Growth Inhibition of Human Lung Cancer Cell Lines by Interleukin 6 in Vitro: A Possible Role in Tumor Growth via an Autocrine Mechanism

Hajime Takizawa, Takayuki Ohtoshi, Ken Ohta, Naomi Yamashita, Shunsei Hirohata, Koichi Hira, Keichi Hiramatsu, and Koji Ito

Department of Medicine and Physical Therapy, Tokyo University School of Medicine [H. T. O., K. O., N. Y., S. H., K. H., K. I.], and Department of Bacteriology, Juntendo University School of Medicine [K. H.], Tokyo, Japan

ABSTRACT

It has been considered that growth of human lung cancer cells, like other malignant cells, is positively and negatively regulated by a variety of growth factors via autocrine as well as paracrine mechanisms. The autocrine mechanism is considered to be important in the autonomy of proliferation of cancer cells. Recently, the role of autocrine growth-inhibiting factors such as transforming growth factor ß attracts special attention for better understanding of growth regulation of malignant cells. Here, we have demonstrated that a multifunctional cytokine interleukin 6 (IL-6) had an inhibitory effect on the proliferation of human non-small cell lung cancer cell lines, as shown by the growth accelerating effect of the specific anti-IL-6 antibody as well as the effect of exogenously added IL-6. Moreover, IL-6 can be expressed and released by human lung cancer cells, and these cells had specific IL-6 receptors on their cell surfaces, suggesting an autocrine mechanism. The growth-inhibitory effect of IL-6 was additive to that of transforming growth factor ß, and could not be neutralized by the addition of anti-transforming growth factor ß antibody. These results suggested that IL-6 may function as another class of autocrine growth-inhibiting factor in the growth regulation of human lung cancer. Relatively lower IL-6 sensitivity of these cells than noncarcinogenic human bronchial epithelial cells also suggested that escape from growth regulation by inhibitory factors such as IL-6 could be involved in lung cancer oncogenesis.

INTRODUCTION

It has been reported that proliferation of human lung cancer is regulated by several growth factors in vitro and in vivo (1). Many of these growth factors such as TGF-α (2), bombesin (3), insulin-like growth factor 1 (4), and TGF-ß (5) have been suggested to exert their effect via autocrine as well as paracrine mechanisms. The autocrine hypothesis is a concept that a cell produces and secretes a growth factor which can interact with specific membrane receptors on its own surfaces to induce effects such as proliferation (6). This has become a central concept to understand the mechanisms of transformation (6, 7), and these autocrine growth factors are now considered to play a role in the cell proliferation of human lung cancer cells. IL-6 is a multifunctional cytokine that can stimulate and inhibit the cell proliferation of several types of cells (8, 9). Moreover, IL-6 has been shown to be an autocrine growth factor for human myeloma cells (10). However, the role of IL-6 in the regulation of the growth of human lung cancer still remains to be studied.

In the present report, we studied the roles of IL-6 in the growth regulation of cells of several human lung cancer cell lines. We also evaluated the effect of IL-6 on growth regulation of noncarcinogenic human bronchial epithelial cells, BEAS-2B (11), to compare the sensitivity to IL-6. We found that IL-6 had an inhibitory effect on cell growth of several lung cancer cells, but these cells were less sensitive than human bronchial epithelial cell line BEAS-2B cells. We further showed the IL-6 receptor expression and release of IL-6 in these cells, suggesting that IL-6 may function as an autocrine growth-inhibiting factor in the proliferation of human lung cancer cell lines.

