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RGD peptides may only temporarily inhibit cell adhesion to fibronectin

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When human fibroblasts were cultured on fibronectin for 4 h in the presence of 0.5 mg/ml of the GRGDSP peptide derived from the fibronectin cell-binding site, they adhered and spread normally and organized talin and integrin α_5 and β_1 subunits into focal adhesions. When the adherent cells were quantitated as a function of time, submaximal peptide concentrations were found to delay cell adhesion on fibronectin, but they had no effect on the maximum. When the cells were plated on vitronectin, however, even relatively low peptide concentrations lowered the maximal amount of cells adhering and abolished cell spreading. The results suggest a different mechanism for cell adhesion on fibronectin and vitronectin

Cell adhesion, Fibronectin, Vitronectin; Integrin; Fibronectin peptide

1. INTRODUCTION

Synthetic peptides, containing the sequence RGD (Arg-Gly-Asp) or some of its variants, have been shown to inhibit adhesion of various cells to several extracellular matrix proteins including fibronectin, vitronectin, von Willebrand factor, fibrinogen, thrombospondin and osteopontin [1]. The sequence was first found in fibronectin [2] and mapped as the main interaction site for the cell surface fibronectin-binding protein, the α_5/β_1 integrin [3]. This protein also interacts with cytoskeletal proteins at the focal adhesions [4], thus regulating the organization of cytoskeleton when cells adhere and spread on fibronectin. RGD peptides have been used to inhibit the function of the α_5/β_1 complex and other integrins in several experimental models, including experimental metastasis [5] and cell migration [6].

2. MATERIALS AND METHODS

Human embryonic fibroblasts (code F84-67) were obtained from a local source, GRGDSP and GRGES peptides were from Multiple Peptide Systems Inc. (San Diego, CA), and the rat monoclonal antibody B1E5 against the α_5 integrin subunit from Dr C. Damsky (San Francisco, CA) [7]. The mouse monoclonal antibody against the β_1 integrin subunit (102DF5) has been characterized earlier [8]. For indirect immunofluorescence microscopy fluorochrome-coupled sheep anti-mouse or goat anti-rat IgG antisera (Cappel, Organon Teknika, West Chester, PA) were used. Adhesion experiments were done in serum-free RPMI 1640 medium on glass coverslips or 96-well multiwell dishes (Nunc, Kamstrup, Denmark) treated for 1 h (at room temperature) with 20 μg/ml of human fibronectin or vitronectin (purified as in [9,10]) followed by treatment with 1% bovine serum albumin solution (Sigma, St. Louis, MO). To quantitate the adhered cells, the cultures were first labelled with 10 μCi/ml of [^{35}S]-methionine (Amersham International, Amersham, UK) for 12 h and then seeded on the multiwell dishes. For some experiments monensin (10 μM; Eli Lilly, Indianapolis, IN) was simultaneously added. After indicated time periods the wells were washed, and the adherent cells were solubilized in 0.5 M NaOH, 1% SDS, and counted in a liquid scintillation counter. The results are shown as mean ± SE values from 4 separate experiments.

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3. RESULTS

When human fibroblasts were plated on fibronectin-treated surfaces in the presence of 0.5 mg/ml of the synthetic GRGDSP peptide, their initial adhesion was diminished with about 50%. However, within 4 h the same amount of cells had been attached as in the control (Fig. 1A), the cells had spread, and the β_1 and α_5 integrin subunits (Fig. 2), as well as talin (not shown) were detected at focal adhesions. At this time the peptide-exposed cells still showed diminished organization of fibronectin fibers (not shown, see [11]) and the organization of the β_1 and α_5 integrins in structures coaligning with these fibers was also less prominent than in the control cells (Fig. 2). 1.5 mg/ml of the peptide completely inhibited cell adhesion on fibronectin (Fig. 1A).

