Eur J Cancer, Vol. 26, No. 3, pp. 231–240, 1990.
Printed in Great Britain

0277–5379/90$3.00 + 0.00

Adhesion, Growth and Cytoskeletal Characteristics of 8701-BC Breast Carcinoma Cells Cultured in the Presence of Type V Collagen*

CLAUDIO LUPARELLO,† ROSARIA SCHILLACI,† IDA PUCCI-MINAFRA† and SALVATORE MINAFRA‡

†*Dipartimento di Biologia Cellulare e dello Sviluppo and* ‡*Istituto di Istologia ed Embriologia, Università di Palermo, Italy*

Abstract—*Type V collagen is one of the minor components of the extracellular matrix (ECM) whose content is increased in cases of ductal infiltrating carcinomas of the breast. In order to clarify its biological role, we have investigated the effect of this molecule, both as substrate and as soluble factor, on the behaviour of a breast carcinoma cell line* (8701-*BC*) *grown* in vitro. *Cell-collagen adhesion was monitored for* 24 h *from plating in the absence or presence of serum. The influence of type V collagen on cell growth was followed during* 9 *days of culture, and the actin–vinculin arrangement was studied by simultaneous fluorescent immuno-staining. The results indicate that type V collagen is not a permissive substrate for neoplastic cell proliferation and dissemination* in vitro.

INTRODUCTION

TYPE V collagen, a minor component of the multigenic collagen family, was originally detected in foetal membranes [1] and later found in several embryonic and adult tissues, where it comprises only a minor fraction of the total collagen (see Ref. [2] for a review). Of significant interest, type V collagen is enhanced in some neoplastic tissues, compared to their normal counterparts [3–6].

Although the molecular and structural characteristics of type V collagen have been thoroughly investigated in the last few years [2], its biological role, both in normal tissues and in cancer, is still obscure.

Our previous investigations of primary ductal infiltrating carcinomas (d.i.c.) of the human breast have shown that the desmoplastic stroma undergoes drastic degradative and productive alterations [6–10], and that the new matrix, in turn, appears to influence some phenotypic aspects of neoplastic cells at the tumor–stroma interface [11].

The most relevant features of the collagen composition of the desmoplastic stroma were the appearance of an embryo-foetal collagen, type I-trimer [7, 8, 12] and an increase in type V [6]. Furthermore, type I-trimer, when used as a substrate for neoplastic cells in culture compared to type I, exerted a promotional effect on cell proliferation, cell migration and cytoskeletal organization [11]. Hence, it appeared to be important to inquire about the influence of type V collagen on tumour cell behaviour.

In the present study we have investigated the effects of type V collagen on the adhesion, growth and cytoskeletal organization of a neoplastic cell line (8701-BC) derived from a primary d.i.c. [13]. It is well known that the invasiveness of malignant tumours, whatever the initiating factor, is associated with two combined aspects of transformed cells, progressive multiplication and cell migration, and that both can be affected to some extent by specific biological substrates, via transmembranous signals to the cytoskeleton [14, 15].

Since the relationship between the cytoskeletal arrangement and metastatic potential of neoplastic cells has been widely recognized [16–20], we have also studied the distribution of two cytoskeletal markers, actin and vinculin, being a probe for microfilament assembly and a component of adhesion plaques respectively [21, 22].

The results suggest that type V collagen apparently exerts an inhibitory effect on cell growth and dissemination, which appears as an antagonist to that exerted by type I-trimer [11].

Accepted 18 October 1989.
Address for correspondence and reprint requests: Prof. S. Minafra, Istituto di Istologia ed Embriologia, Via Archirafi 20, 90123 Palermo, Italy.
*Work supported by grants from AIRC and Ministero della Pubblica Istruzione (R.S.60%).

MATERIALS AND METHODS

Cell culture

A neoplastic cell line, 8701-BC, was obtained from a biopsy fragment of a primary d.i.c. and characterized by morphological, immunological, cytogenetic [13] and ultrastructural parameters [23].

Culture medium was RPMI 1640 (Gibco, Paisley, U.K.), with 10% foetal calf serum (Difco, Detroit, IL, U.S.A.), 5% tryptose-phosphate broth (Difco), 100 u/ml penicillin and 100 μg/ml streptomycin. The cells used for the study were at the 50–58th passages.

Type V collagen

Type V collagen was extracted and fractionated from d.i.c. biopsy fragments according to the procedure described in Ref. [6]. Briefly, after mild pepsin extraction, the collagen solution was exhaustively dialysed against 0.02 M Na_2HPO_4 containing 15% KCl; type V, which is the only collagen remaining in solution, was then precipitated by prolonged dialysis against 0.02 M Na_2HPO_4. Purified type V collagen from human placental tissue, purchased from Sigma (St. Louis, MO, U.S.A.), was also used. The purity of the collagen preparations was tested by SDS-PAGE (Fig. 1).

In a first set of experiments, parallel analyses were performed using placental and tumour-derived collagen. Since similar results were obtained, we have utilized type V collagen obtained from Sigma, as it is readily available in larger quantities.

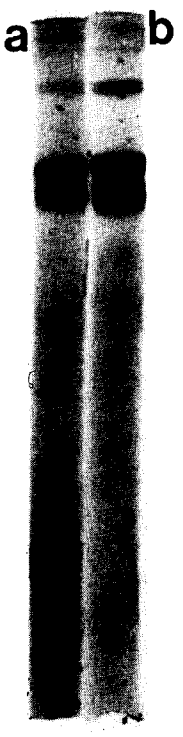

Fig. 1. Coomassie-stained SDS-PAGE of tumoural (a) and placental (b) type V collagen. Gel composition was 5% acrylamide, 0.13% bis-acrylamide, 0.1% sodium dodecylsulphate and 4 M urea.

