Volume 217, number 1, 124–128 FEB 04787 June 1987

Monoclonal antibody to fibronectin which inhibits extracellular matrix assembly

M.A. Chernousov, A.I. Faerman, M.G. Frid, O.Yu. Printseva and V.E. Koteliansky

Institute of Experimental Cardiology, USSR Cardiology Research Center, Academy of Medical Sciences, 3rd Cherepkovskaya Str. 15A, 121552 Moscow, USSR

Received 2 April 1987

A monoclonal antibody L8 specific to fibronectin was shown to inhibit fibronectin incorporation into the fibroblast extracellular matrix. Antibody L8 could not interact with fibronectin complexed with gelatin. The results suggest the existence of a specific site on the fibronectin molecule playing a critical role in the assembly of the fibronectin extracellular matrix. This site is located near the collagen-binding domain.

Monoclonal antibody; Fibronectin; Extracellular matrix assembly

1. INTRODUCTION

Fibronectin is an adhesive glycoprotein present in a soluble form in plasma and in an insoluble form in connective tissues and in association with basement membranes [1–3]. Fibronectin in cell cultures is secreted into the medium or deposited into the extracellular matrix and on the cell surface. The cell surface and extracellular fibronectin is found in close association with collagens, proteoglycans and other components of the extracellular matrix [4,5]. Plasma fibronectin can be incorporated into extracellular matrices of different tissues and cultured cells [6–9]. It was proposed that a special receptor on substrate-attached cells is involved in assembly of soluble fibronectin into insoluble fibrils of the extracellular matrix [8]. Fibronectin matrix formation may also occur through a self-assembly process [9]. In both cases the amino-terminal part of fibronectin was responsible for incorporation of plasma fibronectin into the extracellular matrix [9,10]. To learn more about assembly of the fibronectin matrix we have studied the effect of monoclonal antibody to fibronectin on the formation of fibroblast extracellular matrix. Antibody L8 was shown to be able to prevent extracellular fibronectin fibril formation. Our results suggest the existence of a functional site on fibronectin which is required for assembly of soluble fibronectin into insoluble extracellular matrix.

Correspondence address: M.A. Chernousov, Institute of Experimental Cardiology, USSR Cardiology Research Center, Academy of Medical Sciences, 3rd Cherepkovskaya Str. 15A, 121552 Moscow, USSR

2. MATERIALS AND METHODS

Monoclonal antibody L8 was established after immunization of BALB/C mice with rat aorta smooth muscle cells [11]. Positive clones secreting antifibronectin antibodies were expanded as ascites tumors in BALB/C mice. For the experiments the IgG fraction was isolated from ascites fluid by chromatography on DEAE-cellulose. The obtained monoclonal antibody had a purity greater than 98% according to polyacrylamide gel electrophoresis.

Human skin fibroblasts were grown on glass coverslips in Dulbecco's modified Eagle's medium (DMEM) containing 0.1 mg/ml of different monoclonal antibodies or preimmune IgG, 10% fetal calf serum depleted of fibronectin, and 1

Published by Elsevier Science Publishers B.V. (Biomedical Division)

