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Fibronectin mediates cell attachment to C1q: a mechanism for the localization of fibrosis in inflammatory disease

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SUMMARY

Chronic inflammatory processes frequently lead to the abnormal replacement of normal tissue elements by increased numbers of fibroblasts and fibrous connective tissue, i.e., fibrosis. Since the growth of fibroblasts requires that these cells be attached to an extracellular support, the current study was designed to determine if the interaction between the fibroblast attachment factor fibronectin and the C1q component of complement could support fibroblast attachment and growth and thus could form a basis for the attachment of fibroblasts in abnormal tissue locations in those inflammatory states where C1q is bound. Fibronectin purified from human plasma supported attachment of both Chinese hamster ovary cells and of normal fetal lung fibroblasts (HFL-1) to C1q coated substrates. The attachment activity was approximately twice that of attachment to collagen, and was specific, as no attachment occurred to albumin coated substrates. Cells attached to C1q substrates demonstrated characteristic 'spreading' similar to those on collagen. Moreover, the C1q substrate resembled collagen in its ability to support fibroblast growth. Further, the ability of the interaction between C1q and fibronectin to mediate attachment of fibroblasts to immune complexes was demonstrated by the formation of fibroblast–red blood cell–immune complex rosettes, a process that was dependent on both fibronectin and C1q. Thus, the interaction between fibronectin and C1q could serve as the basis for fibroblast attachment and growth in abnormal tissue sites where immune complexes are formed and could be a contributing factor to the development of fibrosis.

Keywords fibronectin C1q fibrosis immune complexes cell attachment

INTRODUCTION

The reconstruction process associated with chronic inflammatory reactions frequently leads to the development of fibrosis, a state characterized by the accumulation of fibroblasts and their connective tissue products in areas of parenchymal damage. Since inflammatory processes preceding the fibrotic state are known to produce both chemoattractants for fibroblasts (Postlethwaite, Snyderman & Kang, 1976, 1979; Rennard *et al.*, 1981) as well as growth factors for fibroblasts (Wahl, Wahl & McCarthy, 1978; Bitterman & Crystal, 1981; Leibovich & Ross, 1976), current concepts of the accrual of fibroblasts in areas of inflammation suggest that this process

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involves recruitment followed by local replication. However, in addition to the presence of chemoattractants and growth factors, fibroblast accumulation in a local area also requires that the fibroblasts be anchored to components of the extracellular matrix. Under normal circumstances, this requirement is provided by fibronectin, a 440,000 dalton glycoprotein capable of binding both to cell surfaces and to matrix components such as collagen, thus mediating the interaction between fibroblasts and the matrix (Ruoslahti, Engvall & Hayman, 1981; Yamada & Olden, 1978). Interestingly, fibronectin can also bind to C1q, a complement component which is capable of binding to immune complexes (Pearstein, Sorvillo & Gigli, 1982; Menzel *et al.*, 1981; Bing *et al.*, 1982; Atkinson & Frank, 1980). Since immune complexes are present in tissue in many inflammatory processes, these immune complexes, by binding C1q, could provide a nidus for the fibronectin-mediated attachment of fibroblasts, thus providing a basis for the accumulation of these cells at sites of inflammatory reactions.

In this context, the present study was designed to test the hypothesis that C1q bound to immune complexes can mediate the fibronectin-dependent attachment of fibroblasts and provide a basis for fibroblast growth. The results demonstrate: (1) fibroblasts can attach to the C1q component of complement through fibronectin; (2) this attachment is similar to the 'physiologic' attachment of fibroblasts to collagen in that it allows fibroblasts to 'spread and to respond to signals to replicate and (3) that fibronectin and C1q can mediate the attachment of fibroblasts to immune complexes.

MATERIALS AND METHODS

C1q. The C1q component of complement was prepared from normal human serum as described (Yonemasu & Stroud, 1971). Briefly, normal human serum was dialysed three times against a 1:100 excess of 26 mM ethyleneglycol-bis-tetra-acetic acid at 4°C and the resulting precipitate collected by centrifugation and dissolved in 750 mM NaCl, 20 mM Na acetate, 10 mM ethylene diamine tetra-acetate (EDTA), pH 5·0. The resulting solution was then dialysed three times against a 1:100 excess of 60 mM EDTA and the resulting precipitates collected by centrifugation and dissolved in 750 mM NaCl, 5 mM Na phosphate, 10 mM EDTA, pH 5·0. This solution was then reprecipitated by dialysis against 35 mM EDTA. The final precipitate was collected by centrifugation and dissolved in 750 mM NaCl, 10 mM EDTA, pH 7·5. The material was judged to be pure by giving a single band on SDS-polyacrylamide gel electrophoresis (PAGE) and by finding a single band on immunoelectrophoresis against a goat anti-human serum (Meloy).

