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IDENTIFICATION OF A SOLUBLE GM-CSF BINDING PROTEIN IN THE SUPERNATANT OF A HUMAN CHORIOCARCINOMA CELL LINE

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We identified two forms of the receptor for granulocyte-macrophage colony-stimulating factor (GM-CSF) made by the human choriocarcinoma cell line JEG-3 using an affinity-labeling technique. The protein was identified in the detergent-extract was 78 kDa, very similar to that of the membrane-bound GM-CSF receptor α chain expressed in a wide variety of hematopoietic and nonhematopoietic cells, including JEG-3. In contrast, a 62-kDa GM-CSF binding protein, or the soluble GM-CSF receptor, was identified in the supernatant of JEG-3 cells. Utilizing the same affinity labeling technique, we did not detect the soluble GM-CSF binding protein in the supernatant of several hematopoietic cell lines, such as U-937 and KG-1, which express membrane bound α chain as well as β chain. The 62-kDa soluble GM-CSF receptor is produced in abundant amounts by JEG-3, but in very small amounts, if any, by hematopoietic cell lines.

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a cytokine which exerts profound effects on both hematopoietic (for review 1) and nonhematopoietic cells including placental (2,3) and vascular endothelial cells (4). Most of the receptors for cytokines, including GM-CSF, have been molecularly cloned (for review 5). This series of cDNA cloning of the receptors has disclosed the existence of an alternatively spliced form of mRNAs encoding soluble receptors in many cytokine systems (6-11). An mRNA which encodes a soluble receptor for human interleukin 5 (IL-

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5) has recently been identified by Taverinier et al., who reported that the expressed soluble IL-5 receptor protein had an antagonistic effect on its natural ligand, IL-5 (11). On the other hand, a soluble interleukin 6 (IL-6) receptor, which was created by an artificial deletion of a membrane bound form IL-6 receptor, was demonstrated to have an agonistic effect on IL-6 responsive cells (12). However, native soluble receptors have yet been identified only for limited number of ligands such as interleukin 2 (IL-2) (13) and tumor necrosis factor (TNF) (14). In this report, we describe a native binding protein for GM-CSF identified in the supernatant of human choriocarcinoma cell line JEG-3.

MATERIALS AND METHODS

Preparation of radio-iodinated human GM-CSF. Bacterially synthesized recombinant human GM-CSF was kindly provided by Schering Plough Japan Co. Ltd. The iodination procedure was carried out according to the method of Bolton and Hunter (15) with a minor modification as described previously (16). One μCi (37.5 MBq) of Bolton-Hunter reagent (ICN Chemicals, Japan) was used to label 1 μg GM-CSF. The labeled GM-CSF was stored in PBS containing 0.25% gelatin, 0.1% bovine serum albumin and 0.02% NaN_3 at 4°C.

Cell lines. All the cell lines used in this study were obtained from American Type Culture Collection.

Preparation of JEG-3 lysate. JEG-3 cells were solubilized with the buffer contained 25 mM HEPES, pH 7.4, 2 mM EDTA, 200 trypsin inhibitor units/ml aprotinin, 1 mM phenlymethylsulfonyl fluoride and 1.5% Triton X-100, and was stored at -80°C.

Preparation of culture supernatant. JEG-3 cells were grown in Minimum Essential Medium with 10% fetal calf serum (FCS), and U-937 cells and K562 cells were in RPMI1640 medium with 10% FCS. Three days before harvesting the supernatant, the medium was changed and the concentration of FCS was reduced to 1%. The harvested supernatant was then concentrated by 50-fold with MINICON-B (Amicon Division, Grace Co. Ltd., Danvers, MA).

Cross-linking study. [^{125}I]GM-CSF (final concentration 0.5 nM) was incubated with 100 μl of 50-fold concentrated culture-supernatant or 40 μl (approximately 160 μg) of cell lysate in 300 μl phosphate-buffered saline in the presence or absence of 100-fold molar excess of unlabeled GM-CSF for 4 hr at 15°C. Subsequently, disuccinimidyl subrate (DSS; Piece Chemical Co., Rockford, IL) freshly prepared in acetonitrile was added to 0.4 mM. The cross-linking reaction was allowed to proceed on ice for 15 min and then quenched by adding 200 μl of ice cold Tris-buffered saline with 1 mM EDTA on ice for 5 min. The cross-linked materials were precipitated by adding wheat germ agglutinin-agarose. The precipitated materials were then mixed with equal volume of 2-fold concentrated Laemmli's sample buffer and boiled for 3 min. Electrophoresis was performed according to Laemmli (18). Fixed and dried gels were analyzed using Fujix BAS 2000 Bio-image analyzer (Fuji Photo Film Co. Ltd., Tokyo, Japan) (19). A cross-linking study for intact U-937 cells was performed as described previously (16).

RESULTS

A cell-free in vitro cross linking using DSS revealed a 78-kDa GM-CSF receptor present in the detergent-extract of JEG-3 cells. As shown in Fig. 1, lane *a*, a 93-kDa band representing a complex of [^{125}I]GM-CSF and its receptor was demonstrated, which disappeared in the presence of a 100-fold molar excess of unlabeled GM-CSF (lane *b*). Since the molecular weight of the bacterially synthesized GM-CSF is approximately 15 kDa, the detergent-extract of JEG-3 cells contained a 78-kDa GM-CSF receptor. In contrast, a 77-kDa band, which disappeared in the presence of a 100-fold molar excess of unlabeled GM-CSF, was demonstrated when the concentrated supernatant of JEG-3 cells was subjected to the same experimental procedure (lanes *c* and *d*). Subtracting the molecular weight of GM-CSF, we concluded that JEG-3 cells release a 62-kDa GM-CSF binding protein (GMBP) into the culture supernatant.