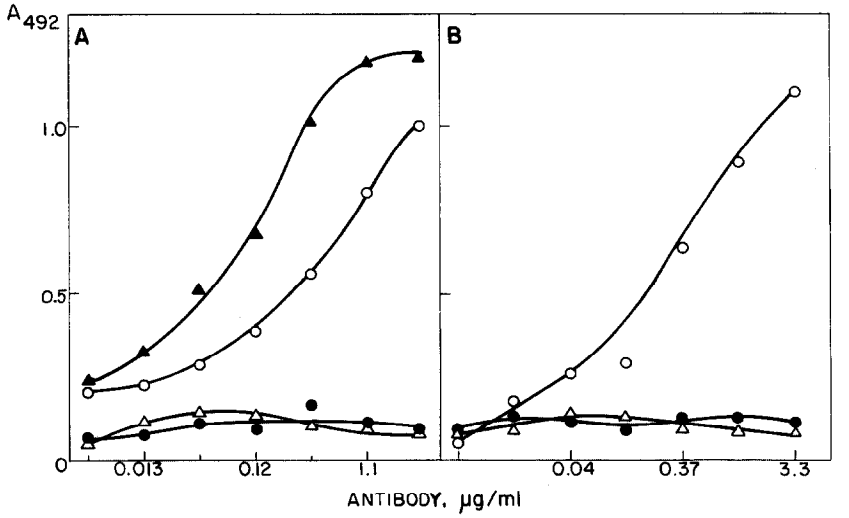


Fig.1. Interaction of monoclonal antibodies L8 with human plasma fibronectin. (A) Polystyrene plates were coated with native fibronectin (▲—▲); bovine serum albumin (●—●); prior to coating fibronectin was incubated in PBS, containing 6 M urea (○—○); prior to coating fibronectin was incubated in PBS containing 0.02 M dithiothreitol (△—△). (B) Polystyrene plates were coated with gelatin (○—○,●—●) or bovine serum albumin (△—△) and fibronectin was allowed to bind to gelatin and albumin. After washing monoclonal antibody L8 (●—●) or other monoclonal antibodies (○—○, △—△) were added into the wells. Rabbit (anti-mouse IgG) IgG conjugated with peroxidase were used to detect monoclonal antibody bound to immobilized fibronectin-gelatin complex.

week after plating the deposition of extracellular matrix fibronectin was detected by immunofluorescence. For immunostaining the coverslips were fixed with 5% paraformaldehyde for 20 min and washed in phosphate-buffered saline, pH 7.4 (PBS). After a wash in PBS samples were incubated for 1 h in the PBS containing affinity-purified rabbit polyclonal antibodies to human plasma fibronectin (0.02 mg/ml). The samples were then incubated for 1 h in solution containing fluorescein-conjugated goat anti-rabbit IgG, 0.05 mg/ml, followed by several washes in PBS. The cells were mounted in 50% glycerol/PBS and viewed with Zeiss photomicroscope III equipped with phase contrast and epifluorescence.

Interaction of monoclonal antibody L8 with fibronectin was tested by enzyme-linked immunoassay [12]. Microtitration plates were coated with proteins (0.01 mg/ml). Unbound protein was washed out and monoclonal antibody in PBS containing 0.05% Tween 20 was added. Antibodies were allowed to interact with protein coating the plate for 60 min at 20°C. Rabbit (anti-mouse IgG) IgG conjugated with horseradish peroxidase were used to detect monoclonal antibody bound to immobilized protein. *o*-Phenylenediamine was a substrate for peroxidase. The absorbance of the reaction products was recorded at 492 nm.

3. RESULTS

Fusion of splenocytes from mice immunized with rat aorta smooth muscle cells with mouse myeloma cells resulted in the establishment of antibody-producing hybridoma clone L8. These monoclonal antibodies reacted specifically with fibronectin (fig.1A). The antigenic determinant for antibody L8 was not disturbed after incubation of fibronectin with 6 M urea (fig.1A). Reduction of fibronectin disulfide bonds by addition of 20 mM dithiothreitol caused the loss of the affinity antibody L8 to denatured fibronectin (fig.1A). ^{1}H-NMR spectroscopic studies of fibronectin structure demonstrated that complete denaturation of the fibronectin occurred only after reduction of disulfide bonds [13]. In solution, containing 6 M urea, fibronectin undergoes only partial unfolding which is not accompanied by the disruption of the