Preparation of type V collagen-coated dishes

Type V collagen (100 μg/ml) was dissolved in 0.5 M acetic acid at 4°C. Falcon dishes (35 mm diameter; Becton Dickinson, Lincoln Park, NJ, U.S.A.) were filled with 1 ml of the solution and the collagen coating obtained by incubating the dishes in a humidified chamber at 37°C for 48 h.

Before use, residual acid was removed and the substrates brought to neutral pH by several washings with medium and then sterilized under U.V. light [11].

Adhesion assay

The procedure is a modification of the method described by Farsi *et al.* [24]. Microwell plates (96 wells, Becton Dickinson) were coated with 3, 10 or 20 μg/well of type V collagen. 8701-BC cells, detached from flasks, were washed three times with serum-free culture medium and plated at a concentration of 5×10^4/well in the same medium. The percentage of attached cells was extrapolated from counts of the non-attached cells. In practice, floating cells residing in the medium were combined with loosely adherent cells, harvested by washing the well with measured aliquots of medium three times. Cells were counted in a Bürker haemocytometer after staining with 0.1% toluidine blue.

Adhesion was assayed in triplicate 2, 4, 6 and 24 h after plating.

Growth assay on type V collagen substrate

8701-BC cells were plated at a concentration of 3×10^3 cm^{-2} on plain and collagen-coated dishes. Cell numbers were measured at different times by removal with EDTA–trypsin solution, staining and counting as described above. Growth parameters were calculated by the following formulae:

$$\text{Growth rate } (\alpha) = (\ln C - \ln C_0)/(t - t_0)$$

where C is the cell concentration at time t and C_0 is the number of cells initially seeded (i.e. at time t_0);

$$\text{Doubling time } (T) = \ln 2/\alpha \text{ [11].}$$

An attachment assay in serum-containing medium was performed in order to elucidate cell behaviour within 24 h from seeding. Cells were plated at the concentration of 2×10^4 cm^{-2} on plain and collagen-coated dishes. Just before processing for the measurement of attachment, cells were observed under a Leitz Diavert inverted microscope and representative fields were photographed

utilizing a Wild photoautomat MPS 55 system and 125 ASA Ilford FP4 films. Attachment was assayed 2, 4, 6 and 24 h from seeding.

Growth assay in the presence of type V collagen as a soluble factor

Type V collagen was dissolved at a known concentration in 0.5 M acetic acid and sterilized as reported in Ref. [25]: the acidic collagen solution was dialysed against acetic acid at the same molarity plus chloroform (2 ml/l) for 2 h in the cold. Subsequently, before use the collagen solution was exhaustively dialysed against a physiological buffer. 8701-BC cells were plated at a concentration of 3×10^3 cm^{-2} on uncoated Falcon dishes and collagen was added at 100, 50, 20, 10, 5 and 2 μg/dish 24 h after seeding. The cell number was counted on the 7th day of culture.

Double immunofluorescence

Cells were seeded at a concentration of 2×10^3 cm^{-2} on plain and collagen-coated glass coverslips and grown for 72–96 h. For immunofluorescent labelling, cells were first extracted with 0.5% Triton X-100 in 50 mM morpholinoethane sulphonate buffer (pH 6), plus 3 mM EGTA and 5 mM magnesium chloride, prewarmed at 37°C for 2 min [16]. Between every treatment during the test, the coverslips were exhaustively washed with PBS (0.13 M NaCl, 5×10^{-3} M magnesium chloride, 1×10^{-4} M calcium chloride and 5×10^{-3} M potassium chloride in 0.02 M phosphate buffer, pH 7.2). After washing at 37°C, cells were fixed with prewarmed 3.5% formaldehyde in PBS (20 min). The subsequent steps were at 4°C. The cells were treated with 0.1 M glycine in PBS to block free aldehyde groups still present (10 min), and then with 2% bovine serum albumin in PBS for 15 min [11].

The immunologic reagents were diluted in PBS according to the manufacturer's instructions as follows:

Primary reagents: rabbit anti-actin serum (A-2668), 1:10; monoclonal mouse anti-vinculin antibodies (V-4505), 1:50. Secondary reagents: fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit IgG F(ab')$_2$ fragments (F-1262), 1:40; tetramethyl rhodamine isothiocyanate (TRITC)-conjugated goat anti-mouse IgG F(ab')$_2$ fragments (T-6528), 1:16.

The fixed cells were incubated with both primary antibodies together for 20 min; after washing, both secondary antibodies were used simultaneously and incubated for 20 min. The coverslips were then washed for 20 min with several changes, mounted on PBS, sealed with nail varnish and observed under a Zeiss III microscope with 63× and 100× plan achro oil immersion objectives. Photographs were taken using a Zeiss MC 63 automated photographic system and 400 ASA Ilford HP5 films.

All reagents were purchased from Sigma.

RESULTS

Previously we have shown that 8701-BC cells are able to adhere directly to collagen type I, type I-trimer and type IV within a few hours [26], and that trimer exerts a preferential long-lasting effect on cell proliferation [11]. When type V collagen was used as substrate for 8701-BC cells, an unexpected effect was observed.

Figure 2 shows the kinetics of cell attachment to substrates coated with 3, 10 and 20 μg of type V collagen, under serum-free conditions, over a period of 24 h. Two hours after seeding, only 20% of cells are firmly attached to wells coated with 3 μg of type V collagen. Under these conditions, cell attachment increases slowly, reaching a maximum value of 43% 6 h after seeding, and it remains steady till 24 h (when virtually all cells are attached to uncoated plastic). The collagen concentration appears more critical at the initial phase of cell attachment, since the increase of collagen from 3 to 10 μg causes a further 60% of decrease in cellular attachment. A small effect was observed increasing the concentration to 20 μg, and from 4 h onwards, the effect of collagen concentration, at least over the range tested, appears irrelevant.