MATERIALS AND METHODS

Cell Culture. Human lung adenocarcinoma cell line A549 (12) (American Type Culture Collection CCL-185) was cultured in RPMI 1640 medium (GIBCO, Grand Island, NY) supplemented with l-glutamine, 10% heat-inactivated FCS (GIBCO) and 1% penicillin-streptomycin (GIBCO) on tissue culture plates (Corning, Corning, NY) at a density of 5 × 10⁵/100-mm plates. Human epidermoid carcinoma cell line A431, lung adenocarcinoma cell lines PC-3 and RERF-LC-MS, and lung squamous cancer cell lines VMRC-LCP and LC-1 sq (generous gifts from Japanese Cancer Research Resource Bank, Tokyo, Japan) were cultured in a similar manner as A549 cells. Human lung small cell cancers RERF-LC-FM and Lu-134-A-H (also generous gifts from Japanese Cancer Research Resources Bank) were cultured in RPMI 1640 supplemented with l-glutamine, 1% penicillin-streptomycin, and 10% FCS in 50-ml cell culture flasks (Corning) at 37°C, 5% CO₂. The media were changed every other day. A human bronchial epithelial cell line BEAS-2B (11) (a kind gift from Drs. J. F. Lechner and C. C. Harris) was cultured on collagen-coated tissue culture plates (Koken, Tokyo, Japan) with hormonally supplemented Ham's F-12 medium as reported previously (13, 14). Five to seven passaged cells were used for experiments.

Quantification of Growth of Human Lung Cancer Cells by Coulter Counter. The effect of human recombinant IL-6 on cell proliferation of non-small cell lung cancer cell lines was evaluated by Coulter Counter. The cells were plated at 5 × 10⁴/35-mm tissue culture plates (Corning) in RPMI 1640-10% FCS. After incubation to allow the cells to attach, the cells were cultured in test media including RPMI 1640-10% FCS, RPMI 1640-10% FCS with 10 ng/ml human recombinant IL-6 (a product of Escherichia coli as described (8), and the activity was evaluated by IL-6-dependent murine fibrosarcoma MH60.BSF2 as described below), with 5 ng/ml TGF-ß (derived from human platelets; Biomedical Technologies, Inc., Stoughton, MA), or with both factors. After different times in culture, the cells were detached by trypsinization and the number of the cells were assessed in triplicate by Coulter Counter (Coulter Electronics, Elk Grove Village, IL) as reported (15).

Colorimetric MTT Assay. For evaluation of the effect of IL-6 on the growth kinetics of human lung cancer cells, a colorimetric MTT assay (Cemonax International, Inc., Temecula, CA) was also used (16). Briefly, the cells were plated at 96-well tissue culture plates (Costar, Cambridge, MA) at 5 × 10⁴/well in 100 μl test media with variable concentrations of IL-6, and cultured for 72 h at 37°C, 5% CO₂. The cells were treated with 10 μl MTT for 5 h at 37°C to yield a dark blue formazan product. Then isopropanol/HCl mixture was added to each well. Absorbance at 570 nm was calculated by automatic ELISA reader (MTP-2, Corona Electric, Tokyo, Japan) and the data were expressed in percentage when the absorbance of the cells cultured in control media was calculated as 100%. The concentration necessary for 20% decrease in cell growth was calculated as IC₅₀ (μg/ml) in each cell line tested. The maximal percentage of decrease of cell growth with up to 500 ng/ml of IL-6 was also noted.

