Differences in adhesion to tissue culture plastic of clonally related transformed and control sublines from an epithelial cell strain

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Summary
Clonally related sublines of the NAL1A lung epithelial cell strain were used in a comparison of the mechanism of attachment and the morphology of control and transformed epithelial cells. The initial attachment and spreading of the control cells on tissue culture plastic was shown to be dependent upon adsorption of serum vitronectin to the substratum. The alpha v subunit of the vitronectin receptor was detected in both the control and transformed cells by immunoprecipitation and immunoblot methods. The spontaneously transformed cells differed from the control cells in that, whereas attachment to tissue culture plastic could occur by binding to adsorbed vitronectin, the transformed cells could also become attached, with time, by a vitronectin-independent mechanism. Attachment by this vitronectin-independent reaction was inhibited by the protein synthesis inhibitor cycloheximide and also by the microtubule-disrupting drugs demicolcemid and nocodazole. The morphologies of attached control and transformed cells cultured on tissue culture plastic were disrupted by treatment with cytochalasin B, demicolcemid or nocodazole, indicating that the shape of these cultured epithelial cells is dependent upon the microtubule system as well as the actin filaments. These results show one important difference between the control and transformed cells, in that the transformed cells can attach to tissue culture plastic by a vitronectin-independent mechanism that involves new protein synthesis by the cell. Another interesting difference is that this vitronectin-independent attachment of the transformed cells was sensitive to inhibition by microtubule-disrupting agents. On the other hand, the attachment of either transformed or control cells to fibronectin- or vitronectin-coated surfaces was not affected by microtubule-disrupting agents.

Key words: vitronectin, cell attachment, microtubules, epithelial cells, demicolcemid, cell shape.

Introduction
One difference often observed between malignant and nonmalignant cells derived from the same cell type is in the shapes that the cells adopt in culture (see Ben-Ze'ev, 1985; Vasiliev, 1985). Having observed a transformation-associated change in shape of cultured epithelial cells derived from pulmonary alveolar epithelium, we have been investigating the mechanism of this change (Smith et al. 1986; Steele et al. 1988, 1990). In the NAL1A culture system that we have been using, nonmalignant control cells derived from normal lung tissue have undergone a spontaneous transformation in culture to become capable of anchorage-independent growth and formation of metastatic carcinomas in mice (Smith and Lykke, 1985; Smith et al. 1986) and to resemble malignant cells cultured from lung adenoma (Smith and Bentel, 1987). The transformation step was initially evident from a change in cell shape and growth pattern; the transformed NAL1A cells growing as islands of tightly associated cells that spread poorly on tissue culture polystyrene (TCP) surface whereas the control cells spread on the surface quite readily when seeded at sparse densities (Smith et al. 1986). In previous studies we have shown that the control NAL1A cells synthesize and accumulate fibronectin (Fn) and laminin into a pericellular matrix, whereas these glycoproteins could not be detected in the transformed cells (Steele et al. 1988, 1990). The control cells show enhanced attachment and spreading on Fn- or laminin-coated substrata. In contrast, the transformed cells can attach to Fn or laminin, but do not show enhanced spreading on these substrata. In contrast to the result with transformed fibroblasts, the shape of the transformed NAL1A cells cannot be made to revert to that of the control cells by seeding on a substratum of Fn or laminin (Steele et al. 1988, 1990). Therefore, although reduced expression of extracellular matrix components is one aspect of the transformed phenotype of NAL1A cells, there must be some additional change in the mechanism controlling the shape of the transformed cells that is also responsible for the refractory nature of the shape of these cells to the culture substratum.
This paper reports the results of experiments to test whether the difference in cell shape of the transformed and control cells following attachment to TCP arises as a result of the use by these cells of different adhesive molecules for attachment to the TCP substratum. The initial attachment and spreading of fibroblasts and endothelial cells seed on TCP is dependent upon adsorption of serum vitronectin (Vn) to TCP (Underwood and Bennett, 1989; Norris et al. 1990; Steele et al. 1991). In this study we have compared the control and transformed NAL1A epithelial cells for their dependence upon serum Vn for the initial attachment and spreading onto TCP. The microtubule-active drugs demicilomcid and nocodazole and the actin-disrupting drug cytochalasin B have been used in a study of the sensitivity of the attachment and shape of the transformed and control NAL1A cells to disruption of microtubules and actin filaments. The results of the experiments show that the transformed cells have a different mechanism for attachment to TCP to that of the control cells.