On vitronectin-coated surfaces 0.5 mg/ml of the GRGDSP peptide almost completely inhibited cell adhesion. 0.005 mg/ml of the peptide, which caused 50% inhibition of cell adhesion within 1 h, also diminished the maximal adhesion reached within 4 h, as did even 0.001 mg/ml of the peptide (Fig. 1B). Even the low concentrations blocked cell spreading on vitronectin (not shown). To study whether the different effects of the peptide to cell spreading on vitronectin and on fibronectin was dependent on cellular secretion of proteins, adhesion experiments on fibronectin were

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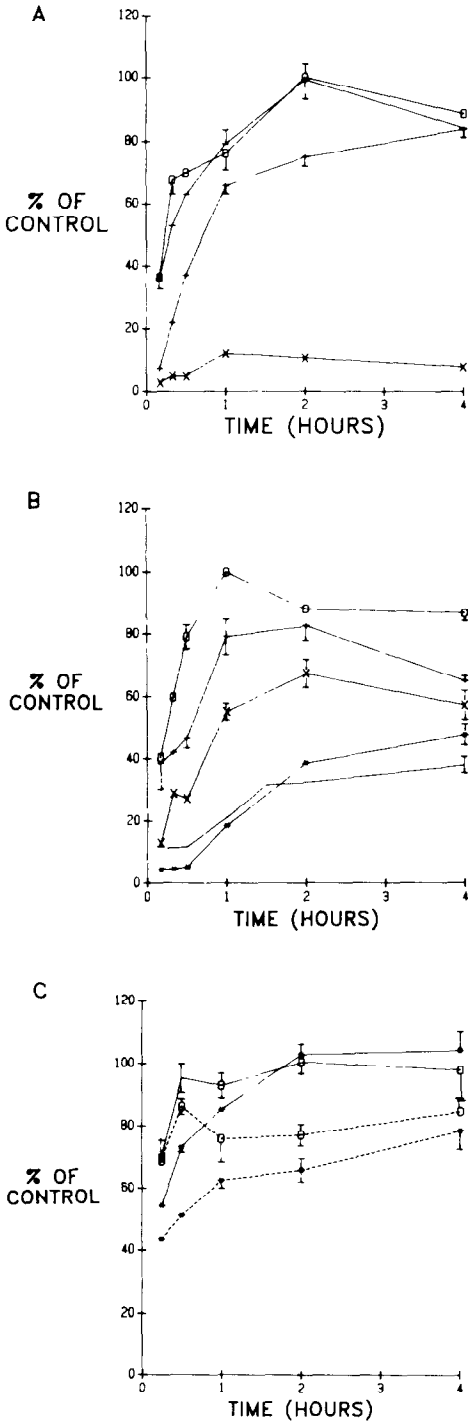

Fig. 1. Adhesion of human fibroblasts on fibronectin (A and C) or on vitronectin (B) in the presence of various concentrations of GRGDSP peptide. In each panel 100% represents the maximal adhesion reached in the particular experiment. (A) (○) Control without the peptide; (−) control with 1.5 mg/ml of the inactive GRGES peptide; (+) 0.5 mg/ml GRGDSP; (×) 1.5 mg/ml GRGDSP. (B) (○) Control without the peptide; (+) 0.001 mg/ml GRGDSP; (×) 0.005 mg/ml GRGDSP; (*) 0.5 mg/ml GRGDSP; (−) background adhesion on BSA-coated surfaces. (C) Adhesion on fibronectin in the presence (dashed line) or absence of monensin (solid line) and without (○) or with (*) 0.25 mg/ml GRGDSP peptide.

also made in the presence of 10 μM monensin, which blocks cellular secretion. Although monensin reduced the amount of adhered radioactivity, it only slightly prolonged the inhibition effect of the peptide, and similar maximal adhesion was reached as in the absence of the peptide and in the presence of monensin (Fig. 1C).