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1 This work was supported by Manabe Medical Foundation.
2 To whom requests for reprints should be addressed, at Department of Medicine and Physical Therapy, Tokyo University School of Medicine, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan.
IL-6 Receptor Assay. Specific binding of 125I-labeled IL-6 to the cells was studied as reported previously (17, 18) with modifications. Briefly, for preparation of 125I-labeled IL-6, 5 ng of recombinant IL-6 dissolved in 10 μl of 0.1 M borate buffer, pH 8.5, were added to 500 μCi of cold Bolton and Hunter reagent (200 Ci/mmol; Amersham Corp., Arlington Heights, IL) and the mixture was agitated for 15 min on ice. The reaction was stopped by 0.2 M glycerin, then the radiolabeled IL-6 was purified by a gel filtration column (PD-10, Pharmacia Fine Chemicals, Piscataway, NJ) (17). The cells cultured in the flasks were washed twice and mixed with 125I-IL-6 with or without a 200-fold excess of unlabeled IL-6 in a final volume of 70 μl of binding medium. Reaction mixtures were incubated on ice for 150 min with frequent agitation. After centrifugation, cell-associated radioactivity was measured by a gamma counter as reported (17). The cells cultured on tissue culture plates were washed twice with Hank's balanced salt solution and incubated for 150 min at 0°C with 125I-labeled IL-6 in the presence or absence of a 200-fold excess of unlabeled IL-6 in a final volume of 180 μl (18). After the medium was removed, the cells were rinsed once and solubilized in 0.5 ml of 1% sodium dodecyl sulfate/100 mM NaOH. The entire volume was transferred to a 200-fold excess unlabeled IL-6 (nonspecific binding). Scatchard plot analysis was performed as reported (17-19).

IL-6 Receptor Gene Expression in Human Lung Cancer Cells. Total cellular RNA was extracted by the method of Chomczynski and Sacchi (20), and electrophoresed on formaldehyde-denatured agarose gel (10 μg/lane), followed by capillary transfer onto Biodyne nylon membrane. Psrl-Xhol fragments of human IL-6 receptor cDNA (1.1 kilobases) (21) were digoxigenin labeled by random priming (DNA Labeling Kit, Boehringer Mannheim GmbH, Mannheim, Germany) and hybridized by the method as described (22) and recommended by the manufacturer (DIG Luminescent Detection Kit for Nucleic Acids, Boehringer Mannheim). Briefly, the blot was hybridized with digoxigenin-labeled DNA at 42°C overnight, then washed at appropriate stringency. After incubation in blocking solution for 1 h, the membrane was reacted with alkaline phosphatase-conjugated anti-digoxigenin antibody for 30 min. After washing twice, 3-(2'-spiradamantane)-4-methoxy-4-(N-phosphoryl-1,2-dioxetane disodium salt was added as substrate for alkaline phosphatase to detect signals by chemiluminescence on Kodak X-ray film. As an internal control, β-actin mRNA transcripts were evaluated with β-actin cDNA probe (pHFβA-1) (23).

IL-6 Assay. Confluent cultures of the cells were rinsed once, and media were replaced by fresh RPMI 1640 medium with a variety of stimuli, including human recombinant interleukin 1α (IL-1α, 5 ng/ml), interleukin 1β (IL-1β, 5 ng/ml), TNF-α, 10 units/ml (IL-1α and IL-1β), and TNF-α were donated by Otsuka Pharmaceutical Co., Osaka, Japan) and no stimulus. The supernatants were harvested at different times in culture. In separate wells protein synthesis inhibitor cycloheximide (GIBCO) (10 μg/ml) was added to the cells with and without IL-1α. Immunoactive IL-6 was measured by IL-6 ELISA kit (Quanti- tine, R&D Systems, Inc., Minneapolis, MN) in duplicates, as recommended by the manufacturer. Biological activity of IL-6 was assessed by IL-6-dependent murine hybridoma MH60.BSF2 (a kind gift from Drs. T. Hirano and T. Kishimoto) as described previously (24, 25).

IL-6 Gene Expression in Human Lung Cancer Cells. Extraction of RNA and preparation of Northern blots were performed as described above. Tagf-3'-untranslated fragment of human IL-6 cDNA (28) (a generous gift from Dr. Kishimoto) was labeled with digoxigenin and hybridized with the blots, followed by enzymatic reaction. The signals were detected as chemiluminescence on Kodak X-ray film.

