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Physical and biological characterization of a growth-inhibitory activity purified from the neuroepithelioma cell line A673

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Epithelial- and haematopoietic-cell growth-inhibitory activities have been identified in the conditioned medium of the human peripheral neuroepithelioma cell line A673. An A673-cell-derived growth-inhibitory activity was previously fractionated into two distinct components which inhibited the proliferation of human carcinoma and leukaemia cells in culture. One inhibitory activity was shown to comprise interleukin-1α (IL-1α). Here, we have purified to homogeneity a distinct activity which inhibited the growth of the epithelial cells *in vitro*. Using a combination of protein-sequence analysis and mass spectrometry, we demonstrated that biological activity can be assigned to a dimeric protein with a molecular mass of 25576 ($\pm$4) Da and an N-terminal sequence identical with that of transforming growth factor-β1 (TGF-β1). Further characterization of the growth inhibitor with TGF-β-isoform-specific antibodies showed that $> 90\%$ of the bioactivity consists of TGF-β1 and not TGF-β2 or TGF-β3. Although A673 cells were growth-inhibited by exogenous TGF-β1, we showed that TGF-β1 in A673-cell-conditioned media was present in the latent, biologically inactive, form which did not act as an autocrine growth modulator of A673 cells *in vitro*.

INTRODUCTION

Cell proliferation may be controlled by secreted growth factors which act as either positive or negative regulators of cell growth, either locally or at a distance. The neoplastic transformation of normal cells can be achieved by constitutive activation of signalling pathways which stimulate cellular proliferation (Cantley et al., 1991) or by loss of negative signalling pathways which prevent cell proliferation (Marshall, 1991). Growth-inhibitory factors, which can function as negative regulators of cell growth, were shown to be secreted into the culture media of the peripheral neuroepithelioma cell line A673 (Iwata et al., 1985; Fryling et al., 1985). The A673 cell line was thought to derive from a rhabdomyosarcoma (Giard et al., 1973), but has recently been reclassified as peripheral neuroepithelioma, in part on the basis of the lack of *myoD* expression (Chen et al., 1991). Growth-inhibitory activity from A673-cell-conditioned media was partially fractionated into two distinct components, which were shown to inhibit potently the growth of an adenocarcinoma of the lung and several mammary carcinomas. Initial comparative studies showed that the two growth-inhibitory activities were most likely to be distinct proteins, differing in apparent molecular mass on gel filtration, in retention time on C_{18} reverse-phase chromatography, and in heat-stability and trypsin-sensitivity. One active component was purified to homogeneity (Fryling et al., 1989) and shown to comprise interleukin-1α (IL-1α), a secreted cytokine which regulates the growth of cell linages derived from all three layers, often synergistically with other growth modulators (Schmidt and Tocci, 1990; Dinarello and Wolff, 1993). A second bioactivity peak (termed TIF-1) remained uncharacterized. The physical and biological identification and characterization of growth inhibitors is of potential therapeutic importance to neoplastic disease. Here, we have addressed two questions. First, what is the nature of the growth-inhibitory factor secreted from A673 cells, and second, what role does this factor play in regulating growth of A673 neuroepithelioma cells *in vitro*?

EXPERIMENTAL

Purification of A673-cell-derived epithelial growth-inhibitory activity (TIF-1)

TIF-1 activity was prepared essentially as described by Todaro and co-workers (Iwata et al., 1985). Serum-free conditioned medium was collected from the human neuroepithelioma cell line A673, concentrated and dialysed against 1 M acetic acid, and freeze-dried. Material was resuspended in 1 M acetic acid and chromatographed by Bio-Gel P100 gel filtration. Bioactive fractions were pooled, freeze-dried, dissolved in 1 M acetic acid and chromatographed by reverse-phase h.p.l.c. using an acetonitrile/0.05% trifluoroacetic acid (TFA) gradient elution system (Iwata et al., 1985).

