Adhesion to Laminin and Expression of Laminin in Clonally Related Transformed and Control Sublines from an Alveolar Epithelial Cell Strain

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ABSTRACT

Clonally related sublines of the NAL1A cell strain were used to examine the expression of laminin and the importance for the attachment and morphology of these control and spontaneously transformed alveolar epithelial cells. A laminin component was detected by immunoblot analysis in extracts of control cells cultured on plastic as a M, 410,000 species consisting of disulfide-linked M, 200,000 components. The laminin content of the malignant cells was reduced at least 40-fold as compared to that of the control cells. Cell attachment to laminin or to a laminin-like neurite promotion factor was compared with attachment to fibronectin and to extracellular matrix from bovine endothelial cells. Both control and transformed cells attached as well to laminin or neurite promotion factor as to fibronectin, in a serum-free adhesion assay. The control cells showed enhanced cell spreading on the surfaces coated with laminin, neurite promotion factor, or fibronectin. The transformed cells had very similar cell shape, as determined by phase contrast and scanning electron microscopy, when cultured on laminin or neurite promotion factor or fibronectin, as on tissue culture plastic.

INTRODUCTION

Pulmonary alveolar type 2 cells are the epithelial cells that synthesize and secrete pulmonary surfactant and that proliferate during alveolar repair (see Refs. 1 and 2). In certain strains of mice, type 2 pneumocytes transform to an alveologenic adenoma, both spontaneously and in response to a range of chemical carcinogens (3–5). A proportion of the adenomas may then progress to carcinomas (3). We have recently developed a series of cell lines that may be used for the study of the transformation of these alveolar epithelial cells. In this NAL1A culture system, nonmalignant control cells derived from normal lung tissue have undergone a spontaneous transformation in culture to become capable of anchorage-independent growth and of forming metastatic carcinomas in mice (6, 7) and to resemble malignant cells cultured from lung adenoma (8). The change in cell growth phenotype was accompanied by a morphological shift that resembled the change observed during transformation of mesenchymal cells (6, 9). In the case of transformed fibroblasts, the altered cell shape is due in part to reduced levels of extracellular matrix components, particularly fibronectin, and to consequential changes in the arrangement of cytoskeletal elements (10–14). The addition of purified Fn* to the culture medium of transformed fibroblasts causes the cells to adopt a more flattened morphology that resembles that of normal fibroblasts (15, 16). A similar mechanism may be involved in the transformation of NAL1A epithelial cells, inasmuch as we have demonstrated that the control of NAL1A cells synthesize and secrete Fn and accumulate Fn in the pericellular matrix, but the transformed cells do not synthesize or accumulate detectable levels of Fn (17). However, the Fn sensitivity of the transformed epithelial cells does not resemble that of transformed fibroblasts inasmuch as the addition of Fn is not sufficient to induce reversion of the shape of transformed NAL1A cells (17). The apparent failure of the transformed NAL1A cells to change shape in response to exogenous Fn was not due to a general refractoriness of NAL1A cells to Fn, because the control cells showed enhanced cell spreading on Fn-coated dishes. One postulate to explain the failure of the transformed NAL1A cells to change shape in response to Fn is that there may be some cytoskeletal differences as compared to the normal cells (18). Another possibility may be that the transformed epithelial cells have a preference for Ln as an attachment factor (19). Ln is a M, 850,000 glycoprotein consisting of three disulfide-linked subunits (A, M, 400,000; B1, M, 215,000; and B2, M, 205,000) and, together with proteoglycans and collagenous glycoproteins, forms the basal lamina which underlies normal epithelia (20–22). In this study we have examined the expression of laminin by control and transformed NAL1A cells. We have also examined the response of the control and transformed NAL1A cells to Ln, which is known to be a potent attachment factor for epithelial cells and neurons, and to a neurite promotion factor which contains Ln components complexed to heparan sulfate proteoglycan (23, 24).