Preparation of A549-conditioned Medium. A549 cells were cultured on 100-mm tissue culture plates until confluency, washed twice, and the supernatant was harvested 48 h after the addition of IL-1α (5 ng/ml). The conditioned medium was dialyzed against 50-fold excess of fresh RPMI medium (the molecular weight cutoff 10,000), and stored at -20°C until use. To test the effect on cell growth, the conditioned medium was added to the cells at different percentages, with and without a variety of pretreatments: with 10 μg/ml anti-IL-6 monoclonal antibody BSF2-166; with anti-TGF-β antibody (polyclonal anti-TGF-β antibody, R&D Systems, Inc.), or with both antibodies. Statistics. Student's t test was used for statistical comparison of the data.

RESULTS

Growth-inhibitory Activity of IL-6 on Human Lung Cancer Cells. Exogenously added human recombinant IL-6 (10 ng/ml) inhibited the growth of A549 cells as shown by direct counting of the cell number (Fig. 1a). This growth inhibitory action was additive to the effect of TGF-β. The effects of IL-6 and TGF-β were not cytotoxic, since cell viability as assessed by trypan blue dye exclusion and lactate dehydrogenase release assay showed no significant changes as compared to controls (data not shown). Colorimetric MTT assay demonstrated that IL-6 inhibited the growth of A549 cells in a dose-dependent fashion (Fig. 1b). The maximal inhibition with IL-6 was 54.5 ± 5.20% at the concentration of 500 ng/ml and IC20 was 0.13 ng/ml. We studied the inhibitory effect of IL-6 at 1000 ng/ml, but the effect was as potent as at 500 ng/ml. Other growth factors and cytokines (IL-1α and IL-1β, IL-2, platelet-derived growth factor and epidermal growth factor) showed no such effect (maximal percentage of decrease of cell growth with up to 500 ng/ml 3.50, 2.20, 7.12, -3.90, and -9.91%, respectively). We further studied the effect of IL-6 on the proliferation of several human lung cancer cell lines
The maximal inhibition (63.3 ± 9.22%) and IC_{20} (0.05 ± 0.01 ng/ml) of IL-6 on BEAS-2B cells was also inhibited by IL-6. The maximal inhibition (63.3 ± 9.22%) and IC_{20} (0.05 ± 0.01 ng/ml) suggested that all the cancer cell lines tested were less sensitive to IL-6 than BEAS-2B cells.

**Specific IL-6R on A549 Cells.** Binding assay with ^125^I-labeled IL-6 to several cancer cells was performed to determine if IL-6 had growth-inhibitory effects via the specific IL-6R on lung cancer cell surfaces. The specific binding of ^125^I-IL-6 to the cells as a function of the concentration of ^125^I-IL-6 was shown in Fig. 2a. Radiolabeled IL-6 bound to A549, VMRC-LCP, and BEAS-2B cells in a saturable manner. Analysis of the binding data by the method of Scatchard revealed only a single linear regression line, suggesting that there was a single set of binding sites on these cells (Fig. 2b). IL-6R affinity and number per cell varied among these cell lines, but seemed not to be correlated with sensitivity to IL-6 effect. Binding of ^125^I-IL-6 to the A549 cells was almost completely inhibited by excess amount of unlabeled IL-6, but not by IL-1α, IL-1β, IL-2, or granulocyte colony-stimulating factor (Fig. 2a), showing that the binding was specific to IL-6R on A549 cells. In two small cell cancer cell lines no significant binding was detected (calculated binding site per cell was less than 10).

**IL-6R Gene Expression in A549 Cells.** To further evaluate the expression of IL-6R on the lung cancer cells, Northern blot analysis was performed to show the specific IL-6R mRNA signals. As shown in Fig. 3, A549 cells showed a constitutive expression of IL-6R mRNA. IL-1α (5 ng/ml) induced a significant increase in IL-6R mRNA levels. More interestingly, IL-6 (10 and 50 ng/ml) itself stimulated the A549 cells to express IL-6R mRNA, suggesting an autocrine regulatory mechanism.

