J. Cell Sci. **86**, 95–107 (1986)

Printed in Great Britain © The Company of Biologists Limited 1986

THE LACK OF ATTACHMENT OF TRANSFORMED EMBRYONIC LUNG EPITHELIAL CELLS TO COLLAGEN I IS CORRECTED BY FIBRONECTIN AND FXIII

M. PAYE AND CH. M. LAPIÈRE*
Laboratoire de Dermatologie Expérimentale, Université de Liège, CHU du Sart-Tilman, B23, B-4000 Sart-Tilman, par Liège 1, Belgium

SUMMARY

PER cells, a transformed pulmonary epithelial cell line that adhered to a large extent to a fibronectin substratum, were found to be attachment-deficient to collagen I. Although fibronectin can bind to collagen I monomers and polymers, the addition of exogenous fibronectin in the attachment medium induced the adhesion of these cells to collagen I polymers but not to monomers. By adding the transglutaminase of blood coagulation, FXIII, in the presence of fibronectin, the attachment of PER cells to collagen I monomers could be recovered while the minimal concentration of fibronectin needed to promote their adhesion to polymers was lowered. These studies indicate that FXIII enhances the fibronectin-mediated attachment of PER cells to collagen I.

INTRODUCTION

Cell–substratum adhesion represents a crucial point in the interaction between the cells and their environment. It is involved in cell migration and proliferation during embryogenesis (Boucaut & Darribere, 1983; Mauger *et al.* 1983), in wound healing (Grinnell, 1984), in tumour cell invasion and metastasis (Terranova *et al.* 1982, 1984; Ruoslahti, 1984) and in other processes.

Eukaryotic cells seem to adhere to their support by means of focal contact of specific membrane components reinforced by secreted attachment protein as proposed by Birchmeier *et al.* (1982). Fibronectin and laminin are well-known attachment proteins, depending on the type of cell and of the nature of the support. Fibronectin binds fibroblasts preferentially to types I and III collagen (Grinnell & Minter, 1978; Grinnell & Bennett, 1981) or to fibrin (Grinnell *et al.* 1980), while laminin mediates the attachment of epithelial cells to type IV collagen (Terranova *et al.* 1980). Some epithelial cells, normally connected to a basement membrane, can however adhere to the connective tissue fibres through fibronectin as epidermal cells do during wound healing before the reconstruction of a basement membrane (Clark *et al.* 1985).

* Author for correspondence.

Key words: epithelial lung cells, cancer, cell adhesion, collagen, fibronectin, FXIII.

The attachment properties of transformed cells are more complex. Virus-infected and transformed fibroblasts may lose their capacity to synthesize and/or deposit fibronectin in the pericellular matrix or at the cell surface (Vaheri & Mosher, 1978; Hayman *et al.* 1981). This mechanism could be involved in the release of metastases (Vaheri *et al.* 1978; Vartio *et al.* 1983). The same process is known to occur in some transformed epithelial cells (Alitalo *et al.* 1982; Keski-Oja *et al.* 1982).

It has been demonstrated that the full biological activity of fibronectin for inducing adhesion of fibroblasts to fibrin is expressed when these proteins are cross-linked by the activated blood coagulation factor XIII (FXIII) (Grinnell *et al.* 1980). FXIII is a transglutaminase that catalyses the formation of covalent bonds between ε-lysyl and γ-glutamyl residues (Duckert, 1973; Henriksson & McDonagh, 1983). Its activity has been demonstrated in the formation of links between fibrin monomers (McKee *et al.* 1970; Kasai *et al.* 1983), reduced fibronectin molecules (Mosher, 1975; Keski-Oja *et al.* 1976), fibrin and fibronectin (Grinnell *et al.* 1980) and fibronectin with collagen I (Mosher & Schad, 1979; Mosher *et al.* 1980; Mosher, 1984).

In order to study the potential involvement of fibronectin in the adhesive functions of epithelial cells, we selected a transformed epithelial cell line lacking attachment properties to type I collagen. This cell line established by Michiels *et al.* (1981) from embryonic rat lung explants is PER. Their phenotype and growth characteristics *in vitro* are described in the accompanying paper (Paye *et al.* 1986). The experiments described in this paper were designed to study the inducing effect of exogenous fibronectin, alone or polymerized by FXIII, on the adhesion properties of PER cells to type I collagen.

MATERIALS AND METHODS

Materials

Fibronectin, laminin and the collagen type I were purified as described below. All other products were from commercial sources: culture medium and trypsin solution (Gibco), foetal calf serum (Flow), culture plates (Nunc), silicon (Sigmacote) and soybean trypsin inhibitor (Sigma), bovine serum albumin (BSA) (Armour), FXIII subunit A (62·5 units ml^{-1}; Behringwerke A.G.), thrombin (30 units ml^{-1}; Behringwerke A.G.) and bis-benzimidazol H 33258 (Hoechst).

Cell cultures

Calf skin fibroblasts were isolated as described (Delvoye *et al.* 1983). Spontaneously transformed pulmonary epithelial rat (PER) cells have been described (Michiels *et al.* 1981) and characterized (Paye *et al.* 1986). Cells were grown in bicarbonate-buffered Dulbecco's modified minimum Eagle's medium (DMEM) supplemented with 10% foetal calf serum (FCS), glutamine (0·3 mg ml^{-1}) and ascorbic acid (50 $\mu g\, ml^{-1}$) and transferred at 1/3 dilution after trypsinization (0·1% trypsin, 0·02% EDTA) when they were subconfluent. For the attachment experiments, subconfluent monolayer cultures between passages 5 and 15 for fibroblasts and between passages 30 and 50 for PER cells were used.

