Expression of the Interleukin 6 Receptor and Interleukin 6 in Prostate Carcinoma Cells

Clay B. Siegall, Gisela Schwab, Richard P. Nordan, David J. FitzGerald, and Ira Pastan

ABSTRACT

We have probed for the presence of interleukin 6 (IL6) receptors in prostatic carcinoma cell lines (LNCaP, DU 145, and PC3) by examining their sensitivity to the cytotoxic effects of a chimeric toxin composed of IL6 and Pseudomonas exotoxin (PE). All three cell lines were killed by IL6-PE664Glu, a version of IL6-PE in which the binding domain of native PE has been mutated to debilitate PE binding to its own receptor. This cytotoxic activity confirmed the presence of IL6 receptors on prostatic carcinoma cells. We have measured the number of IL6 receptors found on these cells and have further determined that they secrete IL6. These data provide evidence that IL6 and its receptor may play an important role in human prostate cancer.

INTRODUCTION

IL6 is a pleiotropic cytokine which has been shown to have many physiological functions. IL6 stimulates activated B-cells to produce immunoglobulins and functions as a hepatocyte-stimulating factor by inducing liver cells to synthesize acute phase proteins (1, 2). IL6 is required for the growth of certain hybridomas or plasmacytomas and can induce neuronal differentiation of PC-12 cells (3-5). IL6 plays a role in hematopoiesis and in T-cell activation (6-8). The IL6-R has been identified on several cancer cell types, most notably in multiple myeloma, and in T-cell activation (6-8). The IL6-R has been identified on both cloned cell lines and fresh isolates from patients (9-11). Other cancer cells which express the IL6-R are histiocytomas, promyelocytic leukemias, astrocytomas, glioblastomas, and hepatomas (10, 12). In the case of multiple myeloma, the existence of an IL6-IL6-R autocrine loop has led to the proposal that this mechanism may be involved in the oncogenesis process (9, 13).

We have described a chimeric toxin, IL6-PE40, which is composed of IL6 fused to a mutant form of PE that is devoid of its native cell recognition domain (amino acids 1-253) (14). This chimeric toxin selectively kills tumor cells displaying large numbers of IL6-R, such as myeloma and hepatoma cell lines (12, 14). IL6-PE40 binds to the IL6 receptor, is internalized into endocytic vesicles, and is translocated into the cytosol where it ADP ribosylates elongation factor 2, thereby arresting protein synthesis and causing cell death. We have recently modified the PE portion of IL6-PE40 to produce reagents which are even more toxic to IL6-R-bearing target cells (15). The most toxic of these chimeric molecules, IL6-PE664Glu, is a mutated form of full-length PE and has been shown to selectively kill certain myeloma and hepatocellular carcinoma cell lines at concentrations as low as 1 ng/ml (11.6 pm). In this study we report that three prostatic carcinoma cell lines express IL6-R, produce IL6, and are sensitive to the cytotoxic effect of a chimeric toxin in which IL6 is linked to Pseudomonas exotoxin.

MATERIALS AND METHODS

Cell Culture. LNCaP, DU 145, and PC3 were purchased from the American Type Culture Collection and cultured in RPMI (5% FCS), minimal essential medium (10% FCS), and Ham's F-12K (7% FCS), respectively. Cells were plated at 1 x 10^5 cells/ml 24 h prior to use. Cytotoxicity Assay. Inhibition of protein synthesis was used to measure the cytotoxic effect of IL6-PE664Glu in LNCaP, DU 145, and PC3 cells. The chimeric toxin was added and the cells were incubated at 37°C for 42 h, followed by a 2-h pulse with [3H]leucine. Incorporation into cellular protein was determined by measuring the trichloroacetic acid-precipitable counts. IL6-PE664Glu (plasmid pCS64G) was expressed in Escherichia coli under control of an inducible T7 late promoter upon addition of 1 mM isopropyl-β-D-thiogalactopyranoside (15). The fusion protein, IL6-PE664Glu, was isolated and purified to near homogeneity from the insoluble fraction (inclusion bodies) of E. coli as described previously (14, 15).

Competition Analysis for the IL6 Receptor. IL6-PE664Glu (100 ng/ml) was added to LNCaP and DU 145 cells (1 x 10^5 cells/ml) with or without 1000 ng rIL6. Cells were incubated for 42 h and protein synthesis was determined as described in Fig. 1. The rIL6 was expressed and purified exactly as described for IL6-PE664Glu except for using the expression plasmid pCS76 (12).

IL6 Receptor-binding Assay. Tumor cells (5 x 10^5 cells/well) were washed three times in binding buffer [25 mM 2-(bis(2-hydroxyethyl)amino]ethanesulfonic acid, pH 6.8-1 ng/ml bovine serum albumin-Dulbecco's modified Eagle's medium and preincubated in binding buffer for 15 min at 37°C. The cells were assayed directly in 24-well culture plates in a volume of 0.2 ml. IL6, purchased from R and D Systems, was iodinated as previously described (12) to a specific activity of 1.5 x 10^4 cpm/mmol. Various amounts of [125I]-IL6 were added to cells with or without 2 μg of unlabeled IL6. Cells were incubated at 0°C for 150 min and washed three times with binding buffer. Cells were lysed and the bound [125I]-IL6 was measured.

IL6 Secretion Analysis. In 96-well plates, serial dilutions of the supernatants harvested from confluent cell cultures were added to IL6-dependent B9 cells (2000/well). After culture for 85 h at 37°C the wells were washed and pulsed with 0.5 μCi/well [3H]hymidine for 4 h, harvested over glass fiber filters, and counted. The amount of IL6 in HGF units is defined as the reciprocal of the dilution required to give 50% of maximum [3H]hymidine incorporation. One HGF unit is approximately 1 pg of native IL6. The specificity of this assay was demonstrated by the ability of a neutralizing monoclonal anti-IL6 antibody (CLB.IL6/8) to completely block the activity present in the cell supernatants (16).

