Collagen Can Modulate Cell Interactions with Fibronectin

KAZUHIRO NAGATA,* MARTIN J. HUMPHRIES,** KENNETH OLDEN,** and KENNETH M. YAMADA*

*Laboratory of Molecular Biology, Membrane Biochemistry Section, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205; and **Howard University Cancer Center, Washington, DC 20060

ABSTRACT We have examined the effects of soluble collagen on the function of fibronectin in baby hamster kidney (BHK) cells. Collagen and its purified α1(I) chain noncompetitively inhibited cell spreading on substrates precoated with fibronectin or a 75,000-D cell-binding fragment of fibronectin. Neither preincubation of cells with collagen followed by washing nor the addition of collagen to previously spread cells had any inhibitory effect on cell spreading, which indicates a requirement for the concurrent presence of collagen during the process of spreading. Treatment of collagen or α1(I) chain with collagenase abolished the inhibitory effect on fibronectin-mediated cell spreading. However, direct attachment of BHK cells to fibronectin-coated or 75,000-D fragment-coated substrates was not inhibited by collagen or by the α1(I) chain. Moreover, the binding of [3H]fibronectin or the 3H-75,000-D fragment to cell surfaces was not inhibited by the presence of soluble collagen, whereas soluble fibronectin inhibited binding. Although the binding of [3H]fibronectin-coated beads to BHK cell surfaces was also not inhibited by collagen, the phagocytosis of such beads was inhibited by the presence of collagen. On the other hand, soluble fibronectin partially inhibited the binding of fibronectin-coated beads but did not inhibit phagocytosis of the beads that did bind. The mechanism of the inhibition of fibronectin function by collagen and the possible interactions of two different kinds of receptors on the cell surface are discussed.

The glycoprotein fibronectin has provided insights into the mechanisms of cell adhesion and cell migration in vitro and in vivo (2, 7, 11, 14, 16, 17, 26, 32, 41, 43, 46). The interaction of cells with fibronectin appears to be mediated by a specific receptor that is saturable and that binds fibronectin with moderate affinity (K_D = 8 × 10^{-7} M; see reference 1). The binding and biological function of the fibronectin receptor can be inhibited by high concentrations of soluble fibronectin (18, 33, 47), a cell-binding fragment of fibronectin (47), and synthetic peptides containing its putative recognition signal for cell adhesion (4, 27, 28, 47, 48).

It is unlikely, however, that cells interact with only one extracellular molecule at a time in vivo. Since collagen is a ubiquitous extracellular molecule, we examined whether collagen might affect the interactions of cells with fibronectin. Native collagen in the form of a gel is known to interact with cells, and cell extension and migration in such gels is well-documented (e.g., 6, 12, 14, 19, 20, 40). Nevertheless, we found in this study that native collagen in the form of a polymerized gel inhibited cell spreading on a fibronectin substrate, even though cells had been permitted to form initial cell–substrate attachments.

Because such collagenous matrices might be inhibiting cell interactions with fibronectin by trivial physical interference, we focused on the effects of soluble collagen chains on cells interacting with such fibronectin substrates. Collagenous proteins were found to inhibit cell spreading on fibronectin noncompetitively, as well as the phagocytosis of fibronectin-coated beads. In contrast, collagen did not appear to inhibit the binding of fibronectin to the cell surface or the initial adhesion of cells to a fibronectin substrate. Our results suggest that the extracellular protein collagen can modulate the interaction of cells with a second matrix protein, fibronectin, by a noncompetitive mechanism.

MATERIALS AND METHODS

Fibronectin and the 75,000-D Cell-binding Fragment: Human plasma fibronectin was purified under nondenaturing conditions according to the methods of Miekka et al. (24). A 75,000-D cell-binding...
fragment was generated by trypsin digestion of human plasma fibronectin and purified exactly as described previously (15). Purity was confirmed by SDS polyacrylamide gel electrophoresis (21).

Collagen and α(1) Chain: Purified, pepsin-solubilized bovine dermal collagen (Vitrogen 100) was obtained from Collagen Corp. (Palo Alto, CA). Vitrogen was prepared at least three times with Dubbecco's modified Eagle's medium (Gibco Laboratories) and incubated at 37°C for 5 min. Trypsinization was terminated by the addition of an equal volume of 0.1% trypsin inhibitor (3x crystallized, Calbiochem-Behring Corp.). The supernatant was collected, filtered, and stored at −20°C.

For the cell-spreading assay, fibronectin (Fig. 1G). Spreading was never observed on control substrates lacking fibronectin (Fig. 1I).