Immunodetection assays

Samples were electrophoresed on a 5–20% polyacrylamide linear gradient gel (Laemmli, 1970). Gels were soaked in transfer buffer containing 10 mM dithiothreitol, and protein was electrophoretically transferred overnight in a cooled transfer apparatus (Towbin et al., 1979). After transfer, the blot was washed in PBS

Abbreviations used: IL-1α, interleukin-1α; TFA, trifluoroacetic acid; TGF-β, transforming growth factor-β; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide; BrdU, bromodeoxyuridine.

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and then in PBS/0.1 % Tween 20 (PBST), incubated in blocking buffer (Tropix Inc., Bedford, MA, U.S.A.) for 1 h at room temperature, washed in PBST and incubated with one of the following antibodies to transforming growth factor-β (TGF-β): 1 μg/ml TGF-β1-specific antibody (ion-exchange-purified chicken polyclonal; R&D Systems Inc., Minneapolis, MN, U.S.A.), TGF-β2-specific antibody (Protein A affinity-purified rabbit polyclonal; R&D Systems Inc.), pan-reactive TGF-β antibody (antigen affinity-purified rabbit polyclonal) or 5 μg/ml TGF-β3-specific antibody (peptide affinity-purified rabbit polyclonal; Oncogene Science Inc., Uniondale, NY, U.S.A.) for 1 h at room temperature. Two washes in PBST were followed by a 30 min incubation with 200 ng/ml alkaline-phosphatase-conjugated goat anti-rabbit antibody (Oncogene Science Inc.) for the TGF-β2, TGF-β3 and pan TGF-β antibodies; 1 μg/ml alkaline-phosphatase-conjugated rabbit anti-chicken antibody (Zymed Labs Inc., S. San Francisco, CA, U.S.A.) was used for the TGF-β1 antibody. Western blots were developed by using the chemiluminescent substrate AMPPD as described by the supplier (Tropix Inc.), sealed in a plastic bag and autoradiographed for 5–60 min.

Bioassay conditions

Growth inhibition of the mink epithelial cell line Mv1-Lu was employed as a standard bioassay which detects TIF-1, but is insensitive to IL-1α. Mv1-Lu cells were grown in Dulbecco's modified Eagle's medium/10 % fetal-bovine serum, trypsin-treated, and seeded in a 96-well plate at a density of 10^3 cells/well. After 4 h, the test material or control factors were added. Cells were incubated for 4 days at 37 °C in air/CO_2 (19:1), before assaying for conversion of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT). For this, 25 μl of MTT solution (2 mg/ml MTT; Sigma) was added and the cells were incubated for 4 h at 37 °C. Medium was aspirated, and ethanol/acetone (1:1, v/v; 100 μl) was added to solubilize the cellular MTT product. Dye incorporation was measured in a Biotech EL320 microplate reader at 540 nm. For the neutralization experiments, bioactive samples were first incubated in media containing 5 μg/ml of the respective neutralizing antibody for 2 h at 37 °C. Bromodeoxyuridine (BrdU) incorporation into actively synthesized DNA was used as measure of cell proliferation. A sensitive 96-well immunoassay (Amersham Corp., Arlington Heights, IL, U.S.A.) was used (5×10^3 cells/well) under conditions specified by the manufacturer. Both the growth-inhibitory assay employing MTT and the DNA synthesis assay utilizing BrdU were shown to reproducibly measure the dose-dependent inhibition of Mv1-Lu cells *in vitro*, for example in response to the addition of exogenous TGF-β1 or TGF-β3.

Elution of bioactivity from SDS/PAGE

Bioactive fractions ($\sim$ 1 μg) were subjected to non-reducing SDS/PAGE (5–20 % gradient gel), stained with Coomassie Blue, destained and washed extensively with deionized water. The sample lane was cut into 3 mm slices, incubated in 100 μl of 50 % acetonitrile/10 mM HCl and ground with a Teflon pestle. Gel fractions were shaken overnight at 4 °C, centrifuged (5 min, 13000 *g*), and the supernatant was analysed for bioactivity in the Mv1-Lu cell-growth inhibition assay with an MTT endpoint.