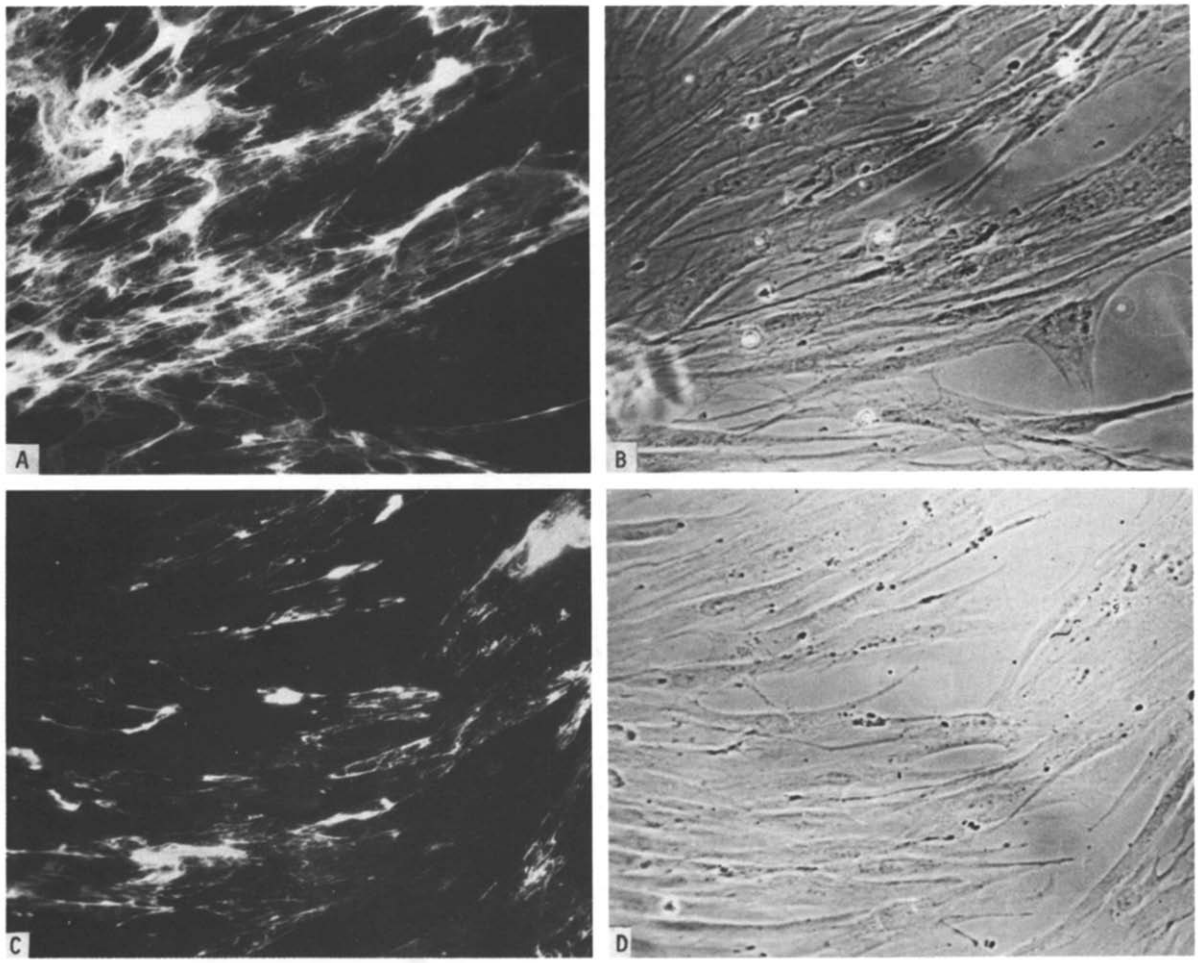
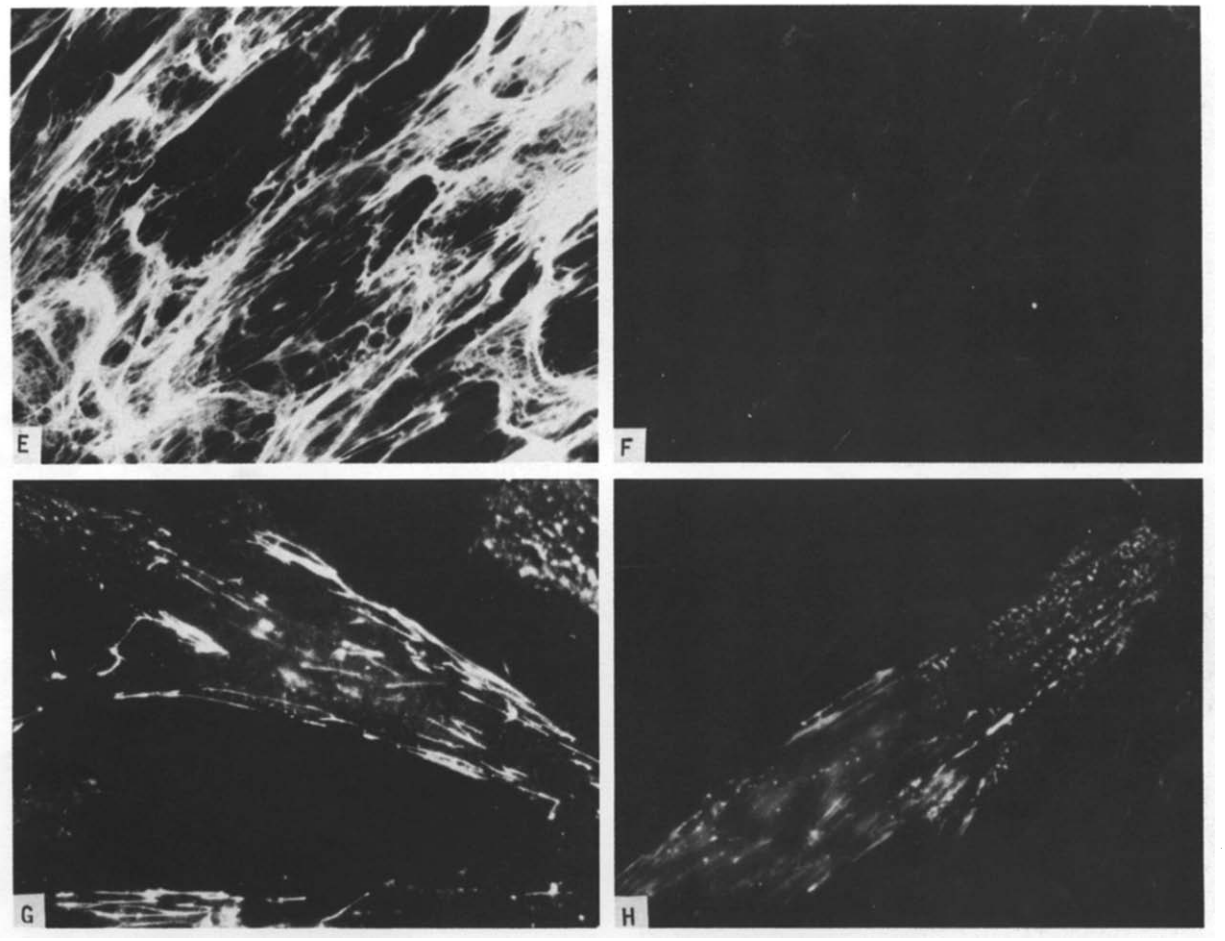