Fibronectin. Fibronectin was prepared from human plasma by gelatin-Sepharose affinity chromatography as described (Engvall & Ruoslahti, 1977). Following elution with 4 M urea, the fibronectin was further purified by heparine-agarose affinity chromatography and eluted with 500 mM NaCl. Samples were adjusted to 2 M urea and frozen in liquid N_2 vapour until use.

Collagen. Collagen prepared from the skin of lathyritic rats was the gift of H. Kleinman.

Culture dishes. All assays were performed on 35 mm plastic petri dishes (Falcon 1008).

Fibronectin-mediated attachment and spreading of fibroblasts on the C1q component of complement. To determine the ability of fibronectin to mediate the attachment and spreading of fibroblasts on a substrate of C1q, cell attachment assays were performed with Chinese hamster ovary cells (CHO, strain K-1, American Type Culture Collection CCL-61) using the method described (Klebe, 1974). Briefly, 10 μg/ml solutions of C1q, collagen, or albumin were prepared and a 1 ml aliquot was pipetted onto a 35 mm plastic petri dish and allowed to air dry. Next, the coated plates were incubated with 1 ml Dulbecco's Modified Eagle's Medium (DMEM) with or without the addition of 10 μg/ml purified human plasma fibronectin for 30 min at 37°C. After rinsing away any unbound fibronectin, approximately 2×10^5 CHO cells suspended in 1 ml of DMEM were added to each plate. Following an incubation of 90 min at 37°C, the petri dishes, together with the cells which had remained in suspension, were then photographed using a phase contrast microscope equipped with an inverted stage (Zeiss). Under these conditions, unspread cells and cells in suspension appear rounded, while spread cells appear larger with a characteristic polygonal or spindle like shape.

Dose-dependent fibronectin-mediated binding of fibroblasts to the C1q component of complement. To quantify the fibronectin dependence of the attachment of fibroblasts to C1q, 35 mm plastic petri

dishes were coated with 10 μg/ml purified human plasma C1q or with 10 μg/ml of collagen. These plates were then incubated for 30 min at 37°C with various concentrations of purified human plasma fibronectin disolved in DMEM. The ability of these plates to support the attachment of CHO fibroblasts was then determined as described above except that unattached cells were removed by rinsing three times with phosphate-buffered saline, following which the CHO cells which had bound to each plate were removed with trypsin and counted in a Coulter counter.

Attachment of human lung fibroblasts to C1q. To demonstrate that fibronectin can mediate the attachment and spreading of normal human fibroblasts to C1q, cell attachment and spreading assays were performed as described above except that the normal diploid strain of human fetal lung fibroblasts (HFL-1, American Type Culture Collection, CCL-153) were used instead of CHO cells. HFL-1 cells were maintained in culture in DMEM supplemented with 10% fetal calf serum and used between passage 10 and 20.

C1q as a substrate for fibroblast growth. In order to demonstrate that C1q could provide an adequate substrate to permit the growth of fibroblasts, plastic petri dishes were coated with C1q as described above. Collagen coated dishes were used as a control. After the dishes had dried, they were sterilized by exposure to u.v. light (2 h) and approximately $1{\cdot}8 \times 10^4$ HFL-1 cells, suspended in DMEM supplemented with 10% fetal bovine serum and 0·6 mg/ml fresh glutamine, were added to each dish. The plates were then incubated at 37°C in an atmosphere of 10% CO_2, 90% air. After 16 h, duplicate plates were rinsed, and the cells attached to each dish were removed with trypsin and counted in a Coulter counter to determine how many cells had attached. The growth of cells on each substrate was monitored by counting the number of cells on replicate plates after various times of incubation.

