Collagen-induced rapid morphogenesis of human mammary epithelial cells: The role of the $\alpha_2\beta_1$ integrin

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Summary

The cell line MTSV1-7, originally derived by immortalizing mammary epithelial cells cultured from human milk, was able to form three-dimensional structures in collagen gel. We have now found that these cells, cultured as a monolayer, are able to undergo rapid morphogenesis forming ridges and balls around collagen fibres, when soluble collagen type I is added to the medium. Monoclonal antibodies to the $\alpha_2$-(P1E6) and $\beta_1$-(mAB13) subunits of VLA-2, but not to the $\alpha_3$-subunit (P1B5) of VLA-3, could block this collagen-induced rapid morphogenesis (CIRM). The effect of the antibodies on cell attachment, spreading, and migration on collagen gels was analyzed to identify $\alpha_2\beta_1$ dependent steps which might be involved in CIRM. The results suggest that while other proteins, besides $\alpha_2\beta_1$, are also involved in cell attachment and migration, cell spreading was specifically blocked by antibodies to the VLA-2, but not to the VLA-3 integrin. The results demonstrate that the $\alpha_2\beta_1$ integrin plays a crucial role in the collagen-induced morphogenesis of human mammary epithelial cells and implicate the process of VLA-2-dependent cell spreading as an important step in this morphogenesis.

Key words: mammary (gland), morphogenesis, integrins.

Introduction

Morphogenesis of the mammary gland is a complex process, controlled by hormones, growth factors and the microenvironment provided by the extracellular matrix (ECM). Studies using mammary epithelial cells taken from the mid-pregnant rodent mammary gland have identified the minimal requirements for morphological and functional differentiation in vitro (Emerman and Pitelka, 1977; Emerman et al., 1977; Burwen and Pitelka, 1980; McGrath et al., 1985; Danielson et al., 1984). In these studies, the crucial role of the extracellular matrix has been clearly demonstrated: to obtain three-dimensional alveolar-like structures, and vectorial secretion of milk proteins the cells have been cultured either in collagen gels or on a reconstituted basement membrane matrix isolated from the Englebreth-Holm-Swarm (EHS) tumour (Lee et al., 1984; Bissell and Hall, 1987; Barcellos-Hoff et al., 1989; Aggeler et al., 1991; Darcy et al., 1991).

The integrins, which are heterodimeric (a$\beta$) receptors for ECM molecules, play a significant role in cell attachment, spreading and migration in vitro (see recent reviews, Albelda and Buck, 1990; Humphries, 1990; Rouslahti, 1991). In vivo, these molecules appear to play an important role in embryo implantation, morphogenesis, wound healing and extracellular matrix assembly. As cell adhesion molecules, integrins are involved in the maintenance of endothelial monolayer integrity in vitro (Lampugnani et al., 1991), and in lymphocyte-lymphocyte and lymphocyte-endothelium interaction (Springer, 1990). Along with their mechanical function as molecular anchors in cell-ECM and cell-cell interactions, integrins have also been recently shown to be involved in signal transduction (Werb et al., 1989; Nojima et al., 1990; Kornberg et al., 1991).

Recently we established novel human mammary epithelial cell lines from milk cells as well as from primary tumours (Bartek et al., 1991; Shearer et al., 1992). One of the normal cell lines, MTSV1-7, which had the characteristics of luminal cells, was able to form compact ball-like structures within a collagen type I gel, while another line (5.3.1.E), derived from a lobular carcinoma, did not have this ability (Berdichevsky and Taylor-Papadimitriou, 1991).

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Materials and methods

Materials

Rat tail collagen (type I) (Vitrogen - 100) was obtained from Celsis Laboratories; Protein A-Sepharose CL-4B was from Pharmacia LKB; Nonidet P-40 was from BDH Ltd; Rabbit serum anti-mouse Ig was from Miles-Yeda Ltd; fluorescein-conjugated goat and rabbit anti-mouse IgG and rhodamine-conjugated swine anti-rabbit IgG were obtained from DAKO Ltd. BSA was from Amersham Int. CITIFLUOR was from Citifluor Ltd. All other reagents were obtained from Sigma.

