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Do cells show an inverse locomotory response to fibronectin and laminin substrates?

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Sixteen cell types from a variety of tissues and from primary and secondary cell cultures and established cell lines were tested for their ability to distinguish between fibronectin and laminin substrates during locomotion *in vitro*. Laminin and fibronectin were presented to the cells as directly adjacent tracks. Most cells, regardless of origin, showed no preference for one substrate over the other. Only two of the cell types tested showed a strong preference for one or other other substrate molecule. Cells were responding to the local substrate, since antibodies directed against one substrate molecule only interfered with locomotion on tracks coated with that molecule. We conclude that many cells simultaneously express functionally active receptors for fibronectin and laminin, and that differential locomotory response to these two molecules cannot be assumed without experimental confirmation.

Key words: cell locomotion/fibronectin/laminin/extracellular matrix

Introduction

The adhesion, spreading and locomotion of several cell types, including established cell lines and primary cell cultures, have been reported to depend on the particular extracellular matrix (ECM) component provided as their substrate (Vlodavsky and Gospodarowicz, 1981; Rovasio *et al.*, 1983). The generalisation has arisen that the adhesion and spreading, and hence locomotion, of epithelial cells is favoured by laminin (LN) substrates, while for mesenchymal cells fibronectin (FN) is preferred (Hogan, 1981; Kleinman *et al.*, 1981; Terranova *et al.*, 1981). Cell locomotion is crucial for morphogenesis, wound healing and the metastatic spread of tumour cells (Carter, 1965; McMinn, 1969; Newgreen, 1982), so evidence of a differential locomotory response mediated *via* an interaction with components of the ECM would give a key to how such locomotion is controlled (Carter, 1967; Ghysen, 1978; Abercrombie, 1979; Vlodavsky and Gospodarowicz, 1981; Newgreen, 1982; Raper *et al.*, 1983; Turner *et al.*, 1983). However, a number of results are in conflict with the generalisation concerning FN or LN preference. Couchman *et al.* (1983) found that mesenchymal fibroblast-like cells attached and spread well on both FN and LN, as did Newgreen (1984) using mesenchymal neural crest cells. Moreover, Palottie *et al.* (1983) found that teratocarcinoma cells (which resemble epithelial-endoderm) adhered better to FN than to LN, and Newgreen (1984) reported that spreading of embryonic endoderm was more rapid on FN than on LN. Recent data suggest that cell locomotion can be controlled *via* cell surface receptors other than those for FN and LN (Goodman *et al.*, 1985).

In an attempt to resolve whether there are, as a rule, cell-class-specific locomotory responses to LN and FN we have investigated the movement *in vitro* of a number of transformed and untransformed cell types derived from epithelia and mesenchyme, using a substrate of parallel and immediately adjacent tracks of LN and FN. We found that most cell types could migrate equally well over both LN and FN substrates and ignored LN/FN boundaries. Thus the control of cell locomotion by differential recognition of FN and LN is not a general phenomenon; nevertheless, we found some cell types which could respond differentially.

Results

Three classes of cell locomotory response to FN and LN

Cells were seeded at high density onto a starting area of LN- or FN-coated substrate, and next to a zone of mutually exclusive and directly adjacent FN and LN tracks that were initially shielded with a coverslip (Figure 1; see Newgreen, 1984). After initial spreading, generally 0.5 – 1 h for mesenchymal cells, 1 – 2 h for epithelially derived cells and up to 4 h for SV3T3 cells, unattached cells were washed away, and adherent cells were allowed to enter the tracked zone by removal of the coverslip. The motile behaviour after 24 – 48 h on FN relative to LN substrates could be compared because directly adjacent tracks of the two molecules were present.

The behaviour of the cells fell into three classes (Table I). Cells in the first class (12 of the 16 tested) showed no observable difference in their ability to locomote on FN and LN (e.g., Figure 1 f – h; Table I) and individual cells were able to span a FN/LN border. In control experiments, where FN or LN tracks were flanked by tracks blocked by coating with bovine serum albumin or haemoglobin alone (Figure 1b,c; Table I), these cells were strongly constrained to the LN and FN tracks. This class included transformed and untransformed cell lines of mesenchymal and epithelial origin, and both primary and secondary cell cultures. The two cell types in the second class, CSG 120 mouse carcinoma and A375 human melanoma, behaved on alternating FN and LN tracks as did the first class, but in control experiments the cells also locomoted over tracks coated with blocking proteins. This behaviour was not due to non-motile cells on the blocked tracks being dragged forward by cells locomoting on flanking FN or LN tracks; the advancing cell front was not curved as one would expect if this were the case, and the cells extended lamellipods onto blocked substrates. Either these cells are remarkably tolerant of their substrates or modify them, e.g., by secreting protease that can negate the effect of adsorbed proteins or by producing adhesive molecules of their own (cf. Avnur and Geiger, 1981). The third class of cells were able to locomote only on one of the ECM molecules (NIH 3T3 on FN and Ru glioblastoma on LN) and formed sharp 'tongues' as they moved off the starting area onto the FN and LN tracks (Table I, Figure 1 i – m). These