Materials and methods

Cell lines and cell culture

The clonal cell strain C4E10 is a subclone of the C4 clone of murine cell strain NALIA, which is derived from type 2 pneumocytes of alveolar epithelium. C4E10 cells are not malignant (Smith and Lykke, 1985; Smith et al. 1986). Clone C4E9 emerged from clone C4 of NALIA as a focus of cells with different morphology and is clonally related to the C4E10 clone but C4E9 cells are malignant (Smith et al. 1986). The cells were grown in CMRL1666 supplemented with 2.5 μg ml⁻¹ fungizone, 100 μg ml⁻¹ kanamycin, 20 mM Hepes at pH 7.4 (Flow Laboratories, Australia) and 10% (v/v) foetal calf serum (FCS).

Cell attachment assays

TCP dishes (well surface area of 3.8 cm²) were precoated with 10 μg ml⁻¹ Fn (from bovine plasma, Sigma Chemicals) or 5 μg ml⁻¹ Vn by incubation at 37°C for at least 1 h, then the dishes were washed twice with phosphate buffered saline (PBS) immediately before seeding of cells. Vn was purified and serum selectively depleted of Vn by passage over an affinity column consisting of anti-bovine Vn monoclonal antibody (mAb) A27 immobilized on Sepharose 4B by the methods reported previously (Underwood and Bennett, 1989; Norris et al. 1990). Polystyrene dishes (well surface area of 3.24 cm², from Sterilin, England) were incubated with Fn or Vn solutions (coating concentrations as shown in Fig. 1C) at 37°C for 1 h, the dishes were washed twice with PBS and residual protein binding sites were blocked by incubation with 10 mg ml⁻¹ bovine serum albumin (BSA) for a further hour.

For cell attachment experiments involving metabolically labelled cells, C4E10 and C4E9 cell cultures that were within one day of confluence were metabolically labelled with 0.05 Ci (1.85 MBq) ml⁻¹ [³⁵S]methionine (SJ1515 from Amersham Radiochemical Centre, UK) for 18–22 h in a culture medium of DMEM containing only 3 mg ml⁻¹ methionine (1 vol. complete DMEM plus 9 vol. DMEM without methionine, Flow Laboratories) and 10% (v/v) FCS. Cells for attachment and spreading analysis were trypsinized with 0.05% (w/v) trypsin in PBS, detached from the culture dish, and then the trypsin was neutralized by addition of soybean trypsin inhibitor (Sigma Chemicals) to a concentration of 0.1% (v/v). As reported previously (Steele et al. 1988), the C4E10 cells detached from the substratum after only a short treatment with trypsin whereas the C4E9 cells required more extensive incubation with trypsin and trituration. Cells were collected by gentle centrifugation and resuspended by gentle titration in CMRL1066 with medium containing fungizone, kanamycin and Heps buffer. Cells were seeded onto different substrata by addition to wells containing an equal volume of medium containing serum or BSA, to give a final concentration of either 10% (v/v) FCS (intact or Fn-depleted) or 10 μg ml⁻¹ BSA (Fig. 1C). After 90 min, 4, 6 h or 16 h incubation (see figure and table legends for details), culture dishes seeded with radiolabelled cells were washed four times with PBS to remove unattached cells, and then adherent cells were removed from the dish by trypsinization and solubilization with 1% (w/v) Nonidet P40 (NP40) and the ³⁵S content was determined by liquid scintillation counting.