Fibronectin-dependent binding of fibroblasts to immune complexes. To determine if fibronectin could mediate the binding of fibroblasts to immune complexes, a qualitative assay using red cell rosettes was performed. Rh^+ human erythrocytes were incubated with subagglutinating amounts of heat-inactivated human anti-Rh serum; these E-A cells were then washed six times with DMEM and were stored for up to 1 week at 4°C. The E-A cells were incubated in DMEM at 37°C for 30 min with or without 10 μg/ml C1q. Unbound C1q was washed away by centrifugation at 2,000*g* for 5 min, the EA-C1q cells were then incubated (30 min, 37°C) with or without 10 μg/ml fibronectin and again washed as described above. Next, CHO cells were added at a ratio of 100:1 (red blood cells to CHO cells) and the mixture was incubated at 37°C. After 16 h, the contents of the incubation were gently suspended and inspected under a phase contrast microscope.

RESULTS

Fibronectin-mediated attachment and spreading of CHO cells on C1q

Fibronectin mediates both the attachment and the spreading of fibroblasts on C1q coated plates but not on albumin coated plates (Fig. 1). Cells which had bound to C1q through fibronectin were indistinguishable from cells attached and spread on collagen. In contrast, cells incubated on C1q in the absence of fibronectin did not demonstrate any cell attachment or spreading. Thus, C1q was able to act as a support for fibronectin-mediated attachment and cell spreading.

Dose-dependence of fibronectin-mediated cell attachment to C1q

It is known that fibronectin-mediated attachment of CHO fibroblasts to collagen shows a dose–response relationship. To determine if the fibronectin-mediated attachment of CHO fibroblasts to C1q was active in a similar dose range, dose–response measurements of fibronectin-mediated attachment of CHO cells to both C1q and collagen were performed. Fibonectin gave half maximal attachment to C1q at 0·6 μg/ml (Fig. 2). In comparison, half maximal attachment to collagen occurred at 1·1 μg/ml (Fig. 2). Thus, under these assay conditions, C1q was at least as good a substrate for fibronectin-mediated attachment of CHO cells as was collagen.

C1q as a substrate for the attachment and spreading of normal human fibroblasts

Although the CHO cell has been an important model system to determine the nature of adhesion of

