Monensin-dependent and -independent mechanisms of cell-matrix adhesion

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Attachment and spreading of human FL cells on a subcellular matrix (SCM) preparation made by treating confluent cell monolayers with deoxycholate are insensitive to the presence of monensin. However, if the cell suspension is surface-iodinated prior to adhesion using the LPO/H_2O_2 system, cell spreading on SCM is inhibited by 1 μM monensin. The suggested interpretation is that cell surface components required for cell spreading on SCM are inactivated by iodination and need replacement from intracellular reserves by a monensin-sensitive pathway. This pathway is not required in the absence of iodination when sufficient surface components (or a monensin-independent pathway of surface expression) are available. Support for this interpretation is obtained by means of double-iodination experiments in which surface-labelled cells adhere and spread, are detached and labelled a second time and then allowed to adhere again to SCM. Cell spreading in the second case is inhibited by $\sim 80\%$, suggesting that both previously expressed and newly recruited receptors are inactivated.

Adhesion *Extracellular matrix* *Monensin* *Surface labeling*

1. INTRODUCTION

Numerous components of extracellular matrices including fibronectin, laminin and the collagens have been shown to be capable of promoting cell adhesion in vitro [1]. Recently, cell surface 'receptors' for matrix molecules have also been isolated [2–6] and it has been suggested that these may be transmembrane entities which, when associated at the external face of the membrane with a matrix component, send signals into the cell to begin reorganization of the cytoskeleton [6,7]. This then gives rise to the change from the rounded morphology of a newly settled adherent cell to the flattened extended shape typical of cells entering the growth cycle [8–10]. Evidence also suggests that adherent cells show non-uniform plasma membrane composition [6,11,12], so that in a monolayer of cells, components associated with adhesion to subcellular matrix or the growth surface may be concentrated in the basal area [13]. Since dissociated cells are expected to settle on a new substratum in random orientations, it may be expected that a reorganization of the plasma membrane occurs during the sequence of events involved in attachment, spreading and the formation of close or focal contacts [1]. Such reorganization may involve lateral diffusion or incorporation of new components into the cell membrane from intracellular reserves.

Molecules present at the external face of the plasma membrane can be analysed by vectorial labelling methods such as lactoperoxidase-catalysed iodination [14]. Intracellular shuttling of membranous components and vesicle-entrapped aqueous phases via the Golgi apparatus are disrupted by the cationophore monensin [15]. In

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Abbreviations: SCM, subcellular matrix; LPO, lactoperoxidase; HBSS, Hank's balanced salt solution

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this study, we have used a combination of these 2 methods to demonstrate that cell adhesion to subcellular matrix can occur either with or without a monensin-sensitive step.

2. MATERIALS AND METHODS

Human FL cells were cultured as described [16]. Subcellular matrix was prepared from confluent cell cultures by treatment with 2% sodium deoxycholate, 0.2 mM EDTA as described [17]. For adhesion assays [16], cells were brought into suspension by treatment with trypsin-EDTA, washed twice and resuspended in medium without a serum supplement except where otherwise indicated. Assays were either quantitated by scoring >250 cells (>8 fields) as either rounded or spread using previously established criteria [16,18] (fig.1), or assessed semi-quantitatively using the following gradations (tables 1 and 2): 4+, 80–100% of cells spread; 3+, 60–80%; 2+, 40–60%; +, 20–40%; (+), 5–20%; −, 0–5%. This method has been shown to be reproducible using 2 independent observers. Monensin (sodium salt, Sigma) was added to the cell suspension immediately prior to the adhesion assay at a final concentration of 1 μM from a 100-fold concentrated stock solution in ethanol. Ethanol alone did not affect cell adhesion.

For iodination of cell surfaces, cells (~10^7) were dispersed with trypsin, washed twice in HBSS, then resuspended in 1 ml HBSS containing 80 μg lactoperoxidase (Boehringer, Mannheim), 10 mM glucose, 0.75 U glucose oxidase (Sigma) and 1 μM KI, and incubated for 10 min at 37°C. 9 ml culture medium were then added and the cells pelleted and washed twice before use in adhesion assays. In experiments not described here, addition of $K^{125}I$ to this mixture led to incorporation of radioactive iodine into a spectrum of FL cell surface glycoproteins (V.M.N., unpublished and [19]).