MATERIALS AND METHODS

Cell Lines and Cell Culture. The clonal cell strain C4E10 is a subclone from the C4 clone of murine cell strain NAL1A, which is derived from type 2 pneumocytes of alveolar epithelium. C4E10 cells are not malignant (6, 7). Clone C4E9 emerged from clone C4 of NAL1A as a foci of cells with different morphology and is clonally related to the C4E10 clone but C4E9 cells are malignant (6). The cell clones derived from NAL1A and NAL1AM were isolated by Bentel and Smith (25). The cells were grown in CMRL1066 supplemented with 2.5 mg/ml Fungizone, 100 mg/ml kanamycin, 20 mm 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid at pH 7.4 (Flow Laboratories), and 10% (v/v) fetal bovine serum, and the culture medium used in this study did not contain dexamethasone.

Cell Attachment Assays. Culture dishes were coated with attachment factors by incubating tissue culture plastic dishes (Costar) with 0.1–10 μg/ml Fn (bovine plasma Fn; Sigma Chemical Co.) or polylysine-coated dishes were incubated with 0.1–100 μg protein/ml (PNPF) (23) or 0.1–10 μg/ml mouse Ln (from EHS tumor) at 37°C for at least 1 h. Residual cell-binding sites on the plastic were then blocked by incubation with 1 mg/ml BSA in DPBS, pH 7.5, for 1 h at 37°C; then the dishes were washed twice with DPBS immediately before seeding of cells.

The PNPF used in this study contains the B chains of laminin and a M, 160,000 entactin/nidogen component but is immunologically distinct from Ln, in that polyclonal anti-Ln antisera do not block the extension of neurites from sensory neurons onto PNPF (23, 24). The laminin in ECM has a similar conformation to that in PNPF but

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The abbreviations used are: Fn, fibronectin; ECM, extracellular matrix; Ln, laminin; DPBS, Dulbecco’s phosphate-buffered saline; DMEM, Dulbecco’s modification of Eagle’s medium with glutamine; NP40, Nonidet P-40 non-ionic detergent; BSA, bovine serum albumin fraction 5; TCP, tissue culture polystyrene plastic; PNPF, polylysine-binding neurite promotion factor; DTT, dithiothreitol.
different from that in purified Ln, inasmuch as neurite extension from sensory neurons onto ECM can be immunoblocked by anti-PNPF antisera but not by anti-Ln antisera (data not shown). The characterization of the polyclonal anti-Ln antisera, which reacts with all 3 chains of Ln, and the anti-PNPF antisera used in this study has been reported previously (24). In the immunoblotting assays, the IgG fractions of the anti-Ln, anti-PNPF, and preimmune sera were prepared by passage through a protein A-Sepharose CL-4B column and the IgG fractions were dialyzed against the culture medium. The IgG fractions were used at a dilution of the antiserum of 1:10, at which level these antisera completely block neurite extension from cultured sensory neurons (24). The antibodies were preincubated with the substrata for 60 min before seeding of the cells.

For cell attachment experiments involving metabolically labeled cells (Fig. 1), C4E10 and C4E9 cell cultures that were within 1 day of confluence were metabolically labeled with 0.050 mcI (1.85 MBq)/ml [35S]methionine (SJ1515 from Amersham Radiochemical Centre, Amersham, United Kingdom) for 12–16 h in a labeling culture medium of DMEM containing only 3 mg/liter methionine (1 volume complete DMEM plus 9 volumes DMEM without methionine; Flow Laboratories) and 10% (v/v) fetal bovine serum. Cells for cell attachment and spreading analysis were trypsinized with 0.25% (w/v) trypsin in DPBS and detached from the culture dish; then the trypsin was neutralized by addition of soybean trypsin inhibitor (Sigma) to a concentration of 0.5% (w/v). Cells were collected by gentle centrifugation and made up in either CMRL1066 medium containing Fungizone, kanamycin, 4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid buffer, and either 10 mg/ml BSA or 10% (v/v) fetal calf serum and seeded onto the different culture substrata. After 1-2 h incubation (for the BSA-medium cultures) or 24 h incubation (medium containing serum), cultures dishes seeded with radiolabeled cells were washed four times with DPBS to remove unattached cells; then the adherent cells were removed from the dish by trypsinization and solubilization with 1% (w/v) NP40 and the 35S content was determined by liquid scintillation counting. In experiments where the cell attachment was measured as the proportion of cells undergoing spreading, randomly selected fields of cultures were photographed using a Nikon phase contrast photomicroscope at various times after seeding with cells, without washing unattached cells from the cultures. The proportion of cells that had spread (deviation from a round morphology and producing cytoplasmic extensions onto the substratum) was counted from the photomicrographs. In other experiments (Figs. 2 and 4), the cells were suspended in complete culture medium made up using fetal calf serum that had been stripped of Fn by passage over a gelatin affinity column and confirmed to be depleted of Fn by immunodot blot analysis (26). Cultures that were photographed 24 h after seeding (Fig. 2, j–m) were washed with DPBS to remove the few unattached cells. For scanning electron microscopy, the fixed cells were treated with 1% (v/v) glutaraldehyde in 0.1 M cacodylate buffer followed by 1% osmium tetroxide for 1 h. Following washing in cacodylate buffer, the fixed cells were treated with 1% tannic acid solution for 1 h and then washed and dehydrated through a series of ethanol solutions, soaked for 10 min with hexamethydisilazane, air-dried, sputter-coated, and viewed in a Cambridge Instruments electron microscope.

