Interleukin-6 Signal Transducer gp130 Mediates Oncostatin M Signaling*

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Oncostatin M (OM) is a multifunctional cytokine that is structurally and functionally related to interleukin 6 (IL-6) and leukemia inhibitory factor (LIF). The specific receptor for OM has been demonstrated (by chemical cross-linking) to be a 150-kDa protein in a number of cell lines. The IL-6 signal transducer, gp130, is also an affinity converter for the LIF receptor. It does not bind to either IL-6 or LIF, but associates with the α subunits of the receptors and transduces the signals. We examined the possible involvement of gp130 in OM binding and signaling. We demonstrate that: (a) anti-gp130 monoclonal antibodies (mAbs) block the inhibitory effect of OM on A375 cell growth, (b) the binding and cross-linking of 125I-OM to H2981 cells are completely abolished by anti-gp130 mAbs, (c) the cross-linked OM-receptor complex is immunoprecipitated by anti-gp130 mAbs, and (d) COS-7 cells transfected with the full-length cDNA encoding gp130 exhibit increased OM binding and cross-linking, which are also blocked by anti-gp130 mAbs. Therefore, we conclude that the 150-kDa OM binding protein previously characterized in a variety of cell lines is gp130. OM is the natural ligand for gp130 and gp130 mediates the biological responses of OM.

Oncostatin M (OM), a 28-kDa glycoprotein, is a newly characterized multifunctional cytokine (1, 2) which shares structural and functional homology to several cytokines including IL-6 and LIF (3, 4). OM acts on a wide variety of cells and elicits diversified biological responses such as growth stimulation, growth inhibition (1, 5), and leukemic cell differentiation (3, 6), acute phase protein induction (7), LDL receptor up-regulation (8), and cell-specific gene expression (6, 7). OM appears to mediate its effects through a specific cellular receptor that has been characterized in a variety of cell lines to be a protein with an approximate molecular mass of 150 kDa (5–10). The biochemical and molecular nature of the OM receptor remains elusive. The overlapping biological effects of OM and IL-6 in many cellular systems, and the isolation of a mutant A375 human melanoma cell line that is resistant to only OM and IL-6, but not to other cytokines (such as TGF-β, II-1, tumor necrosis factor-α and -β, and interferon-γ), suggested that OM and IL-6 share a common component in their signal transduction pathways. Therefore, we investigated the involvement of the IL-6 signal transducer, gp130 (11), in OM binding and signaling. We report here that OM is the natural ligand for gp130 and also that gp130 is a necessary component of OM signaling machinery. Recently, Gearing et al. (12) reported that low affinity OM binding sites are expressed on COS-7 cells transfected with a truncated gp130 cDNA.

EXPERIMENTAL PROCEDURES

Cells and Reagents—The human melanoma cell line A375 (ATCC), the human lung carcinoma cell line H2981 (Bristol-Myers Squibb), and the African green monkey kidney cell line COS-7 (ATCC) were cultured in DMEM medium supplemented with 10% fetal bovine serum. GPX7, GPX22, and GPZ35 are mouse anti-human gp130 monoclonal antibodies. Human recombinant oncostatin M was expressed in Chinese hamster ovary cell supernatants and purified by reverse-phase high performance liquid chromatography.

Cell Proliferation Assays—Cells were incubated overnight in 96-well tissue culture plates (Costar) in DMEM medium (100 µl/well) containing 5% serum at a concentration of 3000 cells/well. Cultures were re-fed and incubated with the same culture medium supplemented with various test factors. Three days later cells were incubated in medium containing 5-125I-Iodo-2'-deoxyuridine (0.1 µCi/well) for 6 h, washed with PBS, fixed in 95% methanol, and air-dried. Cell pellets were solubilized with 200 µl of 1 N sodium hydroxide and the amount of 125I incorporated into DNA was measured using a γ radiation counter. The differences in counts/min incorporated between experimental and control cultures were used as the index for growth. Each data point represents the average from triplicate cultures.