N-terminal sequence, amino acid and mass determinations

The bioactive fractions purified by sequential reverse-phase chromatography were further fractionated by SDS/PAGE, transferred to a polyvinylidene difluoride (PVDF) membrane (Matsudaira, 1987), revealed on the PVDF blot by Coomassie Blue staining (one-tenth normal strength), destained, washed thoroughly and excised. Amino acid analysis was performed without reductive alkylation with 4-vinylpyridine as described by Kligman and Marshak (1985), and N-terminal sequencing was performed by Edman degradation using an Applied Biosystems 475A gas-phase sequencer with on-line PTH amino acid phenylthiohydantoin separation, both according to the manufacturer's specifications.

The molecular mass of the purified bioactive fraction (TIF-1) was measured with matrix-assisted laser desorption mass spectrometry (Hillenkamp et al., 1991) using a time-of-flight mass spectrometer constructed at the Rockefeller University (Beavis and Chait, 1989, 1990). Bioactive h.p.l.c. fractions were pooled and applied to a Mono-S column (Pharmacia) in 50 mM Mes (pH 6.1)/100 mM NaCl, eluted with 50 mM Hepes, pH 7.5, with a linear 0–1 M NaCl gradient and subsequently applied to TGF-β1 antibody coupled to Protein G–Sepharose Fast Flow (Pharmacia), washed with 20 mM ammonium acetate, pH 6, and eluted with 0.1 % TFA. A 1 μl portion of 7 μM purified protein in 0.1 % TFA/acetonitrile (2:1, v/v) was mixed with 4 μl of 3,5-dimethoxy-4-hydroxycinnamic acid [sinapinic acid, 5 g/l in 0.1 % TFA/acetonitrile (2:1, v/v)], and 1 μl of this solution was placed on the mass-spectrometer probe tip and dried with a stream of room-temperature air. The sample on the probe tip was irradiated with 10 ns duration pulses of light (wavelength 355 nm) from a Nd(YAG) laser. The ions created were accelerated in an electrostatic field, and their time-of-flight was measured with a LeCroy 8828D transient digitizer. To improve the statistics, 200 individual spectra were added together. The time-of-flight spectrum was converted into a mass spectrum by using horse myoglobin as an internal calibrant. For purpose of comparison, recombinant TGF-β1 was analysed by mass spectrometry. In this case, the sample was prepared by mixing 1 μl of 8 μM recombinant TGF-β1 with 5 μl of sinapinic acid and applying 1 μl of the solution to the mass-spectrometer probe tip.

RESULTS

Purification and physical characterization of A673-derived epithelial growth-inhibitory activity

A673-conditioned medium was concentrated, dialysed and chromatographed by sequential Bio-Gel P-100 chromatography and reverse-phase h.p.l.c. in accordance with Iwata et al. (1985). Bioactive material eluted at $\sim$ 31 % acetonitrile was isolated and characterized. No additional active fractions were detected. Active protein (1 μg) was further fractionated by SDS/PAGE, and gel slices were eluted with acidified acetonitrile and assayed for bioactivity. Bioactivity was resident only in a silver-stained band of approximate molecular mass 26 kDa (results not shown).

Bioactive fractions from reverse-phase h.p.l.c. were pooled,

TIF-1	A	L	D	T	N	Y	C	F	S	S	T	E	K	N	C	C	V	R	Q					
TGF-β1	A	L	D	T	N	Y	C	F	S	S	T	E	K	N	C	C	V	R	Q					
TGF-β2	A	L	D	a	a	Y	C	r	f	n	v	q	d	N	C	C	l	R	p					
TGF-β3	A	L	D	T	N	Y	C	F	r	n	l	E	e	N	C	C	V	R	p					

Figure 1 N-terminal sequence comparison of A673-derived growth-inhibitory factor (TIF-1) with TGF-β1, TGF-β2 and TGF-β3

Lower-case letters indicate amino acid substitutions.

