Significance of fibronectin in rheumatoid arthritis and osteoarthrosis

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SUMMARY Fibronectin is a glycoprotein secreted by connective tissue cells into their environment and into the blood. Plasma fibronectin has been isolated and used to prepare an antiserum. This has been shown to be specific for fibronectin and unreactive with fibrin(ogen) and collagen, to which fibronectin binds in vitro. The antiserum has been used to examine the distribution of this protein in the synovium in health, in rheumatoid arthritis, and in osteoarthrosis, and to estimate levels in plasma and synovial fluid. The results suggest that fibronectin is synthesised by synovial cells, and the synovial fluid level of fibronectin was found to be about twice the plasma level in rheumatoid arthritis. In long-standing arthritis fibronectin was also found to be codistributed with (presumably by adsorption upon) fibrin and immature collagen in intra-articular structures but was no longer demonstrable in areas where mature collagen had been formed in areas undergoing fibrosis. The possible significance of local fibronectin production within joints in relation to its possible effect on the resolution or continuance of arthritis is discussed.

Fibronectin is a glycoprotein synthesised by connective tissue cells and secreted into their environment. It is also present in plasma and extracellular fluids. In plasma it binds to and coprecipitates with fibrinogen and fibrin in the cold and hence was originally designated as 'cold insoluble globulin' but has since been given other synonyms. Fibronectin also binds to collagen or gelatin, and this property has been utilised as a means of isolating it from plasma or from the medium in which cells are grown in tissue culture. Comparison of the antisera elicited by immunising animals with fibronectin from these different sources suggests that cell-surface fibronectin is closely related antigenically to, and possibly may be the precursor of, plasma fibronectin.

Because fibronectin is a secretory product of connective tissue cells, and because of its mode of interaction with certain components (fibrin, collagen, glycosaminoglycans) concerned in the organisation and repair of damaged or inflamed tissues, it seemed important to investigate the possible role of fibronectin in rheumatic diseases. This is an account of an investigation of fibronectin concentration in plasma and synovial fluid and of its distribution in synovial tissue by immunochemical and immunohistological methods in health and in rheumatoid disease (RA) and osteoarthrosis (OA).

Materials and methods

COLLECTION OF SPECIMENS Operative synovial biopsies were obtained from patients undergoing hip replacements or other joint surgery for RA or OA. Control biopsies were obtained from subjects with noninflammatory conditions necessitating operation (mainly meniscectomies). The 8 patients with RA from whom biopsies were obtained all had classical or definite disease according to the criteria of the American Rheumatism Association. Of the 8 patients with OA similarly yielding biopsy material 7 had polyarticular disease but not all had generalised OA with Heberden's nodes. Details of these patients and the controls are given in Table 1. The biopsies from these patients were snap-frozen by immersion in liquid nitrogen and were stored at −80°C until processed as described below.
Fibronectin in rheumatism

Table 1 Details of patients and controls from whom synovial biopsies were taken

<table>
<thead>
<tr>
<th>No.</th>
<th>Sex (M:F)</th>
<th>Age (range) in years</th>
<th>Disease duration (range) in years</th>
<th>Biopsy sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>5</td>
<td>4:1</td>
<td>32(17-55)</td>
<td>Knee (4)</td>
</tr>
<tr>
<td>OA</td>
<td>8</td>
<td>3:5</td>
<td>61(37-72)</td>
<td>Wrist (1)</td>
</tr>
<tr>
<td>RA</td>
<td>8</td>
<td>1:7</td>
<td>57(26-73)</td>
<td>Hip (7)</td>
</tr>
</tbody>
</table>

*MCP—Metacarpophalangeal.

Table 2 Fibronectin in synovial fluid and plasma

<table>
<thead>
<tr>
<th>No.</th>
<th>M:F</th>
<th>Mean age (± SD) (years)</th>
<th>Fibronectin (mg/100ml) (± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA synovial fluid</td>
<td>18</td>
<td>9:9</td>
<td>58.5±10.7</td>
</tr>
<tr>
<td>RA plasma</td>
<td>17</td>
<td>4:13</td>
<td>61.7±9.8</td>
</tr>
<tr>
<td>Normal plasma</td>
<td>18</td>
<td>9:9</td>
<td>31.7±9.9</td>
</tr>
</tbody>
</table>

Synovial fluid and plasma samples were collected from the patients detailed in Table 2. The samples were collected aseptically into tubes containing ethylene diamine tetracetic acid (EDTA), spun to remove any cellular deposit, and stored at −20°C until analysed. In addition, specimens of 'rice-bodies' aspirated from the joints of 12 individuals were selected as specified in the 'Results' section from a large number of such specimens derived from a series of 45 patients with chronic synovial effusions associated with RA or OA. These specimens were examined as detailed below.