We also examined the possible influence of type V collagen on cell proliferation. To check this, 8701-BC cells were plated at the density given in the methods on type V collagen coated dishes, in the presence of serum, and compared with cells on plain plastic. Figure 3 shows that cells plated on type V collagen decrease during the lag phase (0–2 days); moreover, the attached cells display a very low proliferative activity, resulting in only a 4.75 times increase of the initial cell concentration. This is most evident during the exponential phase (from 4 days) by the low growth rate ($\alpha = 0.27$) and a doubling time of 61.6 h. To verify if the slowing down of cell proliferation was attributable to the lower cell density due to the intial cell decrease, cells were plated on uncoated dishes at 50% of the original density, corresponding to the 24 h cell density on type V collagen. Even under these conditions cell proliferation was rapidly restored (data not shown).

In order to assess if the initial cell decrease observed on type V collagen substrates was due to a defective cell adhesion or to a successive cell detachment, we performed a cell attachment assay to type V collagen compared to plastic over the first day of culture in serum-containing medium. Figure 4 shows micrographs of 8701-BC cells on plain plastic (a–d) and on type V substrate (a'–d') at 2, 4, 6 and 24 h from seeding, and Fig. 5 the

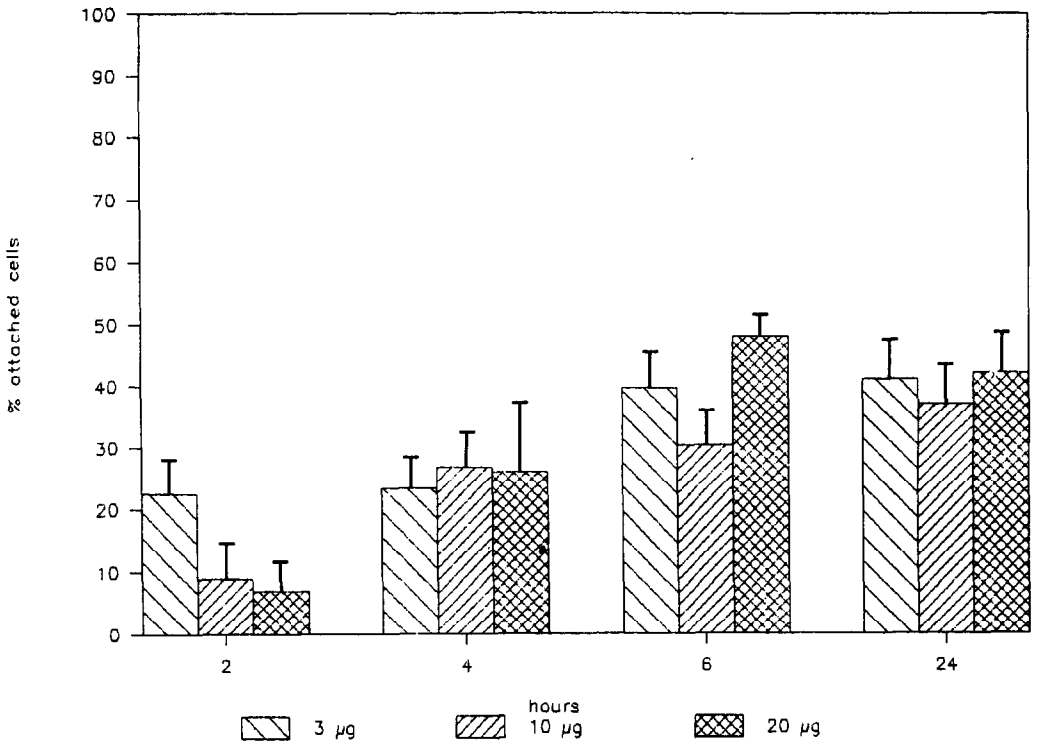

Fig. 2. Adhesion assay of 8701-*BC cells on type V collagen at different concentrations in serum-free medium: after* 2 h *from seeding about* 20% *of cells are firmly spread in the presence of* 3 µg *of substrate. Cell attachment increases slowly up to* 43% *till* 6 h *and then remains steady. An increase in collagen content to* 10 *and* 20 µg *leads to a further cell adhesion decrease of about* 60%. *From* 4 h *onwards the effect of collagen concentration appears to be negligible. Vertical bars indicate the standard deviation (S.D.) of three different experiments made in duplicate.*