Table 1 Amino acid composition of the A673-derived growth inhibitor

	Composition (residues/molecule)	
Amino acid	TIF-1	TGF-β1
D	11.6	10
E	9.8	9
S	8.9	8
G	7.2	5
H	3.2	2
R	5.2	5
T	7.1	7
A	5.7	5
P	7.8	9
Y	8.0	8
V	7.2	8
M	1.2	2
C	5.4	9
I	1.9	2
L	11.0	12
F	2.1	3
K	5.1	4

subjected to SDS/PAGE, transferred to a PVDF membrane, and the N-terminal sequence was determined and the amino acid composition measured. In the absence of reductive alkylation, the repetitive yield comparing Asn-2 and Asn-14 was 91%, with an absolute yield of 64.2% (33 pmol applied, 21 pmol recovered). Upon reduction and alkylation with 4-vinylpyridine, the repetitive yield comparing Leu-2 with Leu-20 was 94%, with an absolute yield of 20% (63 pmol applied, 13 pmol recovered). The first 19 residues were identical with the N-terminal sequence of TGF-β1, but distinct from that of TGF-β2 and TGF-β3 (Figure 1). Amino acid composition data showed similarity to the predicted composition of TGF-β1 (Table 1), for the cysteine composition, which would be expected in the absence of reductive alkylation.

The molecular mass of the active component was determined by mass spectrometry. The matrix-assisted laser desorption mass spectrum is shown in Figure 2(a). The peaks correspond to singly, doubly, triply and quadruply protonated protein species. The measured molecular mass was 25597 ± 22 Da (average of the values obtained from the peaks corresponding to the singly and doubly protonated molecule), which is in agreement (within the experimental error of the determination) with the measured and calculated molecular masses of TGF-β1, respectively 25576 ± 4 Da and 25572 Da. The correspondence between the molecular masses of the bioactive component and TGF-β1 is in accordance with the hypothesis that the two species are identical. In comparison, the predicted molecular masses of TGF-β2 and TGF-β3 are 25422 and 25428 respectively. SDS/PAGE of the protein fraction used in the mass-spectroscopy determination is shown in Figure 2(b).

Detection of TGF-β1 immunoreactivity in bioactive peak fractions

Bioactive material obtained by reverse-phase h.p.l.c. was further fractionated by SDS/PAGE, followed by immunodetection on Western blots developed with TGF-β1-, TGF-β2- or TGF-β3-specific antibodies (Figure 3a). Activity was resident in the fraction recognized by anti-TGF-β1 antibody and was not recognized by anti-TGF-β2 or anti-TGF-β3 antibodies. By comparison with a standardized preparation of TGF-β1 in the

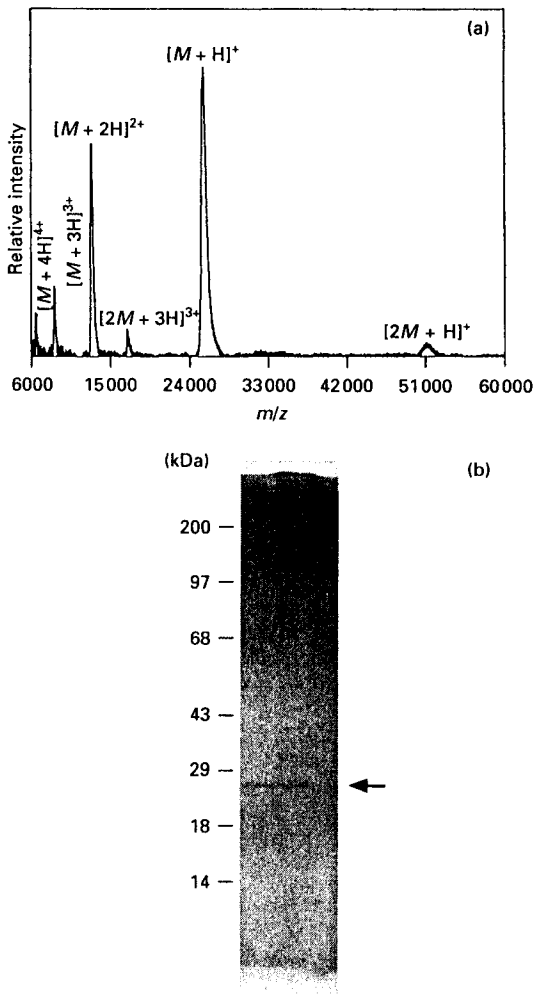

Figure 2 (a) Molecular-mass determination of A673-derived growth-inhibitory factor by matrix-assisted laser desorption mass spectrometry; (b) SDS/PAGE of the protein fraction used for mass-spectrometry determination

In (**a**), $[M+nH]^{n+}$ designates the intact molecule with n protons attached to it. The peak labelled $[2M+H]^+$ corresponds to the singly protonated dimer. In (**b**), the kDa values refer to molecular-mass standards.

