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ESTABLISHMENT OF THE ELISA FOR MURINE SOLUBLE GP130, A SIGNAL TRANSDUCER FOR THE IL-6 FAMILY CYTOKINE, AND ITS DETECTION IN THE ASCITIC FLUIDS OF TUMOR-BEARING MICE

Tadashi Matsuda and Toshio Hirano*

Division of Molecular Oncology, Biomedical Research Center, Osaka University Medical School, 2-2, Yamada-oka, Suita, Osaka 565, Japan

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Summary: Interleukin-6 (IL-6) signal is transduced through a membrane glycoprotein gp130, which associates with the IL-6 receptor α chain (IL-6Rα) in the presence of IL-6. We prepared monoclonal antibodies (mAbs) specific to murine gp130 by immunizing rats and hamsters with a chimeric molecule consisting of the extracellular domain of murine gp130 and human immunoglobulin (Ig) G_1 Fc (m130Ig). Furthermore, we developed a sandwich ELISA for detection of murine soluble gp130 (sgp130) and showed that sgp130 was present in the ascitic fluids of tumor-bearing mice. Because sgp130 can inhibit the biological activities of the IL-6-related cytokine subfamily that can enhance anti-tumor immune response and also has both growth inducing and growth inhibitory activities on various tumors, the results suggest that sgp130 in tumor-bearing host modulates tumor progression.

Interleukin-6 (IL-6) is a pleiotropic cytokine acting on a variety of cells (1-3). IL-6 receptor consists of a 80 kDa IL-6 binding molecule (IL-6Rα) and a signal transducer, gp130. Upon IL-6 stimulation, IL-6Rα associates with gp130 and signal is transduced through gp130 (4, 5 and also see a review 6). Furthermore, gp130 functions as a common signal transducer for the IL-6 family cytokine, including leukemia inhibitory factor (LIF), oncostatin M (OSM), IL-11 and ciliary neurotrophic factor (CNTF) (7-9) that not only act on tumors as either a growth factor or growth inhibitory factor but also

* To whom correspondence should be addressed.

ABBREVIATIONS: IL, interleukin; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; ELISA, enzyme-linked immunosorbent assay; mAb, monoclonal antibody.

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modulate anti-tumor immune response (1-3). Recent work showed that soluble gp130 (sgp130) is detected in human serum and sgp130 inhibits signals through membrane-anchored gp130 (10). In this study, we describe the establishment of monoclonal antibodies (mAbs) against murine gp130 and a sandwich ELISA for murine sgp130. Furthermore, we show the presence of sgp130 in the ascitic fluids of tumor-bearing mice.

MATERIALS AND METHODS

Cell lines. An IL-3-dependent murine pro B-cell line, BAF-B03 (11) and its stable transformant, BAFm130 (12), expressing murine gp130 were maintained in RPMI1640 medium supplemented with 10% fetal calf serum and 10% conditioned medium from WEHI-3B cells as a source of IL-3. For the maintenance of BAFm130, 1 mg/ml of G418 was added to the medium. A human embryonic kidney-derived cell line, 293T was maintained in DMEM medium supplemented with 10% fetal calf serum.

Preparation of m130Ig and m80Ig chimeric proteins. A chimeric molecule consisting of the extracellular domain of murine gp130 and human immunoglobulin (Ig) G_1 Fc (termed as m130Ig) was prepared from the culture supernatants of 293T cells that were transfected with pCDMm130Ig by the calcium phosphate precipitation method (12,13). A chimeric molecule consisting of the extracellular domain of murine IL-6 Rα and human immunoglobulin (Ig) G_1 Fc (termed as m80Ig) was also prepared from the culture supernatant of 293T cells transfected with pCDMm80Ig. m130Ig and m80Ig were affinity purified with protein A-Sepharose column (Pharmacia) from the culture supernatants (13). Protein contents were estimated from their absorbance, based on a value of 10.0 for A_{280} of the 1% solution.

Preparation of monoclonal antibodies against murine gp130. Armenian hamster and Wistar rat were immunized weekly with 100μg of m130Ig. Spleen cells were fused with murine plasmacytoma XA653 using ploy ethylene glycol 1500, and hybridomas were established by conventional HAT selection as described previously (14). Hybridomas producing anti-murine gp130 mAbs were selected by FACS analysis. A hamster anti-murine gp130 mAb, HMβ1 and rat anti-murine gp130 mAbs, RMβ1, RMβ2, RMβ3, RMβ4 were established and purified by Ampure PA column (Amersham).

Immunoprecipitation and immunoblotting. Proteins in the ascites of tumor-bearing mice were immunoprecipitated with HMβ1. The immunoprecipitate was resolved to 4-20% SDS-PAGE and transferred to Immobilon (Millipore). The Immobilon filter was immunoblotted with the mixture of RMβ3 and RMβ4. Immunoreactive proteins were visualized using an enhanced chemiluminescence detection system (Amersham).