A similar inhibition of fibronectin-mediated cell spreading was observed if the collagen was not preheated (Fig. 1I). α(1) Chain, and gelatin using a Vitrogen standard. The concentrations of other proteins were determined by the method of Lowry et al. (22) with BSA as standard.

RESULTS

Inhibition of Fibronectin-mediated Cell Spreading by Collagen

In a standard cell-spreading assay, ~80% of the added BHK cells spread on tissue culture substrates precoated with 2 μg/ml human plasma fibronectin or with 0.6 μg/ml of a purified 75,000-D cell-binding fragment of fibronectin (Fig. 1, A and D). Spreading was never observed on control substrates lacking fibronectin (Fig. 1G). As shown in Fig. 1 and quantitated in Fig. 2a, fibronectin-mediated cell spreading was progressively inhibited by increasing concentrations of collagen added to the adhesion medium. Such dose-dependent inhibition of fibronectin-mediated spreading was observed with collagen preparations before or after salt fractionation to remove traces of type III collagen, and also with a preparation of rat-tail collagen (data not shown).

A similar inhibition of fibronectin-mediated cell spreading was also observed if the collagen was not preheated (Fig. 1I). Native collagen was dialyzed against adhesion medium without heat denaturation. BHK cells were allowed to settle and attach to the fibronectin substrate for 5–10 min, and then the collagen solution was added gently. Such native collagen formed a gel during the 45-min assay period, and it also markedly inhibited cell spreading (Fig. 1I). α(1) Chain purified from collagen was more effective than was collagen in the inhibition of fibronectin-mediated spreading on a weight basis and was approximately equal in activity on a molar basis (Figs. 1 and 2a). In contrast, the inhibitory activity of

Abbreviation used in this paper: BHK, baby hamster kidney.
FIGURE 1 Inhibition of fibronectin-mediated cell spreading of BHK cells by collagen or its isolated α1(I) chain. BHK cells were inoculated onto substrates precoated with 2 μg/ml of fibronectin (A–C, H, and I) or 0.6 μg/ml of the 75,000-D cell-binding fragment of fibronectin (D–F). After a 45-min incubation at 37°C with or without collagenous proteins, the cells were fixed with 2.5% glutaraldehyde in PBS. (A, D, and G) No additions (G was a control substrate without precoating by fibronectin or the 75,000-D fragment). (B and E) 2 mg/ml of soluble collagen was added to the reaction mixture (C and F) 1.5 mg/ml (C) or 1.0 mg/ml (F) of α1(I) chain was added. As a collagenase control, collagen was added to the reaction mixture after preincubation (37°C, 30 min) with 100 U/ml of collagenase (Form III, Advance Biofactures Corp.); it did not inhibit cell spreading (H). To examine the effect of native collagen, cells were permitted to attach to a fibronectin-coated substrate, the adhesion medium was removed, and native collagen in adhesion medium was gently poured into the dish; native collagen also inhibited cell spreading (I). Bar, 100 μm.
gelatin was much lower than that of collagen (Fig. 2a).

To determine whether the inhibition of fibronectin-mediated spreading by collagen was due to its binding to the collagen-binding site on fibronectin, we compared these effects with the effects of collagen on spreading mediated by a 75,000-D cell-binding fragment of fibronectin that lacks the collagen-binding site. Both collagen and the purified $\alpha_1(1)$ chain displayed similar inhibitory dose-response curves for spreading on this fragment as for intact fibronectin (Figs. 1 and 2b).

Fig. 3 shows the time course of spreading on fibronectin-coated substrates in the presence or absence of soluble collagen. Collagen inhibited not only the initial rate of spreading but also the final percentage of spread cells.

Characterization of the Inhibition of Cell Spreading

To examine whether collagen irreversibly altered or dam-

aged the cells, we preincubated BHK cells with various concentrations of collagen at 37°C for 45 min, washed them with adhesion medium, and inoculated them onto substrates precoated with fibronectin. As shown in Fig. 4, fibronectin-mediated spreading was completely unaffected by the preincubation with collagen, indicating that the effects of collagen were reversible and probably not due to cytotoxicity or irreversible binding to cell surfaces.
Preincubation of the fibronectin-coated substrates with collagen or \( \alpha_1(1) \) chain followed by washing with adhesion medium had no effect on subsequent cell spreading of BHK cells (data not shown). This observation excludes the possibility of degradation of the substrate-adsorbed fibronectin by hypothetical proteases in the collagen sample.