PREPARATION OF ANTIFIBRONECTIN ANTISERUM

Human fibronectin was isolated from plasma or the cryoprecipitate (antihaemophilic-factor-containing) fraction of plasma by affinity chromatography using gelatin coupled to cyanogen bromide treated Sepharose 4B (Pharmacia Ltd) according to the method of Engvall and Ruoslahti as modified by Dessau et al. The fibronectin was further purified by the removal of minor contaminating plasma proteins by chromatography on a Sephacryl S300 (Pharmacia Ltd) column and elution with a buffer containing 1M KBr in 0-05 M tris(hydroxymethyl) aminomethane and 0-1 M sodium chloride adjusted with 0-1 N HCl to pH 7-6. The purity of the final product was assessed by sodium dodecyl sulphate polyacrylamide gel electrophoresis and by 1- and 2-dimensional immunoelectrophoresis using antiserum to whole human serum (Burroughs Wellcome Ltd).

The purified fibronectin was homogenised in Freund’s complete adjuvant and injected subcutaneously and intramuscularly into rabbits. Subsequent injections were made intracutaneously without adjuvant in the dosage schedule previously described until positive skin reactions were obtained. In a few instances the antisera obtained still contained unwanted antibodies (to immunoglobulin G and to an unidentified beta globulin) when tested by immunoelectrophoresis. These antibodies were removed by adsorption of the antisera using the fibronectin-free supernatant from the first step of the isolation procedure as absorbant. The final antisera were monospecific when tested against serum or plasma by double immunodiffusion and by 1- and 2-dimensional electrophoresis. The antisera gave single precipitin arcs producing a 'reaction of identity' with 2 reference antisera: (a) a commercial antifibronectin antisem from Hoechst Ltd; and (b) an antisera to plasma fibronectin kindly provided by Dr J. Burns, Department of Pathology, University of Oxford.

QUANTITATIVE ESTIMATION OF FIBRONECTIN

Synovial fluid specimens were incubated with 5% hyaluronidase (1500 IU/ml) for 15 minutes at 24°C. The treated fluids and plasma samples were then assayed by radial immunodiffusion (see below) against the antifibronectin antisem. Tests were carried out in 2% agarose containing 3% polyethylene glycol and 0.1 M phosphate buffer at pH 7.4 against an internal standard of pooled serum (stored at −80°C) previously calibrated against a known concentration of highly purified fibronectin.

HISTOLOGICAL TECHNIQUES

Immunohistological reagents. Samples of the antifibronectin antisem were labelled with fluorescein isothiocyanate as described by Nairn or with horse radish peroxidase by the technique of Nakane and Kawazoe. Monospecific antisera to human fibrinogen related antigen (FRA), immunoglobulin G (IgG) and complement C3 were obtained from the Immunodiagnostic Reagent Laboratory of the Department of Immunology, University of Birmingham, and were used unlabelled or after labelling as described above. Sheep antisem to rabbit immunoglobulin was obtained from the same source and was also used fluorescein labelled or unlabelled. In some instances the distribution of fibronectin and of fibrinogen was also sought by the method employing a peroxidase-antiperoxidase (PAP) complex prepared by the method of Sternberger et al.

Preparation of sections. Frozen sections were prepared from unfixed snap-frozen tissue in the 'Frigistor' apparatus previously detailed. Tissues
were also fixed in cold ethanol and sections prepared by the method of Saint-Marie, or were fixed in 10% neutral formol-saline and taken to wax in the customary manner for the preparation of 6μm sections.

**Immunohistological methods.** The distribution of fibronectin, FRA, IgG, and C‘3 was sought by immunofluorescence, both direct and indirect techniques being used. The appearances obtained were identical. Fibronectin and FRA were detected by the direct and indirect immunoperoxidase techniques and also using the PAP complex method. Again, all 3 methods gave essentially similar results.

**Other histological methods.** The appearances seen with the immunohistological methods detailed above were compared with those seen in corresponding sections stained with: haematoxylin and eosin (H and E); van Gieson’s fluid; Miller’s modification of Weigert’s elastic-tissue stain; Gordon and Sweet’s method for reticulin; the Martius scarletblue (MSB) technique for fibrin described by Lendrum et al.; the picropolychrome method of Herovici; or with phosphotungstic acid haematoxylin (PTAH).

**IMMUNOCHEMICAL METHODS**
Radial immunodiffusion assays were carried out as described by Mancini et al. One-dimensional electrophoresis was performed in 1% agar containing barbiturate buffer at ionic strength 0.05 at pH 8.6 using a modification of the technique of Scheidegger. Two-dimensional immunoelectrophoresis was carried out with Clarke and Freeman’s modification of Laurell’s method.