Detection of Vn receptor subunit alpha subunit

Cultures that were within 1 day of confluence were metabolically labelled with 0.2 μCi (7.4 MBq) ml⁻¹ [³²P]methionine for 16–18 h in the culture medium containing the reduced concentration of methionine. The cells were washed with PBS, then extracted with 1% (v/v) NP40 in 50 mM Tris–HCl, pH 7.6, containing 1 mM 1,2-diaminoethane-N,N,N',N'-tetraacetic acid and 150 mM NaCl. Immunoprecipitation was conducted by preloading of protein A-Sepharose 4B beads (between 100 and 200 μl of a 50% (v/v) slurry of beads from Pharmacia per incubation tube) with either rabbit antisera raised against the alpha subunit of the human vitronectin receptor isolated from human placenta (Telios Pharmaceuticals Inc., catalogue no. A109) or nonimmune rabbit serum. After incubation with shaking for 2 h at room temperature to load the beads with antibody, the beads were washed once with PBS containing 1% (w/v) BSA, pH 7.5, and then the antibody-coated beads were incubated with cell extract (usually 500 μl) for 2 h at room temperature with vigorous shaking. The beads were collected and washed 4 times using 1% NP40 buffer, and then the precipitated proteins were solubilized by boiling in electrophoresis sample buffer and electrophoresed on nonreduced 6.5% (w/v) polyacrylamide gels.

Drugs

Stock solutions of demicilomcid (1 mg ml⁻¹, Sigma Chemicals) in water and of nocodazole (1 mg ml⁻¹, Sigma Chemicals) or cytochalasin B (5 mg ml⁻¹, Sigma Chemicals) in dimethyl sulfoxide (DMSO) were stored at –20°C. Controls in experiments involving nocodazole or cytochalasin B were cultured in medium containing an equivalent concentration of DMSO alone. Cycliclomide (2.5 mg ml⁻¹ in water, Sigma Chemicals) was stored at –70°C.

Immunofluorescence microscopy distribution of tubulin

Cells used for these immunostaining experiments were grown on Fn-coated glass. Cells to be stained for tubulin were cultured for one day either with or without 1 μg ml⁻¹ demicilomcid, washed with PBS and fixed and permeabilized by incubation for 4 h at 4°C with 1% (v/v) solution of 40% (w/v) formaldehyde in PBS, then incubated with 10% (w/v) Triton X-100. The fixed cells were washed twice with PBS, incubated with 10% (w/v) skim milk in PBS (pH 7.2) for 45 min and then incubated with rabbit antisera raised against tubulin from chick embryo brain (1:12 dilution in skim milk solution, code 66–096 from Miles-Yeda Ltd) for 1 h. The cells were washed three times with PBS containing 0.02% (v/v) Tween 20 and then incubated with 70 μg ml⁻¹ fluorescein isothiocyanate (FITC)-conjugated swine IgGs to rabbit IgGs (Dako) in PBS for 1 h in the dark. Following one wash with PBS containing 0.02% (w/v) Tween 20 and two washes with PBS, the cells were viewed at a wavelength of 550 nm using a ×100 objective on a Nikon Diaphot inverted microscope connected to a BioRad MR500 laser confocal microscope.

Cells to be stained for F-actin distribution were cultured for 1 day and then treated with 25 μg ml⁻¹ cytochalasin B for 4 h at 37°C prior to fixation. Cells were fixed with formaldehyde–PBS solution (without Triton X-100) for 15 min at room temperature. Cells were then washed with PBS, permeabilized by 16 min incubation in methanol:acetone (4:1, v/v, at –20°C) and then air dried. The slides were then washed with PBS, incubated for 1 h with 12 units ml⁻¹ rhodamine–phalloidin (Molecular Probes Inc., OR, USA) in the dark, washed three times with PBS, mounted with 50% (v/v) glycerol–PBS and examined with an Olympus IMT-2 microscope at 514 nm, with an immunofluorescence attachment.
Results

Role of serum vitronectin in attachment of C4E10 and C4E9 cells to TCP

We have previously reported that single C4E10 cells attach and spread, within 90 min of seeding in culture medium containing 10% (v/v) serum, on TCP or on TCP coated with Fn. The attachment of clonally related C4E9 cells to TCP is relatively slow, requiring more than 3–12 h after seeding to be complete. The C4E9 cells seed as clumps and grow as islands of cells (Steele et al. 1988).