ELISA for the detection of murine sgp130. 96-well ELISA plates were coated with HMβ1 (5μg/ml) at 100 μl/well. The plates were left overnight at 4°C. After blocking with PBS containing 5% (w/v) non-fat milk, test samples were added to each well and incubated for 2 hours at 37°C. After washing with PBS containing 0.05% Tween 20, RMβ4 (5μg/ml) was added to each well and the plates were incubated for 2 hours at 37°C followed by washing as described above, and 2000-fold diluted alkaline phosphatase-conjugated goat anti-rat IgG (Zymed) was added to each well. The plates were then incubated for 2 hours at 37°C. The color development was performed with phosphatase substrate (Sigma). Absorbance at 405nm was measured with an automated ELISA reader.

FACS analysis. Cells were incubated with the culture supernatant containing mAbs for 20 minutes on ice and washed three times with PBS containing 2% fetal calf serum and 0.02% NaN_3. The cells were then incubated with fluorescein-conjugated mouse anti-rat IgG (Tago) or fluorescein-conjugated goat anti-hamster IgG (Zymed) for 20 minutes on ice. After washing three times, cells were evaluated for membrane immunofluorescence using a FACScan (Becton Dickinson).

RESULTS AND DISCUSSIONS

To prepare mAbs against murine gp130, we made a chimeric molecule consisting of the extracellular domain of murine gp130 and human immunoglobulin (Ig) G_1 Fc (m130Ig) (Fig.1a). By immunizing hamsters and rats with m130Ig, we obtained one hamster (HMβ1) and four rat (RMβ1, RMβ2, RMβ3 and RMβ4) mAbs to murine gp130. Fig.1b shows that one of mAbs to murine gp130, RMβ1 reacted with m130Ig but not m80Ig, showing the specificity of RMβ1. Essentially the same results were obtained with other four mAbs (data not shown). Furthermore, FACS analysis showed that these mAbs against m130Ig reacted with BAFm130 cell line that expresses murine gp130 but not a gp130-negative parent cell line, BAF-B03 (Fig.2). Utilizing

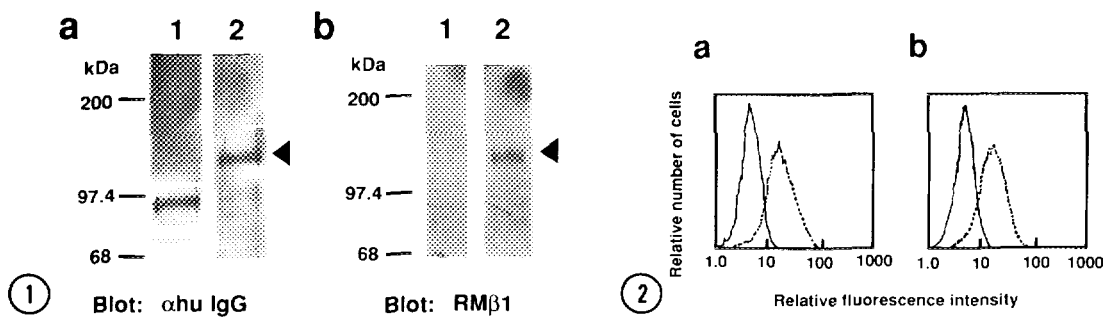

Fig. 1. Western blotting of m80Ig and m130Ig.
Culture supernatants of 293T cells transfected with either pCDMm80Ig (a, b; lane 1) or pCDMm130Ig (a, b; lane 2) were incubated with protein A-Sepharose beads. The beads were extensively washed with PBS containing 0.05% Tween 20 and boiled in an equal volume of sample buffer. The eluted sample was subjected to SDS-PAGE and transferred to Immobilon. The Immobilon filter was then immunoblotted with alkaline phosphatase-conjugated goat anti-human IgG antibodies (a). The same eluted sample was subjected to SDS-PAGE and transferred to Immobilon. The Immobilon filter was then immunoblotted with RMβ1 (b). The arrowheads indicate m130Ig. Molecular weight markers on the left are in kilodaltons.

Fig. 2. FACS analysis of cell surface murine gp130 by HMβ1 and RMβ1.
BAFm130 cells were incubated with the culture supernatant containing HMβ1 (a) or RMβ1 (b) for 20 minutes on ice and washed three times with PBS containing 2% fetal calf serum and 0.02% NaN_3. The cells were then incubated with fluorescein-conjugated mouse anti-rat IgG or fluorescein-conjugated goat anti-hamster IgG for 20 minutes on ice. Dashed and solid lines show the staining with anti-murine gp130 mAbs and control IgG, respectively.

these mAbs, we attempted to develop an ELISA system for the detection of murine sgp130. We used HMβ1 as capture of sgp130 and the mixture of RMβ3 and RMβ4 as the detector antibody. Fig.3 shows that m130Ig could be detected at a concentration of as low as 100ng/ml. The specificity of the ELISA was confirmed because m80Ig was not detected (Fig.3).