**TISSUE CULTURE**
Human skin fibroblasts were obtained as primary cultures from Dr H. W. Davies, Medical Research Council Unit for Experimental Pathology of the Skin, Medical School, University of Birmingham, or as subcultures through the courtesy of Professor D. G. Harnden, Department of Cancer Studies, University of Birmingham. The cells were maintained in RPMI 1640 and Eagle’s medium containing 10% fetal calf serum and HEPE’s buffer at pH 7.2. They were examined as monolayers grown on the surface of glass cover slips immersed in these media for varying periods of time.

**EXPERIMENTAL**
The distinction between fibronectin and other connective tissue components. Fibronectin is known to bind to fibrinogen precipitated from plasma by heparin and other charged polysaccharides; to fibrin during blood clotting; or to collagen and its derivatives. It was necessary, therefore, before seeking to delineate the distribution of fibronectin in synovial fluids or synovial membranes to ensure that the antiserum to be used was truly specific for this glycoprotein and unreactive with other connective tissue components.

**Distinction from fibrinogen or fibrin.** It can be seen from Fig. 1 that when immunoelectrophoresis was carried out against normal plasma and normal serum, using antiserum to FRA and to fibronectin respectively, the following features could be observed: (i) the antiserum to FRA reacted, as expected, with plasma but not with serum, since fibrinogen is completely removed when plasma coagulates; (ii) on the other hand the antiserum to fibronectin reacted with both plasma and serum, presumably because only a part of the fibronectin in plasma is adsorbed on the fibrin clot leaving a sufficient residue in serum to react with the antibody; (iii) the precipitin arcs given by fibrinogen and by fibronectin differed in shape, in position (i.e., in electrophoretic mobility), and crossed over one another to give a reaction of immunological nonidentity. A similar reaction of nonidentity was obtained when each antiserum was tested against the same plasma sample by simple immunodiffusion (not shown).

It was concluded that the antifibronectin antiserum reacted with antigenic component(s) distinct from those present in fibrinogen. Since fibrin is immunologically almost indistinguishable from fibrinogen, it seemed reasonable to assume that fibronectin is also dissimilar to fibrin. These conclusions were of significance when the distribution of fibrin or fibrinogen and that of fibronectin were
compared in tissues by immunohistological methods (see below).

Relation between collagen and fibronectin. The binding affinity of fibronectin for collagen and its derivatives was confirmed by the successful use of the technique described for fibronectin isolation from plasma (adsorption on to a gelatin-Sepharose column—see ‘Methods’). In order to examine the time relation of production of these 2 proteins by a mesenchymal cell known to synthesise both, fluorescein- or peroxidase-labelled antifibronectin was used to examine cultures of human skin fibroblasts grown as monolayers for varying periods of time. The appearances seen varied with the duration of growth and culture of the cells.

In primary cultures composed of individual randomly disposed fibroblasts treatment with labelled antiserum produced a granular but diffuse cytoplasmic staining, while the nucleus failed to stain (Fig. 2). Little or no material reacting with the antiserum was seen extracellularly at this stage.

In older primary cultures composed of larger cell numbers, while apparent cytoplasmic staining was still a feature, it could be inferred that this appearance might also be due in part to surface membrane staining, since a fine fibrillar pattern could now be discerned overlying the negatively reacting nucleus in many cells. This surface staining was also in continuity with extended cell processes and with an irregular extracellular meshwork of fine fibrils which were strongly immunoreactive (Fig. 3).

In primary cultures approaching confluence a much denser extracellular network of fibrils reacted with the antiserum and largely obscured the underlying fibroblasts (Fig. 4). The cells at this stage were, in fact, difficult to descry in antiserum-treated preparations. This was not only because of the intensely staining overlying fibrillar network of extracellular fibronectin, but also because the cells themselves appeared to have lost much of their earlier reactivity with the antiserum. However, the cells could easily be demonstrated to be viable (by dye exclusion) and to be present in large numbers.

In cultures subjected to repeated passage, on the other hand, treatment with labelled antifibronectin demonstrated only extracellular fibrillary staining at all stages, from the initial adherence of single cells to the substratum to the formation of confluent

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**Fig. 2** Primary culture of skin fibroblasts treated with fluorescein-labelled antifibronectin. Note granular fluorescence of cytoplasm of cells but absence of reaction of nuclei. ×630.

**Fig. 3** Primary culture approaching confluence of skin fibroblasts treated with fluorescein-labelled antifibronectin. Fluorescence present apparently in cytoplasm of cells but also in extracellular processes and overlying nuclei. ×630.
The distribution of fibronectin in frozen sections of unfixed synovium was compared with that of FRA, IgG, and C'3 by immunofluorescence. It was also compared with the distribution of fibrin or of collagen in sections prepared from material fixed in cold ethanol or in formalin using the peroxidase-labelled antibody technique for fibronectin or FRA and conventional stains for fibrin, collagen, and other connective tissue components.