To determine the role that the serum Vn may play in the initial attachment and spreading of C4E10 and C4E9 cells on TCP, Vn was selectively removed from the serum used in the culture medium. Fig. 1A shows the effect of depletion of Vn from FCS upon the attachment of C4E10 cells after 90 min and 16 h of culture, and the effect upon C4E10 cell spreading after 90 min is shown in Fig. 1B. Depletion of Vn from the culture medium completely abolished the attachment and spreading of C4E10 cells to TCP during the first 90 min of culture, and continued culture for a 16 h period did not result in cell attachment (Fig. 1A,B). The attachment and spreading of C4E10 cells seeded on Fn–TCP in this Vn-depleted medium was unimpaired (as compared to C4E10 cells seeded on TCP in intact medium), indicating that the Vn-depleted medium was not toxic to the cells. The ability of C4E10 cells to attach to Vn was directly demonstrated by coating

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Fig. 1. Involvement of serum Vn and Fn in attachment of C4E10 and C4E9 cells to TCP. (A and B) Histograms showing proportion of C4E10 (open bars) and C4E9 (hatched bars) cells attached and spread on TCP or Fn–TCP (as indicated) after seeding in culture medium containing intact FCS (FCS) or FCS depleted of Vn (–Vn). A shows cell attachment (% of total cells seeded) 90 min and 16 h after seeding, and B shows spreading of C4E10 cells 90 min, and of C4E9 cells 16 h, after seeding. Mean±s.e.m. (C) Effect of the concentration of Vn (squares) or Fn (circles) used to precoat a polystyrene culture substratum upon the proportion of C4E10 cells (% of total cells seeded) that become attached during 90 min incubation in serum-free medium. Mean±s.e.m. (D) Detection of cellular vitronectin receptor alpha v subunit by immunoprecipitation analysis. C4E10 cells (tracks 1 and 2) or C4E9 cells (tracks 3 and 4) were biosynthetically labelled with [35S]methionine and then the proteins in a cell extract solubilized in Nonidet P-40 were immunoprecipitated with anti-alpha v antiserum (tracks 2 and 4) or preimmune serum (tracks 1 and 3). Immunoprecipitated proteins were separated (without reduction) on a SDS–polyacrylamide gel; the arrows on the right show the calculated M, values (×10^-5) of key bands.

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Expression of vitronectin receptors on C4E10 and C4SE9

The C4E10 and C4SE9 cells were compared for their content of receptors for vitronectin, by immunoprecipitation experiments using polyclonal antisera against the \( \alpha_v \) subunit of human vitronectin receptor (Suzuki et al. 1986). Immunoprecipitated extracts of metabolically labelled C4E10 cells had a major band at \( 152 \times 10^3 \text{M}_r \) and a minor band at \( 173 \times 10^3 \text{M}_r \) (Fig. 1D, track 2). Extracts of C4SE9 cells had a major band at \( 152 \times 10^3 \text{M}_r \) and faint bands at \( 173 \times 10^3 \text{M}_r \) and \( 142 \times 10^3 \text{M}_r \) (track 4). These experiments show that both C4E10 and C4SE9 cells synthesize and contain the \( \alpha_v \) subunit of \( M_r 152 \times 10^3 \), consistent with the mechanism of the initial attachment of these cells to TCP being the binding to serum Vn adsorbed to the TCP.

Sensitivity of the attachment of cultured C4E9 cells by the Vn-independent mechanism to disruption by cytoskeletal-active drugs

The effect of cytoskeletal inhibitors on the attachment to TCP of C4E9 cells by the Vn-independent and Vn-dependent mechanisms was tested, using two drugs that affect the microtubule system (demicolcemid and nocodazole) and an inhibitor of actin filaments, cytochalasin B. The drugs were tested for effects upon the initial attachment of metabolically labelled cells to the substratum when added to the culture medium at the time of seeding. In other experiments, any effects upon cell morphology when the inhibitors were added to established cells attached when seeded in medium containing Vn-depleted serum is expressed relative to attachment to the Fn-TCP surface in the absence of 25 \( \mu \text{g ml}^{-1} \) cycloheximide.

### Table 1. Effect of cycloheximide (25 \( \mu \text{g ml}^{-1} \)) on attachment of C4E9 cells to Fn-TCP, Vn-TCP or TCP over a 6 h incubation in medium containing Vn-depleted serum.