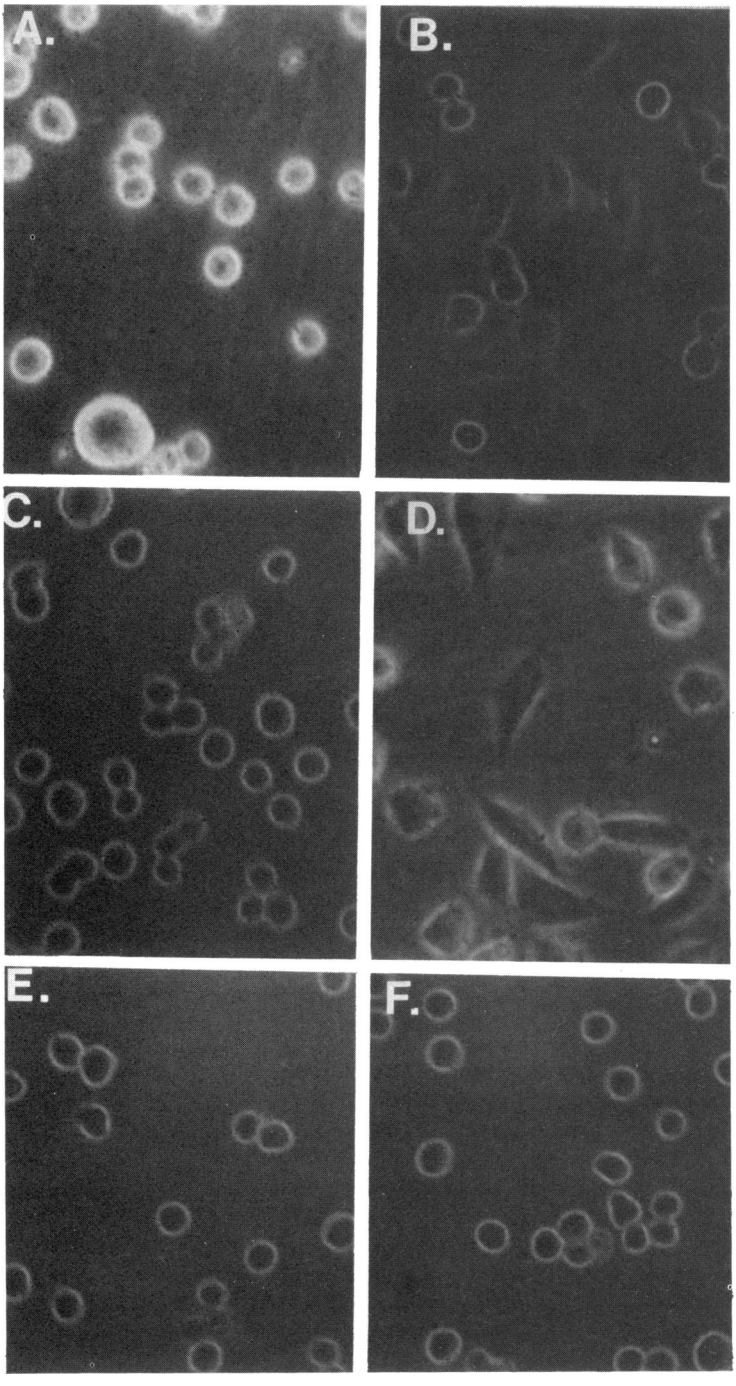

Fig. 1. Fibronectin-mediated attachment and spreading of CHO cells. (A) Attachment to plastic petri dishes coated with C1q; (B) similar to (A), but in the presence of 10 μg/ml fibronectin; (C) attachment to plastic petri dishes coated with collagen; (D) similar to (C) but in the presence of 10 μg/ml fibronectin; (E) attachment to petri dishes coated with albumin; (F) similar to (E) but in the presence of 10 μg/ml fibronectin. All incubations were for 90 min at 37°C and were performed with 2×10^5 CHO cells as described in the Methods.

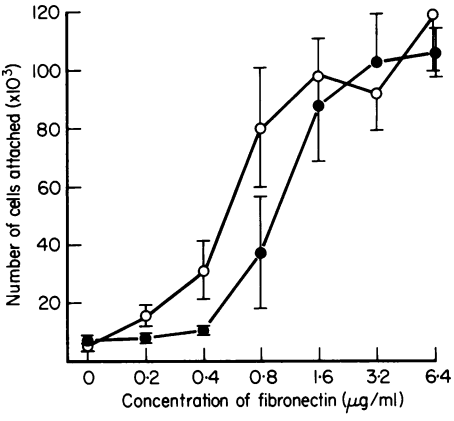

Fig. 2. Comparison of fibronectin-dependent binding of cells to C1q or collagen. Plastic petri dishes were coated with purified C1q (○) or with collagen (●) and then incubated (30 min, 37°C) with the amounts of fibronectin indicated. CHO cells were then added and the number of CHO cells which attached to these dishes in 90 min was counted.

fibroblasts to various substrates, CHO cells are not a normal cell strain. If fibronectin-mediated cell attachment to C1q is to have *in vivo* significance, it is necessary to demonstrate that normal fibroblasts also attach to C1q through fibronectin. For this reason, normal human fetal lung fibroblasts (HFL-1) were tested for the ability to attach to C1q in the presence and the absence of added fibronectin. As with CHO cells, there was little attachment of normal fibroblasts to C1q in the absence of added fibronectin. However, the addition of fibronectin caused the majority of the added HFL-1 cells to attach to C1q and induced prompt spreading of the fibroblasts (Fig. 3).

Growth of normal human fibroblasts on a C1q substrate

Normal lung fibroblasts must attach to a suitable substrate in order to grow at an optimal rate. To determine if the fibronectin-mediated attachment of normal lung fibroblasts to C1q could support fibroblast growth, the ability of HFL-1 cells to grow in the presence of serum on various substrates was measured. As noted by others, bacteriological plastic (in contrast to tissue culture plastic) was a poor substrate for fibroblast growth (Fig. 4). However, when these dishes were coated with C1q, significant fibroblast growth was induced and resembled the growth of these cells on bacteriological plastic dishes coated with collagen.

Binding of fibroblasts to immune complexes

When immune complexes (comprised of antibody coated erythrocytes) were incubated sequentially with C1q, fibronectin, and then CHO fibroblasts, rosettes comprised of red cells bound to the surface of CHO cells were formed (Fig. 5). In contrast, in the absence of either fibronectin, C1q, or both, rosettes did not form. Since omitting either fibronectin or C1q abolished the formation of rosettes, both proteins were required for the binding of CHO fibroblasts to immune complexes.