We examined whether collagen induced rounding or detachment of cells already spread on fibronectin substrates. After incubating cells on fibronectin-coated substrates for 45 min, we added various concentrations (0.5–2.5 mg/ml) of collagen. It was clear that collagen had no effect on previously spread cells, indicating a requirement for the concurrent presence of collagen during the process of cell spreading (Fig. 4).

As a negative control, we examined the effect of high concentrations of BSA on spreading and found no effect on fibronectin-mediated spreading at concentrations up to 10 mg/ml (data not shown).

In another control, when the collagen was hydrolyzed with highly purified collagenase before use, all effects on fibronectin-mediated spreading of BHK cells were abolished (Figs. 1H and 5), suggesting a requirement for an intact collagen polypeptide backbone for the inhibition of spreading.

**Inhibition of Cell Spreading by Collagen Was Not Competitive**

If the inhibition of cell spreading by collagen were competitive in nature, increasing the concentration of fibronectin preadsorbed onto the substrate should overcome the inhibitory effect of soluble collagen. As shown in Fig. 6, this was not the case for the inhibition by collagen, since collagen could still inhibit cell spreading onto substrates precoated with high concentrations of fibronectin or the 75,000-D fragment as effectively as with lower concentrations of fibronectin. Even substrates coated with fibronectin at up to 50 \( \mu \)g/ml showed no decrease in collagen-mediated inhibition of cell spreading (data not shown).

**Attachment of BHK Cells to Fibronectin-coated Substrates Was Not Inhibited by Collagen**

It was important to determine whether collagen also inhibited the process of initial cell attachment or only the event of cell spreading. Fig. 7 shows that collagen had no effect on cell attachment to fibronectin-coated substrates at concentrations of up to 2.5 mg/ml, whereas it almost completely inhibited cell spreading in the same concentration range.

It was possible that cell attachment might be resistant to inhibition because less fibronectin was required for cell attachment than for spreading. In fact, cell attachment could be mediated by lower concentrations of fibronectin preadsorbed onto the substrate than that needed for cell spreading (Fig. 8). Nevertheless, fibronectin-mediated cell attachment was not inhibited by the presence of soluble collagen over the entire range of concentrations of fibronectin tested, including concentrations that yielded only partial cell attachment (Fig. 8).
were incubated at 37°C for 45 rain on fibronectin (2/~g/ml)-coated substrates in the presence of the indicated concentrations of collagen, we performed fibronectin binding assays using radiolabeled fibronectin or the purified 75,000-D cell-binding fragment as a positive control partially inhibited both the binding of [3H]fibronectin and the 75,000-D fragment to BHK cells (Table I). Although collagen inhibited the binding of radioactive fibronectin to BHK cells by a factor of ~0.6, it had no effect on the binding of the radioactive 75,000-D cell-binding fragment. The apparent inhibition of [3H]fibronectin binding to BHK cells could be explained by an artifact: direct binding of collagen to the collagen-binding domain of fibronectin might induce aggregates. In fact, we found that mixing collagen and fibronectin induced precipitable aggregates (data not shown). Thus, it was concluded that collagen had no effect on the binding of the fibronectin cell-binding site to fibronectin receptors on BHK cell surfaces.

Binding and Phagocytosis of Beads Coated with [3H]Fibronectin

The binding of substrate-adsorbed fibronectin to cells can be measured with fibronectin-coated latex beads (10, 23, 35). The binding of [3H]fibronectin-coated beads to BHK cell was not inhibited by the presence of collagen during incubation at 37°C for 90 min, while it was slightly inhibited by fibronectin (Table II). In contrast, the presence of collagen inhibited the uptake of [3H]fibronectin-coated beads to a trypsin-insensitive compartment (a measure of phagocytosis) by more than 50% that of controls (Table II). In a control in which cells were incubated with phosphate-buffered saline lacking trypsin, there was no inhibition by collagen compared with the untreated control, and slight inhibition by fibronectin (Table II). It was of interest that this relatively low concentration of fibronectin slightly inhibited the initial binding of fibronectin-coated beads but did not inhibit the phagocytic process itself.

It was necessary to evaluate the possibility that collagen reduced the amount of cell-bound radioactivity by simply increasing the rate of degradation and excretion of radioactive peptides during the 90-min incubation period and that the initial rate of endocytosis of fibronectin-coated beads might have been normal. The assay period was therefore shortened to 30 min to minimize problems of turnover. Under these conditions for measuring initial rates of phagocytosis, the ratios of endocytosed beads to total beads bound to cells were 10% for cells in control medium without added collagen or fibronectin, 6% for cells incubated in the presence of collagen, and 8.5% for cells with fibronectin. Collagen could, therefore, inhibit phagocytosis into a site inaccessible to trypsin at both short and long incubation times, without affecting the binding of beads to the cell surface.

