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Plasma Inter-α-Trypsin Inhibitor-Related Urinary Glycoprotein EDC1 Inhibits the Growth of a Burkitt's Lymphoma Cell Line

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A homogeneous preparation of a urinary glycoprotein has been isolated from urine of patients with malignant melanoma and advanced adenocarcinomas of colon and lung. This molecule, Mr 30 kDa, is homologous to EDC1, a proteinase inhibitor antigenically related to plasma inter-α-trypsin inhibitor (IATI) originally isolated from the urine of a leukemic patient, E.D. The newly isolated EDC1 inhibits cellular proliferation of a Burkitt's lymphoma cell line, Raji, growing in serum-free medium supplemented with insulin, transferrin, selenium, and linoleic acid. This concentration-dependent inhibitory effect was monitored in terms of change in cell number and ^{3}H-thymidine incorporation. The growth of cells treated with ~3.3 pmol EDC1/ml was 50% that of the control group by both assays. EDC1 was not cytotoxic to the cells because the EDC1-treated cells excluded trypan blue and resumed normal growth after removal of EDC1. In addition, EDC1 treatment of Raji cells prelabeled with ^{3}H-labeled DNA did not release more radioactivity into the conditioned medium than the untreated labeled cells. EDC1 did not affect the growth of Hs2B2, a B-lymphoblast cell line, and Hs294T, a human malignant melanoma cell line. Equimolar and larger quantities of other proteinase inhibitors with inhibitory profiles similar to that of EDC1 (α-1 proteinase inhibitor, soybean trypsin inhibitor, lima bean trypsin inhibitor, and turkey ovomucoid) did not affect the growth of Raji cells. Raji cells have an absolute requirement of transferrin as a nutrient and require insulin to modulate the expression of transferrin receptors. The cells also synthesize interleukin-1 as an autocrine growth stimulator. EDC1 did not form a detectable complex with transferrin, insulin, or any autocrine factor synthesized by the cells.

Key words: IATI, Raji cell line, proteinase inhibitor

The mechanisms of action of various growth factors on their target cells have been extensively investigated in terms of their cell membrane receptors and the cascade of intracellular second messengers generated by this interaction [1–4]. In recent years, a number of growth inhibitors have also been identified, and their role in controlling cellular growth has been demonstrated, although the mechanism of their action is not

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fully understood [5–10]. Of interest in this regard is transforming growth factor-β (TGF-β), which functions as a growth stimulator for certain types of cells and as a growth inhibitor for others [10,11].

We previously reported that EDC1, a glycoprotein of Mr 30 kDa originally isolated by us from the urine of a leukemic patient, E.D. [12], inhibited the proliferation of lectin-stimulated peripheral blood lymphocytes [13]. EDC1 is a proteinase inhibitor that is antigenically related to plasma inter-α-trypsin inhibitor (IATI), Mr 180 kDa [14,15]. Immunoreactive urinary EDC1 was subsequently detected in urine of patients with different types of advanced cancer and certain infections and inflammatory diseases and was tested as an immunodiagnostic tumor marker for a variety of malignancies [16–19]. In 1986, McKeehan et al. [20] reported that an EDC1-like molecule isolated from conditioned medium of a hepatoma cell line stimulated the growth of endothelial cells. In this paper, we report that EDC1, isolated in a homogeneous form from urine of patients with malignant melanoma and adenocarcinomas of colon and lung, inhibits the growth of a Burkitt's lymphoma cell line, Raji, in a concentration-dependent, noncytotoxic manner.