**Release of Immunoreactive as Well as Bioactive IL-6 by Human Lung Cancer Cells.** Since it has been reported that a variety of malignant as well as normal cells are capable of expressing and releasing IL-6 (8), and that IL-6 can affect the cell growth via an autocrine mechanism (10), we hypothesized that IL-6 might be involved as an autocrine growth factor in our system. To test this possibility, we evaluated the release of IL-6 by several lung cancer cells and BEAS-2B cells with the use of immuno- and bio-assay. Human lung adenocarcinoma cell line A549 released a significant amount of IL-6 into culture media in a time-dependent manner as assessed by specific ELISA (Fig. 4a). IL-1α, IL-1β, and TNF-α stimulated the cells to release IL-6 in a time- and dose-dependent manner as assessed by specific ELISA (Fig. 4, a and b). It was markedly abolished by the addition of 10 μg/ml cycloheximide, suggesting a de novo synthesis. Bioactive IL-6 was detected by IL-6-dependent cell line MH60.BSF2, and this activity was neutralized by the anti-IL-6 monoclonal antibody BSF2-166 (IgGl, a kind gift by Dr. T. Kishimoto), but not by control IgGl, showing the specific activity of IL-6 in the culture supernatant (Fig. 4c). Among the other cancer cell lines tested, 4 non-small cell cancers showed a significant release of IL-6 into media (Table 2). BEAS-2B cells also released IL-6 with the stimulation of IL-1α (Table 2).

**IL-6 Gene Expression in Human Lung Cancer Cells.** Northern blot analysis demonstrated that A549 cells expressed a specific message for IL-6 (1.3 kilobases) constitutively when they had been grown in 10% FCS-RPMI medium. IL-1α up-regulated the steady state level of IL-6 mRNA in a dose-dependent manner when analyzed 12 h after the addition of IL-1α (Fig. 5).

**Effect of A549-conditioned Medium on Tumor Growth.** A549 cell-conditioned medium was obtained, dialyzed against fresh media,
and added to A549 cells in culture to see the effect of endogenously released IL-6 into media. The growth-inhibitory effect of the conditioned media was shown by a calorimetric MTT assay (Fig. 6), and moreover, this effect was partially neutralized by the preincubation of the conditioned media with monoclonal anti-IL-6 antibody. This incomplete abolishment of the inhibitory effect of the conditioned media might reflect the only partial blocking of IL-6 activity by the antibody, but also suggested the existence and action of other growth factors such as TGF-β, which is actually known to be released by all mam-

### Table 2: Release of immunoreactive IL-6 by human lung cancer cells

<table>
<thead>
<tr>
<th>Cells</th>
<th>Concentration of IL-6 (pg/ml/10^6) (ELISA)</th>
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<tbody>
<tr>
<td>Non-small cell cancer</td>
<td></td>
</tr>
<tr>
<td>A549</td>
<td>412 ± 3.50</td>
</tr>
<tr>
<td>RERF-LC-MS</td>
<td>95.0 ± 10.9</td>
</tr>
<tr>
<td>VMRC-LC-P</td>
<td>80.6 ± 0.05</td>
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<tr>
<td>LC-1 sq</td>
<td>49.2 ± 9.20</td>
</tr>
<tr>
<td>A431</td>
<td>138 ± 20.1</td>
</tr>
<tr>
<td>Small cell cancer</td>
<td></td>
</tr>
<tr>
<td>RERF-LC-FM</td>
<td>ND*</td>
</tr>
<tr>
<td>Lu-134-A-H</td>
<td>ND*</td>
</tr>
<tr>
<td>BEAS-2B</td>
<td>238 ± 10.2</td>
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</tbody>
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* ND, not detected.

