A Human Monocyte Growth Factor Produced by Lung Cancer Cells

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ABSTRACT

Human lung cancer cell line, T3M-30, has been shown to produce a growth factor that stimulates proliferation of peripheral blood monocytes. In the presence of this factor, human circulating monocytes were able to proliferate in vitro. Gel exclusion chromatography of the conditioned medium revealed a single peak of monocyte growth-promoting activity at an apparent molecular weight of 16,000. The growth-promoting activity was adsorbed to an anion-exchange column, Mono Q, and eluted with a salt gradient as a single peak of bioactivity at 300 mM NaCl. When the sample was applied to a Vydac C4 column, a reverse-phase high-performance liquid chromatography column, a single peak of activity was observed at a concentration of 76% acetonitrile in 0.1% trifluoroacetic acid. The monocyte growth-promoting activity was heat stable at 56°C. It was partially destroyed by trypsin. The activity was lost after treatment with 2-mercaptoethanol.

INTRODUCTION

It has not been known whether human circulating monocytes are capable of undergoing cell division (1, 2). Since some studies reported that human circulating monocytes are minimally labeled with [3H]thymidine (3, 4), it has been generally accepted that they do not divide under normal circumstances. Van Furth et al. (4) suggested that precursors of monocytes in the bone marrow, promonocytes, actively divide but that human circulating monocytes are nondividing under normal circumstances. While studying the effect of steroid hormones on human monocytes, we found that 1,25-dihydroxyvitamin D3 induced proliferation of human circulating monocytes in vitro (5). In addition, human circulating monocytes have been shown to proliferate in vitro in response to conditioned medium of lectin-stimulated lymphocytes (6).

Hematopoietic growth factors, such as colony-stimulating factors, have been purified from several human cancer cell lines (7, 8), which are good sources for large-scale preparation of the factors. We examined several human cancer cell lines to determine whether they produce monocyte growth-promoting activities. In this report, for the first time, we describe production and characterization of a human MoGF using a human cancer cell line.

MATERIALS AND METHODS

Preparation and Incubation of Monocytes

Circulating monocytes were isolated and incubated as described previously (5). In brief, mononuclear cells from healthy young adult volunteers were put into culture plates (Falcon 3047) at the density of 8 x 10^5 cells/well in Ham's F-10 medium containing 1% autologous serum. After the overnight incubation in a humidified atmosphere of 5% CO2-95% air at 37°C, the plates were rinsed with Ham's F-10, and nonadherent cells were removed by washing. More than 95% of the adherent cells were monocytes, as judged by positive α-naphthylbutyrate-esterase reaction and phagocytosis of latex particles. The monocytes were incubated with the sample in Ham's F-10 medium containing 1% autologous serum for 9 days in a 5% CO2 chamber described above.

Measurement of Mitotic Activity of Human Monocytes

Mitotic activity was examined by measuring [3H]thymidine incorporation into cultured monocytes as reported previously (5). During the last 18 h of incubation, the cultured monocytes were pulse labeled with [3H]thymidine (0.5 μCi/well; specific activity, 6.7 Ci/mmol; New England Nuclear, Boston, MA). At the end of incubation, radioactivity in the trichloroacetic acid-insoluble fraction of the harvested cells was measured by a liquid scintillation counter. All determinations were performed in triplicate cultures.

Counting of Nuclei of Cultured Monocytes

The nuclei of cultured monocytes were counted by the method of Nakagawara and Nathan (9). After the medium was removed, the culture wells were treated with 1% Cetavlon in 0.2 M citric acid solution for 20 min. After the pipeting, the cell nuclei were counted with a hemocytometer.

Preparation of Conditioned Medium of T3M-30 Cells

The human lung cancer cell line T3M-30 has been established in this laboratory from a large cell carcinoma of the lung and will be described elsewhere. The cells have been continuously propagated in Ham’s F-10 medium supplemented with 10% FBS for 4 years and produce a monocyte growth-promoting activity. It is serially passaged by trypsinization and grows rapidly to form an adherent monolayer in plastic culture flasks. Routinely, cells are cultured in F-10 medium, supplemented with 10% FBS and antibiotics. For characterization of monocyte growth-promoting activity in T3M-30-conditioned medium, confluent cultures were incubated in F-10 medium containing dimethyl-α-cyclodextrin (200 μg/ml) without FBS. The serum-free conditioned medium was harvested once a week, and the cultures were refed with fresh F-10 with dimethyl-α-cyclodextrin. The cells are able to grow slowly in the serum-free F-10 medium with dimethyl-α-cyclodextrin.