MATERIALS AND METHODS

Materials

All chemicals, enzymes, and reagents were of highest purity available and were obtained from Sigma Chemicals Co. (St. Louis, MO). Fetal bovine serum and RPMI 1640 were from GIBCO (Grand Island, NY). The medium was supplemented with gentamycin 50 mg/liter, amphotericin B 2.5 mg/liter, and sodium bicarbonate 2 g/liter. The supplement ITS Premix Plus, containing insulin, transferrin, selenium, and linoleic acid with bovine serum albumin, was from Collaborative Research (Waltham, MA). ^{3}H-labeled thymidine was from Amersham (Arlington Heights, IL). Antiserum to EDC1 was available in our laboratory [12], and antiserum to human IATI was from Behring Biochemicals (LaJolla, CA). A kit for detection of immunoreactive TGF-β was obtained from Research & Diagnostic Systems, Inc. (Minneapolis, MN). Mycoplasma-free Raji cells and other lymphoblast cell lines were initially obtained from Dr. David Gordon, Department of Medicine, Emory University; later Raji cells were also purchased from ATCC, (Bethesda, MD). The B-lymphoblast nature of the cells was confirmed by examination of Wright's-stained cytospin preparation of these cells. It revealed classic L3 morphology according to French-American-British group criteria [21]. Immunohistochemical analyses revealed that the cells are leu 14-positive and leu 4-negative using commercially available kits (Becton Dickinson, Mountain View, CA). Hs294T human malignant melanoma cells were originally from ATCC and were maintained in serum-free defined medium.

Methods

Isolation of EDC1. The procedure for isolation of a homogeneous preparation of EDC1 and its characterization has been described elsewhere [22]. Briefly, the procedure involves the following: 1) batchwise treatment of urine from an individual patient with advanced malignant melanoma or with advanced adenocarcinomas of colon or lung with a cation exchange resin to adsorb urinary EDC1; 2) treatment of the resin with a buffer pH 3.1 to elute EDC1; 3) gel filtration chromatography on an AcA 44 column; 4) anion exchange chromatography on Mono Q column on FPLC; 5) reverse-

phase C 18 chromatography on HPLC. The purified molecule was tested for homogeneity by various chromatographic and electrophoretic procedures as well as by N-terminal amino acid sequencing on a gas-phase microsequencer according to the procedure of Hunkapiller et al. [23].

Cell growth assays. For long-term culture (>5 weeks), the lymphocyte cell lines Raji and Hs2B2 were grown in RPMI 1640 supplemented with gentamycin, fungizone, and 5% fetal calf serum. For studies lasting <2 weeks, cells were grown in serum-free RPMI 1640 supplemented with insulin 6.25 μg/ml, transferrin 6.25 μg/ml, selenium (as sodium selenite) 6.25 ng/ml, linoleic acid 5.35 μg/ml, and bovine serum albumin 1.25 mg/ml (ITS Plus Premix). Hs294T human malignant melanoma cells were maintained in serum-free Ham's F-10 medium supplemented with gentamycin 50 mg/liter and fungizone 2.5 mg/liter.

Cell number assay. For Raji cells, the lowest cell density capable of growing under serum free conditions was 2×10^5 cells plated in 2 ml RPMI 1640 with ITS Plus in 35 mm^2 Corning tissue culture dishes. The cells were incubated at 37°C in a humid 95% air/5% CO_2 atmosphere. Doubling time was every 48–60 hr. Varying concentrations of EDC1 in 100 μl supplemented medium were added daily for 7 days, eight dishes per concentration. Control cells received 100 μl of supplemented medium alone. The cells also received 1 ml of fresh medium on day 4. On day 7, the cells were counted on a hemocytometer. Since EDC1 did not affect the growth of Hs2B2 cells under these conditions, subsequent studies were conducted only on Raji cells. Other antiproteinases—α-1-proteinase inhibitor, soybean trypsin inhibitor, lima bean trypsin inhibitor, and turkey ovomucoid—were also tested for their effect on the growth of Raji cells at equimolar and higher doses (Table I). Hs294T human malignant melanoma cells were also tested for their response to EDC1 in a similar cell number assay except that these cells were plated (5×10^4 cells) in 2 ml Ham's F-10 plus 10% fetal calf serum and changed to serum free F-10 the next day after attachment.