Detection of Laminin in ImmunobLOTS. Confluent 75-cm² cultures of cells were washed with DPBS and then extracted with 1 ml sodium dodecyl sulfate electrophoresis sample buffer for 30 min at room temperature and then boiled for 5 min. For slot blot analysis, 0.01-ml samples of serial dilutions were applied to a cellulose nitrate membrane and blocked with BSA and incubated with anti-Ln antisera. Samples for electrophoresis and immunoblot analysis were either used without reduction or reduced with DTT and separated by sodium dodecyl sulfate-electrophoresis in 3–8% (w/v) gradient polyacrylamide gels. After electrophoresis, the gels were incubated for 60 min at 37°C in 50 mM DTT; then the proteins were electrophoretically transferred to nitrocellulose (27). In other experiments, 4.5% (w/v) polyacrylamide gels were used to resolve reduced cell extracts. Slot blots and electrophoretic blots were probed with rabbit antisera raised against Ln or nonimmune rabbit serum controls. The immune complexes were detected with 125I-protein A (Amersham Radiochemical Centre) and 125I was detected by autoradiography. Following autoradiography, the gel lane nitrate sheets from the slot blot experiments were dissected and radioactivity in individual slots was determined by direct counting, to obtain more accurate quantitation.

Metabolic Labeling and Immunoprecipitation. Cell cultures that were within 1 day of confluence were metabolically labeled with 0.2 mCi (7.4 MBq)/ml [35S]methionine (SJ1515 from Amersham Radiochemical Centre) for 2–3 h in labeling culture medium. The conditioned medium was collected and the cells were washed with DPBS then extracted with 1% (v/v) NP40 in 50 mM Tris, pH 7.6, containing 1 mM 1,2-di(2- aminoethoxy)ethane-N,N,N',N'-tetraacetic acid and 150 mM NaCl. The conditioned medium and cell extracts were stored at −20°C. 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 anti-Ln antisera [20 μl] or preimmune control serum for a minimum of 2 h. The antibody-coated beads were washed once with DPBS containing 1% (w/v) BSA, pH 7.5, to remove unattached antibodies and then incubated with the cell extracts or conditioned medium (usually 500 μl) for 2 h at room temperature with vigorous shaking. The beads were collected and washed 4 times, using DPBS containing BSA for the beads that were incubated with conditioned medium and using 1% (v/v) NP40 buffer for the beads that had been incubated with cell extracts. Precipitated proteins were solubilized by boiling in reducing electrophoresis sample buffer and electrophoresed on reducing 4.5% (w/v) polyacrylamide gels. Gels were treated with Amplify (Amersham) for fluorography.

RESULTS

Adhesion and Spreading of C4E10 and C4E9 Cells on Laminin and PNPF. We have previously reported that clone C4E10 cells attach and spread within 1–2 h of seeding in culture medium containing 10% serum on TCP or onto TCP-coated with Fn or ECM from bovine endothelial cells. The C4E10 cells grow on each of these surfaces as single cells (17). The attachment of clonally related C4E9 cells to TCP is relatively slow and commences 2–3 h after seeding. The C4E9 cells seed as clumps and grow as islands of cells which have a morphology markedly different than that of the C4E10 cells. The C4E9 cells attach more rapidly to TCP coated with Fn or ECM than to TCP itself (17).