<table>
<thead>
<tr>
<th>Cells</th>
<th>Surface</th>
<th>Cycloheximide</th>
<th>% Cells attached (mean±s.e.m.)</th>
<th>Inhibition</th>
</tr>
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<tbody>
<tr>
<td>C4E9</td>
<td>Fn-TCP</td>
<td>-</td>
<td>100 *</td>
<td></td>
</tr>
<tr>
<td>C4E9</td>
<td>Vn-TCP</td>
<td>+</td>
<td>60±7.0</td>
<td>49.4</td>
</tr>
<tr>
<td>C4E9</td>
<td>TCP</td>
<td>-</td>
<td>20±2.0</td>
<td>79.5</td>
</tr>
<tr>
<td>C4E9</td>
<td>TCP</td>
<td>+</td>
<td>40±0.5</td>
<td>97.2</td>
</tr>
<tr>
<td>C4SE9</td>
<td>Vn-TCP</td>
<td>-</td>
<td>143±4.00</td>
<td></td>
</tr>
<tr>
<td>C4SE9</td>
<td>TCP</td>
<td>+</td>
<td>140±5.0</td>
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</table>

The % cells attached when seeded in medium containing Vn-depleted serum is expressed relative to attachment to the Fn-TCP surface in the absence of 25 \( \mu \text{g ml}^{-1} \) cycloheximide.

The C4E10 and C4SE9 cells were compared for their sensitivity of the attachment of cultured C4E9 cells by the Vn-independent mechanism to disruption by cytoskeletal-active drugs.

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Fig. 2. Histograms showing the effect of demicolcemid (A) and nocodazole (B) on cell attachment to TCP (% of total cells seeded) in medium containing intact serum. Diagonally striped bars, C4E9 cells incubated for 24 h; and open bars, C4E10 cells incubated for 4 h. Mean±s.e.m.
cultures or at the time of seeding were observed. The effects of the drugs upon the attachment to TCP of the control C4E10 cells were also tested in these experiments.

The addition of demicolcemid or nocodazole at the time of seeding of C4E9 cells in medium containing intact serum caused a concentration-dependent, significant but incomplete inhibition of attachment of C4E9 cells to TCP (Fig. 2A and B). To test whether the partial inhibition by demicolcemid or nocodazole of attachment to TCP of C4E9 cells may be related to the ability of these cells to attach to TCP by a Vn-independent mechanism, the attachment experiments were also carried out using medium containing Vn-depleted serum. With this medium, 1 μg ml⁻¹ demicolcemid inhibited Vn-independent attachment of C4E9 cells to TCP by 78% whereas attachment from medium containing intact serum was inhibited by 55% (Fig. 3 and Fig. 2A). Addition of demicolcemid had no effect upon the attachment of C4E9 cells from either of the media to either Fn-TCP (Fig. 3) or Vn–TCP (not shown). The morphology of C4E9 cells seeded on TCP in Vn-depleted serum was sensitive to the addition to the culture medium after 24 h culture of 1 mg ml⁻¹ demicolcemid (Fig. 4A cf. B), which caused retraction of the spread edges of marginally positioned cells. The demicolcemid caused a partial collapse of the fine filamentous network of tubulin microtubules (Fig. 5C and D). On a Fn–TCP substratum, the shape of C4E9 cells seeded in the same medium was unaffected by demicolcemid (Fig. 4C,D).

There was no effect of 25 μM cytochalasin B on the number of C4E9 cells that attached to TCP when seeded in medium containing intact serum (86% of cells attached as compared to controls, mean of 4 experiments) and the
Vn-independent attachment to TCP was not inhibited by addition of cytochalasin B to the culture medium (Fig. 3). When cytochalasin B was added to the culture medium at the time of cell seeding, the spreading of marginally positioned C4SE9 cells on TCP, Fn–TCP or Vn–TCP substrata was inhibited (not shown). Addition of 25 μM cytochalasin B to the culture medium for 3 h caused retraction of the edges of marginally positioned C4SE9 cells and collapse of the F-actin stress fibres (Fig. 5I,J).