DISCUSSION

The reconstruction process following tissue injury associated with chronic inflammatory conditions frequently lead to a local accumulation of fibroblasts and fibrous connective tissue i.e., fibrosis. The present study demonstrates that immune complexes, reaction products frequently associated with chronic inflammatory conditions, can provide a basis for fibroblast attachment thus serving as a nidus for fibroblast growth. In this regard, immune complexes function in this capacity by binding the C1q component of complement which, in turn, binds fibronectin, an attachment factor for fibroblasts. Thus, C1q can function similarly to collagen in providing a substrate for fibroblast

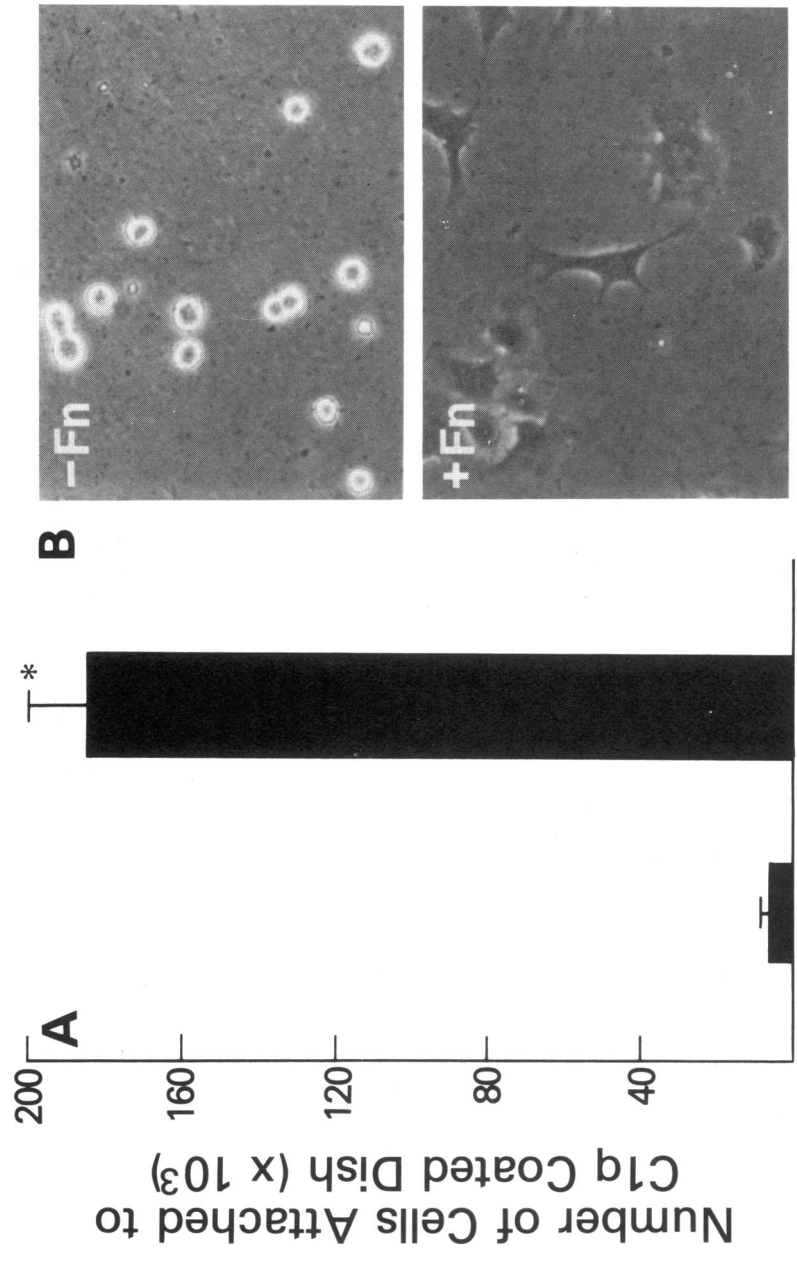

Fig. 3. Fibronectin-mediated attachment and spreading of human fetal lung fibroblasts to C1q. Plastic petri dishes were coated with purified C1q. HFL-1 fibroblasts (2×10^5) were added in the presence or absence of fibronectin (10 μg/ml) and the plates incubated for 90 min at 37°C. (A) Quantification of cell attachment. (B) Phase contrast photomicrograph of cells on C1q substrate after 90 min. −Fn = without fibronectin; +Fn = with 10 μg/ml fibronectin.