In the present study the attachment of C4E10 and C4E9 cells onto a substratum coated with Ln was compared to attachment onto ECM or Fn (Fig. 1). The cells were incubated for 1 h using a serum-free culture medium and each surface, including the control TCP and polylysine-coated, was
blocked with BSA before use. Another substratum tested was neurite promotion factor, PNPF, which contains the B chains of Ln in association with heparan sulfate and a M, 160,000 (entactin/nidogen) component (23, 24). PNPF was included in this study because the conformation of Ln in PNPF is similar to that in ECM but immunologically distinct from that of purified Ln. Fig. 1 shows that the C4E10 cells showed little (less than 10%) attachment to TCP-BSA or polylysine-BSA but attached well to the PNPF or Ln substrata (for PNPF, 86%, and for Ln, 72% of the number of cells attached to the ECM). The C4E9 cells also attached to PNPF (48% of the number of cells attached to ECM, equivalent to C4E9 attached to Fn) and to Ln (24% as compared to ECM). Fig. 1 also clearly shows that attachment of C4E9 cells to PNPF, Ln, or Fn, expressed as a percentage of attachment to ECM, was significantly lower than the attachment of C4E10 cells to these substrate. The addition of either 10 μg/ml Fn or 10 μg/ml Ln to the culture medium did not enhance C4E9 cellular attachment to Fn- or Ln-coated dishes, respective (data not shown).

Comparison of Attachment and Initial Spreading of C4E10 and C4E9 Cells. C4E10 cells spread well onto the PNPF, Ln, Fn, or ECM surfaces during the 1-h assay period with more than 90% of attached cells spread (Figs. 2 and 3). The relatively poor response of the C4E9 cells as compared to the C4E10 cells for attachment to the substrata (Fig. 1), was even more marked in the degree of spreading of the attached cells. The C4E9 cells attached to the PNPF, Ln, or Fn surfaces as clumps of cells which are sufficiently well attached as to resist washing, but the cells did not commence to spread onto the surface during the 1-h period (Fig. 2, c and d). Some spreading of C4E9 cells onto the ECM substratum was seen within 1 h, but most cells had not spread. Because the C4E9 cells failed to spread on PNPF, Ln, or Fn during a 60–90-min incubation, direct quantitative comparison of the C4E10 cells could not be made at this time point (but compare Fig. 2b with Fig. 2d).

Using cell spreading as an assay for C4E10 cell response to the surface, the concentration dependence of coating with PNPF, Ln, or Fn were determined (Fig. 3A). Optimal C4E10 cellular responses were obtained when approximately 50 μg/ml PNPF protein, or greater than 10 μg/ml Ln, or 5 μg/ml or more Fn were used to precoat the polylysine (PL) trays. The ability of anti-Ln and anti-PNPF IgG to block the adhesion of C4E10 cells to PNPF or purified Ln was tested (see Fig. 3B for a representative experiment). Anti-PNPF IgGs inhibited the spreading of C4E10 cells on PNPF and anti-Ln IgGs completely inhibited the attachment and spreading of C4E10 cells on purified Ln. Anti-Fn IgGs did not inhibit C4E10 cell attachment and spreading onto either PNPF or Ln, and anti-PNPF or anti-Ln IgGs did not inhibit C4E10 spreading on Fn (Fig. 3B). The partial inhibition by anti-PNPF IgGs of C4E10 cell spreading on Ln, but failure of anti-Ln IgGs of spreading on PNPF, is not unexpected as immuno-cross-reactivity between the cell attachment sites on PNPF and Ln is very poor (24).