TABLE I. Effect of Addition of Various Proteinase Inhibitors on the Growth of Raji Cells*

Proteinase inhibitor (Concentration μg/ml medium)	Increase in cell number after 7 days ($\times 10^3$)
Control cells	950.9 ± 126.7 (n = 8)
Soybean trypsin inhibitor	
100	1,168.3 ± 71.0 (n = 4)
10	1,057.3 ± 175.0 (n = 4)
1	1,146.8 ± 121.0 (n = 4)
Lima bean trypsin inhibitor	
100	984.1 ± 81.4 (n = 4)
10	914.4 ± 95.4 (n = 4)
1	984.1 ± 86.6 (n = 4)
Turkey ovomucoid	
100	996.6 ± 76.2 (n = 4)
10	975.1 ± 59.9 (n = 4)
α-1-Proteinase inhibitor	
20	829.2 ± 97.0 (n = 3)
16	901.1 ± 79.6 (n = 3)
6	891.6 ± 88.0 (n = 3)
0.5	804.2 ± 30.3 (n = 3)

*No significant decline in cell growth ($P > 0.05$) was observed between the untreated control cells and the cells treated with different proteinase inhibitors.

^{3}H-Thymidine incorporation assay. The assay was modified from our previous procedure [13] and others [22]; 1×10^5 Raji cells/0.2 ml defined medium were cultured in a 96-well Costar plate for 2 days in the absence and presence of varying concentrations of EDC1 added daily in 20 μl culture medium. A control group of cells received only additional 20 μl of culture medium. On day 3, the cells received 1.2 μCi of ^{3}H-thymidine in 50 μl medium along with another aliquot of EDC1. The cells were harvested after 6 hr and washed with 10% trichloroacetic acid and water. Filter discs containing tritiated material were counted in a beta counter.

Other analyses

Viability of EDC1-treated cells. Viability of these cells was tested as described below. First, cells incubated with the highest tested concentration of EDC1 for 8 days were examined for their ability to exclude trypan blue. Second, cells exposed to EDC1 (1.0 μg/ml/day) for 7 days were washed and then replated in EDC1-free culture medium for another 6 days (n = 4). Two control groups—one with and the other without EDC1—were allowed to grow for 13 days. Cells were counted on days 1, 7, 11, and 13. Third, in an experiment based on the procedure of Dell'Aquila and Gaffney [24], Raji cells were incubated with ^{3}H-thymidine in the defined medium for 2 days to label the DNA. The cells were washed repeatedly to remove any extracellular ^{3}H-labeled thymidine. They then received daily additions of EDC1 (1.0 μg/ml) for 3 days. On day 6, the cells were centrifuged and aliquots of the conditioned medium plus the wash from both treated and untreated cells were analyzed for radioactivity [24]. The cell pellets were lysed with 10% trichloroacetic acid solution, and aliquots were analyzed for radioactivity. The control group consisted of cells labeled with ^{3}H-thymidine that were not treated with EDC1.

Analysis of complexed EDC1 in conditioned medium from Raji cells and in fresh medium incubated with EDC1. To determine whether EDC1 formed a complex with a component of the culture medium or with a possible autocrine factor released by Raji cells into conditioned medium, the conditioned medium from cells exposed to 3 μg/ml EDC1 was chromatographed on a gel filtration column on HPLC [19], and the fractions were analyzed for immunoreactive EDC1. In another experiment, 2 ml of fresh culture medium, RPMI 1640 with ITS Plus, was incubated overnight with ^{125}I-labeled EDC1 and then analyzed by gel filtration as above.

Statistical analysis of data. Comparison of increase in cell number and thymidine incorporation in cells treated with various concentrations of EDC1 and the control group was done using single-factor analysis of variance and Student's t test [25].