Preparation of collagen, fibronectin and laminin

Collagen was purified from foetal bovine skin as described (Lapière *et al.* 1977). Before use, it was dialysed against 0·01 M-NaH_2PO_4/Na_2HPO_4, 0·3 M-NaCl, pH 7·4, and adjusted with the same buffer to a concentration of 30 $\mu g\, ml^{-1}$.

Plasma fibronectin was purified using the method of Yamada (1983) by successive chromatographic procedures on Sepharose 6-B, gelatin–Sepharose and heparin–Sepharose. Laminin was extracted from murine Engelbreth-Holm-Swarm tumour according to Timpl *et al.* (1979). The purity of fibronectin and laminin preparations was monitored by sodium dodecyl sulphate (SDS)–polyacrylamide slab gel electrophoresis. Fibronectin was more than 95% pure and the preparation of laminin was shown to contain 10% of entactin. Fibronectin and laminin in solution were stored in 0·01 M-Tris·HCl, pH 7·4, in liquid nitrogen. The protein concentration was determined by measuring the absorbance at 280 nm (fibronectin 1·1 and laminin 1·0 mg ml^{-1} cm^{-1} per O.D. unit) and adjusted at 30 μg ml^{-1} with the storage buffer.

Preparation of attachment supports

The attachment supports were prepared in 24-well plates (Nunclon) by drying overnight 350 μl of the protein solution at 4°C in a desiccator to obtain collagen monomers, and at 37 °C in air for collagen polymers, fibronectin and laminin. Dried salts were removed by two washes with distilled water at room temperature. Plastic and coated surfaces were saturated with 1% solution of heat-denaturated BSA in phosphate-buffered saline (PBS: 0·02 M-KH_2PO_4/Na_2HPO_4, 0·14 M-NaCl, pH 7·4) by incubation for 1 h at room temperature. Wells were then washed twice with distilled water and the plates stored at 4°C.

Activation of FXIII

In each experiment FXIII was used after activation by adding 0·1 unit of thrombin per unit of FXIII in serum-free DMEM at time 0 of the attachment assay.

DNA measurement

DNA was measured by the technique described by Labarca & Paigen (1980). Briefly, attached cells were removed by trypsinization for 10 min at 37 °C in 250 μl of 0·1 % trypsin, 0·02 % EDTA in PBS. After adjusting the volume to 1 ml with PBS, the suspension was sonicated and 1 ml of reagent (bis-benzimidazol, 2 μg ml^{-1} in 4 M-NaCl, 20 mM-Na_2HPO_4/NaH_2PO_4, 0·02% EDTA at pH 7·4) was added. The fluorescence was measured in a Perkin-Elmer fluorimeter (excitation wavelength 356 nm, emission wavelength 458 nm). The linearity of the fluorescence was verified for the DNA content of $0{\cdot}2\times10^5$ to 5×10^5 cells.

Attachment assay

Cells grown on plastic in DMEM and 10% FCS were detached by trypsinization. After neutralization of the trypsin by a 0·25 % solution of soybean trypsin inhibitor in serum-free DMEM and two washes with the same solution, the cells were resuspended in serum-free DMEM at the appropriate density, adjusted after counting the cells on a Thoma's plate. The attachment support was conditioned for 1 h at room temperature with 200 μl serum-free DMEM. After addition of the tested protein to the medium, cells suspension (300 μl) was added to the wells and incubated for the indicated periods of time at 37 °C in a 5 % CO_2/95 % air controlled atmosphere. The incubation was stopped by aspiration of the medium followed by three washings of the wells with 1 ml of PBS. The proportion of attached cells was determined by measurement of DNA in the wells and comparing the result with the DNA content of the initial suspension of cells, defined as 100 %.

For preincubation of the cells with fibronectin and FXIII, cells were suspended in serum-free DMEM containing the indicated concentrations of the tested protein in a siliconized glass tube gently shaken every 2 min. Cells were then washed three times with serum-free DMEM, collected by centrifugation for 5 min at 1000 rev. min^{-1}, resuspended in serum-free DMEM and the attachment assay was performed as described above.

For the preincubation of fibronectin or FXIII with the attachment support, the protein was dissolved and added to the coated wells in 500 μl of serum-free DMEM. After an incubation of 30 min at 37 °C, the wells were washed three times with 1 ml PBS and the attachment experiment was performed immediately.

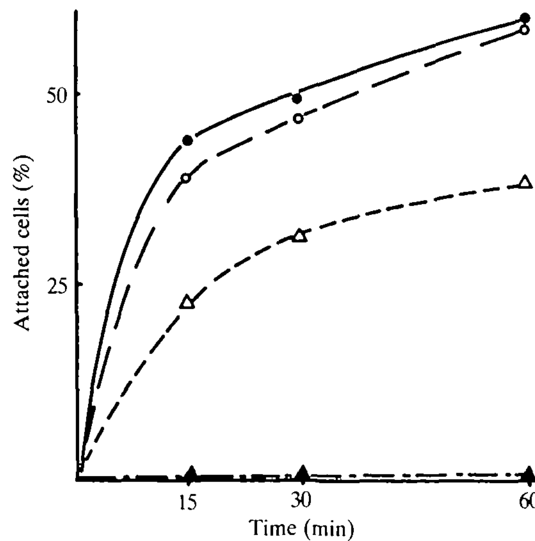

Fig. 1. Influence of denatured BSA on NSF attachment to plastic and to collagen I polymers; BSA was used to saturate plastic alone (▲) and plastic coated with 10 μg polymeric collagen I dried before (○) or after (●) the saturation with BSA. A control without BSA or collagen was included (△) to determine the efficiency of the saturation of free plastic sites. The attachment of NSF was measured after 15, 30 and 60 min of incubation. Each point represents the mean of triplicate assays and the standard deviation was less than 10 %.