3. RESULTS AND DISCUSSION

Confluent FL cell cultures could be dissolved rapidly from plastic surfaces by treatment with 2% deoxycholate. The residual SCM was free of cytoskeletal components and almost free of residual plasma membrane as determined by immunoassay with antibodies to plasma membranes. However, it did retain the ability to promote attachment and spreading in a population of rounded trypsinized cells settling in serum-free medium (fig.1). The kinetics of this process were such that most cells had spread to a polygonal shape after 2 h. This matrix does not contain significant quantities of fibronectin or laminin, but contains a proline-rich glycoprotein of ~70 kDa and a bacterial collagenase-sensitive component in which the adhesion-promoting activity resides. Detailed characterization will be presented elsewhere. When added instead to bare plastic, the cells settled but remained rounded (fig.1).

The presence of monensin at 1 μM during adhesion of FL cells to detergent-generated SCM resulted in an unchanged response in terms of both kinetics and cell morphology (table 1), suggesting that vesicular shuttling via the Golgi apparatus is not a prerequisite for normal adhesion. It was noted in a separate series of experiments involving lactoperoxidase-mediated iodination of externally disposed plasma membrane components ([19] and

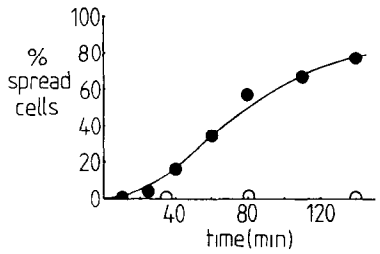

Fig.1. Kinetics of spreading of FL cells after attachment to plastic (○) or SCM deposited by a previous monolayer of cells (●).

Table 1

Spreading of FL cells on subcellular matrix in the presence and absence of monensin (1 μM) and the effect of surface iodination

Monensin	Surface iodination	Cell spreading (2 h)
−	−	4+
+	−	4+
−	+	4+
+	+	+

Based on the kinetics in fig.1, assays were scored after 2 h

V.M.N., unpublished) that incorporation of new iodinatable polypeptides into the cell surface from intracellular membranes occurs during cell-substratum adhesion. Treatment of cell suspensions with LPO/H_2O_2/KI prior to settling did not inhibit cell spreading (table 1) although numerous plasma membrane glycoproteins were labelled (not shown). However, if after surface iodination cells were added to SCM-coated surfaces in the presence of 1 μM monensin, cell spreading was almost completely inhibited (table 1). This result suggests that matrix receptor molecules available at the cell surface are inactivated by iodination, and need to be replaced from an intracellular (Golgi-associated) reservoir. This replacement step is monensin-sensitive. According to this interpretation, normal adhesion to SCM probably utilizes receptors already present at the surface. If, however, these are inactivated, cell spreading may still proceed after rapid recruitment of new receptors to the surface. Published studies of the effect of monensin on endocytotic pathways suggests that internalization of surface moieties may be unaffected [20–22]; unoccupied receptors may even retain the capacity to return rapidly to the cell surface [22]. Recruitment of new molecules from Golgi-associated pools may, however, be interrupted [15].

This interpretation was supported by a further series of experiments in which cells were surface-iodinated, allowed to attach and spread on SCM, then dissociated from the substratum using trypsin, surface-labelled once more, and finally allowed to settle and adhere a second time on SCM. In this way, if an intracellular pool of receptors for SCM is mobilized in the first adhesion step, it is then inactivated by LPO before the second adhesion process. As predicted, a striking (80%) reduction in the ability of the cells to spread in the second stage of adhesion was observed (table 2), suggesting that the intracellular receptor pool is brought to the cell surface in the first stage of adhesion and thus rendered accessible to inactivation by LPO. Residual cell spreading could now also be abolished by monensin (table 2). This residual spreading therefore reflects a small pool of active intracellular receptors still remaining uniodinated.

Table 2

Spreading of twice-iodinated FL cells on SCM and the effect of monensin

Time (h)	Supernatant medium	Control (no added drug)	+ 1 μM monensin
3	MEM/20% FCS	+	−
3	MEM	+	−
20	MEM/20% FCS	4+	−

After subsequent incubation for 20 h in the presence of serum, cells recovered to attach and spread normally on SCM (table 2). Again, membrane recycling is probably involved, because the recovery is sensitive to monensin.

For normal cells, attachment to a surface and spreading are prerequisites for entry into the growth cycle and synthesis of macromolecules [8–10]. The present experiments suggest that if suitable surface components for adhesion are not available, recruitment can take place from cytoplasmic reserves to allow adhesion to occur rapidly (fig.1, table 1). Intracellular shuttling may also be required to redirect plasma membrane moieties to areas where adhesive contact with extracellular matrix occurs. Other studies [23] have shown that initial spreading of human fibroblasts on plastic in serum-free medium is inhibited by monensin, suggesting that monensin sensitivity may vary depending on the cell-substratum recognition system chosen. Details of this variation, which we have confirmed using FL cells, will be published elsewhere.

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