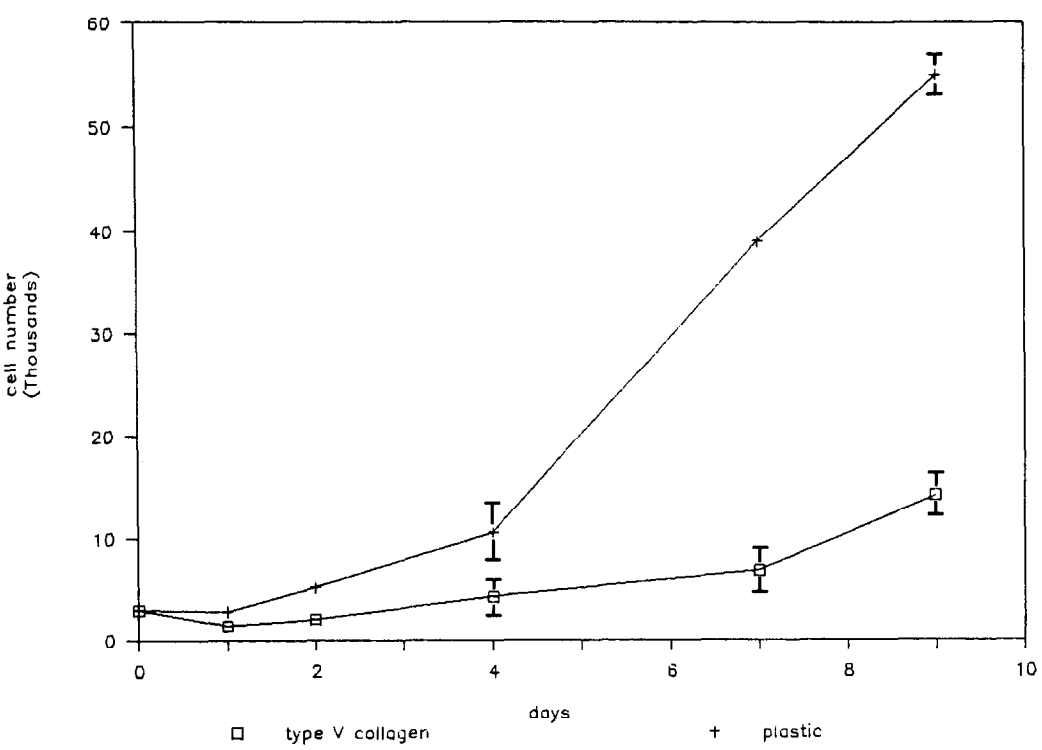

Fig. 3. 8701-*BC cells, trypsinized after the* 51–54*th passages, were plated on type V collagen-coated and uncoated* 35-mm *Falcon dishes at a density of* 3×10^3 cm^{-2}. *Cells were grown in 'stress conditions', i.e. without renewing the medium during the experimental period, in order to avoid any stimulating effect by serum components. Vertical bars represent the S.D. of three different determinations made in duplicate; where not indicated, S.D. is* $< 1 \times 10^3$ cm^{-2}. *In the lag phase cells on type V collagen decrease in number, and the attached fraction acquires a very low proliferative activity in the log phase* ($\alpha = 0.27$, $T = 61.6$ h). *After* 9 *days, the number of cells grown on collagen displays a* 4.75 *times increase with respect to the plating density, versus* 17 *times when grown on plastic.*