8). Cell attachment to substrates coated with the 75,000-D cell-binding fragment was similarly not inhibited by collagen (data not shown).

Binding of Radiolabeled Fibronectin and the 75,000-D Fragment

To determine whether fibronectin binding to putative fibronectin receptors on the cell surface was affected by collagen, we performed fibronectin binding assays using radiolabeled fibronectin or the purified 75,000-D cell-binding fragment as described in Materials and Methods.

The addition of unlabeled fibronectin to the reaction mixture as a positive control partially inhibited both the binding of [3H]fibronectin and the 75,000-D fragment to BHK cells (Table I). Although collagen inhibited the binding of radioactive fibronectin to BHK cells by a factor of ~0.6, it had no effect on the binding of the radioactive 75,000-D cell-binding fragment. The apparent inhibition of [3H]fibronectin binding to BHK cells could be explained by an artifact: direct binding of collagen to the collagen-binding domain of fibronectin might induce aggregates. In fact, we found that mixing collagen and fibronectin induced precipitable aggregates (data not shown). Thus, it was concluded that collagen had no effect on the binding of the fibronectin cell-binding site to fibronectin receptors on BHK cell surfaces.

Effects of Collagen on the Binding of [3H]Fibronectin and the [3H]-75,000-D Cell-Binding Fragment to BHK Cells

| Table 1. Effects of Collagen on the Binding of [3H]Fibronectin and the [3H]-75,000-D Cell-Binding Fragment to BHK Cells |
|---------------------------------------------------------------|
| Binding of [3H]Fibronectin to BHK cells | [3H]-75,000-D fragment |
| [3H]Fibronectin | cpm | cpm |
| Control | 8,884 ± 211 (100) | 4,109 ± 1,264 (100) |
| Collagen, 2 mg/ml | 5,687 ± 108 (64) | 4,114 ± 379 (100) |
| Fibronectin, 2 mg/ml | 5,108 ± 146 (57) | 2,755 ± 755 (67) |

[3H]Fibronectin (specific activity = 1.2 x 10⁶ cpm/mg) was incubated at a final concentration of 0.1 mg/ml with 1 x 10⁷ BHK cells with or without unlabeled collagen or fibronectin. [3H]-75,000-D cell-binding fragment (specific activity = 3.1 x 10⁵ cpm/mg) was incubated at a final concentration of 0.04 mg/ml with 1 x 10⁷ cells. See Materials and Methods for experimental details. Numbers in parentheses indicate percentage of control values.
DISCUSSION

We have described the inhibition of certain functions of the adhesive glycoprotein fibronectin by collagen, another major extracellular matrix protein. Soluble collagen noncompetitively inhibited fibronectin-mediated cell spreading, but it did not inhibit initial cell attachment to fibronectin-coated substrates. Similarly, collagen inhibited phagocytosis of fibronectin-coated beads but did not inhibit their initial attachment to the cell surface. Both collagen and its purified α(I) chain were substantially more active in inhibiting cell spreading than was gelatin. The inhibition was dose dependent, noncotoxic, and reversible, and it could not be mimicked by collagenase-treated collagen.

The concentrations of collagen that were found to be inhibitory were only approximately two- to threefold higher than were the concentrations usually used for cell culture in loose, native collagen gels (6, 12, 19). Such gels inhibited cell spreading on a fibronectin substrate, even when added after initial cell attachment to the substrate had occurred. However, the inhibitory effects could have resulted from simple physical obstruction of lateral cell movement. Consequently, standard assays involved preheating the collagen to 50°C to avoid subsequent gel formation. The retention of inhibitory activity under these conditions and with separated, purified α(I) chains, but not after collagenase treatment, strongly suggests that the native triple-helical configuration of collagen is not necessary for function, but that an intact collagen polypeptide backbone is required. In addition, the inhibition involves the cell-binding function of fibronectin, since cell spreading was also inhibited on a substrate of a purified proteolytic fragment of fibronectin containing this site and lacking sites for binding to collagen or glycosaminoglycans (15).

In studies by Sugrue and Hay (36), soluble extracellular matrix molecules were also found to be active in inducing morphological changes in corneal epithelial cells, including the withdrawal of blebs and cytoskeletal reorganization. They found that heat-denatured collagen was as effective as the native molecule in these effects (36).

