 μl/well) and in-

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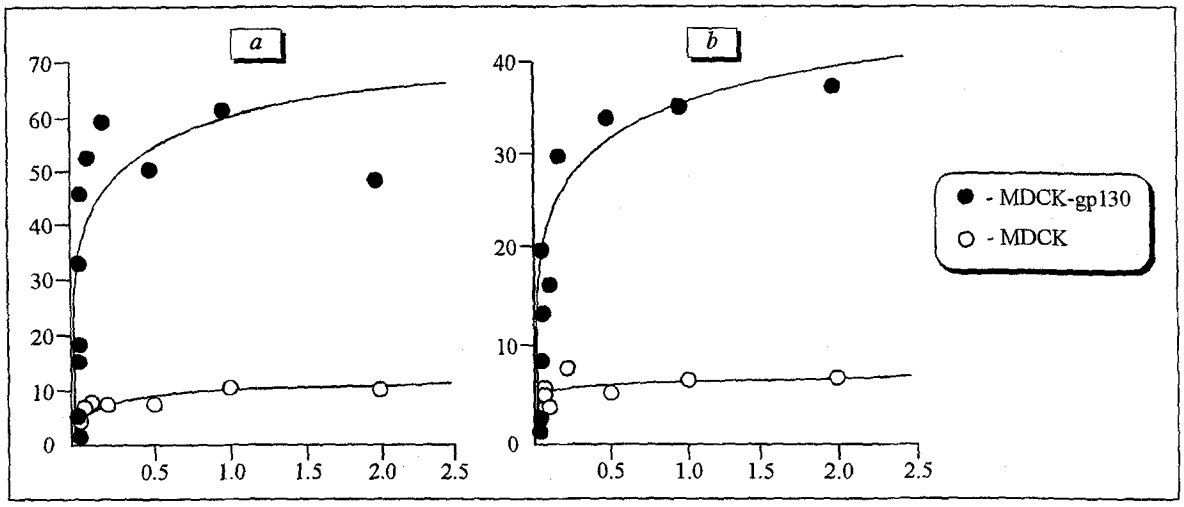

Fig. 1. Binding (*a*) and internalization (*b*) of ^{125}I-soluble interleukin-6 receptor (IL-6R) in MDCK-gp130 and control MDCK cells. Abscissa: IL-6R, ng/ml; ordinate: IL-6R, pg/10^5 cells. Each point is the mean of 5 independent experiments.

cubated at 4°C for 2 h. The cells were washed 3 times with the binding medium, and the surface-bound soluble IL-6R was eluted for 5 min with 0.5 M NaCl, pH 1.0. Under these conditions 94% surface-bound ligand passed into solution.

When studying internalization of the ligand, the cells after the initial binding stage were rapidly heated and incubated at 37°C. The surface-bound ligand was then eluted, and internalized ligand was assayed after the cells were treated with 1 M NaOH for 24 h.

Degradation of labeled ligand was studied in HepG2 cells. To this end, the cells were incubated with the ligand at 4°C for 2 h and at 37°C for 1 h. The ligand was removed, the cells were washed 3 times with warm medium, and aliquots of the same medium were added to the cells and incubated for 0, 3, and 6 h. The cells were then cooled on ice, and degradation products (trichloroacetic acid — TCA-soluble and insoluble radioactivity) were measured.

TABLE 1. Inhibition of ^{125}I-IL-6R in HepG2 cells by Inhibitors of Lysosome Functions

Inhibitors	Incubation time, h		
	0	3	6
Control	0	16.4±1.0	32.6±1.2
Leupeptin, 20 μg/ml	0.1±0.1	10.4±0.2	18.4±0.5
Ammonium chloride, 20 mM	0.4±0.1	4.4±0.2	11.2±0.5
Monensin, 100 μM	0	2.0±0.5	3.4±0.1

Note. Accumulation of acid-soluble degradation products is expressed in % of total radioactivity of HepG2 cells treated with 1.0 M NaOH (100%). Data of 5 independent experiments are processed statistically using the Student's *t* test.

For evaluation of total radioactivity, the cells were solubilized in 1 M NaOH (12 h). Radioactivity was counted in a γ-counter; binding and internalization were expressed in pg labeled IL-6R per 10^5 cells.