Cells and cultures conditions; collagen-induced morphogenesis

The cell line, MTSV1-7 Hg was cultured in Dulbecco’s Modified Eagle’s Medium (DMEM), containing 10% fetal calf serum, hydrocortisone and insulin (Bartek et al., 1991). Morphogenesis was induced by addition of the fresh growth medium, containing different concentrations of ECM components (collagen I, collagen IV, laminin and fibronectin), to cells grown on plastic. Structure formation was observed with phase contrast and photographed on Ilford DANF 135 film after 24, 48 and 72 hours.

Antibodies and probes

Monoclonal antibodies to the α1 (PIB5) and α2 (PIE6) subunits of integrins were from Telios Pharmaceutical, Inc. The monoclonal antibodies directed to the β1 integrin subunit (mAB13 and MAR5) were generously provided by Dr K Yamada (NIH, Bethesda) and Dr M Colnaghi, respectively. The antibody TS2/7, directed to the α1 integrin subunit, was from Dr M Hemler (Dana-Farber Cancer Institute, Boston). Rabbit polyclonal anti-type I collagen was a gift from Dr W Lankes (Stanford University, Stanford).

Inhibition of cell-substrate adhesion and spreading

Collagen gel-coated 24-well dishes were prepared as described by the manufacturer and blocked with heat-inactivated BSA (HSAB) for 2 h at room temperature. Cells (1-3 x 10^4) were preincubated with monoclonal antibodies for 1 h at room temperature and plated onto the collagen gel and incubated at 37°C. At intervals, non-adherent cells were removed by washing three times (each wash for 30 s on rotary shaker) with PBS and the alkaline phosphatase activity of adherent cells was measured as previously described (Pignatelli and Bodmer, 1988). Briefly, cells were incubated in alkaline-phosphatase buffer (100 mm NaCl; 50 mM Tris-HCl, pH 9.0, 5 mg MgCl_2 containing 0.5 mg/ml 4-methyl-umbelliferyl-phosphate at 37°C for 2 h and aliquots of the reaction were transferred to 96-well Dynatech microtitre plates, the fluorescence was measured using Dynatech Microfluor TM reader (Dynatech, Cambridge, MA). Under the above conditions the assay was linear with cell numbers over the range of 10^4 to 5 x 10^5. In experiments on the inhibition of spreading, cells were preincubated with monoclonal antibodies for 1 h at room temperature and plated on collagen gel-coated dishes for 16 h.

Immunofluorescent staining

Cells were grown on 35 mm dishes, washed twice with PBS supplemented with 1 mM MgCl_2 and 1 mM CaCl_2 (PBS) and fixed in PBS buffer, containing 5% sucrose and 2% paraformaldehyde for 20 min at room temperature. After permeabilization by 0.5% Triton X-100 in PBS for 5 min at room temperature, cells were washed three times with PBS and blocked with 20% FCS for 30 min. Immunostaining was performed as previously described (Berdichevsky and Taylor-Papadimitriou, 1991). In double fluorescent staining fixed cells were incubated for 1 h with the combination of mouse anti-integrin monoclonal antibodies and rabbit polyclonal serum against collagen type I at the relevant dilutions in PBS. After washing with PBS (3 times; 5 min) a combination of FITC-conjugated goat anti-mouse IgG and rhodamine-conjugated swine anti-rabbit IgG was added for 1 h at 37°C. After washing with PBS cells were mounted with CITIFLUOR and immunofluorescence analysed with a Zeiss Axioskop microscope.

Cell labelling and immunoprecipitation

Cells were labelled in monolayers with 125I as previously described (Hubbard and Cohn, 1972); protein extraction and immunoprecipitation were performed according to the protocol of Adams and Watt (1990). Immunoprecipitated samples were loaded onto 5%/7.5% SDS-PAGE gel in non-denatured sample buffer (Laemml). Radioactive bands were visualized using Kodak O-XHR film.

Time-lapse cinemicroscopy

The cinemicroscopy apparatus consisted of four Olympus time lapse units, 16 mm Bolex H16 cameras, and inverted Olympus IMT microscopes which were fitted with 10x phase objectives and were enclosed within a controlled environment (Riddle, 1990). Kodak Infopack AHU microfilm was used and the cultures were filmed, directly after plating, for 24 h at the rate of one frame every four min. The films were viewed using an analytical projector (NAC), and the motility of the cells was calculated over the first 20 h period by digitisation using a GP7 digitiser (Science Accessories Corporation, Southport, Connecticut, USA) linked to a BBC B microcomputer.