Another series of experiments was conducted in which the cells were seeded in culture medium which contained serum that had been depleted of Fn, permitting the time course to be extended to observe attachment after 3 h and enabling more direct comparison of cell spreading of C4E10 and C4E9 cells. In these experiments using medium containing serum, the TCP and other substrata were not pretreated with BSA. The C4E9
cells failed to attach to TCP during a 3-h incubation whereas C4E10 cells were well attached (Fig. 2, e and f) (17). The C4E9 cells did attach to PNPF, with cell spreading onto the PNPF evident at 3 h although the reaction was not as extensive as with C4E9 cells attached to ECM (Fig. 2, g and h). This response of the C4E9 cells to PNPF was blocked by anti-PNPF IgGs (Fig. 2i) but not by anti-Fn IgGs, indicating that any contribution to the attachment reaction from other serum components such as vitronectin (28) was not significant. Preimmune IgGs and direct counting of individual slots showed that the C4E10 immunoprecipitates after 10 weeks of fluorographic exposure, suggesting that the rate of Ln synthesis in C4E10 cells is relatively low (data not shown); similar results were reported for primary cultures of type 2 cells (34).

Effect of Laminin on the Morphology of Cultured C4E10 and C4E9 Cells. C4E10 cells cultured for 24 h on Ln or PNPF substrata showed markedly enhanced cell area compared to control cells cultured on TCP. Quantitation of the effect of substratum on the cell area of individual C4E9 cells cannot be reliably conducted as the cells seed as clumps (17). It is obvious from Fig. 2, l and m, that Ln or PNPF did not have a marked effect on the morphology of C4E9 cells during 24 h culture, as compared to that on TCP. The C4E9 cells had the same island-like pattern of attachment and growth on each of these different laminin substrata as on TCP, and the morphology of the cells was identical, as judged by phase contrast microscopy (see Fig. 2m for example of PNPF substratum).

The morphology of the C4E10 and C4E9 cells on Ln or PNPF substrata was further examined by scanning electron microscopy (Fig. 4). As compared to C4E10 cells grown on TCP, the C4E10 cells grown on Ln, PNPF, or Fn were better spread and flatter, and many cells showed ruffled membranes, suggestive of increased motility of these surfaces. The C4E9 cells grown on Ln, PNPF, or Fn retained a morphology that was generally similar to that of these cells grown on TCP. In summary, the phase contrast microscopy and scanning electron microscopy show that for each of the different substrata, the cell shape of C4E9 cells was quite different from that of C4E10 cells on the same substratum, with the C4E10 cells showing clearly enhanced spreading on Ln or PNPF as compared to TCP.

Synthesis of Laminin by C4E10 and C4E9 Cells. To determine whether C4E10 and C4E9 cells synthesize and secrete Ln, cell proteins were separated by electrophoresis on gradient polyacrylamide gels, transferred to cellulose nitrate, and then probed with anti-Ln antiserum. The C4E10 cells contained a M, 410,000 immunoreactive component (not reduced) which electrophoresed at M, 205,000 following reduction with dithiothreitol (Fig. 5A). From the molecular weight values, before and after reduction, the M, 410,000 (not reduced) band is likely to be a disulfide-linked dimer of the B chains of Ln (20). In the nonreduced extract of C4E10 (Fig. 5A, track 1) there was also a M, 180,000 band which increased to M, 205,000 following reduction; this would correspond (within experimental error) to the behavior expected for the Ln B chain monomer. A faint band of immunoreactive material was observed at M, 100,000, which is almost certainly Ln which has been partially degraded by protease activity either prior to or during extract preparation. No M, 850,000 component was detected in the nonreduced C4E10 extracts. This is unlikely to have been due to inefficient transfer of large glycoproteins to the cellulose nitrate sheet, because standard nonreduced Ln was transferred and readily detected on the blots. It is likely that these C4E10 cells resemble RN22 schwannoma cells, fibrosarcoma cells, human epidermal cells, and dermal fibroblasts (29–33) in being poor at synthesis of the Ln A chain, and the failure to see a M, 400,000 band in the reduced extracts would be consistent with this interpretation. In C4E9 cells, the M, 410,000 and M, 180,000 (not reduced) and M, 205,000 (reduced) component was either not present or was present at a much lower cellular content level (Fig. 5A). The relative cell content levels of Ln components were also demonstrated in slot blot immunoanalysis (Fig. 5B) and direct counting of individual slots showed that the C4E10 cells had at least 40 times more immunoreactive Ln components than the C4E9 cell extracts. In other experiments, C4E10 cells were metabolically labeled with [35S]methionine and immunoprecipitated with anti-Ln antiserum, in order to directly demonstrate synthesis of the A and B chains of Ln. Although the labeling conditions used were suitable for detection of Ln synthesis in bovine corneal endothelial cells and murine F9 teratocarcinoma cells, only very faint bands of specifically immunoprecipitated B chains of Ln could be seen in the C4E10 immunoprecipitates after 10 weeks of fluorographic exposure, suggesting that the rate of Ln synthesis in C4E10 cells is relatively low (data not shown); similar results were reported for primary cultures of type 2 cells (34).