4. DISCUSSION

GRGDSP [12] or GRGDS [13] peptides have been shown to be the most active among RGD-containing peptides in the inhibition of cell adhesion on fibronectin. Some differences in the inhibitory ability have been noted between different cells [13], and the peptides are commonly more potent inhibitors of cell adhesion on vitronectin than on fibronectin [12,13]. The inhibitory activity of the peptides has usually been measured in short term (30 min–1 h) adhesion assays [12,13]. Here I have shown that, at least in human fibroblasts, peptide concentrations that may cause 50% inhibition of cell adhesion in short term assays only delayed the adhesion on fibronectin, but caused a more permanent inhibition of adhesion and spreading on vitronectin. This difference cannot be explained by that the cells would use the secreted fibronectin for adhesion, since (i) the cells most probably secrete similar amounts of fibronectin either when cultured on fibronectin or on vitronectin, but on vitronectin the inhibitory effect of the RGD peptide does not cease within 4 h, and (ii) blocking the secretion had no prominent effect on the inhibition. The possibility of different degradation rates of the peptide on the two culture substrata is also quite unprobable. Monensin, however, slightly decreased the amount of radioactivity adhered to the substratum, but this might be explained by its nonspecific effects.

The observed difference in the long term inhibition effect of the RGD peptide suggests that fibroblasts might use different mechanisms of adhesion on fibronectin than on vitronectin. Apparently, several co-operative cell-fibronectin interaction sites [14,15] are used and the adhesion is less sensitive to blocking of the RGD site, while such co-operative sites have not been described for vitronectin.

The present finding may be important, when various applications of the RGD peptides are developed. It is possible that the phenomenon reported here is cell-type specific. Anyway, it seems that when fibroblasts are allowed to attach on fibronectin under stationary conditions, huge amounts of GRGDSP or related peptides are needed to inhibit the adhesion process. Such concentrations may be highly toxic in vivo. On the other hand, under dynamic conditions, in which the rate of initial attachment might be the delimiting step in cell adhesion to fibronectin, as well as in the cases where other matrix proteins than fibronectin are the promi-