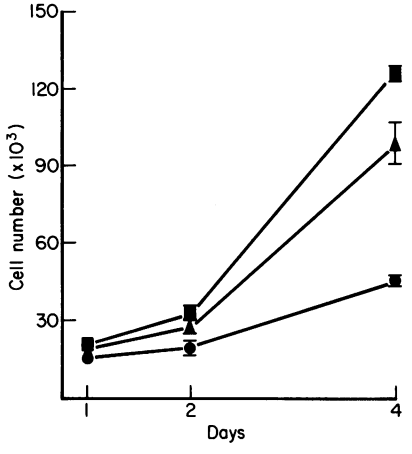

Fig. 4. Comparison of the growth of human fetal lung fibroblasts on plastic, C1q, and collagen. Plastic petri dishes were coated with C1q or collagen as described in methods and sterilized by u.v. iradiation for 2 h. HFL-1 cells ($1{\cdot}8 \times 10^4$) were plated on these dishes in DMEM supplemented with fresh glutamine and 10% fetal calf serum. Growth was measured by counting the cells at various times after plating (●=plastic; ▲=C1q; ■=collagen).

attachment, thereby prividing a scaffolding for tissue reconstruction following inflammatory reactions. Specifically, the fibronectin-mediated attachment of fibroblasts to C1q is accompanied by a characteristic flattening and spreading of these cells which is indistinguishable from the spreading of fibroblasts on collagen. Moreover, C1q also resembles collagen in providing an adequate substrate for fibroblast growth. Thus the interaction of fibroblasts–fibronectin–C1q immune complexes can provide a nidus for fibroblast growth in inflammatory conditions associated with presence of immune complexes.

The origin of the fibronectin and C1q in inflammatory sites is unclear, but there are several possible sources. Both fibronectin and C1q-r-s complex, from which C1q is derived, are present in plasma (Ruoslahti *et al.*, 1981; Yamada & Olden, 1978; Atkinson & Frank, 1980). It is likely that the increased permeability which occurs in many inflammatory states allows these large molecules access to extracellular sites (Wilhelm, 1971). In addition, both fibronectin and C1q could be produced locally by inflammatory cells or by fibroblasts (Muller, Hananske-Abel & Loss, 1978; Al-Adnani & McGee, 1976). In this regard, alveolar macrophages recovered from patients with fibrotic lung disease and mononuclear cells recovered from inflammed joints of patients with rheumatoid arthritis produce larger amounts of fibronectin than normal (Rennard *et al.*, 1981, Lavietes *et al.*, 1982). Moreover, fibroblast fibronectin release can be stimulated by PGE_2, a prostaglandin thought to be an important modulator of immune reactions (Rennard *et al.*, 1982). Thus, in addition to transudation from plasma, local sources of fibronectin and C1q may be of importance in the development of fibrosis.

Not only is fibrosis characterized by the growth of fibroblasts within tissues, but also by the fact that normal tissue architecture is often severly disrupted with corresponding loss of function. The ability of immune complexes to provide a nidus for fibroblast attachment and an adequate matrix for fibroblast growth at morphologically abnormal sites could contribute significantly to this tissue derangement. Under normal circumstances, the collagenous meshwork of the extracellular matrix provides a definition for tissue architecture (Ruoslahti *et al.*, 1981; Kleinman, Klebe & Martin, 1981). Since attachment of fibroblasts to C1q is at least as effective as attachment to collagen, the widespread deposition of immune complexes within a tissue would provide a basis for the attachment of fibroblasts at abnormal tissue sites. An example of the role for immune complexes in the development of fibrosis may be the development of intra-alveolar buds, a unique form of fibrosis consisting of local growth of fibroblasts and fibrous connective tissue in the normally acellular, air filled alveolar lumen of the lung (Kawanami *et al.*, 1982). These structures are most