Fig.2. Effect of antifibronectin monoclonal antibody L8 on extracellular matrix formation. Human skin fibroblasts were cultured in medium, containing antifibronectin monoclonal antibody L8 (C,D,G,G) or other antifibronectin monoclonal antibodies (A,B), or preimmune mouse IgG (E,F). In (F) instead of rabbit antifibronectin polyclonal antibodies non-immune rabbit IgG were added. A,C,E,F,G,H, immunofluorescence; B,D, phase contrast.

compact structural domains [13]. The antibody L8-binding site seems to be a structural domain and disulfide bonds play an important role in maintaining the tertiary structure of this domain. We have studied the effect of monoclonal antibody L8 upon the organization of fibronectin in fibroblast culture. Fibroblasts cultured in fibronectin-depleted medium deposited fibronectin as a network of interconnecting fibrils in the extracellular matrix (fig.2A,E). Fibroblasts cultured in the presence of antifibronectin antibody L8 (0.1 mg/ml) has very little detectable fibrillar extracellular fibronectin (fig.2C,G,H); fibronectin was present on the cell surface in short, fine fibrils. Fibroblasts plated in fibronectin-depleted medium containing antibody L8 attached and spread normally on glass substrate; there were no differences in cell density, morphology (fig.2B,D) and fibronectin synthesis. To exclude the possibility that antibody L8 blocked immunostaining of the extracellular matrix with polyclonal antifibronectin antibody we showed that binding of polyclonal antibody to fibronectin was not affected by an excess of antibody L8.

To define the position of the antibody L8-binding site on the fibronectin molecule we



have studied the effect of gelatin on binding of antibody L8 to fibronectin. The data shown in fig.1B demonstrate that antibody L8 could not interact with fibronectin if fibronectin was bound to gelatin. This result suggests that the monoclonal antibody-binding site is located very close to the collagen-binding domain in the NH_2-terminal part of the fibronectin molecule.