RESULTS

Characterization of EDC1

The urinary glycoprotein was characterized in terms of its Mr (30 kDa), its inhibitory characteristics, its homogeneity, and its partial N-terminal amino acid sequence [22]. These data were indistinguishable from EDC1 isolated from leukemic urine and HI30, an IATI related proteinase inhibitor isolated from pooled normal urine (Table II). The final homogeneous preparation of EDC1 (5 μg sample) did not have any detectable immunoreactive TGF-β when tested according to the double-antibody immunochemical procedure suggested by the manufacturers of the kit; the lower limit of detection of TGF-β in this method was 3 ng. Figure 1 shows the single band obtained after sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis of the purified homogeneous EDC1.

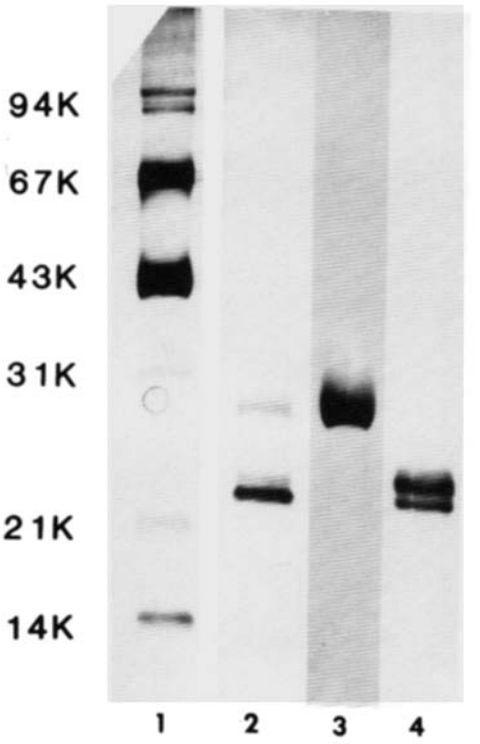

Fig. 1. SDS-PAGE analysis of EDC1 at various stages of purification. A 16 cm × 15 cm × 0.75 mm gel (3% stacking and 9% resolving) was used under reducing conditions. Analyses of samples under nonreducing conditions showed identical patterns. Samples were electrophoresed at 11 mA constant current in stacking gel and at 22 mA in the resolving gel at 15°C until the tracer dye was within 1 cm of the lower edge. The gel was treated with methanol:acetic acid:water mixture and then silver stained according to the manufacturer's instructions. Lane 1 is the standard mixture of proteins, and lane 2 is the fraction obtained after gel filtration. This shows three bands: 30 kDa EDC1 and a 22 kDa/20 kDa doublet. Lane 3 is the 30 kDa EDC1 obtained after anion exchange and reverse-phase chromatographic analyses. The single band reflects ~10 μg of the glycoprotein loaded onto the gel. The doublet separated from EDC1 after anion exchange and reverse-phase steps is shown in lane 4.

TABLE II. Comparison of the Partial Covalent Structure (31 N-Terminal Amino Acids) of Cancer Patient Urine-Derived Proteinase Inhibitor EDC1 With- the Corresponding Region of the Normal Urine-Derived HI30*

EDC1	
	A.V.X.P.Q.E.E.E.G.G.G.G.G.Q.L.V.X.E.V.X.K.K.E.D.S.X.Q.L.G.Y.S.
HI30	
	A.V.L.P.Q.E.E.E.G.S.G.G.G.Q.L.V.T.E.V.T.K.K.E.D.S.C.Q.L.G.Y.S...

*The complete covalent structure of HI30 (total residues 143) has been reported [Gebhard W, Hochstrasser K: In: 'Proteinase Inhibitors. New York: Elsevier, 1986, pp 389–410].

Cell growth assays

Raji cells were capable of short-term growth in the ITS Plus-supplemented medium as well as in medium supplemented with transferrin alone. However, the growth rate in transferrin-supplemented medium was approximately 50% that in medium

supplemented with ITS Plus. No other single component of the ITS plus could either support the growth of Raji cells by itself or add to the growth rate seen with transferrin alone. Apotransferrin could not support the growth of these cells.