Our studies also indicated that treatment of cells with collagen did not inhibit the binding of fibronectin to cells as determined by assays for the binding of the cell-binding region of fibronectin to cells, for the attachment of cells to a fibronectin-coated substrate, and for the binding of fibronectin-coated beads to the cell surface. In contrast, the subsequent step of active cell spreading over the fibronectin substrate to attain a normal flattened morphology was inhibited by collagen. Grinnell and others have suggested that phagocytosis of fibronectin-coated beads is analogous to cell spreading, since the latter can be conceptualized as a cell attempting to engulf a bead of infinite diameter (11). In agreement with that hypothesis, collagen treatment also partially inhibited phagocytosis of fibronectin-coated beads.

The effects of collagen can be contrasted to the antiinhibitory effect on fibronectin function of high concentrations of fibronectin itself, its fragments, and its biologically active synthetic peptides (27, 47, 48). In fibroblasts, fibronectin fragments and synthetic peptides readily inhibit both fibronectin-mediated cell attachment and cell spreading (47). In platelets, fibronectin synthetic peptides inhibit platelet attachment to substrates and platelet aggregation, as well as the binding of fibronectin to the cell surface in a competitive fashion (7a, 13a). Other synthetic peptides corresponding to the COOH terminus of the γ-chain of fibrinogen also competitively, rather than noncompetitively, inhibit the binding to platelets of fibronectin, fibrinogen, and von Willebrand factor, each of which are quite distinct proteins thought to be necessary for platelet adhesion (29, 39).

The effects of collagen on fibronectin function appear to be distinct from these competitive effects because (a) collagen did not inhibit cell attachment to fibronectin, (b) the inhibition of cell spreading was not competitive in that increasing the concentration of substrate-adsorbed ligand did not overcome the inhibition, and (c) collagen added to cells that had previously spread on fibronectin-coated substrates induced neither rounding nor detachment.

The inhibitory relationship between collagen and fibronectin could be mutual. For example, the presence of fibronectin when cells are migrating into three-dimensional gels of native collagen in vitro can inhibit the migration of human skin fibroblasts while stimulating the migration of melanoma cells (34, see also reference 42). In these studies, however, there was no quantitative examination of the effect of fibronectin on cell adhesion to collagen or vice versa. More generally, effects of one ligand on the receptor for another ligand have been described in the literature (e.g., reference 5). The ligation of fibronectin receptors with fibronectin at the basal surface of cells activates C3-receptor-mediated phagocytosis at the apical cell surface of cultured human monocytes (30, 44, 45). Culturing melanoma cells in the presence of fibronectin reduces the subsequent ability of these cells to bind laminin (38).

The mechanism by which collagen inhibits fibronectin function is not known, although competition for the same or related receptors appears to be ruled out as discussed above. Although other mechanisms may be possible, a simple hypothesis that explains all of our results is that occupation of the fibroblast collagen receptor (9) inhibits the mobility and

### Table II: Effects of Collagen on the Binding and Phagocytosis of [3H]Fibronectin-coated Beads

| Total sedimented radioactivity | Cell-beads complex incubated with | Phagocytosis |
|-------------------------------|----------------------------------|-------------|
| Polysaccharide beads with [3H]Fibronectin incubated with BHK cells in suspension for 90 min in the presence or absence of unlabeled collagen (2.0 mg/ml) or fibronectin (2.0 mg/ml) as described in detail in Materials and Methods. At the end of the incubation period, aliquots were trypsinized to determine the proportion of internalized radioactivity. See Materials and Methods for experimental details. |
| Beads alone | 84 | 84 | 84 | 84 |
| Beads + collagen | 177 | 177 | 177 | 177 |
| Beads + cells | 3,633 ± 107 | 3,119 ± 125 | 1,146 ± 68 | 31.5 |
| Beads + cells + collagen | 3,929 ± 880 | 3,340 ± 541 | 576 ± 100 | 14.7 |
| Beads + cells + fibronectin | 3,240 ± 32 | 2,739 ± 102 | 997 ± 139 | 30.8 |
| PBS | | | | |
| Trypsin | | | | |

The inhibition of certain functions of the adhesive glycoprotein fibronectin by collagen, another major extracellular matrix protein. Soluble collagen noncompetitively inhibited fibronectin-mediated cell spreading, but it did not inhibit initial cell attachment to fibronectin-coated substrates. Similarly, collagen inhibited phagocytosis of fibronectin-coated beads but did not inhibit their initial attachment to the cell surface. Both collagen and its purified α(I) chain were substantially more active in inhibiting cell spreading than was gelatin. The inhibition was dose dependent, noncytotoxic, and reversible, and it could not be mimicked by collagenase-treated collagen.