Purification of rabbit anti-fibronectin antibodies

Antibodies against purified human plasma fibronectin were raised in rabbits. The specificity of the antiserum was tested by Western blotting as described by Towbin *et al.* (1979) and by enzyme-linked immunoassay according to Rennard *et al.* (1980). The immunoglobulins were precipitated with $(NH_4)_2SO_4$ (between 30 and 50 %, w/v) collected by centrifugation, solubilized in PBS and dialysed against the same buffer.

RESULTS

Determination of the optimum attachment conditions for skin fibroblasts and PER cells

The experimental conditions were established using normal calf skin fibroblasts (NSF). The optimal amount of collagen polymers needed to coat the surface of the wells was found to be between 5 and 10 μg. The non-specific attachment of the cells to plastic was adequately prevented by saturation with denaturated BSA. Fig. 1 shows that fibroblasts attached up to 40 % to non-coated culture plastic after 30 min of incubation whereas there was less than 2 % of attachment after saturation with BSA. Treatment with BSA could be performed before or after drying collagen without a significant difference in the interaction between cells and support. The proportion of attached cells did not vary between 10^5 and 5×10^5 seeded cells on 10 μg collagen and was 45 ($\pm$3) % in 30 min.

The effect of trypsin treatment on fibroblast attachment was monitored for increasing periods of time and the proportion of cells adhering to collagen I polymers

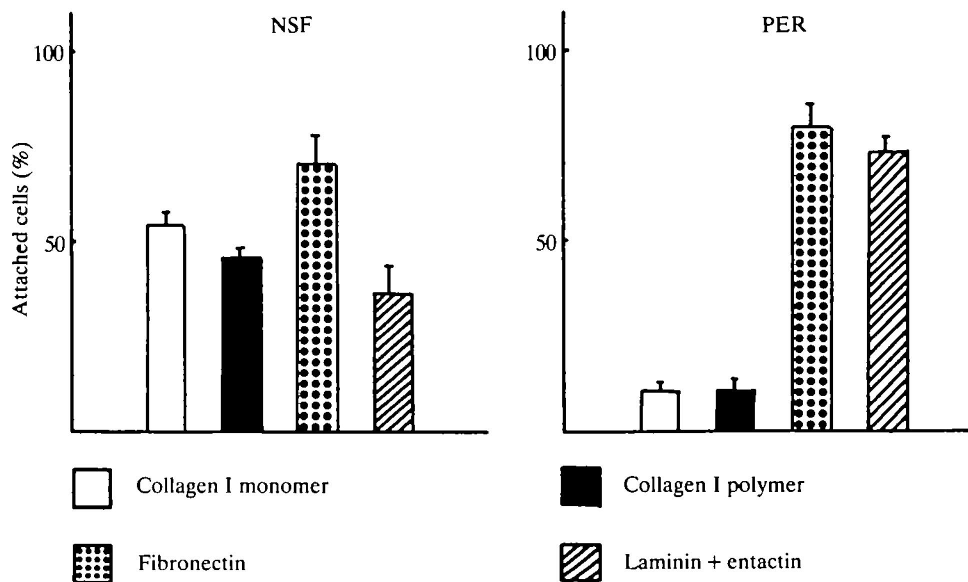

Fig. 2. Attachment of NSF and PER cells in 30 min to 10 μg of dried collagen I monomers, polymers, fibronectin and laminin. Each bar represents the mean of triplicate assays ± 1 S.D.

in 30 min was measured. Trypsin treatment for 10 min or less did not modify the attachment to collagen. To determine time 0, cells were detached with 0·02% EDTA. For all the following experiments, trypsinization was for 2 min.

Using the conditions described above the attachment of PER cells at passage 25 to collagen I matrices, monomeric as well as polymeric, was very low whereas these cells adhere successfully to fibronectin and to laminin (Fig. 2). Calf skin fibroblasts, used as a known reference, attached to monomeric and polymeric collagen type I, to fibronectin and less to laminin. Higher-passage PER cells that formed multilayers adhered to fibronectin and laminin and also to monomeric and polymeric collagen I (not illustrated).

Effect of exogenous fibronectin and laminin on the attachment of PER cells to collagen I

The time-course measured for the binding of NSF and PER cells to collagen I supported the lack of attachment of PER cells to this substrate under either its monomeric or its polymeric form (Fig. 3). Addition of increasing amounts of fibronectin to the attachment medium did not modify the attachment of PER cells or of NSF to monomeric collagen I. When added to wells coated with polymeric collagen I, fibronectin increased the attachment of NSF very little but that of PER cells to a large extent (Fig. 4). A concentration of 5 $\mu g\,ml^{-1}$ fibronectin was found to produce the maximum effect.

Preincubation of the collagen support with fibronectin and washing instead of adding it with the cell suspension produced a similar effect on the attachment of PER cells to polymeric collagen I (Fig. 5). Preincubation of PER cells with fibronectin

and washing before contact with polymeric collagen did not improve the attachment (not illustrated). The action of fibronectin in the correction of the attachment was inhibited by antibodies directed against fibronectin (Table 1). Laminin did not affect the attachment of PER cells or NSF to collagen I.