Cell number assay. Figure 2 summarizes the data regarding change in cell number in EDC1-treated cells compared with controls. The change in cell number for both treated and control groups was calculated as the number of cells on day 7 minus the number of cells on day 1. The percent change in treated groups was calculated as shown in the legend to Figure 2 and is plotted against the concentration of EDC1. Significantly lower growth rate in cell number was observed in cells treated with >0.03 μg EDC1/ml medium ($P < 0.01$). A 50% decline in cell growth was observed in cells treated with ~0.13 μg/ml or 3.3 nM EDC1. In the cell number assay, EDC1 did not significantly affect ($P > 0.05$) the growth of the B-lymphocyte cell line Hs2B2 or the human malignant melanoma cell line Hs294T at a concentration of 1.0 μg EDC1/ml/day. The change in cell number ($\times 10^3$) for EDC1-treated Hs294T cells (n = 5; average ± SD) was 10.5 ± 2.9 vs 10.8 ± 1.5 for untreated cells. α-1-Proteinase inhibitor, turkey ovomucoid, lima bean trypsin inhibitor, and soybean trypsin inhibitor also did not affect the growth of Raji cells (Table I).

^{3}H-thymidine incorporation assay. Figure 3 summarizes the data regarding ^{3}H-thymidine incorporation; percent of labeled thymidine incorporation by treated cells is plotted against the concentration of EDC1. Significantly less ($P < 0.01$) incorporation of ^{3}H-thymidine, compared to the control cells, was noted in cells treated with ≥0.03 μg EDC1/ml medium. A 50% decline in incorporation of ^{3}H-thymidine was observed in cells treated with ~0.14 μg EDC1/ml medium or ~3.3 nM. These values are identical to those observed in the cell number experiments.

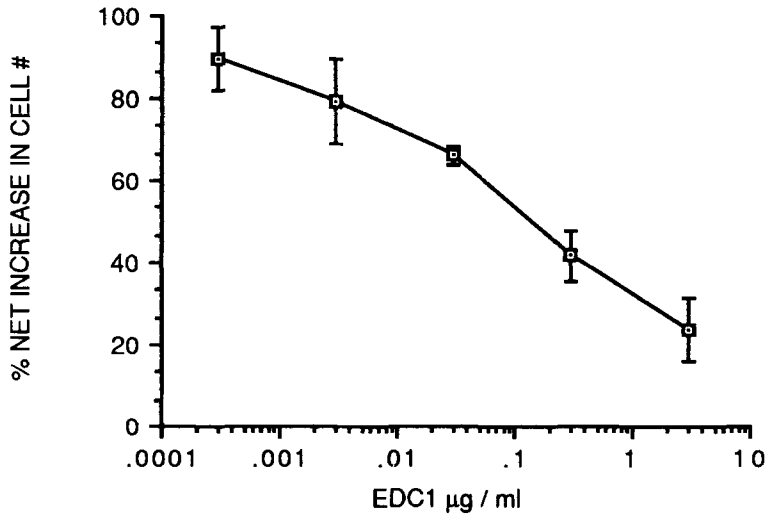

Fig. 2. Change in cell number of Raji cells treated with varying amounts of EDC1 for 7 days in serum-free defined medium. Change in cell number over 7 days is expressed (Y axis) as a percent of the change of the control group. Each point is an average ± SD of eight observations. A significant inhibition ($P < 0.01$) of growth was observed at concentrations of 0.03, 0.3, and 3.0 μg EDC1/ml. A 50% decrease in cell growth was observed at ~0.13 μg or 3.3 pmol/ml. The percent change in cell number was determined as follows: Y = 100 × (change in cell number for EDC1 treated group/change cell number of control group).