The concentrations of collagen that were found to be inhibitory were only approximately two- to threefold higher than were the concentrations usually used for cell culture in loose, native collagen gels (6, 12, 19). Such gels inhibited cell spreading on a fibronectin substrate, even when added after initial cell attachment to the substrate had occurred. However, the inhibitory effects could have resulted from simple physical obstruction of lateral cell movement. Consequently, standard assays involved preheating the collagen to 50°C to avoid subsequent gel formation. The retention of inhibitory activity under these conditions and with separated, purified α(I) chains, but not after collagenase treatment, strongly suggests that the native triple-helical configuration of collagen is not necessary for function, but that an intact collagen polypeptide backbone is required. In addition, the inhibition involves the cell-binding function of fibronectin, since cell spreading was also inhibited on a substrate of a purified proteolytic fragment of fibronectin containing this site and lacking sites for binding to collagen or glycosaminoglycans (15).

In studies by Sugrue and Hay (36), soluble extracellular matrix molecules were also found to be active in inducing morphological changes in corneal epithelial cells, including the withdrawal of blebs and cytoskeletal reorganization. They found that heat-denatured collagen was as effective as the native molecule in these effects (36).

Our studies also indicated that treatment of cells with collagen did not inhibit the binding of fibronectin to cells as determined by assays for the binding of the cell-binding region of fibronectin to cells, for the attachment of cells to a fibronectin-coated substrate, and for the binding of fibronectin-coated beads to the cell surface. In contrast, the subsequent step of active cell spreading over the fibronectin substrate to attain a normal flattened morphology was inhibited by collagen. Grinnell and others have suggested that phagocytosis of fibronectin-coated beads is analogous to cell spreading, since the latter can be conceptualized as a cell attempting to engulf a bead of infinite diameter (11). In agreement with that hypothesis, collagen treatment also partially inhibited phagocytosis of fibronectin-coated beads.

The effects of collagen can be contrasted to the antiinhibitory effect on fibronectin function of high concentrations of fibronectin itself, its fragments, and its biologically active synthetic peptides (27, 47, 48). In fibroblasts, fibronectin fragments and synthetic peptides readily inhibit both fibronectin-mediated cell attachment and cell spreading (47). In platelets, fibronectin synthetic peptides inhibit platelet attachment to substrates and platelet aggregation, as well as the binding of fibronectin to the cell surface in a competitive fashion (7a, 13a). Other synthetic peptides corresponding to the COOH terminus of the γ-chain of fibrinogen also competitively, rather than noncompetitively, inhibit the binding to platelets of fibronectin, fibrinogen, and von Willebrand factor, each of which are quite distinct proteins thought to be necessary for platelet adhesion (29, 39).

The effects of collagen on fibronectin function appear to be distinct from these competitive effects because (a) collagen did not inhibit cell attachment to fibronectin, (b) the inhibition of cell spreading was not competitive in that increasing the concentration of substrate-adsorbed ligand did not overcome the inhibition, and (c) collagen added to cells that had previously spread on fibronectin-coated substrates induced neither rounding nor detachment.

The inhibitory relationship between collagen and fibronectin could be mutual. For example, the presence of fibronectin when cells are migrating into three-dimensional gels of native collagen in vitro can inhibit the migration of human skin fibroblasts while stimulating the migration of melanoma cells (34, see also reference 42). In these studies, however, there was no quantitative examination of the effect of fibronectin on cell adhesion to collagen or vice versa. More generally, effects of one ligand on the receptor for another ligand have been described in the literature (e.g., reference 5). The ligation of fibronectin receptors with fibronectin at the basal surface of cells activates C3-receptor-mediated phagocytosis at the apical cell surface of cultured human monocytes (30, 44, 45). Culturing melanoma cells in the presence of fibronectin reduces the subsequent ability of these cells to bind laminin (38).

The mechanism by which collagen inhibits fibronectin function is not known, although competition for the same or related receptors appears to be ruled out as discussed above. Although other mechanisms may be possible, a simple hypothesis that explains all of our results is that occupation of the fibroblast collagen receptor (9) inhibits the mobility and
the redistribution of fibronectin receptors, which is necessary for cell spreading. Previous studies suggest that fibronectin receptors are mobile and are redistributed to the ventral (lower) surfaces of cells undergoing spreading, at least by functional assays (11, 31; Fig. 9). The inhibition of cell attachment and spreading by soluble fibronectin and its fragments (27, 47, 48) competes directly for fibronectin receptors, preventing binding (1).