Mv1-Lu cell bioassay, it was estimated that A673-conditioned media contained $\sim 6 (\pm 1)$ ng/ml of TGF-β-like activity.

Electrophoretic mobility was examined under reducing or non-reducing conditions (Figure 3b). The electrophoresed proteins were transferred to nitrocellulose and probed with pan-reactive TGF-β antibody. As would be expected for a member of the TGF-β family, the bioactive component appeared to be a disulphide-linked dimer, with an apparent molecular mass of 26 kDa shown by non-reducing SDS/PAGE and 13 kDa by reducing SDS/PAGE. The bioactive h.p.l.c. fraction (lane 4) and TGF-β1 (lane 6) were shown to migrate identically under reducing conditions. In contrast, TGF-β3 (lane 5) migrated with slightly faster mobility.

Bioactivity is selectively neutralized by TGF-β-specific antibody

A673-conditioned medium was preincubated with antibodies which selectively neutralize TGF-β1, TGF-β2 or TGF-β3 before

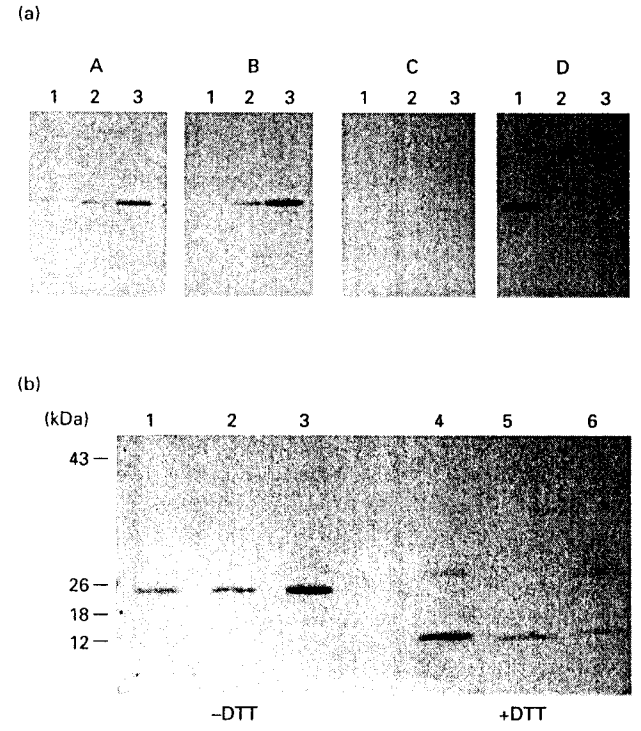

Figure 3 (a) Recognition of A673-derived growth-inhibitory factor by TGF-β1-specific polyclonal antibody; (b) electrophoretic mobility under reducing and non-reducing conditions

In (**a**), TGF-β3 (lane 1), TGF-β1 (lane 2) and TIF-1 (lane 3) (~ 26 kDa) were electrophoresed by SDS/PAGE, transferred to nitrocellulose and incubated with pan TGF-β, TGF-β1-specific, TGF-β2-specific or TGF-β3-specific antibodies. In (**b**), TGF-β1 (lanes 1 and 6), TGF-β3 (lanes 2 and 5) or the bioactive fraction (lanes 3 and 4) were electrophoresed under reducing (10 mM dithiothreitol; DTT) or non-reducing conditions. Molecular-mass (kDa) markers are indicated.

bioassay (Mv1-Lu growth inhibition; Figure 4a). TGF-β1-neutralizing antibody specifically abolished the growth-inhibitory activity. TGF-β2- or TGF-β3-neutralizing antibodies had little effect on bioactivity. Control incubations of TGF-β1, -β2 or -β3 proteins with their respective neutralizing antibody established the selectivity of these antibodies (Figure 4b). In order to verify that during purification we had not removed other, additional, growth-inhibitory activities secreted by A673 cells, unfractionated acidified conditioned medium was preincubated with pan TGF-β neutralizing antibody. Growth-inhibitory activity was reversed by antibody addition (Table 2).