Utilizing the ELISA system described above, we measured amounts of sgp130 in mice bearing plasmacytoma, XA653. Fig.4 shows that large amounts of sgp130 were detected in the ascitic fluids in 7 out of 17 tumor-bearing mice. No measurable level of sgp130 was detected in normal murine sera (data not shown). Furthermore, sgp130 protein was detected by western blotting utilizing anti-murine gp130 mAbs: Fig.5 shows the presence of sgp130 proteins with molecular weight of around 100 kDa in the ascitic fluids of tumor-bearing mice.

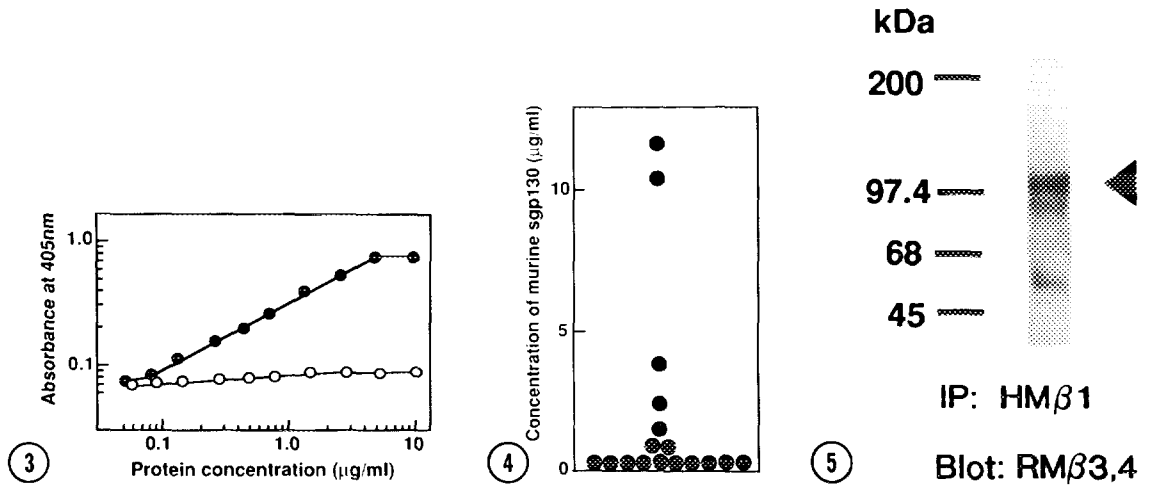

Fig.3. Standard curves for the ELISA for murine sgp130.
96-well ELISA plates were precoated with 5μg/ml of HMβ1. Purified m130Ig (●) or m80Ig(○) that was diluted with various concentrations in PBS was added. ELISA was performed as described in MATERIALS AND METHODS. Averages of duplicate measurements are shown.

Fig.4. Soluble gp130 levels in the ascitic fluids of plasmacytoma bearing mice. Murine sgp130 levels were measured by the ELISA. Averages of duplicate measurements are shown.

Fig.5. Western blotting of murine sgp130 in the ascitic fluids.
100μl of acites was immunoprecipitated with HMβ1-conjugated Sepharose beads. The beads were extensively washed with PBS containing 0.05% Tween 20 and boiled in an equal volume of sample buffer. The eluted sample was subjected to SDS-PAGE and transferred to Immobilon. The Immobilon filter was then immunoblotted with the mixture of RMβ3 and RMβ4. The arrowhead indicates murine sgp130. Molecular weight markers on the left are in kilodaltons.