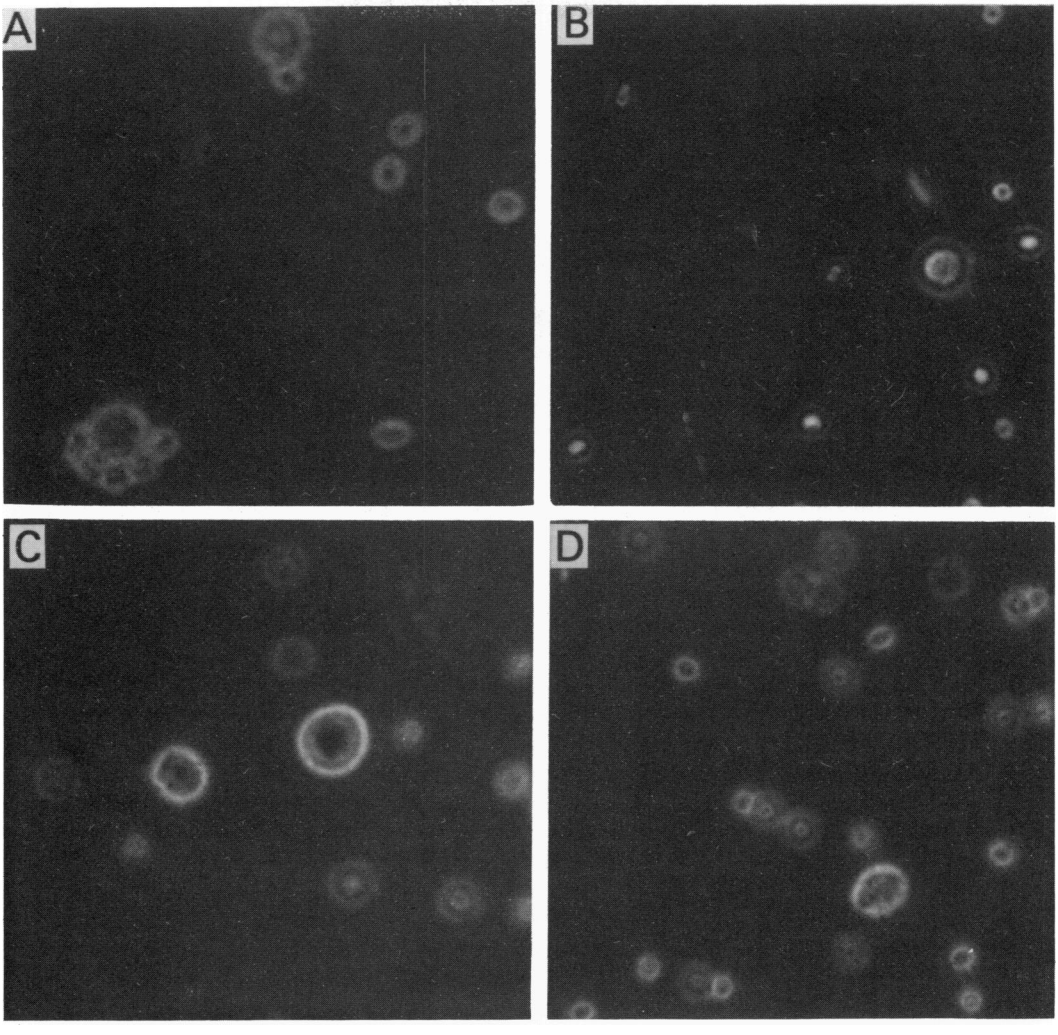

Fig. 5. Fibronectin-C1q-dependent binding of fibroblasts to immune complexes. (A) CHO cell binding to erythrocytes coated with anti-erythrocyte antibody–C1q–fibronectin complex. In sequence, heat-inactivated human anti-Rh antibody, purified human C1q, and purified human plasma fibronectin were added in subagglutinating amounts to Rh^+ normal human erythrocytes. CHO cells were then added and the mixture incubated for 16 h at 37°C. (B) Similar to (A) but with fibronectin omitted. (C) Similar to (A) but with C1q omitted. (D) Similar to (A) but with both C1q and fibronectin omitted.

commonly found in diseases such as hypersensitivity pneumonitis and idiopathic pulmonary fibrosis, conditions frequently associated with intra-alveolar immune complexes. By providing a nidus for fibroblast attachment and growth, the immune complexes within the alveolar lumens in these disorders may contribute to the invasion of the airspaces by fibroblasts.

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