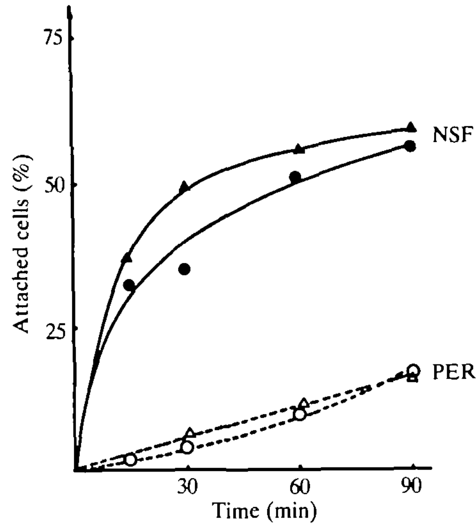

Fig. 3. Time course of attachment of NSF (●, ▲) and PER cells (○, △) to type I collagen monomers (△, ▲) and polymers (○, ●) in the absence of added factors. Each point represents the mean of three experiments and the variability between experiments was less than 10%.

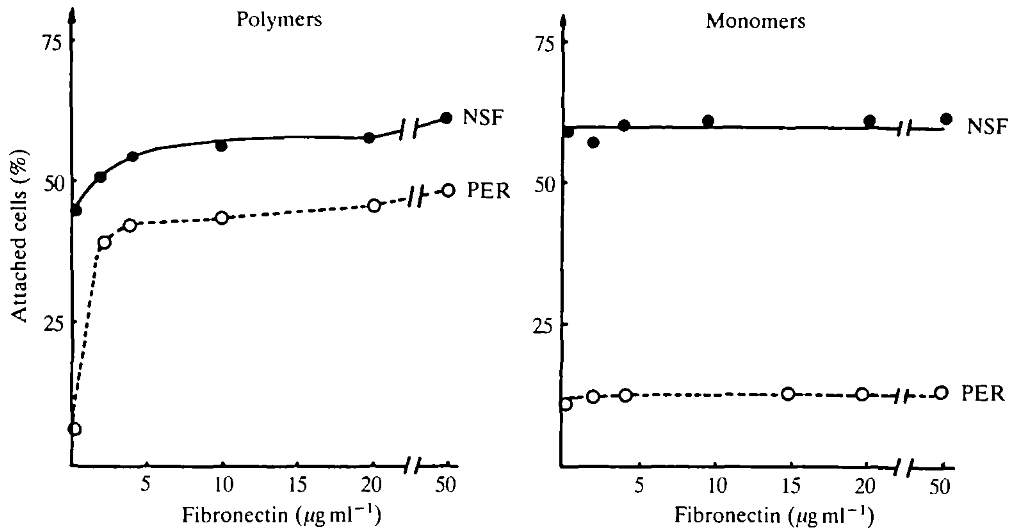

Fig. 4. Effect of increasing concentrations (in $\mu g\, ml^{-1}$) of exogenous fibronectin on the attachment of NSF (●) and PER cells (○) to collagen I monomers and polymers. Fibronectin was added to the attachment medium at the same time as the cells, and the percentage of attachment was determined after 30 min of incubation. Each point represents the mean of triplicate assays and the standard deviation was less than 10%.

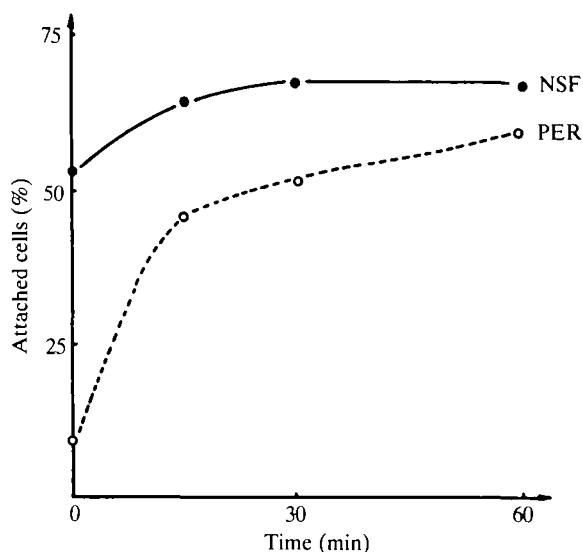

Fig. 5. Attachment of NSF (●) and PER cells (○) to collagen I polymers in 30 min. The attachment support was preincubated for the indicated periods of time with fibronectin at $10\,\mu g\,ml^{-1}$ in $500\,\mu l$ serum-free DMEM before performing the attachment assay.

Improvement of the attachment of PER cells to collagen I monomers by fibronectin and FXIII

The attachment of PER cells to collagen I monomers was not modified by fibronectin alone ($20\,\mu g\,ml^{-1}$) or FXIII alone ($1\,unit\,ml^{-1}$), whereas it was stimulated when fibronectin and FXIII were added together in the attachment medium (Fig. 6). The addition of thrombin in concentrations similar to or higher than those used for the activation of FXIII, with or without fibronectin, did not modify the attachment. For a constant concentration of FXIII ($1\,unit\,ml^{-1}$) the adhesion of PER cells to monomeric collagen I was enhanced as a function of increasing concentrations of fibronectin (Fig. 6A). For a constant concentration of fibronectin ($40\,\mu g\,ml^{-1}$) the maximum attachment was achieved by adding very low concentrations of FXIII (Fig. 6B). The minimum concentration of FXIII needed to

Table 1. *Effect of anti-fibronectin antibodies on the attachment of PER cells to collagen I polymers*

Attachment performed in the presence of	% of attachment ± S.D.
0	10·6 ± 1·8
Fn	34·6 ± 5·3
Fn+anti-Fn IgG	19·2 ± 4·1
Fn+NRS IgG	44·3 ± 3·6

The cell attachment was determined after a 30-min incubation on collagen I polymers in serum-free DMEM (0) and in the presence of fibronectin ($4\,\mu g\,ml^{-1}$) alone or with IgG collected from anti-fibronectin antiserum (anti-Fn, $1\,mg\,ml^{-1}$ of protein) or from non-immunized rabbit serum (NRS, $1\,mg\,ml^{-1}$ of protein) as control. Each point represents the mean of triplicate assays ± 1 S.D.

produce the maximum stimulation of the fibronectin-mediated attachment of PER cells to collagen I monomers was determined to be 0·01 unit ml^{-1}.