**Fig. 4.** Production of IL-6 by A549 cells in vitro. A, A549 cells released immunoreactive IL-6 into culture media. Upon confluence, human recombinant IL-1α (5 ng/ml), IL-1β (5 ng/ml), and TNF-α (10 units/ml) were added and the supernatant was harvested 48 h after the treatment: Immuneactive IL-6 was detected and 3 stimuli up-regulated the release of IL-6. Cycloheximide (CHX) markedly inhibited IL-6 release, suggesting a de novo synthesis. b, IL-1α and IL-1β stimulated IL-6 release from A549 cells in a dose-dependent fashion. The supernatant was harvested 48 h after the treatment with different concentrations of IL-1α and IL-1β, and immunoreactive IL-6 was measured by ELISA. c, A549 cells released bioactive IL-6. Upon confluence, the A549 cells were treated with and without 5 ng/ml IL-1α. The conditioned medium was harvested 48 h after the treatment and bioactivity of IL-6 was evaluated by MTT assay by using MHE60.BSF2 cells. Specific monoclonal anti-IL-6 antibody abolished this activity, whereas control IgG1 had no effect.

**Fig. 5.** Northern blot analysis for IL-6 gene expression in A549 cells. Lane 1, no stimulus; Lane 2, 24 h after the stimulation of 5 ng/ml IL-1α; Lane 3, 50 ng/ml IL-1α. 1.3 kilobase specific signals for IL-6 were observed without stimulus, and IL-1α showed dose-dependent stimulatory effect on IL-6 mRNA expression. β-actin mRNA transcripts (2.0 kilobases) were shown as controls.

**Fig. 6.** A549-conditioned medium (CM) significantly inhibited the growth of A549 cells. A549-CM was harvested 48 h after confluence with IL-1α, dialyzed against fresh RPMI medium (molecular weight cutoff range, 10,000) and added to A549 cells, 40% CM added to the wells showed a significant inhibition of growth as assessed by MTT assay (P < 0.001). Preincubation of CM with anti-IL-6 antibody (BSF2-166, 10 μg/ml) or anti-TGF-β antibody (10 μg/ml) showed a significant abolishment of the inhibitory activity and CM treatment with both antibodies almost completely blocking the growth-inhibitory activity of A549-CM (P > 0.05 as compared to baseline).

Table 2: Release of immunoreactive IL-6 by human lung cancer cells

<table>
<thead>
<tr>
<th>Cells</th>
<th>No stimulus</th>
<th>IL-1α (5 ng/ml)</th>
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</thead>
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<tr>
<td>Non-small cell cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A549</td>
<td>412 ± 3.50</td>
<td>815 ± 25.3</td>
</tr>
<tr>
<td>RERF-LC-MS</td>
<td>95.0 ± 10.9</td>
<td>122 ± 9.20</td>
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<tr>
<td>VMRC-LC-P</td>
<td>80.6 ± 0.05</td>
<td>150 ± 0.80</td>
</tr>
<tr>
<td>LC-1 sq</td>
<td>49.2 ± 9.20</td>
<td>99.3 ± 2.75</td>
</tr>
<tr>
<td>A431</td>
<td>138 ± 20.1</td>
<td>322 ± 12.9</td>
</tr>
<tr>
<td>Small cell cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RERF-LC-FM</td>
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</tbody>
</table>

* ND, not detected.
media contained an active form of TGF as well as IL-6, and that IL-6 affected tumor growth via a different pathway from that of TGF-β.

Accelerating Effect of Anti-IL-6 Antibody on Growth of A549 Cells. To further determine the role of IL-6 in the growth regulation of the cells, we studied the effect of anti-IL-6 antibody on the cell proliferation at various cell densities. When the cells were initially plated at 5 × 10^3/well, anti-IL-6 antibody showed no effect on tumor growth (data not shown). Since it seemed possible that IL-6 release and IL-6R expression depended on cell density, we studied the effect of anti-IL-6 antibody at higher cell density (5 × 10^5/well). We also evaluated the effect of FCS concentration, because FCS was the likely source of growth factors which could affect the potency of endogenous IL-6. The results showed that anti-IL-6 antibody itself could increase the growth of A549 cells when the cells were plated at high cell density, further suggesting a role of endogenously released IL-6 on cell proliferation. The accelerating effect was more prominent in the cells with a lower concentration of FCS (2.5%) than a high concentration (2.5% FCS: 66.6 ± 9.20% versus 10% FCS: 36.0 ± 2.51%, P < 0.01) (Fig. 7).