Chemical Cross-linking—The confluent monolayers were washed once and removed from the culture plates by incubation with cell removal buffer (50 mM Hepes, pH 7.4, 1 mM EDTA, 5 mM glucose, 5 mM KCl, 125 mM NaCl, 2 mg/ml bovine serum albumin, and 1 µg/ml each leupeptin, aprotinin, and pepstatin A) for 10 min at 37°C. The lysis of OM and cross-linking to cells was conducted as essentially described (9). Immune precipitatin—Approximately 1.7 × 10^7 H2981 cells were cross-linked to 125I-OM and subsequently extracted with 1 ml of digitonin extraction buffer (11) for 5 h at 4°C. The debris was removed by centrifugation at 7000 × g for 10 min and supernatants were then incubated with antibodies for 15 h. The immunoreactive complexes were then immunoprecipitated by protein A-Sepharose and analyzed by SDS-PAGE (9). COS Cell Transfections and Binding Assay—COS-7 cells grown in tissue culture flasks (Nunc, Inc.) were transfected with plasmid DNA by the DEAE-dextran-chloroquine method (14). Forty-eight hours after transfection, cells were incubated with 125I-OM (2000 Ci/ mm) for 2 h at room temperature and then extensively washed. The autoradiographic analysis of transfected cells was performed as described (15).
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RESULTS AND DISCUSSION

To investigate the possible involvement of the IL-6 signal transducer, gp130, in OM signaling, we examined the effects of monoclonal antibodies (mAbs) against the β subunit of the IL-6 receptor (gp130) on the growth inhibition of A375 cells by OM, IL-6, and TGF-β. We observed that anti-gp130 mAbs, GPX7, GPX22, and GPZ35 was used in this and subsequent experiments. The control mouse mAb was anti-CD4 mAb. Cells were pulsed with [125I]iododeoxyurine (0.1 μCi/ml) for an additional 6 h. The amount of radioactivity incorporated into DNA was determined, and the data was normalized to the percent of the growth of the culture relative to cells not treated with the factors, medium alone (○), with anti-gp130 mAbs (△), + control mouse mAb (□).

Neither IL-6 nor LIF bind to the human lung carcinoma cell line H2981; however, these cells possess 1–3 × 10^6 binding sites/cell for OM and express mRNA encoding the gp130 protein. In order to clarify the role of gp130 in OM-receptor interactions we examined the ability of anti-gp130 mAbs to block the binding of radiolabeled OM to H2981 cells. Binding of [125I]-OM to H2981 was completely blocked by 10 μg/ml of anti-gp130 mAbs but was unaffected by the control mouse mAb (Fig. 2). This suggested that OM binds directly to gp130. Ligand-receptor cross-linking studies with [125I]-OM and H2981 cells were carried out to biochemically characterize the OM receptor on these cells. SDS-PAGE analysis of OM-binding proteins on H2981 cells showed one major species with an apparent molecular mass of 150 kDa (180-kDa labeled complex minus 30-kDa OM) and another minor species with an apparent molecular mass of 300 kDa. The cross-linking of OM to these two molecules was inhibited by unlabeled OM or anti-gp130 mAbs, but not by IL-6 or control mouse mAb (Fig. 3). At present, the relationship between these two OM binding species is not clear. However, we believe that the 300-kDa species could be a receptor complex that includes OM, gp130, and another subunit or the dimer of gp130. It is possible that the 150-kDa protein cross-linked to OM is not gp130 but is instead a closely related molecule that shares a common binding epitope for the anti-gp130 mAbs. However, we think this is highly unlikely because four different monoclonal anti-gp130 mAbs (AM64, AM66, GPX7, GPX22) individually blocked the cross-linking of OM to the 150-kDa protein. To further demonstrate the relationship between the OM receptor and gp130, [125I]-OM was cross-linked to its receptor on H2981 cells, and the cells were subsequently washed, solubilized with a lysis buffer containing digitonin, and then immunoprecipitated with the anti-gp130 mAbs or with the control mouse mAb. Analysis of this material by SDS-PAGE showed that anti-gp130 mAbs were able to immunoprecipitate the radiolabeled protein of 180 kDa (150-kDa + 30-kDa OM).