Transmission electron microscopy

Cells were cultured and collagen I added as described above. After morphogenesis had occurred they were fixed with 2.5% glutaraldehyde in 0.1 M Sorenson’s phosphate buffer (pH 7.4) for 2 h at room temperature. After postfixation for 2 h with 1% osmium tetroxide in 0.1 M Sorenson’s phosphate buffer, the cells were dehydrated through graded ethanolos and impregnated and embedded in Araldite. Ultrathin sections were cut at 70-100 nm, stained with uranyl acetate/lead citrate and viewed on an Zeiss EM10 electron microscope.

Results

Expression of collagen-binding integrins in MTSV1-7 cells

We have previously reported (Berdichevsky and Taylor-Papadimitriou, 1991) that the human mammary epithelial cell line, MTSV1-7, when embedded in collagen type I gels formed compact ball-like structures. These structures, which develop from single cells, appear after 2 weeks, and develop “spikes” on further incubation (Fig. 1). Sections of the balls show that only some develop internal cavities or lumen. Since the structure formation within collagen gels requires the cell-collagen fibrils interaction, we analyzed the expression of integrins, potential receptors for collagen, in MTSV1-7 cells.

Immunoprecipitation of 125I-labelled protein extracts with the anti-α-subunit-specific monoclonal antibodies (Fig. 2) showed significant levels of the α2β1 and α3β1...
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Fig. 1. Growth of MTSV1-7 cells in collagen gels. Within gels the cells formed organized balls in 2 weeks (A), while some of them developed branches after 4 weeks (B).

integrins and barely detectable level of the $\alpha_1\beta_1$ integrin. This observation is in agreement with the derivation of the MTSV1-7 cells from normal luminal epithelial cells, since these cells have been shown to express the $\alpha_2\beta_1$ and $\alpha_3\beta_1$ in sections of the normal human mammary gland (Zutter et al., 1990). The distribution of integrin receptors in cell monolayers was examined using indirect immunofluorescent staining. Antibodies to the $\alpha_2$, $\alpha_3$, and $\beta_1$ subunits of integrins stained the outer membrane of MTSV1-7 cells particularly in cell-cell contact regions (Fig. 3). Again this reflects the staining pattern of luminal epithelial cells in sections of the normal human mammary gland.

Fig. 2. Expression of integrins by MTSV1-7 cells. Cells were surface labelled with $^{125}$I using lactoperoxidase/glucosidase. Integrins immunoprecipitated with monoclonal antibodies TS2/7(1) to $\alpha_2$, P1E6(2) to $\alpha_3$- and P1B5(3) to $\alpha_5$-subunits were resolved under non-reducing conditions.

Collagen-induced rapid morphogenesis

The inhibition of integrin functions with specific antibodies has been used in various studies as a major approach to understanding a role of these molecules in differentiation and morphogenesis in vivo and in vitro (Jeffredo et al., 1988; Lein et al., 1991). However, the collagen embedding technique requires relatively long incubation periods, when collagen fibrils surrounding cells could prevent their binding to blocking antibodies, making this an inappropriate system for examining the effect of blocking antibodies on the morphogenesis of MTSV1-7 cells. In order to overcome this problem, a new approach described recently by Jackson and Jenkins (1991) has been used to analyze further the role of integrins in collagen-induced morphogenesis. These workers showed that collagen type I added directly into a growth medium induced rapid (6 h) capillary-like structure formation on the monolayer of human endothelial cells (Jackson and Jenkins, 1991). Using this technique we found that the mammary epithelial cell line MTSV1-7 also responded to the presence of collagen fibrils by forming ridges and ball-like structures (Fig. 4).

Acid soluble collagen type I forms fibrils in neutral solutions and when growth medium containing collagen type I was added to monolayers of MTSV1-7 cells, fibrils were visible after 4-6 h when they could be seen at the bottom of the dish, apparently stuck to the cell monolayer. After 24-36 h, ridges appeared which seemed to represent single cell layers surrounding the collagen fibril. This is illustrated in Fig. 5 where the cells surrounding the collagen fibre are stained with an antibody to the $\beta_1$ integrin subunit (Fig. 5A); the collagen fibre can be seen in Fig. 5B stained with an antibody to collagen I. After 72-96 h the ridges increased in size and the layers covering the fibrils
Fig. 3. Immunofluorescent localization of \( \alpha_2\beta_1 \) and \( \alpha_3\beta_1 \) integrins in MTSV1-7 cells. Cells were cultured on plastic dishes, treated as described in Materials and Methods and stained with monoclonal antibodies against \( \alpha_2 \) (A), \( \alpha_3 \) (B) and \( \beta_1 \) (C) integrin subunits. There is extensive accumulation of integrin subunits in cell-cell contacts.