To assess the effect of TGF-β1 on A673-cell proliferation, TGF-β1-neutralizing antibody (5 μg/ml) was added to the culture media for a 24 h time period. Neutralization of TGF-β1 activity had no significant effect on the fraction of A673 cells in S-phase, as analysed by Student's t test. E.l.i.s.a. assay of BrdU incorporation showed an A_{410} of 0.222 (±0.001) for the control A673 cultures, compared with 0.236 (±0.011) for the neutralizing-antibody-treated cells.

When conditioned medium from A673 cells was maintained at neutral pH, and subsequently assayed for inhibition of Mv1-Lu cell proliferation, no growth-inhibitory activity was observed (Figure 5). In contrast, acidification of A673-conditioned medium unmasked statistically significant bioactivity ($P < 0.0004$), which could be neutralized by the anti-TGF-β1 antibody. We note that the 4-day growth-inhibition assay (MTT endpoint) and the 24 h DNA-synthesis assay (BrdU endpoint) utilized here are essentially equivalent and show similar IC_{50} plots for standard preparations of TGF-β1 or TGF-β3.

DISCUSSION

Growth-inhibitory factors are postulated to play a critical role in the autocrine and paracrine regulation of normal and tumour cell growth. Here we have isolated and characterized a growth inhibitor derived from A673 cells and have shown that at least 90% of this activity consists of TGF-β1. Mass spectrometry and Edman sequencing techniques were utilized to assign activity physically to TGF-β1.

We have shown that the bioactive component has exact protein sequence identity with TGF-β1 over the N-terminal 22 amino acids (Figure 1). The measured amino acid composition was also consistent with that of TGF-β1 (Table 1). Matrix-assisted laser desorption mass spectrometry established the molecular mass to

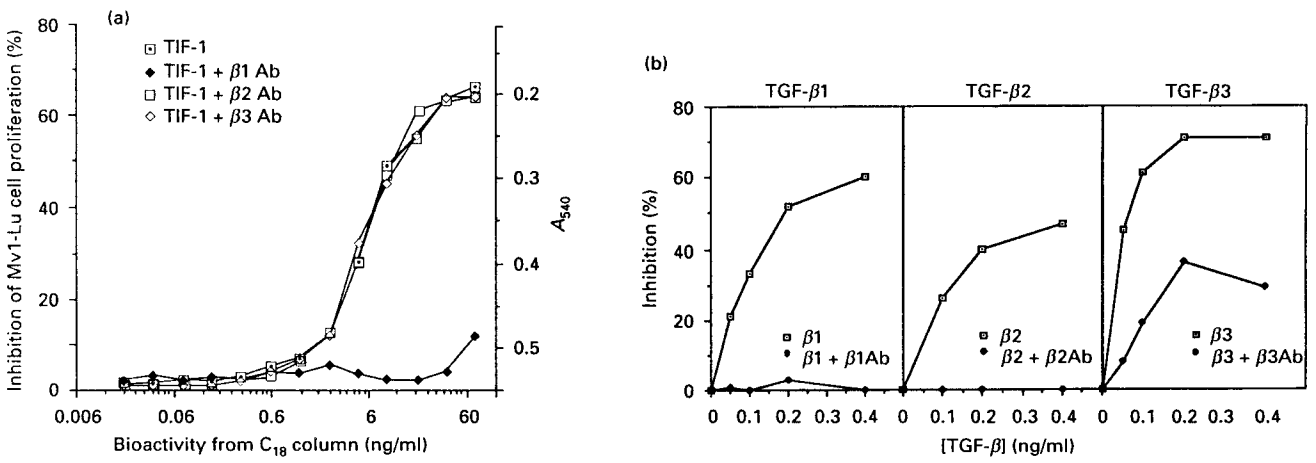

Figure 4 (a) Specific neutralization of bioactivity (TIF-1) eluted from C_{18} column (acetonitrile/TFA) by TGF-β1-specific antibody; (b) neutralization of TGF-β1, -β2 and -β3 bioactivity by anti-TGF-β1, -β2 or -β3 antibodies (Ab) respectively

(**a**) Inhibition of Mv1-Lu cell proliferation as a function of A673-conditioned medium concentration in the presence or absence of TGF-β1, -β2 or -β3-specific neutralizing antibody. (**b**) Percentage inhibition of Mv1-Lu cell growth by TGF-β1, TGF-β2 or TGF-β3 (0–0.4 ng/ml) in the presence or absence of the corresponding specific neutralizing antibody.