RESULTS

Sensitivity of Prostatic Carcinoma Cell Lines to IL6-PE664Glu.

To determine whether the three prostatic carcinoma cell lines, (LNCaP, DU 145, and PC3) were sensitive to IL6-PE664Glu, we measured the rate of protein synthesis in these cells after treatment with the chimeric toxin. The cell line LNCaP was most sensitive to IL6-PE664Glu, with an ID50 of 1.8 ng/ml in a 44-h assay (Fig. 1). The cell lines DU 145 and PC3 had ID50 values of 25 and 1400 ng/ml, respectively. The cytotoxic effect...
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Fig. 1. Cytotoxicity of prostatic carcinoma cells treated with various amounts of IL6-Pseudomonas toxin. The toxicity of IL6-PE66\textsuperscript{4Glu} was determined by measuring the level of protein synthesis in treated versus nontreated cells. □, LNCaP; O, DU 145; Δ, PC3; numbers in parentheses, ID\textsubscript{50} values.

Fig. 2. Competition of the cytotoxic effect of IL6-Pseudomonas toxin with rIL6. Cells were incubated in the presence or absence of rIL6 (1000 ng/ml) and IL6-PE66\textsuperscript{4Glu} (100 ng/ml).

IL6 Binding to Prostatic Carcinoma Cells. The demonstration that the killing of prostatic carcinoma cells by IL6-PE66\textsuperscript{4Glu} is specifically blocked by excess IL6 indicates that these cells possess IL6 receptors. To determine the number of IL6 receptors expressed, we performed IL6 receptor-binding assays using \textsuperscript{125}I-IL6 (12). The binding data shows a single type of binding site (LNCaP: 1300 IL6-R/cell, \(K_d = 1 \times 10^{-9}\) M; DU 145: 2550 IL6-R/cell, \(K_d = 5 \times 10^{-9}\) M; PC3: 400 IL6-R/cell, \(K_d = 5 \times 10^{-9}\) M) (Fig. 3; data not shown).

It is interesting to note that the cell line LNCaP, which is most sensitive to IL6-PE66\textsuperscript{4Glu}, has about 50% fewer receptors than DU 145 (1300 versus 2550, respectively). However, the receptors found on LNCaP have a 5-fold higher affinity for IL6 than the receptors found on DU 145. The IL6 receptor is composed of more than one subunit and the difference in binding may reflect different forms of the IL6 receptor in different cell lines (17).

Endogenous IL6 Secretion. To determine whether IL6 was produced by these prostatic cell lines, we analyzed cell culture supernatants for IL6 using the sensitive B9/HGF biological assay (18). This assay is based on the IL6-dependent proliferation of the murine B-cell hybridoma, B9. The three prostatic carcinoma cell lines all produced IL6 with levels ranging from 50 to 240 units/ml (Table 1). To prove that the stimulation of the B9 cells was due to IL6, a monoclonal antibody to IL6, CLB/IL6.8, was added to the supernatants and found to block completely the mitogenic response.

DISCUSSION

In this study, we have demonstrated that the prostatic carcinoma cell lines LNCaP, DU 145, and PC3 are sensitive to the...
cytotoxic action of a chimeric toxin composed of IL6 and Pseudomonas toxin (Fig. 1). The specificity of the chimeric toxin for these cells was shown by competition using rIL6 (Fig. 2). We further examined these cells by measuring the number of IL6 receptors which they express (Fig. 3; data not shown). Additionally, we have identified IL6 in the cell supernatants, indicating that these cells secrete IL6 (Table 1).

The finding that prostatic carcinomas express IL6 receptors and secrete IL6 raises the possibility that IL6 has a role in the growth of these tumors. Myeloma cells have been shown to be stimulated by IL6 in an autocrine fashion (9, 13); it is possible that prostatic carcinoma cells may behave in a similar way. The addition of rIL6 to the cells cultured for 48 h in media supplemented with 0.5% FCS increased DNA synthesis but not near to the levels seen when cultured in medium containing normal amounts of fetal calf serum (data not shown). This suggests that IL6 is only one of the factors that contributes to the growth of prostatic carcinomas.

Prostatic carcinoma characteristically metastasizes to lymph nodes, bone, and liver (19). All of these tissues are known to be sites where IL6 is present. In the bone, it has been shown that monocytes secrete significant amounts of IL6 (20). This source of IL6 is thought to stimulate the growth of malignant plasma cells in multiple myeloma and on the basis of our data may also have a role in prostatic carcinomas. The infiltration of prostatic adenocarcinoma cells into the liver may also be due to local levels of IL6.

Prostatic carcinoma is a common cause of death in adult men (19). Although there are many modes of treatment for metastatic disease, including prostatectomy, irradiation, hormone therapy, and chemotherapy, no single therapy is more than marginally effective in delaying tumor growth. If the IL6 receptor is important for the growth of prostatic carcinoma cells, it may be possible to use the receptor to target drugs or toxins to these carcinomas with elevated numbers of receptors. This could be accomplished by attaching a toxin to IL6, as has been done here, or to use antibodies directed at the IL6 receptor which could deliver various cytotoxic agents.

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REFERENCES


Table 1

<table>
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<th>Experiment</th>
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<td>Cell line</td>
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<tr>
<td>PC3</td>
<td>160</td>
</tr>
<tr>
<td>LNCaP</td>
<td>160</td>
</tr>
<tr>
<td>DU 145</td>
<td>80</td>
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* HGF units detected in the supernatants of three prostate cancer cell lines.
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