In contrast, the noncompetitive inhibition by collagen of spreading and phagocytosis on fibronectin substrates, but not of direct binding to fibronectin, could be explained by an inhibition of fibronectin receptor redistribution. Some other effect on cytoskeletal elements, of course, is also possible. These hypotheses would be testable once antibodies to the functional assays (11, 31; Fig. 9). The inhibition of cell spreading, at least by receptors are mobile and are redistributed to the ventral (lower) surfaces of cells undergoing spreading, at least by fibronectin that retain attachment-promoting activity. These current concepts of its structure, interactions and biological roles. N. Akiyama, S. K., and K. M. Yamada. 1985. The interaction of plasma fibronectin with fibroblastic cells in suspension. J. Biol. Chem. 260:4902–4905.

2. Agarwal J. D., and R. C. Hughes. 1983. Complex carbohydrates of the extracellular matrix. Structure, interactions and biological roles. Biochim. Biophys. Acta 784:375–418.

3. Bonnistein, M. B. 1973. Organotypic mammalian central and peripheral nerve tissue. In Tissue Culture Methods and Applications, P. F. Kruse and M. K. Patterson, editors, Academic Press, Inc., New York. 86–92.

4. Boucicaut, J.-C., T. Darribère, T. J. Poole, H. Aoyama, K. M. Yamada, and J. P. Thiery. 1984. Biologically active synthetic peptides as probes of embryonic development. A competitive peptide inhibitor of fibronectin function inhibits gastrulation in amphibian embryos and neural crest cell migration in avian embryos. J. Cell Biol. 99:1822–1830.

5. Edelman, G. M., I. Yahara, and J. L. Wang. 1973. Receptor mobility and receptor-cytoskeletal interactions in lymphocytes. Proc. Natl. Acad. Sci. USA. 70:1442–1446.

6. Elsdale, T., and J. Bard. 1972. Collagen substrate for studies on cell behavior. J. Cell Biol. 54:626–637.

7. Furcht, L. T. 1983. Structure and function of the adhesive glycoprotein fibronectin. Med. Cell Biol. 1:13–117.

8. Ginsburg, M., M. D. Pierschbacher, E. Rouslahti, G. Mangeri, and E. Plow. 1985. Inhibition of fibronectin binding to platelets by proteolytic fragments and synthetic peptides which support fibroblast adhesion. J. Cell Biol. 260:3931–3938.

9. Gia, J. 1953. A micro biuret method for protein determination. Determination of total protein in cerebrospinal fluid. Scand. J. Clin. Lab. Invest. 5:218–222.

10. Goldschmidt, R. B. 1979. Binding of soluble type I collagen molecules to the fibroblast plasma membrane. J. Cell Biol. 16:263–275.

11. Grinnell, F. 1978. Attachment and spreading of baby hamster kidney cells with latex beads coated by cold insoluble globulin (plasma fibronectin). J. Cell Biol. 75:483–488.

12. Grinnell, F., and D. Minter. 1978. Attachment and spreading of baby hamster kidney cells to collagen substrata: effects of cold-insoluble globulin. Proc. Natl. Acad. Sci. USA. 75:4084–4092.

13. Grinnell, F., D. G. Hays, and D. Minter. 1977. Cell adhesion and spreading factors: partial purification and properties. Exp. Cell Res. 110:175–190.

14. Hay, E. D. 1981. Cell Biology of Extracellular Matrix. Plenum Publishing Corp., New York. 417 pp.

15. Hayashi, M., and K. M. Yamada. 1983. Domain structure of the carboxyl-terminal half of human plasma fibronectin. J. Biol. Chem. 258:3332–3340.

16. Hynes, R. O. 1981. Fibronectin and its relation to cell structure and behavior. In Cell Biology of Extracellular Matrix, E. D. Hay, editor, Plenum Publishing Corp., New York. 295–334.

17. Hynes, R. O., and K. M. Yamada. 1982. Fibronectins: multifunctional modular glycoproteins. J. Cell Biol. 95:369–377.

18. Johansson, S., and M. Höök. 1984. Substrate adhesion of rat hepatocytes on the mechanism of attachment to fibronectin. J. Cell Biol. 98:810–817.

19. Kluppner, H. K., E. B. McGowan, S. N. Grinnell, and G. R. Martin. 1979. Preparation of collagen substrates for cell attachment: effect of collagen concentration and phosphate buffer. Anal. Biochem. 94:308–312.