Table 2 Neutralization of Mv1-Lu cell-growth inhibitory activity from A673-conditioned medium with anti-TGF-β antibody

A673 medium, which was acidified and neutralized, was preincubated with pan TGF-β antibody and applied to Mv1-Lu cells in culture. Mv1-Lu cell growth was measured after 4 days by MTT assay. Samples were as follows: (1) control unconditioned culture medium; (2) A673-conditioned medium (A673CM) (diluted 1:20); (3) A673-conditioned medium (diluted 1:20) preincubated with pan TGF-β antibody (Ab); (4) A673-conditioned medium (diluted 1:40); (5) A673-conditioned medium (diluted 1:40) preincubated with pan TGF-β antibody; (6) TGF-β1 (0.25 ng/ml); and (7) TGF-β1 (0.25 ng/ml) preincubated with pan TGF-β antibody. Statistical significance (*P*) was determined by Student's *t* test.

Sample	MTT (A_{540})	MTT (% of control)	*P*
(1) Mv1-Lu control	0.54 (±0.02)	100	
(2) Mv1-Lu + A673CM (1:20)	0.09 (±0.00)	17	0.0008
(3) Mv1-Lu + A673CM (1:20) + pan TGF-β Ab	0.49 (±0.04)	91	
(4) Mv1-Lu + A673CM (1:40)	0.12 (±0.01)	22	< 0.0001
(5) Mv1-Lu + A673CM (1:40) + panTGF-β Ab	0.48 (±0.02)	90	
(6) TGF-β1 control (0.25 ng/ml)	0.12 (±0.01)	23	< 0.0001
(7) TGF-β1 + panTGF-β Ab	0.49 (±0.02)	91	

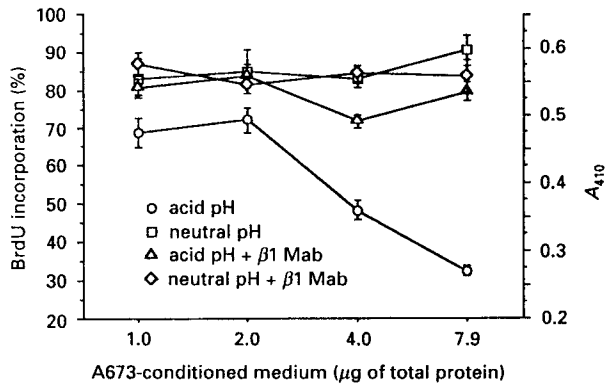

Figure 5 Effect of A673-conditioned medium on Mv1-Lu cell proliferation

A673-conditioned medium, which was either acidified with 1 M acetic acid and then neutralized, or maintained at neutral pH, was incubated with Mv1-Lu cells for 24 h. DNA synthesis was measured by BrdU incorporation using a two-step e.l.i.s.a. for BrdU. Conditioned-medium samples also were preincubated with neutralizing TGF-β1 monoclonal antibody and applied to Mv1-Lu cells for 24 h before measurement of DNA synthesis (in triplicate).

be 25597 (±22) Da and the molecular mass of purified TGF-β1 to be 25576 (±4) Da (Figure 2a), in agreement with the molecular mass of TGF-β1 calculated from its known sequence. These data extend previous work suggesting a TGF-β-like activity in conditioned media derived from A673 cells (Iwata et al., 1985; Dart et al., 1985). Bioactive fractions in both gel-filtration and reverse-phase h.p.l.c. steps were selectively recognized by an anti-TGF-β1 antibody (Figure 3a). Under reducing and non-reducing conditions, activity migrated on SDS/PAGE with mobility identical with that of TGF-β1 (Figure 3b). The growth-inhibitory activity was abolished by preincubation with TGF-β1-specific neutralizing antibody, but not with control antibodies (Figure 4a). Finally, a pan TGF-β neutralizing antibody effectively abolishes the Mv1-Lu cell inhibitory activity in A673-conditioned media (Table 2).