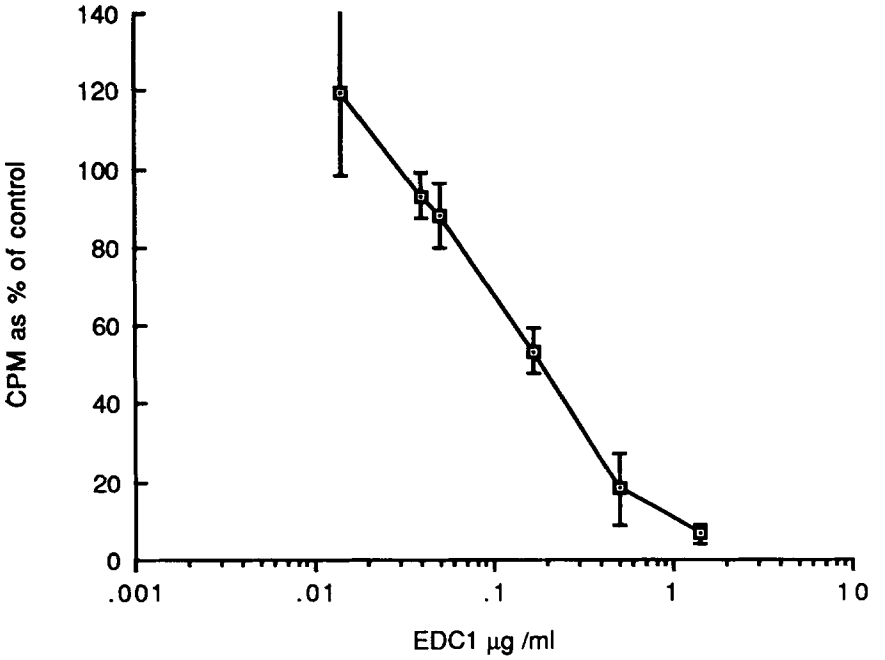

Fig. 3. ^{3}H-thymidine incorporation by Raji cells incubated with varying concentrations of EDC1. ^{3}H-thymidine incorporation is expressed on Y axis as percent of the DPM of the control groups. Significantly less incorporation ($P < 0.01$) of ^{3}H-thymidine was noted in cells treated with 0.03, 0.05, 0.12, 0.15, and 0.4 µg EDC1/ml medium. The concentration of EDC1 required for a 50% decrease in incorporation of labeled thymidine was ~0.13 µg/ml or 3.3 pmol/ml. Nonspecific incorporation of labeled thymidine was 5–12% of the total and was subtracted from the total to obtain the specifically incorporated ^{3}H-thymidine. Nonspecific binding of ^{3}H-thymidine to the filter disc was also computed and subtracted from the total counts to obtain radioactivity specifically incorporated by the cells. The percent of change in incorporation of ^{3}H-thymidine in cells grown in the presence of EDC1 was calculated as above: = 100 × (DPM incorporated in EDC1 treated group/DPM incorporated in the control group)

Other Analyses

Viability of EDC1-treated cells

Dye exclusion. Nearly 90% of the control cells and ~88% of the EDC1-treated cells at the three highest concentrations of EDC1 excluded the dye.

Reversibility. The effect of EDC1 on Raji cells could be reversed by washing them and plating them in EDC1-free medium. The data are summarized in Figure 4. During days 1–7, the increase in cell number in the control group and for the cells grown in the presence of EDC1 was 1,844 ± 86.1 $\times 10^3$ vs. 1,330 ± 107.1 $\times 10^3$; $P < 0.01$ (average ± SD). During days 7–13, the increase in cell number for the control group and of the group previously treated with EDC1 but now on the regular defined medium was 2,011 ± 165.9 $\times 10^3$ vs. 2,124 ± 161.7 $\times 10^3$; $P > 0.05$.

The cells that continued to receive EDC1 from day 7 to 13 increased by 751.8 ± 104.3 $\times 10^3$. This demonstrates that the growth inhibitory effect of EDC1 was reversible.