Effect of monoclonal antibodies to \( \alpha \) subunits of integrins on CIRM of MTSV1-7 cells

In order to understand the role of \( \alpha_2\beta_1 \) and \( \alpha_3\beta_1 \) integrins (the two major integrins of the \( \beta_1 \) family in MTSV1-7 cells) in CIRM, monolayers of MTSV1-7 cells were pretreated with different non-toxic concentrations of monoclonal antibodies to \( \alpha_2^- \), \( \alpha_3^- \) and \( \beta_1^- \) subunits of integrins and acid-soluble collagen type I was sequentially added to the growth medium. In these experiments, structure formation was suppressed in the cell monolayer preincubated with the monoclonal antibody directed to the \( \alpha_2^- \) and \( \beta_1^- \) integrin subunits. Neither anti-\( \alpha_2^- \) (up to 1:5 dilution of ascites) nor the anti-mucin antibody HMFG-1 (0.5 mg/ml) had any discernible effect on CIRM. The effect of antibody pretreatment on morphogenesis of MTSV1-7 cells is illustrated in Fig. 7.

Inhibition of cell adhesion, spreading and migration by integrin-specific monoclonal antibodies

Cell attachment, cell spreading and migration on the collagen fibrils could all be important steps in CIRM. Since \( \alpha_2\beta_1 \) integrin function was found to be crucial in the collagen induced morphogenesis of MTSV1-7 cells, we further analyzed the effect of anti-VLA-2 monoclonal antibodies on these activities. Inhibition of attachment of MTSV1-7 cells by pretreatment with antibodies to the \( \alpha_2^- \) and \( \beta_1^- \) subunits was only observed when the cells were allowed to attach for short periods of time. As the time allowed for attachment was increased beyond 2 hours however, this inhibition was lost, suggesting that while the \( \alpha_3\beta_1 \) integrin is involved in the rapid attachment of the cells to collagen type I gels, other collagen-binding proteins are involved in the slower attachment which proceeds with time. These results are illustrated in Fig. 8. Antibodies to the \( \alpha_3^- \) integrin subunit had no significant effect on cell attachment measured at any time.

The effect of monoclonal antibodies on cell spreading was examined under a light microscope, and using time-lapse videomicroscopy. Cell spreading on collagen gels was first detected after 5-6 h and about 85% of cells which had attached to the collagen gel were found to be spread after 16 h (Table 1). However, when cells were preincubated with monoclonal antibodies against \( \alpha_2^- \) appeared multilayered. At this time also, ball-like structures such as that shown in Fig. 5C usually appeared. The multilayered nature of the structures probably prevents the accessibility of the collagen fibre to the anti-collagen antibody (Fig. 5D). That the MTSV1-7 cells are indeed forming ridges around collagen fibres is shown by the electron micrograph illustrated in Fig. 6.

The induction of morphogenesis in MTSV1-7 cells appeared to be specific for collagen I since neither laminin, fibronectin nor collagen type IV in a range of concentrations could induce a morphogenetic response in these cells (data not shown). We have therefore referred to the phenomenon as collagen-induced rapid morphogenesis (CIRM).
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and $\beta_1$ subunits of VLA-2 before plating only about 30% and 2% of attached cells respectively, could spread on the top of collagen gels (Fig. 9, Table 1). Anti-mucin (HMFG-1) and anti-$\alpha_5\beta_1$ monoclonal antibodies showed only minor effects on the spreading of MTSV1-7 cells on collagen gels.

An examination of the effect of antibodies to $\alpha_5\beta_1$ and $\alpha_2\beta_1$ on cell migration showed that, in contrast to the results with cell spreading, both integrins appeared to be involved in migration on collagen. Time-lapse video microscopy of MTSV1-7 cells plated on the gel in the presence of integrin $\alpha$- and $\beta$-specific monoclonal antibodies revealed that cell motility was impaired by the monoclonal antibodies (Table 2). The anti-mucin control monoclonal antibody (HMFG-1) actually stimulated the rate of cell migration.

The above analysis suggests that the specific inhibitory effect of anti-VLA2 monoclonal antibodies on

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**Fig. 4.** Structure formation induced by collagen type 1 fibrils on a monolayer of normal human mammary epithelial cells (MTSV1-7 cells).