As shown in Fig. 2, two major cross-linked species were demonstrated when the intact U-937 cells were subjected to a cross-linking study. The arrow and the arrow-head indicate cross-

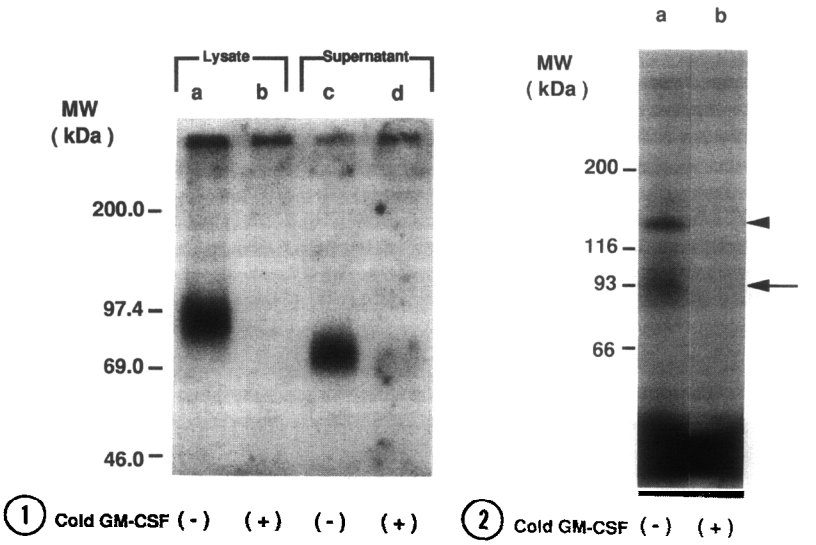

Figure 1. Cross-linking of [^{125}I]GM-CSF to the JEG-3 lysate and supernatant. Binding was performed in the presence of 100-fold molar excess of unlabeled GM-CSF (lanes *b* and *d*) or absence (lanes *a* and *c*) as described in MATERIALS AND METHODS.

Figure 2. Cross-linking of [^{125}I]GM-CSF to the intact U937 cells. Binding was performed in the presence of 100-fold molar excess of unlabeled GM-CSF (lane *b*) or absence (lane *a*) as described in MATERIALS AND METHODS.

Table 1. Identification of GM-CSF receptor

	membrane bound receptor		soluble receptor
	α 78kDa	β 135kDa	62kDa
JEG-3	+	−	+
U937	+	+	−
K562	−	ND	−

linked complexes formed by α chain and β chain of the GM-CSF receptor, respectively. However, we could not identify GMBP in the supernatant of U-937 cells by the same experimental procedure used to identify the GMBP in the supernatant of JEG-3 cells. An erythroleukemia cell line K562, which does not exhibit detectable GM-CSF binding, also did not release the GMBP at a detectable level into the culture supernatant. These results are summarized in Table 1.

DISCUSSION

Many cDNAs encoding receptors for immune-hematopoietic cytokines have been cloned since 1989. Along with the cDNA cloning of such receptors, the existence of alternatively spliced mRNAs encoding putative soluble receptors has gradually become apparent (6-11). However, the biological significance of this type of mRNAs is unknown, since the products of these mRNAs have not yet been well characterized. Although soluble receptor for several cytokines such as interleukin 2 (IL-2) (13), interleukin 6 (IL-6) (20) and tumor necrosis factor (TNF) (14) has been described, all of these were identified with specific antibodies against each receptor, and some such as the soluble IL-2 receptor (Tac antigen) have been proven to be a protein cleaved from an originally membrane-bound receptor.

In the present study we used a cell-free in vitro affinity labeling system to identify a 78-kDa GM-CSF receptor in the detergent-extract of JEG-3 cells. This protein is assumed to be identical to the membrane bound GM-CSF receptor α chain, which has been shown to be present in various types of cells, including hematopoietic (3,16) and nonhematopoietic (3,21) cells. Utilizing

the same technique, we have also identified a 62-kDa protein, which retains binding activity against GM-CSF, in the culture supernatant of JEG-3 cells. Very recently, Raines et al. described the existence of an mRNA which may encode the putative secreted human GM-CSF receptor in a choriocarcinoima cell line BeWo (22). The molecular weight of the deduced protein encoded by this mRNA is consistent with that of the GMBP described in this paper, suggesting that the GMBP is a product of the mRNA identified by Raines. However, several other possibilities can also be raised. The GMBP may be a cleaved protein of an originally membrane-bound GM-CSF receptor α-chain. Alternatively, the mRNA encoding the GMBP may be the transcript of a distinct gene. A specific antibody against the extracellular portion of the GM-CSF receptor α chain, which is not presently available, could elucidate the latter possibility. We did not detect the GMBP in the supernatant of the U-937 cells, which express membrane bound α chain as well as a component of the high affinity receptor β chain (3,23). We previously described that the JEG-3 cells express the GM-CSF receptor α chain at a level one order of magnitude higher than the U-937 cells. Therefore, the level of the GMBP in the supernatant of the U-937 cells may simply be a reflection of the low expression level of the membrane bound α chain in the U-937 cells compared to the JEG-3 cells. Another possibility is that the GMBP does not exist in U-937 cells, because the GMBP expression is differently regulated from the membrane bound α chain, regardlss of whether or not the GMBP is encoded by the gene for α chain. The physiological role of the soluble cytokine receptors still remains unknown, but they will provide us a new approach in understanding the ever complex cytokine network.

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