4. DISCUSSION

A monoclonal antifibronectin antibody L8 was obtained which inhibited the fibronectin organization in the fibroblast extracellular matrix. The epitope for these antibodies is very close to the collagen-binding domain of fibronectin. Thus, it appears that the region of fibronectin molecule near the collagen-binding domain plays a critical role in the assembly of the fibronectin extracellular matrix. Previously, it was demonstrated that in matrix assembly assay polyclonal antibody to whole fibronectin or specific to a 60 kDa collagen-binding fragment of fibronectin disrupted the fibronectin organization in the fibroblast extracellular matrix [14,15]. Recently, the presence of a new fibronectin cell-binding site was shown [8,10]. This site is located in the 70 kDa fibronectin NH_2-terminal collagen-binding fragment and is distinct from the well-characterized cell-adhesion site [10]. The 70 kDa fragment blocked incorporation of fibronectin into the extracellular matrix [10]. These data also indicate that there is a new matrix assembly site on fibronectin. There are two models explaining the incorporation of fibronectin into the extracellular matrix and the role of the new matrix assembly site in this process. The fibronectin may be incorporated into the extracellular matrix via special cell-surface receptors

and these cell matrix assembly receptors interact with the fibronectin matrix assembly site [8,10]. Additionally to a cell-dependent mechanism of fibronectin fibril formation, exogenous fibronectin becomes incorporated into preexisting extracellular matrix through a self-assembly process [9]. The NH_2-terminal part of fibronectin, containing the 70–60 kDa collagen-binding fragment, is responsible for fibronectin-fibronectin interaction during fibronectin fibril formation [9]. It was found that the gelatin-binding fragment could also interact with intact fibronectin, and therefore contains a site involved in fibronectin-fibronectin associations [16,17]. All these data suggest the possibility that the matrix assembly receptor for fibronectin is fibronectin itself. Now it is difficult to imagine how the interaction of fibronectin with the cell-surface matrix assembly receptor can lead to the incorporation of fibronectin into the extracellular matrix. However, in any case a specific site on the fibronectin molecule is involved in matrix assembly and this site can be recognized by the monoclonal antibody L8 described here.

REFERENCES

[1] Mosher, D.F. (1980) Prog. Hemostas. Thromb. 5, 111–151.
[2] Ruoslahti, E., Engvall, E. and Hayman, E.G. (1981) Coll. Res. 1, 95–128.
[3] Hynes, R.O. and Yamada, K.M. (1982) J. Cell Biol. 95, 369–377.
[4] Hay, E.D. (1981) Cell Biology of Extracellular Matrix, Plenum, New York.
[5] Kleinman, H.R., Klebe, R.J. and Martin, G.R. (1981) J. Cell Biol. 88, 573–585.
[6] Hayman, E.G. and Ruoslahti, E. (1979) J. Cell Biol. 88, 352–357.
[7] Oh, E., Pierschbacher, M. and Ruoslahti, E. (1981) Proc. Natl. Acad. Sci. USA 78, 3218–3221.
[8] McKeown-Longo, P.J. and Mosher, D.F. (1983) J. Cell Biol. 97, 466–472.
[9] Chernousov, M.A., Metsis, M.L. and Koteliansky, V.E. (1985) FEBS Lett. 183, 365–369.
[10] McKeown-Longo, P.J. and Mosher, D.F. (1985) J. Cell Biol. 100, 364–374.
[11] Printseva, O.Yu., Faerman, A.I. and Thurmin, A.V. (1987) Exp. Cell Res., in press.
[12] Rennard, S.Y., Berg, R., Martin, G.R., Foidart, J.M. and Robey, P.G. (1980) Anal. Biochem. 104, 205–214.
[13] Bushuev, V.N., Metsis, M.L., Morozkin, A.D., Ruuge, E.K., Sepetov, N.F. and Koteliansky, V.E. (1985) FEBS Lett. 189, 276–280.
[14] Kurkinen, M. and Vaheri, A. (1977) Cell Biol. Int. Rep. 1, 469–475.
[15] McDonald, J.A., Kelley, D.G. and Broekelmann, T.J. (1982) J. Cell. Biol. 92, 485–492.
[16] Ehrisman, R., Roth, D.E., Eppenberger, H.M. and Turner, D.C. (1982) J. Biol. Chem. 257, 7381–7387.
[17] Homandberg, G.A. and Erickson, J.W. (1986) Biochemistry 25, 6917–6925.