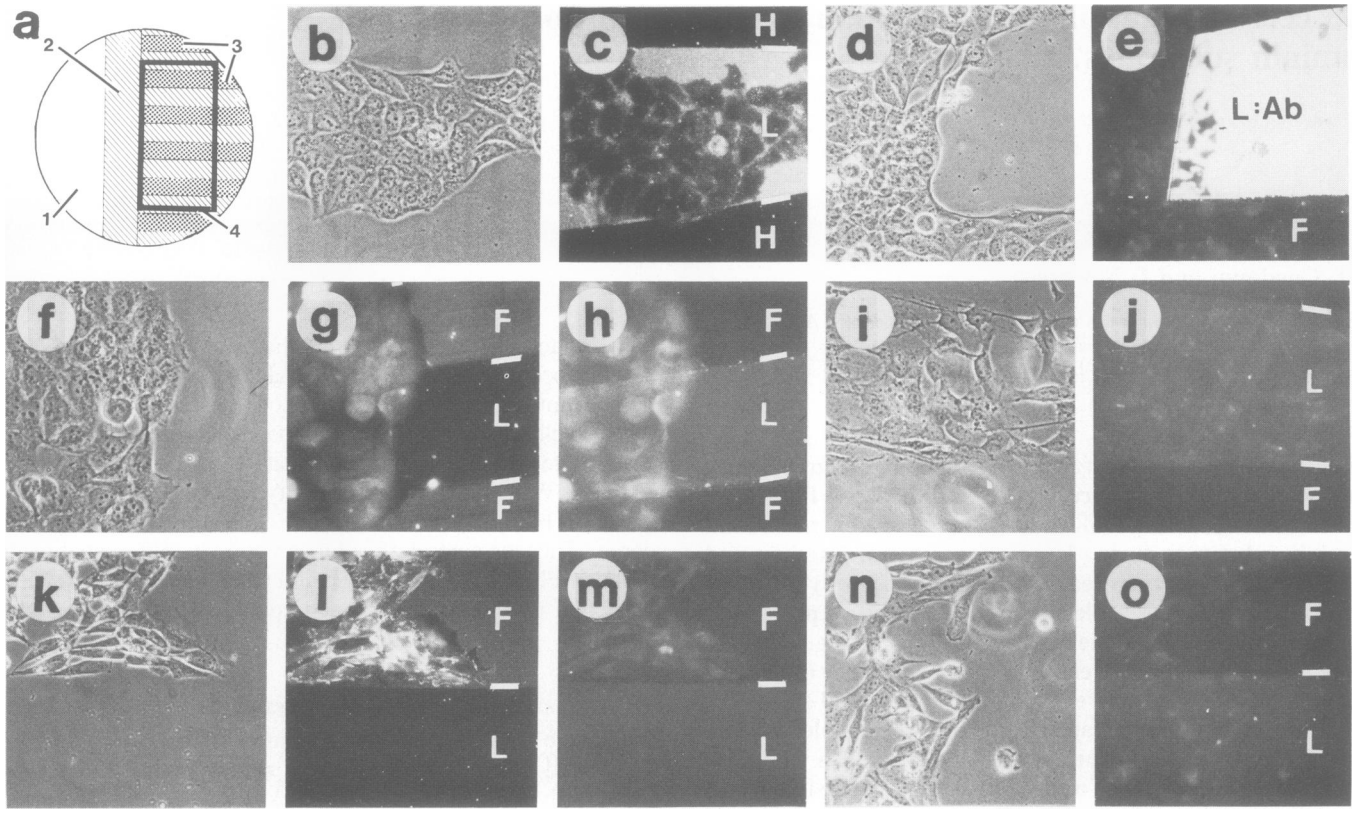

Fig. 1. The locomotion of cells on tracked substrates. **(a)** Shows the experimental set-up: round glass coverslip (1), starting zone where cells were plated (2), scraped tracks (3), shielding chip of coverslip (4). **(b – o)** Show phase contrast and IF pairs of fixed unpermeabilised cells after migration. **(b – h)** TR126 human carcinoma cells: migration on alternating tracks of **(b,c)** LN and haemoglobin, or **(d – h)** LN and FN; **(b,d,f)** phase contrast **(c,e,h)** IF for LN, **(g)** IF for FN; in **(d,e)** the LN tracks were pre-blocked with a polyclonal rabbit anti-LN. **(i,j)** Ru human glioblastoma cells migrating on alternating tracks of LN and FN; **(i)** phase contrast **(j)** IF for LN. **(k,l,m)** NIH 3T3 murine fibroblasts migrating on alternating tracks of FN and LN; **(k)** phase contrast **(l)** IF for FN **(m)** IF for LN. **(n,o)** SV40-transformed NIH 3T3 cells migrating on alternating tracks of LN and FN; **(n)** phase contrast **(o)** IF for LN. Migration times were 24 h **(b – m)** or 48 h **(n,o)**. All photographs at 166 × magnification.