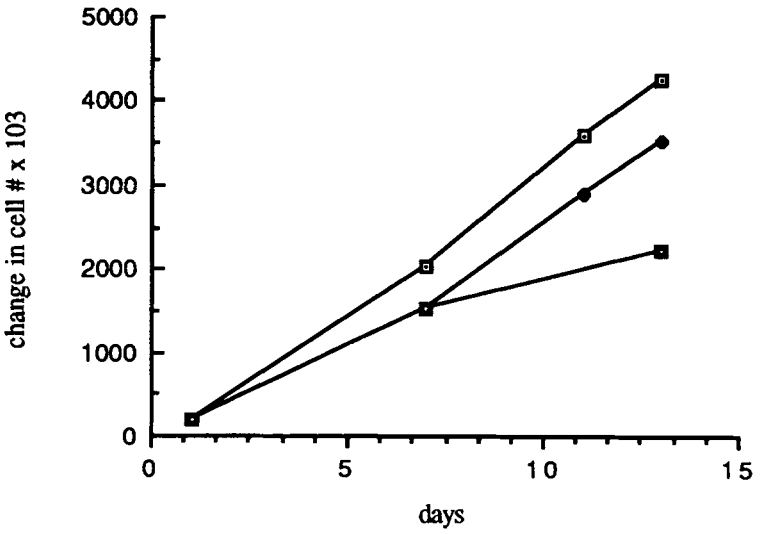

Fig. 4. Reversibility of the effects of EDC1 on Raji cells. Cells were cultured with or without EDC1 for 7 days. Thereafter, cells were centrifuged, washed, and allowed to grow in EDC1-free medium for another 6 days (total 13 days). Two control groups of cells were grown in the presence and absence of EDC1 for the entire duration of the experiment.

TABLE III. Release of ^{3}H Thymidine (DPM) by Labeled Raji Cells Into Conditioned Medium Upon Treatment With EDC1*

	Treated cells	Control (no EDC1 treatment)
Media	17,854 ± 946	18,690 ± 1,517
Cells	124,519 ± 19,527	127,927 ± 20,805

*All values are average ± SD of six observations. No significant difference ($P > 0.05$) was observed between radioactivity of treated and untreated cells.

Release of labeled DNA from Raji cells upon treatment with EDC1. The amount of radioactivity released by labeled Raji cells upon treatment with EDC1 is shown in Table III. The EDC1-treated group released 12.5% ± 0.67% (average ± SD) of the total radioactivity incorporated by the cells vs. 12.77% ± 1.0% released spontaneously by the control cells. No significant difference ($P > 0.1$) in the amount of labeled DNA released into the conditioned medium was noted between the two groups, suggesting that EDC1 was not cytotoxic to the cells.

Analysis of complexed EDC1 in conditioned media and in fresh media incubated with EDC1. On gel filtration and analysis of fractions, all immunoreactive EDC1 eluted at ~30 kDa. Similar results were obtained by analysis of ^{125}I-EDC1 incubated with the fresh culture medium. These observations indicate the absence of any detectable high-Mr EDC1 complexed with a component of the medium or any factor released by the cells into the conditioned medium.

DISCUSSION

The biologic functions or physiologic target enzymes of the IATI-related antiproteinases are not known. However, the present study indicates that EDC1 may have a role in modulating the growth of B lymphocytes. In this study only cells capable of growing in a serum-free defined medium were tested so that the effect of EDC1 would not be masked by related molecules in the bovine serum. The Kd of ~3.3 nM in both assays is of the same order of magnitude as reported for the growth stimulatory effect of EDC1 on endothelial cells [20] and of thrombin on fibroblasts [26,27]. The possibility that the inhibitory effect was due to any copurifying TGF-β was ruled out because the final homogeneous preparation of EDC1 did not have any detectable TGF-β. In addition, other workers have shown that TGF-β does not affect the proliferation of Raji cells [28]. We examined the following mechanisms to account for the antimitotic effects of EDC1.