Soluble cytokine receptors modulate the biological activity of cytokines: most soluble cytokine receptors act as a competitive inhibitor, whereas some are agonistic and in certain cases, the complex of cytokine and its soluble cytokine receptor exert different biological activity from the original cytokine (6,15). Because cytokine regulates immune response, hematopoiesis and inflammation and acts as a growth factor or a growth inhibitory factor for various tumor cells, soluble cytokine receptor may affect tumor progression by either modulating the host defense reaction against tumor or by directly acting on tumor growth. Soluble IL-6Rα was shown to potentiate the biological activities of IL-6. In contrast, sgp130 inhibits functions of the IL-6 family cytokine and may impair the host defense reaction. IL-6 enhances the generation of cytotoxic T lymphocytes and anti-tumor response (1-3). IL-6, LIF and OSM upregulate the expression of ICAM-1 on melanoma cells (16-18). The high expression of ICAM-1 on the melanoma cells was shown to enhance anti-tumor response and reduce metastasis. IL-6, IL-11, LIF, OSM and CNTF are growth factor for myeloma (3,19,20). On the other hand IL-6, LIF and OSM act as growth inhibitory factor for several tumor cells, such as myeloleukemic cells and breast carcinoma (1-3,21). Therefore, sgp130 may modulate the tumor progression by impairing host anti-tumor response or directly affecting the tumor growth. There are evidence that soluble cytokine receptors are related to inflammatory disease and malignancy. For example, soluble IL-2Rα and soluble erythropoietin receptor were found in the serum of some patients with ovarian cancer and acute erythropoiesis, respectively. The elevated levels of sIL-6Rα were found in the serum of HIV-infected patients and aged autoimmune-prone mice (15). The ELISA system for the detection of murine sgp130 described in this study should be useful for further understanding the biological significance of sgp130 in health and disease in relation to IL-6-related cytokines.

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REFERENCES

1 Sehgal, P. B., Grienger, G., and Tosato, G. (Editors) Regulation of the Acute Phase and Immune Responses: Interleukin-6. (1989) Vol.557, pp.1-583 Ann New York Acad Sci, New York.

2 Van Snick, J. (1990) Annu Rev Immunol.8,253-278.
3 Hirano, T. (1992) Chemical Immunol. 51,153-180.
4 Taga,T., Hibi, M., Hirata, Y., Yamasaki, K., Yasukawa, K., Matsuda, T., Hirano, T., and Kishimoto, T.(1989) Cell 58,573-581.
5 Hibi, M., Murakami, M., Saito, M., Hirano, T., Taga, T., and Kishimoto, T. (1990) Cell 63,1149-1157.
6 Hirano, T., Matsuda, T., and Nakajima, K. (1994) Stem Cell,in press.
7 Gearing, D. P., Comeau, M. R., Friend, D. J., Gimpel, S. D., Thut, C. J., Mcgourty, J., Brasher, K. K., King, J. A, Gills, S., Mosley, B., Ziegler, S. F. , and Cosman, D. (1992) Science 255,1434 -1437.
8 Ip, N. Y., Nye, S. H., Boulton, T. G., Davis, S., Taga, T., Li, Y., Birren, S. J., Yasukawa , K., Kishimoto, T., Anderson, D.J., Stahl, N. and Yancopoulos, G. D. (1992) Cell 69,1121-1132.
9 Yin, T., Taga, T., Tsang, M. L.-S., Yasukawa, K., Kishimoto, T. , and Yang, Y.-C. (1993) J Immunol 151,2555-2561.
10 Narazaki,M., Yasukawa,K., Saito,T., Ohsugi,Y., Fukui, H., Koishihara, Y., Yancoupoulos, G. D., Taga, T., and Kishimoto, T. (1993) Blood 82,1120-1126.
11 Hatakeyama, M., Mori, H., Doi, T.,and Taniguchi, T. (1989) Cell 59,837-845.
12 Matsuda, T., and Hirano, T. (1994) Blood 83,3457-3461.
13 Matsuda, T., Yamanaka, Y., and Hirano, T. (1994) Biochem. Biophys. Res. Commun. 200, 821-828.
14 Matsuda, T., Hirano, T., and Kishimoto, T. (1988) Eur. J. Immunol. 18,951-956.
15 Heaney, M. L., and Golde, D. W. (1993) Blood 82, 1945-1948.
16 Vanky, F.,Wang, P., Patarroyo, M., and Klein, E. (1990) Cancer Immunol. Immunother. 31,19-27.
17 Anichi, A., Mortarini, R., Supino, R., and Parmiani, G. (1990) Int. J. Cancer 46,508-515.
18 Giavazzi, R., Chirivi, R. G. S., Garafalo, A., Rambaldi, A., Hemingway, I., Pigott, R., and Gearing, A. J. H. (1992) Cancer Res. 52, 2628-2630.
19 Zhang, X.-G., Gu, J.-J., Lu, Z,-Y., Yasukawa, K., Yancopoulos, G. D., Turner, K., Shoyab, M., Taga, T., Kishimoto, T., Bataille, R., Klein, B. (1994) J. Exp. Med. 179, 1337-1342.
20 Nishimoto, N., Ogata, A., Shima, Y., Tani, Y., Ogawa, H., Nakagawa, M., Sugiyama, H., Yoshizaki, K., and Kishimoto, T. (1994) J. Exp. Med. 179, 1343-1347.
21 Chen, L., Mory, Y., Zilberstein, A., and Revel, M. (1988) Proc. Natl. Acad. Sci. USA. 85, 8037-8041.