cells were, like class one, strongly constrained by the blocking proteins.

The behaviour of the cells reflects a recognition of substrate-bound molecules

The position of motile cells with respect to the tracks was assessed by indirect immunofluorescence using antibodies to FN and LN. This indicated that there was a very low degree of cross-contamination of one substrate with the other (e.g., Figure 1c,e). To confirm the functional independence of adjacent tracks, cell translocation was examined after pre-incubating the substrate with rabbit polyclonal antibodies directed against LN or FN. Anti-LN completely inhibited cell locomotion on LN tracks, but cell behaviour on immediately adjacent FN tracks was unaffected (Figure 1d,e). Anti-FN strongly, but not completely, inhibited locomotion on FN but not on LN tracks. The limited residual locomotion could possibly be due to cell-surface FN being cross-linked by antibody to the substrate thereby allowing some cell attachment and spreading. The functional independence of the tracks was further revealed by NIH 3T3 and Ru glioblastoma which clearly could distinguish LN from FN.

Discussion

In this investigation we have studied various cell types, some of which had been in culture for extended periods while some were only recently established or were primary or secondary cultures. We have tested them for their ability to show a preference for FN or LN as substrates for locomotion. Most cell types (14/16) could use both substrates equally well, and their locomotion over FN and LN could be independently inhibited by the respective monospecific polyclonal antibodies to these substrate molecules. A minority (2/16) of cell types recognise either FN or LN but not both. From this we conclude that, (i) the blocking procedures used to mask one track from another were sufficient to produce tracks mutually exclusive to a level exceeding the discriminating ability of the cells, and (ii) the locomotion of the cells, with the exception of CSG 120 and A375, on each substrate reflects a true recognition of that substrate, and not a response to underlying serum-coated glass. Our data, especially the observation that cells can routinely span FN/LN borders, suggest that most of the cells that we used, be they of epithelial or mesenchymal origin, either have or can rapidly express cell-surface 'receptors' for both FN and LN simultaneously. These observations are probably not due to the passage of cells into tissue culture as primary and secondary cultures, and established cell lines could all show these responses.

Many of the differences in behavioural response to FN and LN reported in the literature may be due to the type of assay (Rovasio *et al.*, 1983; Newgreen, 1984). For B16 cells in filter assays LN and FN stimulate the same degree of haptotactic crossing of the filter (McCarthy and Furcht, 1984). Here we are concerned only with true cell locomotion on FN and LN, where the cells move up to 500 μm; this may be an appropriate model for cells moving over the ECM *in vivo*. Most assays comparing these two molecules, however, test the rate of attachment to a substrate of cells settling from suspension (see e.g., Vollmers and Birch-

Table I. The locomotion of cells on tracked substrates

Cell[a] type	Transformed or normal	Species	Tissue[b] origin	Ability to locomote over FN/bl	LN/bl	FN/LN
Class 1						
SV3T3	T	Mouse	M	+/−	+/−	+/+
MRC5	N	Human	M	++/−	++/−	++/++
Strömer	T	Human	M	++/−	++/−	++/++
WI38	N	Human	M	++/−	++/−	++/++
TR126	T	Human	E	++/±	++/±	++/++
HeLa	T	Human	E	++/−	++/−	++/++
BHK	N	Hamster	M	++/−	++/−	++/++
MDCK	N	Dog	E	+/−	+/±	+/+
CEF[5-11]	N	Chicken	M	++/−	++/−	++/++
SR-1	T	Chicken	M	++/−	++/−	++/++
NQNc	N	Quail	M	++/−	++/−	++/++
NQScl	N	Quail	M	++/−	++/−	++/++
Class 2						
CSG 120	T	Mouse	E	++/++	++/++	++/++
A375	T	Human	M	++/++	++/++	++/++
Class 3						
3T3	N	Mouse	M	++/−	±/−	++/−
RuGli	T	Human	M	±/−	++/−	±/++