DISCUSSION

The observation that a “switch off” of synthesis of extracellular matrix components such as Fn and Ln occurs during transformation has been well established from studies of transformed mesenchymal cells (10, 11, 14). There have been com-
comparatively few studies, however, of epithelial/carcinoma model systems to determine whether a similar coordinate down-regulation of expression of extracellular matrix components occurs in those cell types and is responsible for the abnormalities of the basal lamina often observed in carcinoma tumors (35). Such studies should be valuable, inasmuch as both the inability of cells within a primary tumor to accumulate a pericellular matrix as well as the ability of circulating tumor cells to bind to extracellular matrix components in other sites such as the subendothelial matrix have been implicated in the process of invasion and metastasis (reviewed in Refs. 12 and 36). The NAL1A alveolar cell system is a suitable cell model for such studies in epithelial cells, because the clonal malignant cells may be compared to clonally related control cells (6, 17, 25). The control cells are capable of serial subculture but are anchorage dependent and are not tumorigenic, whereas the transformed cells are anchorage independent and form s.c. carcinomas and metastatic lung deposits in mice (6–8, 25). We have shown previously that the control NAL1A cells synthesize and secrete Fn and accumulate Fn into a pericellular matrix, whereas synthesis and secretion of Fn could not be detected in the transformed cells (17). As part of a continuing investigation of this model system, we have studied the expression of Ln components in the control and transformed cells. The transformed and control cells also differ in cellular content of Ln: the control cells had an immunoreactive Mr 410,000 component which almost certainly consists of disulfide-linked B chains of Ln, whereas the transformed cells had at least a 40-fold reduced cellular content of this component. Therefore the cellular accumulation of Ln in our model system is similar to that found in transformed mesenchymal cells, where a coordinate change in expression of Fn and Ln upon transformation was observed (14). In other rodent cell culture systems, however, an unchanged or enhanced expression of Ln was observed with chemically transformed murine AKR fibroblasts or rat liver TRL1215 epithelial cells (37, 38). There are several studies of the effect of transformation on Ln expression on human cells of epithelial origin (39, 40) showing that with human keratinocytes, both normal keratinocytes and squamous cell carcinoma cells (whether derived from primary or secondary tumors) synthesized and secreted Ln (39). In view of the role that cellular interaction with basal lamina components such as Ln are believed to play in the cellular phenotype of anchorage-dependent growth (41, 42) and disturbance of the normal stasis during metastatic processes (12), further studies of Ln expression in rodent metastatic carcinoma model systems and in human cell lines of epithelial origin should be valuable.

During the transformation of NAL1A cells in culture, the cells underwent a morphological change analogous to that observed during transformation of mesenchymal cells (6, 9, 25). Various studies of mesenchymal models of transformation have shown that the addition of Fn to transformed cells restored the normal cell shape, indicating an important role played by the extracellular matrix in the control of mesenchymal cell shape (15, 16). In our previous study (17) we showed that the control shape of transformed NAL1A cells differs from transformed mesenchymal cells in that although the control NAL1A cells showed enhanced cell spreading on a Fn as compared to TCP, the malignant cells had the same cell shape on Fn as on TCP. Because Ln is a potent attachment factor for epithelial cells with impact upon epithelial cell morphology and polarity (19, 20, 43), we have now compared the attachment and spreading of control and transformed NAL1A cells on Ln and a laminin-like neurite promotion factor, PNPF. PNPF was included in this study because we have found that for neurite extension on Ln, PNPF, or ECM, the PNPF more closely reflected the conformation of Ln that is found in ECM than purified Ln (data not shown). PNPF may differ from Ln by having a cellular attachment site additional to those on purified Ln (44, 45), or else the conformation of the sites on Ln may be altered on PNPF as a consequence of association with heparan sulfate or entactin (24). As part of these experiments, we were able to test whether the transformed NAL1A cells showed a preference for Ln as compared to Fn, as has been observed with epithelial cells during studies of the PAM212 cell line (19). This study shows that Ln or PNPF was as effective as Fn for the attachment of transformed or control NAL1A cells, albeit with higher coating concentrations of these molecules being required for