The attachment of C4E10 cells to TCP was not inhibited by the addition of demicolcemid or nocodazole to the culture medium at the time of seeding (Fig. 2A and B). The shape of C4E10 cells cultured on either TCP or Fn–TCP was sensitive to the presence of demicolcemid in the culture medium. This sensitivity to demicolcemid was particularly evident when the cells were cultured in the presence of the drug and on a Fn–glass substratum for immunostaining of tubulin distribution (Fig. 5A vs B). There was no effect of 25 μM cytochalasin B on the number of C4E10 cells that attached to TCP (89% of cells attached as compared to controls, mean of 3 experiments). Addition of 1 μM cytochalasin B to established cultures of C4E10 cells caused retraction of the cell borders and rounding up of the cells during 1–3 h of treatment and disruption of the F-actin stress fibres (Fig. 5G,H). C4E10 cells that attached to TCP or Fn–TCP in the presence of cytochalasin B did not display the shape polarity that was seen in the control cells (not shown).

Taken together, the results of the drug experiments show that the C4SE9 cells differ from the C4E10 cells in the effect of disruption of their microtubule system on initial cell attachment to TCP. The initial attachment of C4SE9 cells to TCP was sensitive to disruption of the microtubules. The effects of the inhibitors were different for the different mechanisms of their attachment to TCP: the initial attachment of C4SE9 cells to TCP by a Vn-independent mechanism was almost completely inhibited by poisoning of the microtubules whereas the attachment
of C4SE9 cells to either Fn-TCP or Vn-TCP was not inhibited by disruption of the microfilaments. The initial attachment to TCP of C4E10 cells (which must attach to TCP by a Vn-dependent mechanism) was unaffected by microtubule-active drugs.

Discussion

The NAL1A alveolar cell system is an attractive cell model for studies of the mechanism of neoplastic transformation of epithelial cells, as the transformed cells may be compared to nontransformed cells derived from the same clonal cell source. The transformed cells arose from the clonal control cells as the result of a spontaneous event, initially evident as a spontaneous morphological change during culture; the resultant transformed cells have the stable phenotype of cells that attach to TCP as islands, with the marginally positioned cells showing poor spreading on the culture substratum (Smith and Lykke, 1985; Smith et al. 1986; Steele et al. 1988, 1990). In previous studies of the altered morphology of the transformed C4SE9 cells and clonally related control C4E10 cells, it has been shown that the C4E10 cells grown on TCP can assemble an extracellular matrix containing Fn and laminin, whereas the C4SE9 cells do not. This failure of C4SE9 cells to secrete Fn and laminin is not the sole factor causing the altered shape of these malignant cells, however, as these cells retain a poorly spread morphology (as compared to C4E10 cells) when seeded onto Fn-, laminin- or extracellular matrix-coated substrata (Steele et al. 1988, 1990). In this study we have examined the mechanism by which the control and transformed cells initially attach to TCP, and have used cytoskeletonally disruptive drugs to look for differences in the cytoskeletal-cell surface-TCP substratum linkages of these cells.

The attachment of C4E10 cells to TCP occurs during the first 90 min of culture and is due to cell binding to serum Vn adsorbed to the substrata. This mechanism is the same as that demonstrated for the initial attachment to TCP of fibroblasts and endothelial cells (Underwood and Bennett, 1989; Norris et al. 1990; Steele et al. 1991). The selective depletion of Vn from the serum completely inhibited the attachment of C4E10 cells to TCP, indicating that it is Vn, rather than any other serum-adhesive protein, that becomes adsorbed to TCP in sufficient quantity to permit initial attachment of C4E10 cells. It was shown previously that in the presence of serum concentrations greater than 1 % (v/v), adsorption of Fn to TCP is inhibited and at 10 % (v/v) very little Fn adsorbs to TCP (Grinnell and Feld, 1982; Underwood and Bennett, 1989). As a result of this inhibition, the relative amounts of Fn and Vn that adsorb to TCP during a 90 min incubation with medium containing 10 % (v/v) FCS are 38 ng cm\(^{-2}\) Fn and 227 ng cm\(^{-2}\) Vn (J. G. Steele, G. Johnson and P. A. Underwood, unpub-
lished data). This initial attachment of C4E10 cells to adsorbed serum Vn was not inhibited by actin- or microtubule-active drugs, although other studies suggest that integrin receptors are linked at the cytoplasmic face of the plasma membrane to the actin filaments, either directly or indirectly (Horwitz et al. 1986; Burridge et al. 1988; Otey et al. 1990). Following the initial binding step, C4E10 cells spread rapidly and quite extensively on the substratum. This spreading of attached C4E10 cells was sensitive to disruption of either the actin filaments (by cytochalasin B) or the microtubules (by nocodazole or demicolcemid). Similar sensitivity of the shape of cultured epithelial cells to microtubule-disruptive drugs has been observed with other cell lines (Dominina et al. 1985) but not with primary cultures (Middleton et al. 1988), suggesting that the importance of microtubules for the shape of epithelial cells may be acquired as a result of adaptation to cell culture (see Middleton et al. 1988, for discussion).