Effect of FXIII on the attachment of PER cells to polymeric collagen

By adding a constant concentration of FXIII (1 unit ml^{-1}) to increasing concentrations of fibronectin, it was observed that FXIII displayed the maximum

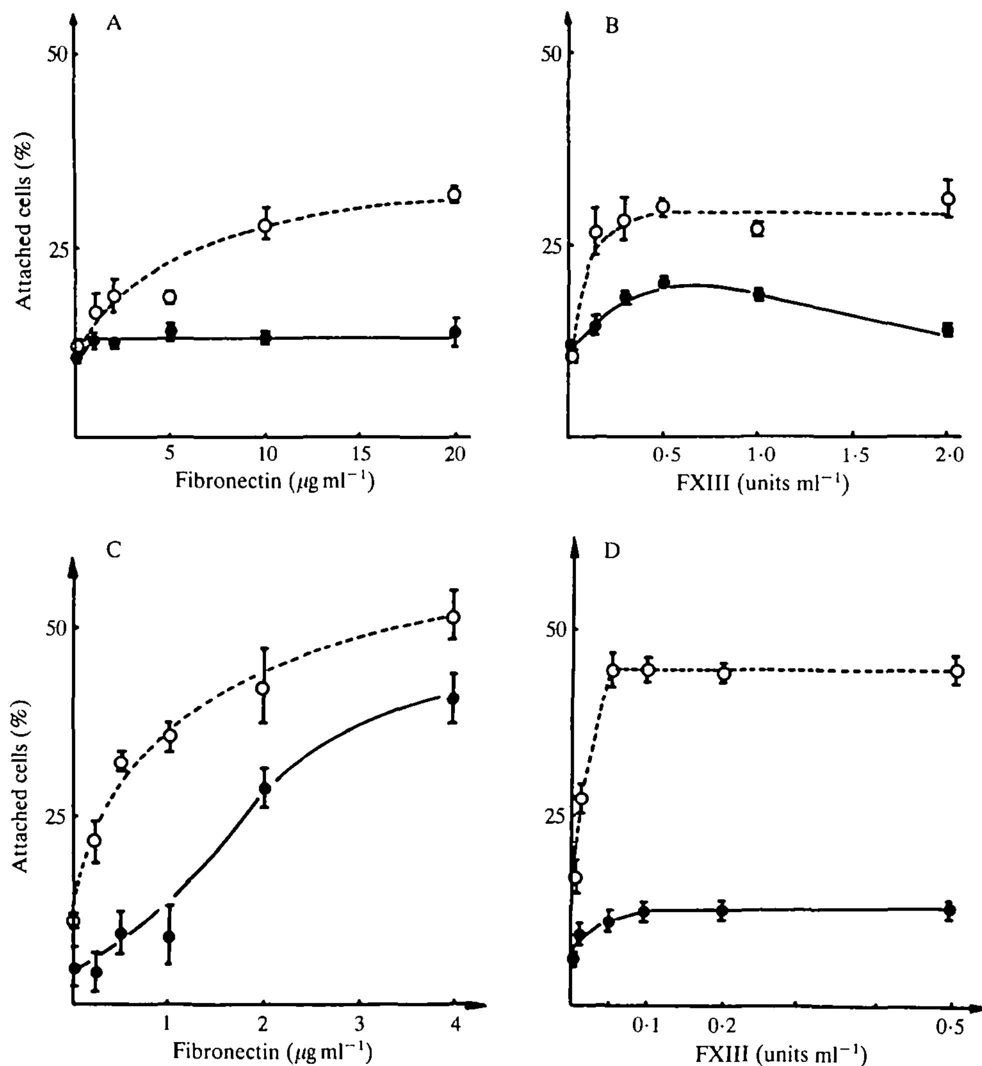

Fig. 6. FXIII dependence of the fibronectin-mediated adhesion of PER cells to collagen I monomers (A,B) and polymers (C,D). The cells were incubated on their support for 30 min at 37°C: A,C, with increasing fibronectin concentrations in the absence (●) or in the presence (○) of FXIII (1 unit ml^{-1}); and B,D, with increasing concentrations of FXIII in the absence (●) or in the presence (○) of fibronectin (40 $\mu g\,ml^{-1}$ on monomers (B) and 1 $\mu g\,ml^{-1}$ on polymers (D)). Each point represents the mean of triplicate assays ± 1 S.D.

effect on the attachment to collagen I polymers mainly at low concentrations (0·5–1 μg ml^{-1}) of fibronectin (Fig. 6C). At higher concentrations of fibronectin the effect of FXIII on the attachment of PER cells to collagen I polymers was less pronounced. At a low concentration of fibronectin (1 μg ml^{-1}) in the attachment medium small amounts of FXIII strongly improved the adhesion of PER cells to collagen I polymers (Fig. 6D). FXIII at a concentration of 0·05 unit ml^{-1} was sufficient to produce the maximum effect.