Fig. 1. Prevention of growth inhibitory effects of OM and IL-6 by anti-gp130 mAbs. A375 cells (3000 cells/well) in 96-well tissue culture plates were incubated for 2 days in DMEM containing 5% fetal bovine serum and various amounts of purified recombinant OM (A), IL-6 (B), and TGF-β (C) with and without 4 μg/ml of anti-gp130 mAbs or control mouse mAb. A mixture of anti-gp130 mAbs GPX7, GPX22, and GPZ35 was used in this and subsequent experiments. The control mouse mAb was anti-CD4 mAb. Cells were pulsed with [125I]iododeoxyurine (0.1 μCi/ml) for an additional 6 h. The amount of radioactivity incorporated into DNA was determined, and the data was normalized to the percent of the growth of the culture relative to cells not treated with the factors, medium alone (○), + anti-gp130 mAbs (△), + control mouse mAb (□).

Fig. 2. Blocking of [125I]-OM binding to H2981 cells by anti-gp130 mAbs. A, H2981 cells (1.5 × 10^5 cells/well) in 48-well tissue culture plates were incubated with binding medium (RPMI, 20 mM Hepes, 2.5% bovine serum albumin, 0.2% sodium azide) alone (○) or supplemented with 10 μg/ml anti-gp130 mAbs (△) for 60 min at room temperature. Then various amounts of [125I]-OM were added with or without an excess of unlabeled OM and the cells were incubated for another 90 min. The monolayers were washed with PBS and solubilized with 1 N NaOH. The specific binding is expressed as the difference between total binding and nonspecific binding. B, total binding of [125I]-OM to H2981 cells was conducted in the absence (○) or presence of anti-gp130 mAbs (△), or the presence of control mouse mAb (□).

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Fig. 3. Cross-linking of 125I-OM to H2981 cell surface protein. H2981 cells (5 x 10^6 cells) were incubated with 1 nM 125I-OM alone, or supplemented with 100-fold unlabeled OM, 50-fold unlabeled IL-6, 10 μg/ml anti-gp130 mAbs, or 10 μg/ml control mouse mAb for 90 min at room temperature. To cross-link, disuccinimidyl suberate (0.065 mM) was added to the cells for 15 min at 4 °C. The cells were subsequently washed with PBS and extracted with 2% Triton X-100/PBS for 1 h, and proteins in the supernatants were analyzed by SDS-PAGE. Gels were dried and autoradiographed on Kodak X-AR 5 film.

Fig. 4. Immunoprecipitation of 125I-OM cross-linked 150-kDa protein with anti-gp130 mAbs. H2981 cells (1.5 x 10^7) were cross-linked with 1 nM 125I-OM, then solubilized with digitonin lysis buffer. After spinning briefly in a microcentrifuge, the supernatant was divided equally into three parts and immunoprecipitated with medium alone, anti-gp130 mAbs, or control mouse mAb respectively, and analyzed by SDS-PAGE. Experimental details were as described (12).

This radiolabeled protein was not immunoprecipitated by the control mouse mAb (Fig. 4). To illustrate the direct interaction between OM and the β subunit of the IL-6 receptor, COS cells were transfected with a mammalian expression vector containing a full-length cDNA encoding gp130. Two days post-transfection, the COS cell transfectants grown on glass slides were incubated with 125I-OM (ranging in concentration from 0.1 to 5 nM), fixed, and dipped in photographic emulsion. After a 48-h exposure, the slides were developed and examined by phase contrast and dark field microscopy. Slides containing COS cells transfected with the plasmid carrying the gp130 cDNA displayed positive cells as identified by the presence of autoradiographic grains. The binding of 125I-OM to the COS cell transfectants was blocked by excess unlabeled OM or anti-gp130 mAbs but was not blocked by unlabeled IL-6 or the control mouse mAb (Fig. 5).