In mammals, three homodimeric forms of TGF-β have been identified (TGF-β1, -β2 and -β3), which share significant amino acid sequence similarity (Derynck et al., 1985; de Martin et al., 1987; ten Dijke et al., 1988). In their mature bioactive forms, TGF-βs are acid- and heat-stable disulphide-linked homodimeric proteins (26 kDa), with each monomer consisting of 112 amino acids (Sporn and Roberts, 1990). TGF-βs are secreted in a biologically inactive form, which may be activated *in vivo* by plasmin or *in vitro* by acidic pH (Figure 5). The secretion of TGF-β1 in a latent, inactive, form may explain how proliferating A673 cells express this negative growth regulator, yet are growth-inhibited, albeit at high concentration, by exogenous activated TGF-β1. It should be noted that, although A673 cells are not autocrinely regulated by TGF-β1 *in vitro*, it is clearly possible for such regulation to operate *in vivo*, where sources of TGF-β1-activating factors may be present (e.g. plasmin).

A673 tumour cells are relatively insensitive to growth inhibition by TGF-β1 (IC_{50} > 100 pM) as compared with primary normal human epithelial cells (IC_{50} ~ 2 pM) Abrogation of growth inhibition by TGF-β1 has been shown to result from mutation, deletion and/or loss of expression of genes critical for the TGF-β-activated signal-transduction cascade. The growth-inhibitory activity of the TGF-βs has been shown to be transduced through a heteromeric transmembrane serine/threonine protein kinase (Chen et al., 1993) and requires the presence of at least one retinoblastoma (Rb) tumour suppressor protein family member (Kimchi et al., 1988). TGF-β-mediated cell-cycle arrest in G1 phase has been shown to correlate with failure to assemble the active cyclinE/cdk2 kinase complex (Koff et al., 1993) and requires the presence of the cyclin/cdk inhibitor $p27^{kip1}$ (Polyak et al., 1994). Attenuation of cellular responsiveness to TGF-β has been postulated to contribute to neoplastic transformation in haematopoietic and epithelial-derived malignancies, and can derive from a decrease in the number of TGF-β receptors and/or impairment in downstream signalling pathways thought to be regulated by tumour suppressor genes, for example Rb or p53 genes (Marshall, 1991), which are often deleted or inactivated in human cancers. In this regard, A673 cells have been shown to lack detectable p53-gene expression (Chen et al., 1991).

In vivo, TGF-βs can act as negative regulators of epithelial and haematopoietic progenitor-cell proliferation (Moses, 1992; Ruscetti et al., 1991). For example, TGF-β1 can inhibit mammary-duct formation and hepatocyte proliferation *in vivo* (Silberstein et al., 1992; Russell et al., 1988). Similarly, in transgenic-mouse models where active TGF-β1 is overexpressed, hypoproliferation of epithelial tissue and/or haematopoietic progenitor cells are observed (Pierce et al., 1993; Sellheyer et al., 1993; Jhappan et al., 1993). Conversely, in TGF-β1-null mice, hyperproliferation of the haematopoietic and epithelial compartments correlates with death due to multifocal inflammatory

lesions (Schull et al., 1992; Kulkarni et al., 1993). Not surprisingly, autocrine TGF-β1 has been shown to act as a suppressor of early-stage colon and breast carcinoma proliferation *in vivo* (Wu et al., 1992, 1993; Hsu et al., 1994), and in transgenic models TGF-β1 can act as a suppressor of tumour formation (D. F. Pierce and H. L. Moses, personal communication). Furthermore, tumour progression and metastasis have been shown to correlate with a loss of cellular responsiveness to TGF-β. The increased synthesis of TGF-β by tumour cells *in vitro* may represent a homoeostatic response to the loss of normal growth control by TGF-β.

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