The conditioned medium was concentrated 20-fold on a hollow-fiber apparatus equipped with an Aff 1010 hollow fiber (molecular weight cutoff of 6000) (Asahi Kasei Co., Ltd., Tokyo, Japan).

Characterization of Monocyte Growth Factor Activity

The concentrate of T3M-30-conditioned medium was diazylated against phosphate-buffered saline and used for characterization studies. Trypsin. The samples were incubated with insoluble trypsin (50 units/ml gel; Sigma Chemical Co.), and the reaction was terminated by removing the insoluble enzyme by centrifugation.

2-Mercaptoethanol Treatment. The samples were exposed to 2-mercaptoethanol (0.5%) for 60 min at room temperature to examine the sensitivity to reducing agents.

Thermal Effect. Heat stability was tested by separate treatment of aliquots of the samples at 25°C (room temperature) for 24 h, 56°C for 60 min, and 100°C for 10 min.

Size-Exclusion Chromatography. The concentrate (0.5 ml) was applied to a Superose 12 column (HR 10/30, Pharmacia) in 10 mM Tris-HCl buffer, pH 7.2. The column was eluted at a flow rate of 1.0 ml/min and monitored with absorbance at 280 nm. Fractions (1.0 ml) were collected and assayed for monocyte growth-promoting activity. The column was calibrated using catalase (Mr, 232,000), ovalbumin (Mr, 30,000), and aprotinin (M22, 6500).

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1 To whom requests for reprints should be addressed.

2 The abbreviations used are: MoGF, monocyte growth factor; FBS, fetal bovine serum; HPLC, high-performance liquid chromatography; CSF, colony-stimulating factor.

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43,000), chymotrypsinogen A (M, 25,000), and ribonuclease (M, 13,700).

Ion-Exchange Chromatography. The concentrate was applied to a Mono Q column (HR 5/5; Pharmacia). Superose 12 and Mono Q were attached to a fast-performance liquid chromatography apparatus (Pharmacia) and operated at room temperature. The column was eluted at a flow rate of 1.0 ml/min with a gradient of 0–400 mM NaCl in 10 mM Tris-HCl, pH 7.2. Absorbance at 280 nm was monitored. Fractions of 1.0 ml were collected and tested for monocyte growth-promoting activity.

Reverse-Phase High-Performance Liquid Chromatography. Reverse-phase HPLC was performed on a Vydac C4 column (4.6 x 250 mm) on a Shimazu LC6A HPLC system (Shimazu Seisakusho Ltd., Tokyo, Japan). The column was washed with 0.1% trifluoroacetic acid, and bound proteins were eluted with a linear gradient of 0–90% acetonitrile in 0.1% trifluoroacetic acid for 30 min. Fractions of 4.0 ml were collected and assayed for monocyte growth-promoting activity.

RESULTS

Production of Monocyte Growth-promoting Activity by Lung Cancer Cells. Exposing the monocytes to supernatants from T3M-30 cells resulted in a marked increase in monocyte proliferation rates. In the sample shown, whereas cell number increased approximately 200% with the supernatants from T3M-30 cell cultures, monocytes exposed to supernatants from other cancer cells or control medium alone decreased in cell number by 30% in the same time period (Fig. 1A). A marked increase in the incorporation of [3H]thymidine into DNA was also observed (Fig. 1B). These observations suggest that T3M-30 cells produce monocyte growth-promoting activity.

Characterization of Monocyte Growth-promoting Activity. When a concentrate of supernatants from T3M-30 cells was incubated with trypsin, the monocyte growth-promoting activity was partially destroyed (Table 1). The activity was lost after treatment with 2-mercaptoethanol. A slight decrease in the activity was observed at 56°C. The activity was destroyed by heating at 100°C for 10 min.

To determine the approximate size of the molecule(s) responsible for the monocyte growth-promoting activity, a concentrate of the supernatants was loaded on a Superose 12 fast-performance liquid chromatography column. As shown in Fig. 2, the activity was detected as a single peak at an approximate molecular weight of 16,000. When a concentrate was subjected to Mono Q anion-exchange chromatography, the activity was eluted with a salt gradient as a single peak at 300 mM NaCl (Fig. 3). The sample was also applied to a Vydac C4 reverse-phase HPLC column, and the bound proteins were eluted with a linear gradient of 0–90% acetonitrile. This procedure yielded a single peak of biological activity at 76% acetonitrile (Fig. 4).