The attachment of C4E9 cells to TCP can occur by both Vn-dependent and Vn-independent mechanisms. The mechanism of attachment of C4E9 cells to TCP occurred relatively slowly compared with that of C4E10 cells (Steele et al. 1990 and this study) and those C4E9 cells that attach within the first 90 min bind to Vn adsorbed to TCP. We show here that with incubation beyond 90 min the C4E9 cells can attach by a Vn-independent mechanism. This Vn-independent mechanism is dependent upon protein synthesis, but other biochemical details of the mechanism remain to be determined. It seems likely that the Vn-independent mechanism requires cellular secretion of some adhesive molecule, as we have observed the Vn-independent attachment reaction to BSA-blocked TCP in serum-free medium (data not shown). In demonstrating this ability of C4E9 cells to attach in a Vn-independent reaction, we show that there is a clear difference between the control and transformed NAL1A cells in the mechanism of attachment to TCP in the presence of FCS. In experiments with microtubule inhibitors, the Vn-independent attachment of C4E9 cells to TCP was highly sensitive to inhibition by the microtubule-active drugs demicolcemid and nocodazole whereas attachment to a Fn- or Vn-coated surface was not. Furthermore, whereas the shape of the C4E9 cells attached to TCP by the Vn-independent mechanism was quite sensitive to demicolcemid, when these cells were cultured on Fn–TCP or Vn–TCP their shape was relatively insensitive to demicolcemid. It is not known whether the mechanism of demicolcemid sensitivity of cell attachment to inhibitors of microtubules is the same as that underlying the Vn-independent attachment of C4E9 cells to TCP. One hypothesis to explain the inhibition could be that there is a direct linkage between the microtubules and a cell surface adhesive molecule. Alternatively, the Vn-independent attachment reaction and the sensitivity of that reaction to microtubule-disrupting drugs may be due to different mechanisms of attachment to inhibitors of microtubules. The mechanism of this attachment may be cross-reactive with murine receptors (and so monoclonal antibodies are unlikely to be useable).

Numerous studies have pointed to a 'switch off' of the synthesis of extracellular matrix components upon neoplastic transformation of fibroblasts, and a similar effect is observed in the NAL1A epithelial cell system (Alitalo and Vaheri, 1982; Hayman et al. 1982; Ruoslahti, 1984; Steele et al. 1988, 1990). The specific aim of this study was to compare the mechanisms of attachment and shape control of the transformed and control NAL1A cells on TCP, in order to identify the mechanism that altered the morphology of the transformed cells (Smith and Lykke, 1985; Smith et al. 1986; Steele et al. 1990). The study shows that the transformed NAL1A cells have another attachment mechanism, in addition to those described for the control cells. The working hypothesis that arises from these results is that the transformed NAL1A cells synthesize a cell surface attachment molecule and that the expression of this (as yet unidentified) molecule by these transformed cells permits attachment to TCP without a requirement for exogenous attachment factors such as serum Vn or Fn. We suggest that this Vn-independent, microtubule-related attachment mechanism may be responsible for the altered shape and spreading behaviour of the transformed cells compared to the controls. In further studies in this cell system we shall work towards the identification of the Vn receptor in the transformed cells to determine the exact role that it plays in the control of the shape and growth of the transformed NAL1A cells and to determine whether it is present in normal tissue and other carcinoma cells.

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Epithelial cell adhesion and shape