Effect of pretreatment of the cells or the collagen monomers with fibronectin and FXIII

From the data in Table 2, it is obvious that preincubation of the cells with FXIII or fibronectin alone or fibronectin and FXIII together did not promote the attachment of PER cells to monomeric collagen. A slight increase in the attachment was observed by preincubating the layer of monomers of collagen with fibronectin or fibronectin and FXIII. The addition of fibronectin and FXIII together with the cells in the attachment assay induced maximum adhesion.

DISCUSSION

In physiological conditions epithelial cells are attached to a basement membrane containing, among other components, laminin as an adhesion protein (Terranova

Table 2. *Interactions required for improving the attachment of PER cells to collagen I monomers*

Preincubation		Attachment assay:	
Cells	Monomers	monomers+cells+	% Attachment
0	0	Fn+FXIII	100
0	0	0	26
Fn	0	0	21
FXIII	0	0	28
Fn+FXIII	0	0	29
0	0	0	37
0	Fn	0	51
0	FXIII	0	41
0	Fn+FXIII	0	48

The cells in suspension were preincubated for 30 min in serum-free DMEM (0), with fibronectin (Fn, 20 μg ml^{-1}) and FXIII (1 unit ml^{-1}) alone or together, washed three times, collected by centrifugation and resuspended in serum-free DMEM before performing the attachment assay on monomeric collagen I. Monomeric collagen was preincubated with serum-free DMEM (0), with fibronectin (Fn) and FXIII (FXIII) alone or together at the concentrations shown above. The attachment of PER cells, performed in serum-free DMEM in the absence (0) or in the presence of fibronectin and FXIII (Fn+FXIII) at the concentrations shown above, is expressed as % of the attachment measured under optimum corrective conditions, taken as 100 % (= 42 % of cells attached within 30 min). The determinations were performed in triplicate with a mean standard deviation of 12 %.

et al. 1980; Alitalo *et al.* 1980). In some circumstances, however, epithelial cells have to interact directly with their supporting connective tissue, as in wound healing or during the process of invasion and metastasis of cancer.

The transformed epithelial cells that we used (PER cells) are derived from embryonic lung (Michiels *et al.* 1981). Like several other types of epithelial cells, they attach to laminin, and like keratinocytes (Takashima & Grinnell, 1984; Clark *et al.* 1985), corneal epithelial cells (Nishida *et al.* 1984) and hepatocytes (Johansson & Hook, 1984), they also adhere to fibronectin coated on a rigid support. They are however unable to adhere to type I collagen in either monomeric or polymeric form. The addition of fibronectin during the attachment assay corrects this inability only when polymeric collagen is used as substrate but not with monomeric collagen. Preincubation of fibronectin with the cells or with the matrix enabled us to demonstrate that fibronectin must coat the collagen polymers to be able to promote adhesion.

The lack of correction of the attachment to monomeric collagen I by fibronectin can be related to other studies demonstrating that the biological activity of fibronectin is strongly dependent on its orientation (Grinnell & Feld, 1982) or on its three-dimensional configuration (Schwarz & Juliano, 1984). Fibronectin is indeed a large glycoprotein made up of several functional domains (Hayashi & Yamada, 1981, 1983) that might display different conformations when bound to collagen monomers or polymers. The accessibility of the cell binding site might be modulated by the conformation of the molecule and/or by self-association and formation of complexes with other macromolecules. Indeed, the cell binding site of fibronectin is not operational when the glycoprotein is in solution and becomes functional when it is bound to fibrin, collagen or proteoheparan sulphate (Johansson & Hook, 1984; Osterlund *et al.* 1985).

Activated factor XIII of blood coagulation (FXIII) is a transglutaminase known to be able to crosslink fibronectin to various proteins including collagen. It was used in this study to modify the interaction mediated by fibronectin in the attachment of the PER cells to monomeric collagen type I. In such conditions, fibronectin and FXIII permitted the adhesion of PER cells. On collagen I polymers the addition of FXIII reduced the fibronectin concentration required to permit the attachment of the cells. Very low concentrations of FXIII were sufficient to produce the maximum effect. It would have been interesting to test the induction of adhesion to collagen by transglutaminases of different origins. Enzymes displaying the transglutaminase activity that exists in many types of cells and tissues are known to be reduced following transformation (Birckbichler *et al.* 1977).

During wound healing, platelets from the blood clot release various growth factors and FXIII. In the absence of this factor the formation of the scar is delayed and its mechanical properties are impaired (Beck *et al.* 1961). Mosher (1984) showed that FXIII is able to cross-link fibronectin covalently to collagen but the physiological role of such an interaction has not yet been demonstrated. Grinnell *et al.* (1980) showed that the binding of fibroblasts to fibrin required fibronectin and this adhesion was greatly improved when fibronectin was covalently cross-linked to fibrin by

FXIII. Epithelial cells might also need FXIII to attach to the newly formed connective tissue before reconstruction of the basement membrane during reformation of the epithelium.

From our preincubation experiments (Table 2) it is obvious that the mechanism of action of FXIII is not mediated through the binding of soluble exogenous fibronectin to a cell membrane component by a stable link. It also appears that the binding of fibronectin to collagen I monomers by FXIII is not sufficient, since the maximum effect is obtained when fibronectin, FXIII, cells and the collagen matrix are present simultaneously. These findings indicate that FXIII might act on both fibronectin–matrix and fibronectin–cell surface interactions or on the interaction between cell-bound fibronectin and collagen-bound fibronectin to promote fibronectin-mediated attachment of PER cells to collagen I monomers.

Experiments using specific domains of the fibronectin molecule and immunological measurements of the binding of fibronectin and FXIII to collagen are now in progress in our laboratory in order to define better the mechanisms involved in these interactions.