DISCUSSION

The present study demonstrated that the human circulating monocytes were induced to proliferate in the presence of MoGF(s). Proliferation of cultured monocytes was shown by both an increase in the cell number and [3H]thymidine incorporation into cultured cells. MoGF activity was detected in the culture medium from human lung cancer cells, T3M-30. The MoGF activity was heat stable, trypsin sensitive, and destroyed by 2-mercaptoethanol. These results suggest that MoGF is a protein, which appears to contain at least one essential disulfide bond. Size-exclusion chromatography revealed that MoGF had an apparent molecular weight of 16,000. It was adsorbed to a column of Mono Q anion-exchange chromatography and eluted as a single peak of biological activity with a salt gradient. The MoGF was adsorbed to a Vydac C4 reverse-phase HPLC column and eluted as a single peak of activity at 76% acetonitrile. These observations suggest that T3M-30 cells produce a protein growth factor that stimulates proliferation of human circulating monocytes.

The generation of monocyte-macrophages from immature hematopoietic progenitor cells depends on the presence of several hormone-like glycoproteins, CSFs (10). There are several classes of CSFs and they can be distinguished by the kind of mature cells that they produce in semisolid media in vitro. To determine whether the MoGF from T3M-30 cells stimulates proliferation of hematopoietic progenitor cells, CSF activity of
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Table 1  Stability of monocyte growth-promoting activity

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Residual activity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>100</td>
</tr>
<tr>
<td>Heat 25°C, 24h</td>
<td>80.9</td>
</tr>
<tr>
<td>25°C, 60 min</td>
<td>75.4</td>
</tr>
<tr>
<td>100°C, 10 min</td>
<td>6.4</td>
</tr>
<tr>
<td>Trypsin 120 min</td>
<td>40.7</td>
</tr>
<tr>
<td>2-Mercaptoethanol 60 min</td>
<td>2.4</td>
</tr>
</tbody>
</table>

* % residual activity = (dpm in treated sample − dpm in controls) / (dpm in untreated sample − dpm in controls) × 100%.

Fig. 2. Gel filtration profile of the supernatants on Superose 12. A concentrate of the supernatants was loaded on a Superose 12 column and eluted with 10 mM Tris-HCl buffer (pH 7.2). The elution was monitored by absorption at 280 nm (---) and each fraction was tested for MoGF activity (O—O). Standards include catalase (Mr, 232,000), ovalbumin (Mr, 43,000), cymotrypsinogen A (Mr, 25,000), and ribonuclease (Mr, 13,700).

Fig. 3. Ion exchange of the supernatants was applied to an anion-exchange column of Mono Q. After a wash with 10 mM Tris-HCl buffer (pH 7.2), bound proteins were eluted with a linear gradient of 0-400 mM NaCl in the same buffer as indicated (---). The elution was monitored by absorption at 280 nm (---), and each fraction was tested for MoGF activity (O—O).

Fig. 4. Reverse-phase high-performance liquid chromatography. A concentrate of the supernatants was applied to a Vydac C4 column equilibrated with 0.1% trifluoroacetic acid. After a wash with 0.1% trifluoroacetic acid, the bound proteins were eluted with a 30-min linear gradient of 0-90% acetonitrile in 0.1% trifluoroacetic acid at room temperature at a flow rate of 0.5 ml/min. Fractions (4.0 ml) were collected and aliquots of each fraction were assayed for MoGF activity (O—O). ——, absorbance at 280 nm; ——, % acetonitrile.

Fig. 5. Thymidine incorporation (dpm) in adherent cells. The partially purified MoGF from Superose 12 size-exclusion chromatography was examined, using both human and mouse bone marrow cells as targets as described previously (7, 8).

Human granulocyte CSF, granulocyte-macrophage CSF, or interleukin 3 (human multi-CSF) stimulated the colony formation in soft agar culture of human bone marrow cells. Human macrophage CSF was able to stimulate the colony formation of mouse bone marrow cells. However, the active fraction from Superose 12 column chromatography was ineffective against the human or mouse bone marrow cells (data not shown). Together with the biochemical properties of CSFs (10), these observations suggest that the MoGF from T3M-30 cells is different from these colony-stimulating factors.

Whether the MoGF is a novel growth factor for human circulating monocytes or is related to the known hematopoietic growth factors will have to be ascertained by further studies. Purification studies are under way in our laboratory.

REFERENCES

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