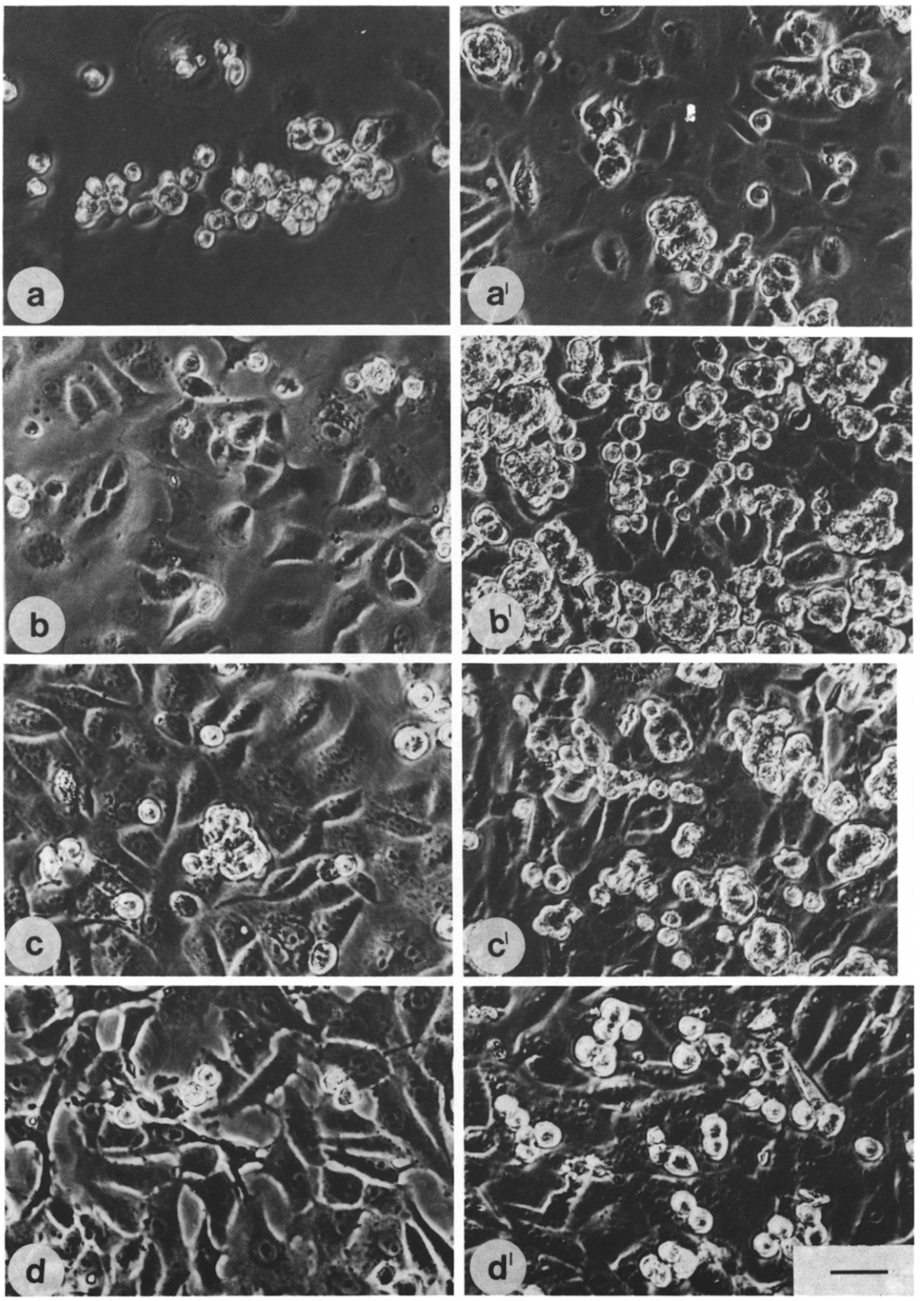

Fig. 4. Qualitative cell attachment assay in serum-containing medium: the micrographs show 8701-*BC cells on plastic (a–d) and on type V collagen (a'–d'), after* 2 h *(a, a'),* 4 h *(b, b'),* 6 h *(c, c') and* 24 h *(d, d') from seeding. After* 2 h, *cells on plastic are almost entirely unattached, while an aliquot begins to adhere on type V. After* 6 h, *the adhesion process is nearly completed on plastic, whilst at* 24 h *from plating a conspicuous fraction of cells is still rounded and unattached to collagen. However, cells attached on type V appear more cohesive than cells on plastic. The measurement of cell attachment is reported in Fig. 5. Bar =* 50 μm.

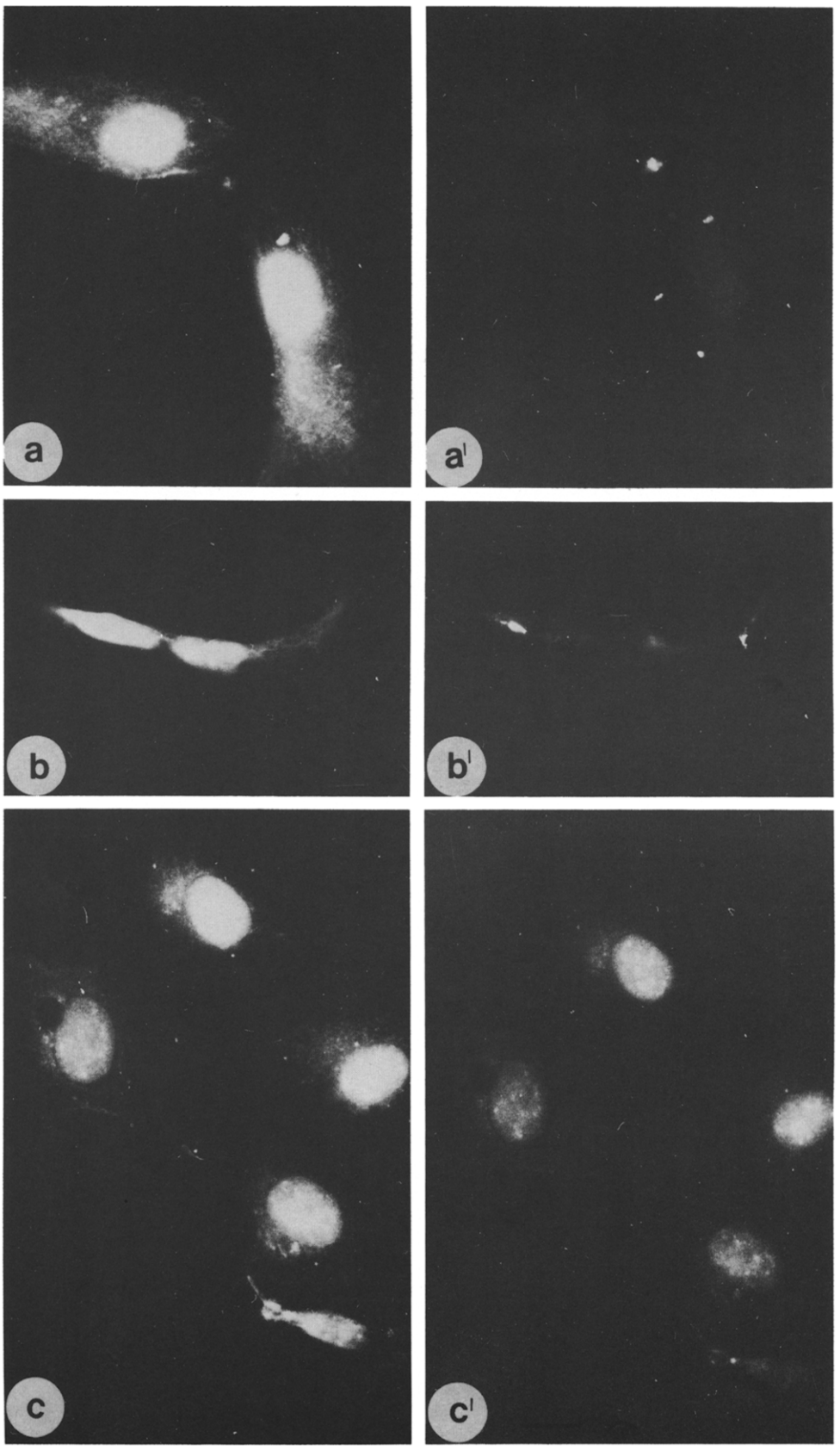

Fig. 6. Actin (a–c) and vinculin (a'–c') double immunolocalization in 8701-BC cells grown on plastic (a, a') and type V (b–c'). Cells on plastic display a diffuse arrangement of the actin network and a very faint reaction to vinculin. In the presence of type V collagen, no significant reorganization of the actin component is observed, whilst a slightly higher positivity to vinculin can be detected. Vinculin appears to be concentrated in few peripheral plaques in fusiform cells (b') and scattered (mostly co-localized with the microfilamentous apparatus) in the polygonal cells (c'). Bar = 14 μm (a–b'), 20 μm (c, c').