**Fig. 5.** Double immunofluorescence labelling of MTSV1-7 cells for the $\beta_1$ subunit of integrins (A,C) and collagen type 1 (B,D). The Figure shows the formation of ridges (A,B) and balls (C,D) around collagen fibrils.
Fig. 6. Electron micrograph of an ultrathin section through a ridge of MTSV1-7 cell formed around a collagen fibril (C). Arrow indicates a collagen fibril surrounded by epithelial cells. ×3000

Table 1. Spreading of MTSV1-7 cells on collagen gels and effect of antibodies to integrins

<table>
<thead>
<tr>
<th>Cells and antibodies</th>
<th>Spread cells % (attached cells)</th>
<th>Number of cells analyzed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control MTSV1-7 cells</td>
<td>84.9±4.1</td>
<td>929</td>
</tr>
<tr>
<td>MTSV1-7 cells+PlB5 (α2)</td>
<td>85.1±2.5</td>
<td>891</td>
</tr>
<tr>
<td>MTSV1-7 cells+PlE6 (α5)</td>
<td>30.5±3.5</td>
<td>878</td>
</tr>
<tr>
<td>MTSV1-7 cells+mAB13 (β1)</td>
<td>1.9±0.2</td>
<td>890</td>
</tr>
<tr>
<td>MTSV1-7 cells+HMFG-1</td>
<td>91.9±4.0</td>
<td>872</td>
</tr>
</tbody>
</table>

Cells were added to the substrate either alone or in the presence of monoclonal antibodies to β1 (20 µg/ml), α2 (1:10 dilution of the ascites), α5 (1:10 dilution of the ascites) and to a polymorphic epithelial mucin (100 µg/ml). Cells were cultured overnight and the number of spread cells estimated from two independent experiments from at least 10 independent fields.

Table 2. Migration of MTSV1-7 cells on collagen gels and effect of antibodies to integrins

<table>
<thead>
<tr>
<th>Cells and antibodies</th>
<th>Speed of movement* (µm/hour)</th>
<th>Number of cells analyzed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control MTSV1-7 cells</td>
<td>9.6±1.40</td>
<td>27</td>
</tr>
<tr>
<td>MTSV1-7 cells+PlB5 (α2)</td>
<td>4.2±0.70</td>
<td>26</td>
</tr>
<tr>
<td>MTSV1-7 cells+PlE6 (α5)</td>
<td>6.5±0.64</td>
<td>31</td>
</tr>
<tr>
<td>MTSV1-7 cells+mAB13 (β1)</td>
<td>2.7±0.23</td>
<td>30</td>
</tr>
<tr>
<td>MTSV1-7 cells+HMFG-1 (PEM*)</td>
<td>13.1±1.20</td>
<td>32</td>
</tr>
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</table>

The migration of individual cells for 20 h was recorded by time-lapse video microscopy and rates of migration were measured as described in Materials and Methods. The figures in the Table represent the average of three separate experiments.

*During first 20 hours after plating

Polymorphic epithelial mucin

Discussion

In this report, we have used a new approach for studying the morphogenesis of human mammary epithelial cells induced by collagen type I fibrils and have obtained evidence for a crucial role of the α2β1 integrin receptor in this collagen induced morphogenesis (CIRM). Originally applied to human umbilical vein endothelium (Jackson and Jenkins, 1991), the method describes a simple and rapid procedure for the induction of three-dimensional structure from cell monolayers in response to the addition of soluble collagen. When a normal human mammary epithelial cell line (MTSV1-7) was tested in the CIRM assay, ridges and ball-like structures appeared on the cell monolayer after 1-3 days, the new multicellular structures being formed only around collagen fibrils. In previous studies we have shown that normal human mammary epithelial cells form well-organized compact structures when grown within collagen gels, while cell lines developed from primary human breast carcinomas grow as single cells or as disorganized clumps. We find that the behaviour of normal and tumour derived cell lines in the rapid morphogenesis assay correlates well with the behaviour of the cells in collagen gels, i.e. only the normal cell lines form structures (data not shown).