First, is the inhibitory effect of EDC1 on Raji cells due to cytotoxicity? This mechanism was ruled out because EDC1-treated cells remained viable as determined by dye exclusion and by their ability to resume normal growth after removal from the EDC1 containing medium. In addition, Raji cells with ^{3}H-labeled DNA incubated in the presence of EDC1 did not release more radioactivity into the medium than did controls. These criteria have been used by others to rule out cytoxicity as a mechanism of peptide growth inhibitors [5,23,29]. Also, EDC1 did not affect the growth of another B-lymphoblast cell line (Hs2B2) and a malignant melanoma cell line (Hs294T). This, coupled with McKeehan's observations that EDC1 stimulated the growth of endothelial cells and had no effect on the growth of human hepatoma cells, smooth muscle cells, and fibroblasts [20], strongly argues against EDC1 being a nonspecific cytotoxic growth inhibitor. Possibly, the growth-regulatory effect of EDC1 is observed only in cells that express a specific receptor for this glycoprotein.

Second, is the antimitotic effect of EDC1 related to its antiproteolytic characteristics? Usually proteinases are believed to stimulate and antiproteinases inhibit growth of a number of cells [30]. Thrombin functions as a classic growth factor for fibroblasts via a receptor-mediated mechanism that involves proteolytic activation of a membrane protein by the receptor-bound thrombin. Antithrombin III and protease nexin counteract the stimulatory effects of thrombin on fibroblasts [26,27,31]. Plasmin and plasminogen activator have a role in tissue remodeling and oncogenesis; several cell lines, e.g., human monocyte U937 and human adenocarcinoma SW 1116, have specific receptors for these enzymes [32,33]. In addition, a T-cell-associated serine proteinase is capable of stimulating the growth of B cells in serum-free medium in the absence of any cytokines or growth factors [34]; aprotinin and phenylmethylsulfonyl fluoride neutralize this activity. EDC1 could exert its growth-inhibitory effect by neutralizing the activity of a cellular proteinase required for the growth of Raji cells. However, other proteinase inhibitors with a similar profile of proteinase inhibition did not mimic the effect of EDC1. This can be explained as follows: 1) The antiproteolytic activity of EDC1 may not be required for its growth regulatory activity. 2) EDC1 may neutralize the activity of a unique cellular proteinase that the other tested inhibitors cannot. 3) EDC1 may first bind with a cell membrane receptor through a binding domain and then utilize its antiproteolytic domain(s) to inhibit the activity of a putative cellular proteinase—surface bound or intracellular—involved in the mitosis of Raji cells. Other proteinase inhibitors may lack the binding domain and may therefore fail to have any effect on cell growth.

Third, does EDC1 form a complex with an essential nutrient (transferrin), a hormone (insulin), or an autocrine growth factor interleukin-1 (IL-1) in the defined medium and thus arrest the growth of Raji cells? Normal resting B lymphocytes require exogenous factors for their activation, proliferation, and differentiation [35]. However, Epstein Barr virus (EBV)-transformed cells, including Raji cells, are free from the constraints of exogenous cytokines and do not require such factors for their growth and proliferation [35–37]. Nevertheless, these cells do have an absolute requirement for transferrin, which is internalized via a specific receptor. Insulin probably modulates the expression of transferrin receptors [38,39]. In addition, EBV-transformed cells, including Raji, synthesize an IL-1-like autostimulating factor for autocrine growth [37,40]. On gel filtration analysis of the conditioned medium from EDC1-treated cells or of fresh medium incubated with EDC1, no high-molecular-weight complex of EDC1 with these factors was detected, and all immunoreactive EDC1 was localized around 30 kDa. In addition, ^{125}I-labeled EDC1 was incubated with the culture medium and then chromatographed on a gel filtration column. All radioactivity eluted in the 30 kDa region. These experiments do not support formation of a complex of EDC1 with a nutrient or an autocrine growth factor as the basis of the growth inhibitory effect of EDC1.

This study, along with the reported growth-stimulatory effects of an EDC1-like molecule on endothelial cells [20], suggests that EDC1 may have a role in controlling cellular growth and that this role may be more significant than its antiproteolytic activity. Further work is underway to establish the mechanism of action of the urinary proteinase inhibitor on EBV-transformed B cells.

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