RESULTS

Binding and internalization of the IL-6—IL-6R complex were studied on MDCK-gp130 cells stably transfected with a gp130-encoding vector. Nontransfected MDCK cells served as the control. MDCK-gp130 cells effectively bond iodine-labeled IL-6R, the binding depending on the concentration of IL-6 (Fig. 1). Control MDCK cells bond IL-6R almost 6-fold less effectively (Fig. 1, *a*). This is due to endogenous expression of gp130 in MDCK cells [6]. In both cases the binding was saturable at an IL-6 concentration of 1000 ng/ml (Fig. 1, *a*). This concentration was used in the study of internalization. In these experiments, the cells after the initial binding period were incubated at 37°C, and the content of internalized receptor (residual radioactivity after treatment with 0.5 M NaCl, pH 1.0) was measured. About 50% surface-bound ligand was internalized during 1 h in both MDCK-gp130 and control MDCK cells (Fig. 1, *b*).

Human hepatoma cells HepG2 synthesize both IL-6R and gp130 [5,8]. Binding and internalization of ^{125}I-labeled soluble receptor in these cells similarly to MDCK-gp130 cells depended on the concentration of IL-6 (Fig. 2), but the total amount of bound ^{125}I-IL-6R was lower (Figs. 1 and 2).

The internalized ligand underwent intracellular (most probably, intralysosomal) degradation, as evidenced by the rise of TCA-soluble radioactivity (Table 1). At the same time, TCA-insoluble radioactivity

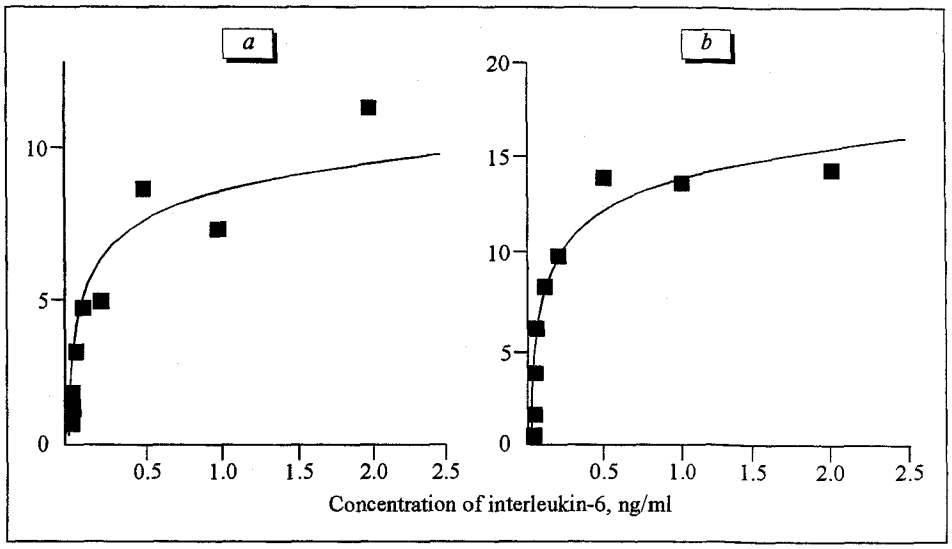

Fig. 2. Binding (*a*) and internalization (*b*) of ^{125}I-soluble interleukin-6 receptor (IL-6R) in human hepatoma cells (HepG2). Ordinate: IL-6R, pg/10^5 cells. Each point is the mean of 5 independent experiments.

only slightly increased (5%), implying some damage to lysosomes during incubation with labeled ligand [1].

Generally, degradation of ^{125}I-labeled IL-6—IL-6R complex is a slow process: 32.6% internalized ligand was cleaved over a period of 6 h (Table 1). The causes of slow intracellular proteolysis of this complex remain unknown.

Intralysosomal degradation of IL-6—IL-6R complex was suppressed by inhibitors of lysosome functions; most effective inhibitors were ammonium chloride and monensin which reduced the rise of proteolysis products 3-4- and 8-10-fold, respectively (Table 1), leupeptin being less effective (1.5-1.7-fold inhibition). Similar differences have been previously noted for intralysosomal degradation of other ligands, in particular, asialoglycoprotein receptor, but their causes remain unclear [3].

Since ammonium chloride inhibits protein catabolism in lysosomes [1,3], its effect on the degradation of IL-6R substantiates the role of lysosomes in its degradation. Lysosomal degradation of IL-6R is also confirmed by pronounced inhibiting effect of monensin, an ionophore which increases pH in lysosomes, endosomes, and cysternae of the Golgi apparatus.

Thus, interaction of IL-6R with cells is mediated through receptor-dependent endocytosis. Internalized ligand undergoes intralysosomal degradation. Our experiments verify the hypothesis that gp130 is required for IL-6 endocytosis. It can be assumed that cells expressing surface gp130 are able to internalize IL-6R in the presence of IL-6.

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