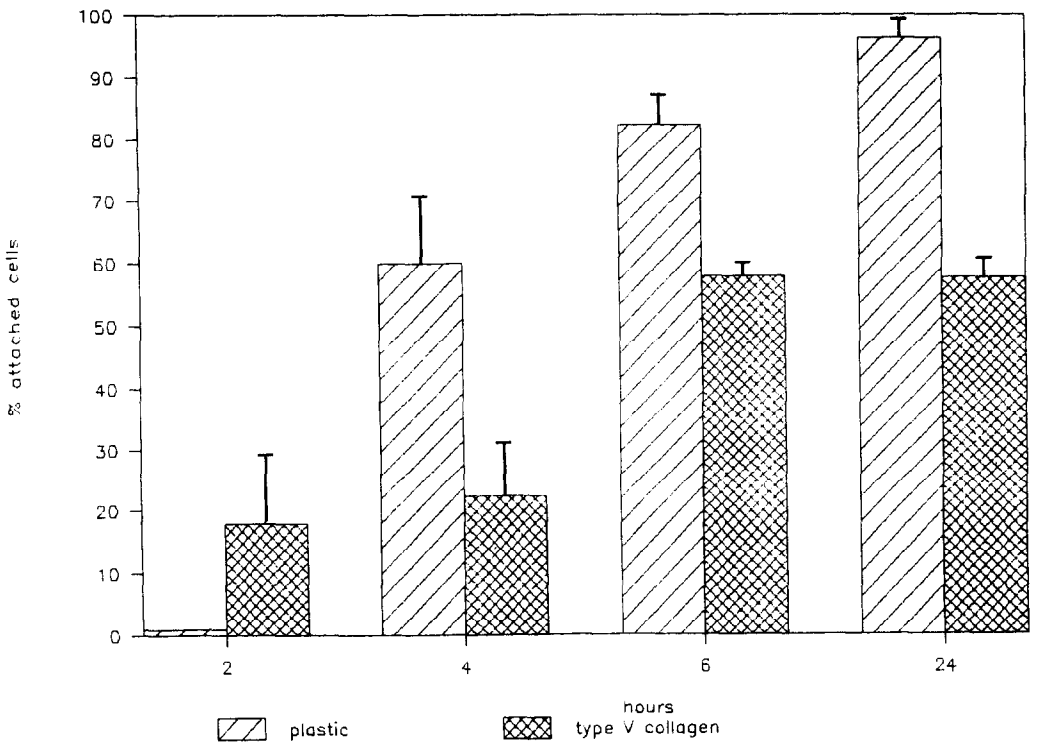

Fig. 5. Cell attachment assay in serum-containing medium: after 2 h *from seeding*, 17.85 ± 15.6% *of cells are already spread onto collagen, versus* 1% *on plastic. Subsequently, the attachment on plastic is more rapid (spread cells are* 82.5 ± 6.5% *after* 6 h, *and* 96.3 ± 3.3% *after* 24 h). *As already shown in Fig. 4, only a fraction of cells* (57.5 ± 5%) *is firmly attached to collagen after* 24 h. *Vertical bars represent the S.D. over three different determinations made in duplicate.*

quantitative assay performed in parallel with the microscopic observations. Two hours after seeding the majority of cells are still floating on the plastic substrate, while about 18% begin to adhere to collagen. From 4 h onwards, an inversion of the adhesion values was observed: cells on plastic progressively adhered to the substrate, completing their attachment process during the 24 h, while cell adhesion to type V collagen slowly increased within 6 h and remained steady over the 24 h, with a maximum value of 57.5 ± 5%. These results indicate that the presence of serum has a little, if any, influence on the attachment to type V collagen. In addition cells spread on type V collagen apparently display tighter cell-to-cell and cell–substrate aggregation patterns (compare Figs 4d and 4d′). The generation of stronger adhesion forces in the presence of type V is also indirectly suggested by the low susceptibility of cells to EDTA–trypsin treatment during experimental manipulations, compared to cells on uncoated dishes.

In order to determine if the slowing down of the growth rate, observed in the preceding experiments, was to be ascribed to the physical nature of type V collagen as a substrate, or to its chemical effect on cell behaviour, aliquots of soluble collagen were added to the culture and cells counted after 7 days incubation. The inhibitory effect of collagen was already detectable at low doses, since 2 μg of type V collagen resulted in 19% inhibition of growth. Besides, the inhibitory effect of type V increases progressively, being 29, 56, 90 and 96% for 10, 20, 50 and 100 μg/dish respectively. This finding suggests that the negative influence of type V collagen on cell growth is not merely due to shape variations following the attachment to the substrate, but, more specifically, to its chemical interaction with the cell surface.

We then investigated the actin and vinculin arrangement in cells grown for 72–96 h on type V collagen substrate. Triton extraction preceding fixation removes the cytoplasmic soluble pool of the two cytoskeletal proteins, allowing a better identification of their organized components [27].

Figure 6 shows the double-immunostaining of cells incubated with anti-actin and anti-vinculin. Cells on plastic display a diffuse arrangement of the actin network (Fig. 6a), as expected for transformed cells lacking stress fibres [16–20], and relatively few adhesion plaques (Fig. 6a′). Cells seeded on type V collagen (Figs 6b and 6c) underwent no significant change of their actin organization, as compared to that seen in cells grown on type I-trimer [11]. A slightly higher positivity was detected for the anti-vinculin reaction, which appears either diffuse in cells with polygonal morphology (Fig. 6c′) or concentrated in few peripheral adhesion plaques in fusiform cells (Fig. 6b′). The presence of more than one morphological cytotypes in 8701-BC cell cultures has been previously reported [13, 23].