We thank Dr H. Karges (Behringwerke A.G.) for the gift of FXIII and thrombin, and Dr H. Karges and B. V. Nusgens for suggestions during the experimental work and correction of the manuscript. The helpful typographical assistance of Mrs I. Leclercq is acknowledged. This work was supported in part by a grant no. 3.4512.85 from the Belgian Fonds de la Recherche Scientifique Médicale and a grant from the Fonds Cancérologique de la Caisse Générale d'Epargne et de Retraite. M.P. is a doctoral fellow supported by Behringwerke, Marburg, F.R.D.

REFERENCES

ALITALO, K., KESKI-OJA, J., HEDMAN, K. & VAHERI, A. (1982). Loss of different pericellular matrix components of rat cells transformed with a T class ts mutant of Rous sarcoma virus. *Virology* **119**, 347–357.

ALITALO, K., KURKINEN, M., VAHERI, A., KRIEG, T. & TIMPL, R. (1980). Extracellular matrix components synthesized by human amniotic epithelial cells in culture. *Cell* **19**, 1053–1062.

BECK, E., DUCKERT, F. & ERNST, M. (1961). The influence of fibrin stabilizing factor on the growth of fibroblasts in vitro and wound healing. *Thromb. Diath. Haemorrh.* **6**, 485–491.

BIRCHMEIER, W., LIBERMANN, T. A., IMHOF, B. A. & KREIS, T. E. (1982). Intracellular and extracellular components involved in the formation of ventral surfaces of fibroblasts. *Cold Spring Harbor Symp. quant. Biol.* **46**, 755–760.

BIRCKBICHLER, P. J., ORR, G. R., CONWAY, E. & PATTERSON, M. K. JR (1977). Transglutaminase activity in normal and transformed cells. *Cancer Res.* **37**, 1340–1344.

BOUCAUT, J. C. & DARRIBERE, T. (1983). Fibronectin in early amphibian embryos. Migrating mesodermal cells contact fibronectin established prior to gastrulation. *Cell Tiss. Res.* **234**, 135–145.

CLARK, R. A. F., FOLKVORD, J. M. & WERTZ, R. L. (1985). Fibronectin, as well as other extracellular matrix proteins, mediate human keratinocyte adherence. *J. invest. Derm.* **84**, 378–383.

DELVOYE, P., NUSGENS, B. & LAPIÈRE, CH. M. (1983). The capacity of retracting a collagen matrix is lost by dermatosparactic skin fibroblasts. *J. invest. Derm.* **81**, 267–270.

DUCKERT, F. (1973). The fibrin stabilizing factor, factor XIII. *Blut* **26**, 177–179.

GRINNELL, F. (1984). Fibronectin and wound healing. *J. Cell Biochem.* **26**, 107–116.

GRINNELL, F. & BENNETT, M. H. (1981). Fibroblast adhesion on collagen substrata in the presence and absence of plasma fibronectin. *J. Cell Sci.* **48**, 19–34.

GRINNELL, F. & FELD, M. K. (1982). Fibronectin adsorption on hydrophilic and hydrophobic surfaces detected by antibody binding and analyzed during cell adhesion in serum-containing medium. *J. biol. Chem.* **257**, 4888–4893.

GRINNELL, F., FELD, M. & MINTER, D. (1980). Fibroblast adhesion to fibrinogen and fibrin substrates: requirement for cold insoluble globulin (plasma fibronectin). *Cell* **19**, 517–525.

GRINNELL, F. & MINTER, D. (1978). Attachment and spreading of baby hamster kidney cells to collagen substrata: effects of cold-insoluble globulin. *Proc. natn. Acad. Sci. U.S.A.* **75**, 4408–4412.

HAYASHI, M. & YAMADA, K. M. (1981). Differences in domain structures between plasma and cellular fibronectins. *J. biol. Chem.* **256**, 11 292–11 300.

HAYASHI, M. & YAMADA, K. M. (1983). Domain structure of the carboxyl-terminal half of human plasma fibronectin. *J. biol. Chem.* **258**, 3332–3340.

HAYMAN, E. G., ENGVALL, E. & RUOSLAHTI, E. (1981). Concomitant loss of cell surface fibronectin and laminin from transformed rat kidney cells. *J. Cell Biol.* **88**, 352–357.

HENRIKSSON, P. & MCDONAGH, J. (1983). Factor XIII activation and interactions. In *Factor XIII and Fibronectin* (ed. R. Egbring & H. G. Klingemann), pp. 1–14. Marburg, West Germany: Die Medizinische Verlagsgesellschaft mbH.

JOHANSSON, S. & HOOK, M. (1984). Substrate adhesion of rat hepatocytes: on the mechanism of attachment to fibronectin. *J. Cell Biol.* **98**, 810–817.

KASAI, S., KUNIMOTO, T. & NITTA, K. (1983). Cross-linking of fibrin by activated factor XIII stimulates attachment, morphological changes and proliferation of fibroblasts. *Biomed. Res.* **4**, 155–160.

KESKI-OJA, J., GAHMBERG, C. G. & ALITALO, K. (1982). Pericellular matrix and cell surface glycoproteins of virus-transformed mouse epithelial cells. *Cancer Res.* **42**, 1147–1153.

KESKI-OJA, J., MOSHER, D. F. & VAHERI, A. (1976). Cross-linking of a major fibroblast surface associated glycoprotein (fibronectin) catalyzed by blood coagulation FXIII. *Cell* **9**, 29–35.

LABARCA, C. & PAIGEN, K. (1980). A simple, rapid, and sensitive DNA assay procedure. *Analyt. Biochem.* **102**, 344–352.