COS cell transfectants expressing gp130 were cross-linked to 125I-OM. This material was analyzed by SDS-PAGE and compared with that obtained from mock transfectants and H2981 cells cross-linked with 125I-OM. Fig. 6A shows that 125I-OM cross-linked protein migrated with an apparent molecular mass of 150 kDa in all cells; however, the intensity of signal was much higher in gp130 transfectants than in mock transfectants. Most importantly, all the cross-linked proteins in COS cells were competed out by anti-gp130 mAbs (Fig. 6B). These data, again, strongly suggest that the 150-kDa protein cross-linked to 125I-OM is gp130. The 150-kDa band from

Fig. 5. Visualization of radiolabeled OM binding to COS-7 cells transfected with human gp130 cDNA. COS-7 cells grown on microscope slides were transfected with the COS cell expression vector containing gp130 cDNA (A) or with empty vector (B) and then incubated with 1 nM 125I-OM alone (A), plus unlabeled IL-6 (C), plus unlabeled OM (D), plus mouse mAb (E), or plus anti-gp130 mAbs (F), for 90 min at 20 °C, then washed 5 times with cold PBS, fixed, and dipped in photographic emulsion. The slides were exposed at 4 °C in the dark for 48 h and developed. Photographs were taken under dark field illumination.

Fig. 6. Cross-linking of 125I-OM to COS-7 cells transfected with gp130 cDNA. A, samples containing 5 x 10^6 cells of H2981, gp130 cDNA, or mock-transfected COS-7 cells were cross-linked to 1 nM 125I-OM individually (plus or minus excess unlabeled OM), and the cross-linked materials were analyzed by SDS-PAGE. B, chemical cross-linkings of 125I-OM to COS-7 cells transfected with gp130 cDNA or mock-transfected were performed in the presence or absence of anti-gp130 mAbs or control mouse mAb. The cross-linked materials were analyzed by SDS-PAGE.
COS cells ran slightly diffused as compared with that of H2981, which may be due to differences in glycosylation of gp130 molecules in the two cell types.

Recently, Gearing et al. (12) reported that OM binds to COS-7 cells transfected with a cDNA encoding a truncated form of gp130 with an affinity of 7.7 × 10⁻⁸ M, which is significantly lower than the low affinity OM binding expressed by H2981 and COS-7 cells (Kₐ = 8 ± 2 × 10⁻⁸ M).⁴ Studies with COS cell transfectants expressing a full-length gp130 protein show that the complete gp130 binds OM with the same affinity as the low affinity OM binding receptor (data not shown), suggesting that the cytoplasmic domain of gp130 modulates the binding affinity of the extra-cellular domain of gp130. We identified the nature of the OM receptor (data not shown), suggesting that the cytoplasmic domain of gp130 mediates OM signaling. Although the OM-gp130 interaction is necessary for OM-elicited biological responses it might not be sufficient for initiating OM signals. It is very likely that the OM receptor, like other cytokine receptors, is a dimer or oligomer consisting of gp130 and other subunit(s), which associates with gp130 to generate the functional receptor. There is, however, no experimental evidence for the existence of the putative β subunit of OM receptor. Nevertheless, the following facts suggest the contribution of another nonbinding subunit in OM signaling: (a) gp130 is widely expressed in a variety of cells, but most of the hematopoietic cells do not respond to OM; (b) BAF.03 cells transfected with gp130 cDNA fail to respond to OM under the conditions where IL-6 is able to transduce mitogenic signals; (c) OM does not bind to B9 cells but binds to LIF receptor transfected B9 cells with an affinity lower than the binding to native OM receptor (16); (d) the majority of receptors for other members of the cytokine super family are dimers. Multiple receptor subunits and shared receptor subunits could provide a rational basis for the pleiotropy and redundancy of OM function. The OM-gp130 interaction represents a unique system where gp130, the signal transducer and affinity converter for IL-6 and LIF, is the ligand binder for OM. Cytokines IL-3, IL-5, and granulocyte macrophage-colony stimulating factor also share a common signal transducer named KH97 (17–20). All of the ligands tested so far have failed to bind KH97, although future studies might reveal the ligand for KH97. Recently, OM has been shown to be the major growth factor for Kaposi’s sarcoma-derived cells whose growth is also stimulated by IL-6 and other cytokines (21, 22). Since gp130 is a shared and essential component of IL-6-, OM-, and LIF-signaling pathways, the interruption of gp130-mediated signals by anti-gp130-neutralizing mAbs or antisense gp130 RNA may be useful inhibitors of the growth of Kaposi’s sarcoma cells in vitro and in vivo.

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