Fig. 4. Cell morphology of C4E10 and C4E9 cells viewed by scanning electron microscopy. C4E10 cells (a–e) and C4E9 cells (d–f) were cultured for 24 h in medium containing Fn-depleted serum on TCP (a, d), PNPF (b, e), or Fn (c, f). Bar, 50 μm.
concentrations (ng/ml) of purified Ln. Optimal activity. These results are consistent with reports of relative Ln content of extracts of confluent cultures of C4E10 cells (Column/) and C4SE9 cells (Column 2). Successive tracks are 2-fold dilutions of each of the extracts of C4E10 (Tracks 1 and 2) or reduced (Tracks 3 and 4) conditions, transferred to cellulose nitrate and protein blots, probed with Ln antiserum. Arrows, positions of standard proteins. The Ln column shows the reaction obtained with the indicated concentrations (ng/ml) of purified Ln.

Both Ln and Fn are important adhesion molecules for the transformed NAL1A epithelial cells as well as for the control cells. The failure of these transformed cells to assemble a pericellular matrix containing Ln and Fn would therefore be expected to have important consequences for the malignant phenotype including the metastatic dissemination of tumor cells to form secondary tumors (see reviews in Refs. 12 and 36 for further discussion). In further studies we will examine whether the greatly decreased level of cell-associated Ln in malignant C4E9 cells as compared to the control cells is due to a switch-off of transcription and translation from the Ln genes. Other possible lesions in Ln accumulation in C4E9 cells could be either a defect in the cell surface receptor leading to a failure to assemble Ln into a pericellular matrix (see Ref. 49) or proteolytic degradation of newly synthesized Ln as a result of secretion of proteases by the C4E9 cells (see Ref. 50). Irrespective of the outcome of those studies, it is clear from the attachment experiments in this and the previous study (17) that the altered morphology of the transformed cells cannot be attributed solely to a defect in the production and assembly of an extracellular matrix containing Ln and Fn. The consequences of the observed inability of the transformed cells to change cell shape in response to ECM components could extend to both altered growth control and the increased metastatic behavior of the malignant cells (41, 42, 51). It will therefore be interesting to test in future studies whether the difference in the behavior of the malignant cells (41, 42, 51) will be attributed solely to a defect in the production and assembly of an extracellular matrix containing Ln and Fn. The consequences of these results with reports of optimal activity. These results are consistent with reports of epithelial cells and hepatocytes responding to immobilized Ln by enhanced attachment and spreading (reviewed in Refs. 20 to 22). The binding of epithelial or other nonneuronal cells to PNPF has not been reported previously, but the attachment of C4E10 and C4E9 cells to PNPF is consistent with the Ln content of PNPF, with recent results indicating that there are multiple binding sites on purified Ln for nonneuronal cell attachment (44).

In contrast to the enhanced spreading of control C4E10 cells on Ln or PNPF substrata, the attachment of the transformed cells to Ln or PNPF does not lead to a change in cell shape. The response of the transformed cells to exogenous Ln or Ln-like substrata is therefore similar to the attachment of these cells to Fn and in contrast to the cell spreading of the control cells on surfaces coated with either Ln or Fn. Summarizing the results of this and our previous study (17), the transformed and control NAL1A cells have similar ability to attach to Ln or to Fn, but maintain a distinctive cell shape from each other on these substrata. The results of the attachment assay show that the transformed cells can bind to Fn, Ln, or ECM components and so the failure of the transformed cells to revert to the shape of the control cells when cultured on these substrata is not due to these cells being entirely refractory to these molecules [cf. our previous study (17) and in comparison with TRL1215 cells (47, 48)].

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