Cells migrated on parallel tracks of fibronectin (FN) and laminin (LN) in direct apposition (FN/LN), or in apposition to tracks blocked with a blocking protein (bl; bovine haemoglobin or serum albumin) to which most cells were poorly or non-adherent (FN/bl; LN/bl; see Figure 1a).
++ indicates motility over the substrate comparable with NQNc;
+ indicates lower but still substantial locomotion; ± indicates poor locomotion; − indicates no locomotion.
[a]For cell derivations see Vollmers and Birchmeier (1983) and Lane *et al.* (1982).
[b]Immediate antecedent tissue type.
Transformed=T; normal=N; epithelial=E; mesenchymal=M; CEF=chick embryo fibroblast secondary culture (same results observed at passage 5, 7, 9 and 11); SR-1=Rous sarcoma transformed CEF; NQNc=normal quail embryo neural crest; NQScl=normal quail embryonal sclerotome: both primary culture. MRC5, WI38=diploid human embryonic fibroblast (mortal); 3T3=NIH 3T3 mouse fibroblasts; SV3T3=SV40 transformed 3T3; TR126, HeLA=human carcinoma; CSG 120=murine carcinoma; Strömer, A375=human melanoma. BKH, MDCK=kidney lines; RuGli=gioblastoma.

meier, 1983) or their longer term differentiation and biosynthetic activities.

There are several possible interpretations of our data. Firstly, it could be that FN and LN substrates elicit different locomotory responses from adult cells, but cells of embryonal origin (such as MRC5, WI38, CEF, NQScl and NQNc) may have not yet acquired an ability to distinguish between FN and LN as preferred substrates. Fully transformed cells may have lost any ability that they once possessed and are re-expressing a more flexible, embryonal phenotype. This view is supported by the fact that the FN-favouring NIH 3T3 cell, when transformed with SV40, became able to locomote on LN too (Figure 1n,o), and that mesenchymally derived transformed cells (e.g., Strömer) recognise LN, while transformed cells of epithelial origin recognise FN. Secondly, it is possible that LN and FN have little or no intrinsic differential control over cell translocation, so that most cells will locomote equally well over both when given a choice. The control may perhaps lie in whether the cells are given a choice; restricted access to adhesive substrata *in vivo* may be a critical controlling factor. Finally, it is possible that other, as yet undiscovered molecules at the substrate and their corresponding receptors at the cell surface can control cell locomotion.

We conclude that either FN or LN may be necessary for cell locomotion on otherwise non-adhesive substrata and that some cell types do have a strong preference for one molecule or the other. Thus the generalisation that locomotory control can be exerted by a response to the LN, permissive only for epithelial cells, or to FN, permissive only for mesenchymal cells, does not seem justified. We have shown here that many cell types can locomote over both molecules.

Materials and methods

Preparation of the substrates

The experimental set-up is shown in Figure 1a. FN (20−80 μg/ml) was adsorbed (30 min, 37°C) onto a round glass coverslip. After washing, the surface was blocked with bovine haemoglobin or heat treated (80°C, 5 min) bovine serum albumin (10 mg/ml, 30 min, 37°C) to prevent adsorption of other proteins. After washing, tracks were scraped free of protein with a plastic scriber, washed, and a second protein [either LN (20−80 μg/ml) or blocking protein] was adsorbed onto the scraped tracks. Proteins were dissolved in 150 mM NaCl, 10 mM sodium phosphate, pH 7.4 (PBS) which was also used for the washings. The surface of the coverslip was never allowed to dry. In some cases the sequence of application of FN and LN was reversed. In immunoblocking experiments, the coverslip was then exposed to 100 μg/ml rabbit polyclonal anti-FN or anti-LN antibody (Bethesda Research Laboratories) in PBS plus 1 mg/ml BSA (1 h, 37°C). FN was prepared by affinity chromatography on immobilised gelatin of bovine plasma (Engvall and Ruoslahti, 1977). Haemoglobin and serum albumin were purchased from Sigma, and LN and polyclonal antibodies from Bethesda Research Laboratories.

Cell culture and immunofluorescence

The tracked region was shielded with a chip of coverslip, and cells were plated (3×10^5 cells/cm^2) in Dulbecco's modified Eagle's medium containing 3% heat inactivated and FN-depleted (Engvall and Ruoslahti, 1977) fetal calf serum ('medium'). After 0.5−4 h non-adherent cells were washed away with medium and the chip removed, allowing the cells to migrate from the starting area into the tracked region. Cultures were observed for 24 h (48 h for SV3T3 cells) before fixation and indirect immunofluorescence. With the exception of NIH 3T3 and Ru glioblastoma which only plated on FN and LN, respectively, the composition of the starting area and the order in which LN and FN were applied to produce tracks had no influence on subsequent cell behaviour. For further details and indirect immunofluorescence procedure, see Newgreen (1984).

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