Effect of Anti-TGF-β Antibody on Growth Inhibition by IL-6. TGF-β had a growth-inhibitory effect on A549 cells as shown previously (5) and by our present data. Therefore, the next question would be how IL-6 inhibited the growth of lung cancer cells and whether this effect was via the effect of TGF-β or not. In this context, we studied the effect of various dilutions of anti-TGF-β antibody (original stock solution, 100 µg/ml) on growth inhibition by exogenous IL-6. The results showed that the inhibitory activity of IL-6 was not via the action of TGF-β possibly secreted by these cells (Table 3).

### DISCUSSION

In the present report, we have demonstrated that (a) exogenously added human recombinant IL-6 had a growth-inhibiting effect on human lung cancer cell lines: namely, the inhibitory effect of exogenous IL-6 on cell proliferation was ascertained in a human lung adenocarcinoma cell line A549 and less markedly in other cell lines, including VMRC-LCP, REFLC-MS, LC-1 sq, and A431 cells by MTT assay, as well as the direct enumeration of the cells; (b) A549 cells, VMRC-LCP, and transformed bronchial epithelial cells expressed specific IL-6R. The affinity to IL-6 (dissociation constant, approximately 10^-10 m) corresponded to the concentration necessary to affect the tumor growth; (c) human lung cancer A549 cells expressed and released significant amounts of both immunologically and functionally active IL-6 at the concentration comparable to that capable of inhibiting cell growth. Cells of several other lung cancer cell lines released less, but a significant amount of IL-6. Moreover, A549-conditioned media, which contained active IL-6, had a growth-inhibitory effect, and anti-IL-6 antibody partially neutralized its activity; (d) finally, addition of anti-IL-6 antibody itself accelerated the growth kinetics of the densely plated cells, further supporting the possibility that endogenous IL-6 plays a role in the regulation of tumor growth. These observations suggested that IL-6 might be involved as a potent growth-inhibitory peptide in the growth of human lung cancer cells via an autocrine mechanism. Although the conditioned media harvested 48 h after addition of IL-1α contained high levels of IL-6 to inhibit the growth, exogenous IL-1α itself did not inhibit its growth by a MTT assay. Different concentrations of IL-6 at the initial plating conditions (A549-conditioned media contained ~1000 pg/ml IL-6) might account for this discrepancy.

The autocrine secretion of growth factors can be a central mechanism in the process of malignant transformation (1). The peptide growth factors which function via an autocrine mechanism in lung cancer cells include TGFα (2), bombesin and related peptides (3), IGF-1 (4), and TGF-β (6, 7). Among those, TGF-β now attracts attention as a well-documented autocrine growth-inhibiting peptide in the regulation of normal and malignant cells (5). Since the identification of this peptide, the autocrine hypothesis was extended to include the concept that malignant transformation may be the result not only of excess production, expression, and action of positive autocrine growth factors, but also of the complete or relative failure of the cells to synthesize, express, or respond to specific negative growth factors they normally release to control their own growth (5). Our present report has suggested that another class of growth factor peptide IL-6 may function as a growth inhibitor via an autocrine mechanism in several human lung cancer cell lines. Exogenously added IL-6, however, could not completely suppress growth of the lung cancer cells, therefore this cytokine might be involved as one of the growth regulators in lung cancer growth.