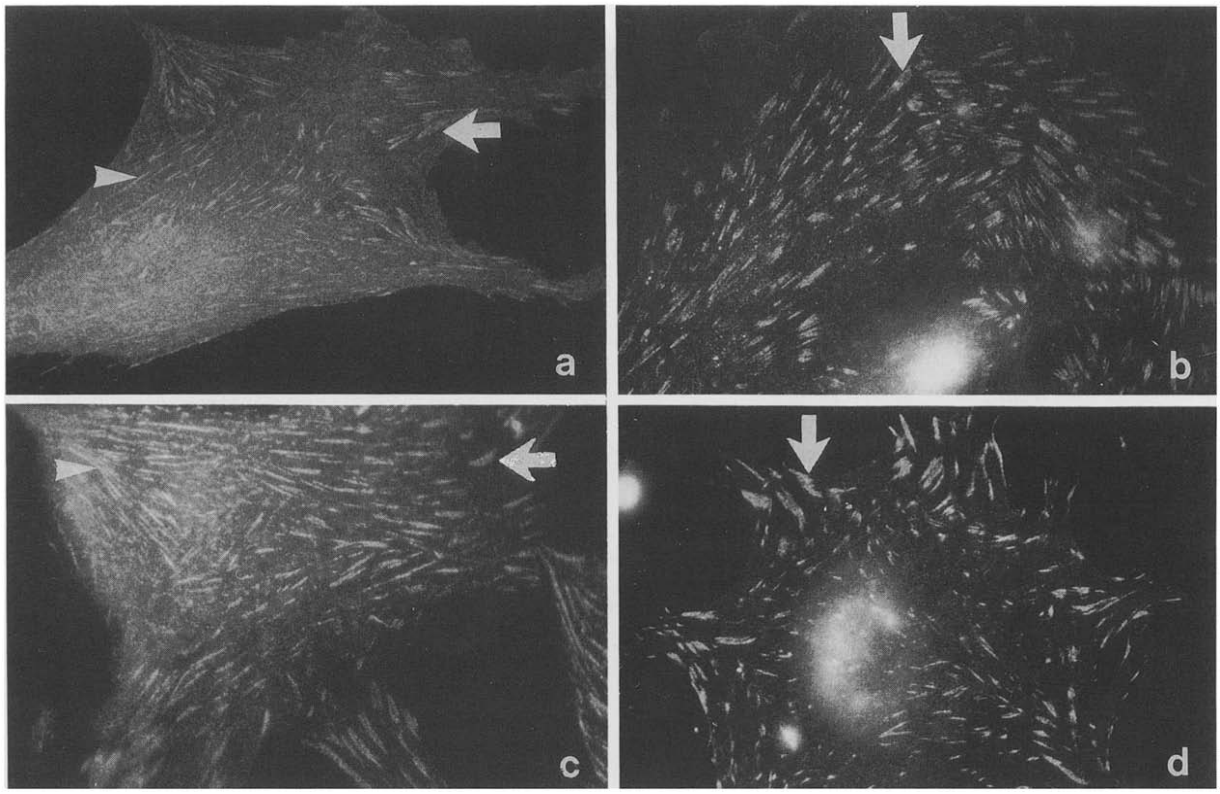

Fig. 2. Localization of the β_1 (a, b) and α_5 (c, d) integrin subunits by indirect immunofluorescence microscopy. Human fibroblasts were cultured for 4 h on fibronectin in the absence (a, c) or presence of 0.5 mg/ml GRGDSP peptide (b, d). Arrows point at the focal adhesions which can be seen in each case, and arrowheads (a, c) at the fibrillar structures, which are less prominent in the presence of the peptide (b, d).

nent adhesive components, the peptides may be used in much lower concentrations.

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REFERENCES

[1] Ruoslahti, E. and Pierschbacher, M.D. (1987) Science 238, 491–497.
[2] Pierschbacher, M.D. and Ruoslahti, E. (1984) Proc. Natl. Acad. Sci. USA 81, 5985–5988.
[3] Pytela, R., Pierschbacher, M.D. and Ruoslahti, E. (1985) Cell 40, 191–198.
[4] Buck, C.A. (1987) Annu. Rev. Cell Biol. 3, 179–205.
[5] Humphries, M.J., Olden, K. and Yamada, K.M. (1986) Science 233, 467–470.
[6] Akiyama, S.K., Yamada, S.S., Chen, W.-T. and Yamada, K.M. (1989) J. Cell Biol. 109, 863–875.
[7] Werb, Z., Tremble, P.M., Behrendtsen, O., Crowley, E. and Damsky, C.H. (1989) J. Cell Biol. 109, 877–889.
[8] Ylanne, J. and Virtanen, I. (1989) Int. J. Cancer 43, 1126–1136.
[9] Engvall, E. and Ruoslahti, E. (1977) Int. J. Cancer 20, 1–5.
[10] Yatohgo, T., Izumi, M., Kashiwagi, H. and Hayashi, M. (1988) Cell Struct. Funct. 13, 281–292.
[11] McDonald, J.A., Quade, B.J., Broekelmann, T.J., Lachance, R., Forsman, K., Hasegawa, E. and Akiyama, S. (1987) J. Biol. Chem. 262, 2957–2967.
[12] Pierschbacher, M.D. and Ruoslahti, E. (1987) J. Biol. Chem. 262, 17294–17298.
[13] Yamada, K.M. and Kennedy, D.W. (1987) J. Cell Physiol. 130, 21–28.
[14] Obara, M., Kang, M.S. and Yamada, K.M. (1988) Cell 53, 649–657.
[15] Humphries, M.J., Akiyama, S.K., Komoriya, A., Olden, K. and Yamada, K. (1986) J. Cell Biol. 103, 2637–2647.