20. Kluppner, H. K., E. R. Kiefe, and G. R. Martin. 1981. Role of collagenous matrices in the adhesion and growth of cells. J. Cell BioL 88:473–485.

21. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (Lond.) 227:680–685.

22. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein determination with the Folin phenol reagent. J. Biol. Chem. 193:265–275.

23. McKeever, D. D., and F. Grinnell. 1983. Fibronectin binding and phagocytosis of polyethylene latex beads by baby hamster kidney cells. J. Cell Biol. 97:1515–1523.

24. Minick, S. I., K. C. Ingham, and D. Mesache. 1982. Rapid methods for isolation of human plasma fibronectin. Thromb. Res. 17:1–14.

25. Miller, E. J., and R. K. Rhodes. 1982. Preparation and characterization of the different types of collagen. Methods Enzymol. 82:33–64.

26. Mosher, D. F. 1984. Physiology of fibronectin. Annu. Rev. Med. 35:561–575.

27. Pierschbacher, M. D., and E. Rouslahti. 1984. Cell attachment activity of fibronectin can be duplicated by small synthetic fragments of the molecule. Nature (Lond.) 309:19–22.

28. Pierschbacher, M. D., and E. Rouslahti. 1984. Variants of the cell recognition site of fibronectin that retain attachment-promoting activity. Proc. Natl. Acad. Sci. USA 81:5983–5988.

29. Plow, E. F., A. H. Srouji, D. Meyer, G. Marguerie, and M. H. Ginsberg. 1984. Evidence that three adhesive proteins interact with a common recognition site on activated platelets. J. Biol. Chem. 259:5388–5394.

30. Pommier, C. G., S. Inada, L. F. Fries, T. Takahashi, M. M. Frank, and E. J. Brown. 1983. Competition of bacteriophage T4 factor and synthetic tetrapeptide derived from the cell binding domain of fibronectin. Blood. In press.

31. Sugrue, S. P., and E. D. Hay. 1981. Response of basal epithelial cell surface and protein in cerebrospinal fluid. Stand. J. Clin. Lab. Invest. 5:195–208.

32. Terranova, V. P., J. E. Williams, L. A. Liotta, and G. R. Martin. 1985. Modulation of multidimensional gels of native collagen fibres. J. Biol. Chem. 259:8842–8847.

33. Terranova, V. P., J. F. Williams, L. A. Liotta, and G. R. Martin. 1985. Modulation of...
the metastatic activity of melanoma cells by laminin and fibronectin. Science (Wash. DC). 226:982-985.

39. Timmons, S. M. Kloczewiak, and J. Hawiger. 1984. ADP-dependent common receptor mechanism for binding of von Willebrand factor and fibrinogen to human platelets. Proc. Natl Acad Sci. USA. 81:4935-4939.

40. Tomasek, J. J., and E. D. Hay. 1984. Analysis of the role of microfilaments and microtubules in acquisition of bipolarity and elongation of fibroblasts in hydrated collagen gels. J. Cell Biol. 99:536-549.

41. Treistad, R. L., editor. 1984. The Role of Extracellular Matrix in Development. Alan R. Liss, Inc., New York. 643 pp.

42. Tucker, R. P., and C. A. Erickson. 1984. Morphology and behavior of quail neural crest cells in artificial three-dimensional extracellular matrices. Dev. Biol. 104:390-405.

43. Wartiovaara, J., and A. Vaheiri. 1980. Fibronectin and early mammalian embryogenesis. Dev. Mamm. 4:233-266.

44. Wright, S. D., L. S. Craigmyle, and S. C. Silverstein. 1983. Fibronectin and serum amyloid P component stimulate C3b- and C3bi-mediated phagocytosis in cultured human monocytes. J. Exp. Med. 158:1328-1343.

45. Wright, S. D., M. R. Licht, L. S. Craigmyle, and S. C. Silverstein. 1984. Communication between receptors for different ligands on a single cell: ligation of fibronectin receptors induces a reversible alteration in the function of complement receptors on cultured human monocytes. J. Cell Biol. 99:336-339.

46. Yamada, K. M. 1983. Cell surface interactions with extracellular materials. Annu. Rev. Biochem. 52:761-799.

47. Yamada, K. M., and D. W. Kennedy. 1984. Dualistic nature of adhesive protein function: fibronectin and its biologically active peptide fragments can autoinhibit fibronectin function. J. Cell Biol. 99:29-36.

48. Yamada, K. M., and D. W. Kennedy. 1985. Amino acid sequence specificities of an adhesive recognition signal. J. Cell. Biochem. In press.