DISCUSSION

Studies by many investigators over the past decade have modified the concept of the extracellular matrix (ECM) from an inert and passive scaffold, into a flexible and probably multi-functional system [28, for review].

During embryogenesis, the ECM is in a highly dynamic state and takes part in morphogenetic events [29, 30]. In adult organs, the basal lamina in concert with the underlying connective tissue is responsible for the maintainance of tissue architecture and for functional cyto-differentiation [31].

Moreover, the collagenous stroma in adult organs provides a barrier against the penetration of large molecules [32] and prevents cell migration. This equilibrium is broken down in malignant tumours. Paradoxically, some highly invasive carcinomas, such as d.i.c. of the breast, produce large amounts of collagen, which appear to be ineffective for restraining neoplastic cell penetration.

Traditionally, the hyper-production of ECM components in d.i.c. was considered a defensive host reaction against the cancer cells [3, 33]. We have previously reported that the stroma in d.i.c. undergoes local lytic degradations [10], which probably preceed the deposition of a new collagenous matrix. In addition, the neoplastic cells themselves are capable of synthesizing type I-trimer collagen but apparently not type V [34], whose production is to be searched for within the host's mesenchymal compartment. These findings are in good agreement with other reports in the literature, stressing the relevance of degradative and compositional matrix alterations in carcinogenesis [28, 35–38 for reviews]. However, the specific effect of individual matrix components on gene expression, phenotypical modulations and clonal selections of a neoplastic cell population within primary tumours are far from being understood.

Our present results have demonstrated an unexpected inhibitory effect exerted by type V collagen on the proliferation and dissemination of 8701-BC cancer cells, which appears as an antagonist to that exerted by type I-trimer on the same system. The most impressive data are the progressive decrease of the cell population grown in the presence of type V collagen, both as soluble factor and as a substrate compared to plastic, especially when contrasted to an average 35% growth increase induced by type I-trimer collagen [11]. Interestingly, some authors [39] have recently observed a similar inhibitory effect by type V collagen on endothelial cells.

A point which will require further study is the identification of the receptors for type V collagen and their distribution within the heterogeneous malignant cell population.

Type V collagen appears to be unable to promote a dynamically oriented reorganization of the cytoskeleton, as observed in the presence of type I-trimer [11]. On the contrary, type V collagen appears to slightly enhance vinculin expression, as is typical for cells in a more stationary state [16]. This, together with the inhibitory effect on cell multiplication, suggests type V collagen as an ECM component more suitable for restraining than promoting the spread of malignant cells.

Taking our previous and present results together we can formulate the hypothesis that the collagenous matrix newly deposited in the desmoplastic stroma of d.i.c. produces at least two antagonist local signals which may alternatively direct the neoplastic cells towards the development of a more or a less malignant phenotype. Our hypothesis, however, does not exclude the existence of other concomitant influences from the microenvironment and the host tissue.

Acknowledgements—Claudio Luparello is a research fellow of the Italian Association for Cancer Research (AIRC); Rosaria S-chillaci is a research fellow of the Sicilian Regional Government (Ass. Sanità).

REFERENCES

1. Burgeson RE, El Adli FA, Kaitila JJ, Hollister DW. Fetal membrane collagen: identification of two new collagen α chains. *Proc Natl Acad Sci USA* 1976, **73**, 2579–2583.
2. Miller EJ, Gay S. The collagens: an overview and update. In: Cunningham LW, ed. *Methods in Enzymology*. Orlando, Academic Press, 1987, Vol. 144, 3–41.
3. Barsky SH, Rao CN, Grotendorst GR, Liotta LA. Increased content of type V collagen in desmoplasia of human breast carcinoma. *Am J Pathol* 1982, **108**, 276–283.
4. Yamamoto M, Sumiyoshi H, Nakagami K, Tahara E. Distribution of collagen types I, III and V in fibrotic and neoplastic human liver. *Acta Pathol Jap* 1984, **34**, 77–86.
5. Marian B, Danner MW. Skin tumor promotion is associated with increased type V collagen content in the dermis. *Carcinogenesis* 1987, **8**, 151–154.
6. Luparello C, Rizzo CP, Schillaci R, Pucci-Minafra I. Fractionation of type V collagen from carcinomatous and dysplasic breast in the presence of alkaline potassium chloride. *Anal Biochem* 1988, **169**, 26–32.
7. Minafra S, Pucci-Minafra I, Tomasino RM, Sciarrino S. Collagen composition in the ductal infiltrating carcinoma of the human breast. *Cell Biol Int Rep* 1984, **8**, 79–85.