LAPIÈRE, CH. M., NUSGENS, B. & PIERARD, G. E. (1977). Interaction between collagen type I and type III in conditioning bundles organization. *Connect. Tiss. Res.* **5**, 21–29.

MAUGER, A., DEMARCHEZ, M., HERBAGE, D., GRIMAUD, J. A., DRUGUET, M., HARTMANN, D. J., FOIDART, J. M. & SENGEL, P. (1983). Immunofluorescent localization of collagen types I, III, IV, fibronectin and laminin during morphogenesis of scales and scaleless skin in the chick embryo. *Wilhelm Roux Arch. Devl Biol.* **192**, 205–215.

MCKEE, P. A., MATTOCK, P. & HILL, H. L. (1970). Subunit structure of human fibrinogen, soluble fibrin, and cross-linked insoluble fibrin. *Proc. natn. Acad. Sci. U.S.A.* **66**, 738–744.

MICHIELS, F., DUVERGER, A. & CHOUROULINKOV, I. (1981). Correlation between preneoplastic lesions in rat embryo lung treated with B(a)P or CSC in organ culture and tumour production *in vivo*. *Carcinogenesis* **2**, 885–896.

MOSHER, D. F. (1984). Cross-linking of fibronectin to collagenous proteins. *Molec. cell. Biochem.* **58**, 63–68.

MOSHER, D. F. (1975). Cross-linking of cold-insoluble globulin by fibrin stabilizing factor. *J. biol. Chem.* **250**, 6614–6621.

MOSHER, D. F. & SCHAD, P. E. (1979). Cross-linking of fibronectin to collagen by blood coagulation factor XIIIa. *J. clin. Invest.* **64**, 781–787.

MOSHER, D. F., SCHAD, P. E. & VANN, J. M. (1980). Cross-linking of collagen and fibronectin by factor XIIIa. Localization of participating glutaminyl residues to a tryptic fragment of fibronectin. *J. biol. Chem.* **255**, 1181–1188.

NISHIDA, T., NAKAGAWA, S., NISHIBAYASHI, C., TANAKA, H. & MANABE, R. (1984). Fibronectin enhancement of corneal epithelial wound healing of rabbits *in vivo*. *Archs Opthal. N.Y.* **102**, 455–456.

OSTERLUND, E., ERONEN, I., OSTERLUND, K. & VUENTO, M. (1985). Secondary structure of human plasma fibronectin: conformational change induced by calf alveolar heparan sulfates. *Biochemistry* **24**, 2661–2667.

PAYE, M., PIERARD, D., ETIEVANT, C., NUSGENS, B. & LAPIÈRE, CH. M. (1986). Characterization of a spontaneously transformed pulmonary embryonic rat (PER) epithelial cell line. *J. Cell Sci.* **86**, 83–93.

RENNARD, S., BERG, R., MARTIN, G. R., FOIDART, J. M. & GEHRON-ROBEY, P. (1980). Enzyme-linked immunoassay (ELISA) for connective tissue components. *Analyt. Biochem.* **104**, 205–214.

RUOSLAHTI, E. (1984). Fibronectin in cell adhesion and invasion. *Cancer Metast. Rev.* **3**, 43–52.

SCHWARZ, M. A. & JULIANO, R. L. (1984). Surface activation of the cell adhesion fragment of fibronectin. *Expl Cell Res.* **153**, 550–555.

TAKASHIMA, A. & GRINNELL, F. (1984). Human keratinocyte adhesion and phagocytosis promoted by fibronectin. *J. invest. Derm.* **83**, 352–358.

TERRANOVA, V. P., LIOTTA, L. A., RUSSO, R. G. & MARTIN, G. R. (1982). Role of laminin in the attachment and metastasis of murine tumor cells. *Cancer Res.* **42**, 2265–2269.

TERRANOVA, V. P., ROHRBACH, D. & MARTIN, G. R. (1980). Role of laminin in the attachment of PAM 212 (epithelial) cells to basement membrane collagen. *Cell* **22**, 719–726.

TERRANOVA, V. P., WILLIAMS, J. E., LIOTTA, L. A. & MARTIN, G. R. (1984). Modulation of the metastatic activity of melanoma cells by laminin and fibronectin. *Science* **226**, 982–985.

TIMPL, R., ROHDE, H., GEHRON-ROBEY, P., RENNARD, S., FOIDART, J. M. & MARTIN, G. R. (1979). Laminin a glycoprotein from basement membranes. *J. biol. Chem.* **254**, 9933–9937.

TOWBIN, H., STAEHELIN, T. & GORDON, J. (1979). Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. natn. Acad. Sci. U.S.A.* **76**, 4350–4354.

VAHERI, A., ALITALO, K., HEDMAN, K., KESKI-OJA, J., KURKINEN, M. & WARTIOVAARA, J. (1978). Fibronectin and the pericellular matrix in normal and transformed adherent cells. *Ann. N.Y. Acad. Sci.* **312**, 343–353.

VAHERI, A. & MOSHER, D. F. (1978). High molecular weight, cell surface associated glycoprotein (fibronectin) lost in malignant transformation. *Biochem. biophys. Acta* **516**, 1–25.

VARTIO, T., VAHERI, A., DE PETROP, G. & BARLATI, S. (1983). Fibronectin and its proteolytic fragments. Potential as cancer markers. *Invest. Metast.* **3**, 125–138.

YAMADA, K. M. (1983). Isolation of fibronectin from plasma and cells. In *Immunochemistry of the Extracellular Matrix* (ed. H. Furthmayr), vol. 1, pp. 111–123. Boca Raton, Florida: CRC Press.

(Received 22 April 1986 – Accepted 26 June 1986)