IL-6 is a multifunctional cytokine, which is now recognized as a growth factor that may play an important role in the growth regulation of several types of cells (8). For example, IL-6 can be an autocrine growth stimulator in human myeloma cells (10). In contrast, IL-6 is a growth inhibitor for human breast cancer cell lines and certain lymphoma leukemia cell lines (9). These observations showed that IL-6, like TGF-β, has dual effects, stimulating growth of some cells while inhibiting that of others. However, the exact role of this cytokine in the regulation of growth of nonhematopoietic malignancies remains unelucidated. Miki et al. (27) reported that IL-6 functioned as an in vitro positive autocrine growth factor in human renal cell carcinomas. Recently, Serve et al. (28) evaluated effects of IL-6 on the growth of 26 different nonhematopoietic cell lines, including two breast carci...
nomas and four renal carcinomas, several of which were the same cell lines previously studied (9, 27). They found no significant influence on any cell lines tested. They also studied its effect on CCL185 (A549) cells with no effect. The reason for these discrepancies are unknown; however, there are several possibilities. First, we utilized methods of direct counting of the cell numbers and MTT assay which correlate well with each other (16). It is known that thymidine incorporation assay measures the number of cells synthesizing DNA, but not always dividing. Thus, our methods could potentially differ from the radiolabeled nucleotide incorporation method used by Serve et al. Another possible reason could be the cell culture conditions used. Cell attachment to the culture plates with or without attachment matrix can change the receptor affinity and number to certain growth factors. Moreover, the cell density may be another important factor regulating receptor expression as shown by Beckmann et al. in bovine bronchial epithelial cell proliferation (29). Finally, components in the serum which had growth factor activities might influence the results.

Normal cell growth seems regulated by the dual actions of growth factors via an autocrine as well as paracrine mechanism in local environments. Hugett et al. (30) reported that IL-6 had inhibitory activity on the proliferation of primary hepatocytes and transformed liver cells. Our preliminary studies suggested that normal human bronchial epithelial cells in primary culture could express IL-6 and that IL-6 inhibited their proliferation in vitro. It is proposed that non-small cell lung cancer such as epidermoid cancer and adenocarcinoma cells are derived from airway epithelial cells. It is important to note that human lung cancer cell lines tested were relatively less sensitive to the inhibitory effect of IL-6 on cell growth as compared with BEAS-2B cells, a noncancrogenic human bronchial epithelial cell. This fact gives some reality to a scenario that relative loss of responsiveness to IL-6, an autocrine growth inhibitor, might be involved in human lung carcinogenesis. The mechanism for this decrease in sensitivity to IL-6 is unknown, but a similar finding has been reported with TGF-Ø. Masui et al. (31) reported that the decreased responsiveness to TGF-Ø found in human lung cancer cell lines was not due to changes in receptor affinity or numbers. In the present report, we studied IL-6 binding to lung cancer cell lines and IL-6R gene expression. All 3 cell lines tested, including A549, VMRC-LCP, and BEAS-2B cells, had specific receptors for IL-6. Northern blot analysis demonstrated a constitutive IL-6R mRNA expression. More interestingly, IL-6 itself up-regulated IL-6R expression, further supporting a hypothesis of the autocrine mechanism of IL-6 action. The affinity and receptor number per cell of each tumor varied, but were not correlated to the sensitivity to IL-6 action. Kawano et al. (10) demonstrated that IL-6R number per cell was not always associated with the sensitivity to IL-6 in human melanoma cells. A novel class of autocrine growth inhibitor for the growth control of bronchial epithelial surfaces. This is the first report proposing a concept that IL-6 may be a novel class of autocrine growth inhibitor for the growth control of non-small cell lung cancer cell lines, and thus may play a role in the carcinogenesis of human lung cancer. Studies with more human lung carcinoma cell lines and their comparison to those with normal airway epithelial cells might be necessary to better clarify its importance. In vivo experiments might also be useful to address this problem.

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INHIBITORY EFFECT OF IL-6 ON LUNG CANCER GROWTH


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Hajime Takizawa, Takayuki Ohtoshi, Ken Ohta, et al.


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