8. Pucci-Minafra I, Luparello C, Sciarrino S, Tomasino RM, Minafra S. Quantitative determination of collagen types present in the ductal infiltrating carcinoma of the human mammary gland. *Cell Biol Int Rep* 1985, **9**, 291–296.
9. Pucci-Minafra I, Minafra S, Tomasino RM, Sciarrino S, Tinervia R. Collagen changes in the ductal infiltrating ('scirrhous') carcinoma of the human breast. A possible role played by type I-trimer collagen on the invasive growth. *J Submicrosc Cytol* 1986, **18**, 795–805.
10. Pucci-Minafra I, Luparello C, Schillaci R, Sciarrino S. Ultrastructure evidence of collagenolytic activity in ductal infiltrating carcinoma of the human breast. *Int J Cancer* 1987, **39**, 599–603.
11. Schillaci R, Luparello C, Minafra S. Type I and I-trimer collagens as substrates for breast carcinoma cells in culture. Effect on growth rate, morphological appearance and actin organization. *Eur J Cell Biol* 1989, **48**, 135–141.
12. Luparello C, Pucci-Minafra I. Different behavior of type I and type I-trimer collagen in neutral sodium chloride solutions. *Anal Biochem* 1986, **155**, 325–327.
13. Minafra S, Morello V, Glorioso F *et al.* A new cell line (8701-BC) from primary ductal infiltrating carcinoma of human breast. *Br J Cancer* 1989, **60**, 185–192.
14. Vlodavski I, Lui GM, Gospodarowicz D. Morphological appearance, growth behavior and migratory activity of human tumor cells maintained on extracellular matrix versus plastic. *Cell* 1980, **19**, 607–616.
15. Watt FM. The extracellular matrix and cell shape. *Trends Biochem Sci* 1986, **11**, 482–485.
16. Raz A, Geiger B. Altered organization of cell–substrate contacts and membrane-associated cytoskeleton in tumor cell variants exhibiting different metastatic capabilities. *Cancer Res* 1982, **42**, 5183–5190.
17. Volk T, Geiger B, Raz A. Motility and adhesive properties of high- and low-metastatic murine neoplastic cells. *Cancer Res* 1984, **44**, 811–824.
18. Di Renzo MF, Tarone G, Comoglio PM, Marchisio PC. Organization of cytoskeleton and fibronectin matrix in Rous Sarcoma Virus (RSV)-transformed fibroblast lines with different metastatic potential. *Eur J Cancer Clin Oncol* 1985, **21**, 85–96.
19. Zachary JM, Cleveland G, Kwock L *et al.* Actin filament organization of the Dunning R3327 rat prostatic adenocarcinoma system: correlation with metastatic potential. *Cancer Res* 1986, **45**, 926–932.
20. Curschellas E, Matter A, Regenass U. Immunolocalization of cytoskeletal elements in human mammary epithelial cells. *Eur J Cancer Clin Oncol* 1987, **23**, 1517–1527.
21. Burridge K, Molony L, Kelly T. Adhesion plaques: sites of transmembrane interaction between the extracellular matrix and the actin cytoskeleton. *J Cell Sci* 1987, Suppl 8, 211–229.
22. Geiger B, Volk T, Volberg T, Bendori R. Molecular interactions in adherens-type contacts. *J Cell Sci* 1987, Suppl 8, 251–272.
23. Pucci-Minafra I, Minafra S, Alessandro R, Faccini AM. An ultrastructural evaluation of cell heterogeneity in invasive ductal carcinomas of the human breast. II. An *in vitro* study. *J Submicrosc Cytol Pathol* 1989, **21**, 489–499.
24. Farsi JMA, Sodek J, Aubin JE. Fibronectin-independent attachment of human gingival fibroblasts to interstitial and basement membrane collagens. *Exp Cell Res* 1985, **161**, 473–483.
25. Kleinman HK, Luckenbill-Edds L, Cannon FW, Sephel GC. Use of extracellular matrix components for cell culture. *Anal Biochem* 1987, **166**, 1–13.
26. Ghersi G, La Fiura AM, Minafra S. Direct adhesion to type I and homotrimer collagens by breast carcinoma and embryonic epithelial cells in culture: a comparative study. *Eur J Cell Biol* 1989, **50**, 279–284.
27. Geiger B. Microheterogeneity of avian and mammalian vinculin. Distinctive subcellular distribution of different isovinculins. *J Molec Biol* 1982, **159**, 685–701.
28. Van den Hooff A. Stromal involvment in malignant growth. *Adv Cancer Res* 1988, **50**, 159–196.
29. Hay ED. Collagen and embryonic development. In: Hay ED, ed. *Cell Biology of Extracellular Matrix*. New York, Plenum Press, 1981, 379–409.
30. Spooner BS, Thompson-Pletscher HA, Stokes B, Bassett KE. Extracellular matrix involvement in epithelial branching morphogenesis. In: Steinberg MS, ed. *Developmental Biology. A Comprehensive Synthesis*. New York, Plenum Press, 1986, Vol. 3, 225–260.
31. Cunha GR, Bigsby RM, Cooke PS, Sugimura Y. Stromal–epithelial interactions in adult organs. *Cell Diff* 1985, **17**, 137–148.
32. Pearce RH, Laurent TC. Exclusion of dextrans by meshworks of collagenous fibers. *Biochem J* 1977, **163**, 617–625.
33. Azzopardi JG. Problems in breast pathology. In: *Major Problems in Pathology*. London, WB Saunders, 1979, 388–394.
34. Minafra S, Luparello C, Rallo F, Pucci-Minafra I. Collagen biosynthesis by a breast carcinoma cell strain and biopsy fragments of the primary tumour. *Cell Biol Int Rep* 1988, **12**, 895–905.
35. Poste G, Fidler IJ. The pathogenesis of cancer metastasis. *Nature* 1980, **283**, 139–146.

36. Nicolson GL. Cancer metastasis. Organ colonization and the cell surface properties of malignant cells. *Biochim Biophys Acta* 1982, **695**, 113–176.
37. Woolley DE. Proteolytic enzymes of invasive cells. In: Mareel MM, Calman KC, eds. *Invasion. Experimental and Clinical Implications*. Oxford, Oxford University Press, 1984, 228–251.
38. Gorstein F. The dynamic extracellular matrix. *Hum Pathol* 1988, **19**, 751–752.
39. Fukuda K, Koshihara Y, Oda H, Ohyama M, Ooyama T. Type V collagen selectively inhibits human endothelial cell proliferation. *Biochem Biophys Res Commun* 1988, **151**, 1060–1068.