Since CIRM was specifically associated with collagen type I we investigated the role of collagen-binding integrins in the phenomenon. Immunoprecipitation and immunofluorescent staining revealed that MTSV1-7 cells expressed α2β1 and α5β1 integrins at a high level. A role for the VLA-2 integrin in collagen induced morphogenesis of MTSV1-7 cells was directly demonstrated by showing that CIRM could be blocked with α2
Fig. 7. Effect of monoclonal antibodies to integrin subunits on CIRM. Monolayers of MTSV1-7 cells were pretreated with medium alone (A) or with monoclonal antibodies against α2- (B), α3- (C) and β1- (D) subunits of integrins and collagen type 1 was added to the growth medium. The formation of ridges and balls was efficiently blocked by the monoclonal antibodies to α2- and β1-integrin subunits.

Fig. 8. Inhibition of MTSV1-7 cell adhesion to collagen gels. Cells were plated on top of collagen gels and allowed to attach for 30 min, 1 h and 2 h in the presence or absence of monoclonal antibodies against α2- (P1E6), α3- (P1B5) and β1- (monoclonal antibody 13) integrin subunits and against mucin (HMFG-1). Non-adherent cells were removed by washing. Alkaline phosphatase activity of adherent cells was measured as described in Materials and Methods. Alkaline phosphatase activity of cells bound to collagen gel in the absence of antibodies is indicated as 100%. Adhesion was measured in quadruplicate in three independent experiments.
collagen gels and in the generation of the isometric forces which cause gel contraction. On the other hand, it has been shown that contraction of the collagen gel itself can induce morphological changes and functional differentiation of mammary epithelial cells grown on the top of collagen gels (Emerman et al., 1979). Based on these observations, one hypothesis is that in the collagen induced morphogenesis described here, the MTSV1-7 cells spread over the collagen fibrils using the $\alpha_2\beta_1$ integrin and thus induce mechanical forces in the fibrils: These forces in turn could then reorganize the cell monolayer leading to the appearance of ridges and ball-like structures. This mechanism postulates a crucial role for cell spreading in CIRM. Therefore, we analyzed in detail the attachment, spreading and migration of the cells on collagen gels.

The data obtained from cell attachment to collagen suggest that in human mammary epithelial cells, $\alpha_2\beta_1$, but not $\alpha_3\beta_1$, is specifically responsible for the primary recognition and cell attachment to polymerized collagen. However the data suggest that other proteins can also be involved in cell adhesion to collagen as has been previously shown for rat mammary epithelial cells (Wirl and Pfaffle, 1988), and HBL-100, another human mammary cell line (Elenius et al., 1990). Thus, it seemed unlikely that the inhibitory effect of these antibodies on morphogenesis was mediated by inhibition of attachment of MTSV1-7 cells to collagen. Further analysis of the separate functions of spreading and migration on collagen, led to the conclusion that the VLA2 integrin was specifically involved in cell spreading. On the other hand, although migration of MTSV1-7 cells on collagen was also inhibited by antibodies to the $\alpha_2$ integrin subunit, a similar inhibition was observed with antibodies to the $\alpha_3$ integrin. Since $\alpha_3$-specific antibodies did not inhibit cell attachment to collagen gels, the mechanism of cell migration utilizing $\alpha_3\beta_1$ integrin might involve a deposition of another extracellular matrix molecule, which could be the substrate for VLA-3 (Carter et al., 1990; 1991). An alternative to the
hypothesis is that newly formed collagen fibrils on the cell monolayer could induce a mitogenic signal and rapid proliferation of underlying cells leading to the appearance of multicellular three-dimensional structures.

Although monoclonal antibodies to α2β1 integrin could block collagen-induced morphogenesis of the human mammary cells in vitro the relevance of VLA2 to mammary gland development in vivo remains to be elucidated. In the in vitro system described here, the rapid reorganization of the cell monolayer apparently involves cell proliferation, spreading and migration of the epithelial sheet as a whole along the collagen fibrils. This process might imitate the stages of the mammary bud formation in the mammary gland development (Sakakura, 1987; Russo and Russo, 1987) and may provide a convenient model system for a study of these early events in the mammary gland morphogenesis.

In this report we have concentrated on analyzing the role of the collagen binding integrins in CIRM. However, using this simple in vitro system, it should now be possible to examine the role of other surface molecules which may also play a role in collagen induced morphogenesis.

The authors would like to thank Dr M Hemler, Dr K Yamada, Dr E Ruoslahti, Dr W Lankes and Dr M Colnaghi for the antibodies. We are also grateful to the ICRF Electron Microscopy Unit for technical assistance.

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(Received 19 March 1992 - Accepted 1 May 1992)