**Distribution by immunofluorescence.** On examining control biopsies with antifibronectin by either the direct or indirect techniques diffuse fluorescence was seen on the surface of synovial villi and in the scanty cytoplasm of some of the synovial cells. There was also evidence of a loose network of fluorescence in the lamina propria of villi and reactivity with the basement membranes surrounding small blood vessels. These tissues showed no corresponding reactivity with antisera to IgG or C'3 or FRA.

An increased distribution of specific fluorescence for fibronectin was seen in the thickened and hypertrophic synovia from the patients with RA, and patchy areas of reactivity were seen on some villi in the biopsies from the cases of OA.

In villi covered by several layers of proliferating sheets of cells. In primary cultures, or in subcultures, brought to confluence extracellular fibronectin was often abundantly present at a time when there was no evidence of mature collagen formation as judged by conventional histochemistry and light microscopy. Cultures at this stage were also examined by Dr C. J. Morris (Department of Investigative Pathology, University of Birmingham) in the electron microscope, and the absence of fibrils with the characteristic banded morphology of mature collagen was confirmed.

It appeared from these observations that fibronectin is indeed an intrinsic secretory product of human fibroblasts but one that is distinct from, and which precedes the production of, mature collagen, the later major product of these cells.

**Results**

**FIBRONECTIN IN PLASMA AND SYNOVIAL FLUID**

With the antifibronectin antiserum thus characterised the levels of this protein were assayed by radial immunodiffusion in plasma and in synovial fluids. The results obtained are summarised in Table 2.

**Fig. 4** Subculture at confluence of skin fibroblasts treated with fluorescein-labelled antifibronectin. Note fluorescence reveals only extracellular network of fibres. ×400.

**Fig. 5** Synovial biopsy, from case of rheumatoid arthritis, treated with fluorescein-labelled antifibronectin. Note specific fluorescence in cytoplasm (but not nuclei) of superficial synovial cells and diffusely in environment of synovial cells, as network in lamina propria of villus and around blood vessels. ×1000.
synovial cells there was evidence of specific fluorescence for fibronectin in the cytoplasm (but not the nucleus) of these cells and in a diffuse pericellular distribution (Fig. 5). A fine network of fluorescence was also visible in the lamina propria, round the smooth muscle cells in the wall of small blood vessels, and along the basement membranes of these vessels (Fig. 6A and B). In some respects this distribution was reminiscent of that previously seen in cultures of fibroblasts as described above. On the other hand little or no fluorescence was seen within the lamina propria of villi in the clusters of cells which corresponding H and E stains revealed to be composed of lymphocytes and plasma cells. Staining of the latter was seen, however, with antisera to IgG and C'3.

In the pathological biopsies an additional feature was seen which was absent from control biopsies. This was the occurrence of areas of intense fluorescence for fibronectin presenting as deposits of irregular shape and size, often enclosing cells reacting negatively. These deposits were frequently present on the surface of villi (Fig. 7B) but in some instances appeared to have become incorporated into the superficial lining of villi. On examining corresponding sections from the same specimens with antifibrinogen, the areas reactive for fibronectin we found equally intense reactivity for FRA (Fig. 7A).

In biopsies from subjects with long-standing chronic arthritis (both RA and OA) roughly spherical microscopic nodules were sometimes seen within the hypertrophied superficial lining of villi. These micronodules reacted variably for fibronectin. Some reacted strongly, in marked contrast with unreactive cells embedded within the nodules (Fig. 8). Other apparently similar nodules reacted weakly or patchily, while some were largely or completely unreactive (Fig. 9). None of such nodules stained

**Fig. 6** Portion of villus from biopsy from case of rheumatoid arthritis. A: Stained with haematoxylin and eosin to show proliferation of superficial synovial cells and presence of lymphoid cells and blood vessels in lamina propria. ×220. B: Adjacent section treated with antifibronectin. Note reactivity of synovial cells and lining of blood vessels but negative reaction of lymphoid cells. ×220.

**Fig. 7** Synovial biopsy from case of rheumatoid arthritis. A: After treatment with fluorescein-labelled antifibrinogen; B: Corresponding field in adjacent section from same block after treatment with fluorescein-labelled antifibronectin. Note codistribution of FRA and fibronectin but negative reactions of cells entrained in fibrin. ×800.
uniformly in the way freshly formed fibrin does with the MSB (red) or PTAH (purple) stains. Nevertheless, nodules reactive with antifibronectin were also reactive for FRA. With van Gieson's fluid nodules showed a pale fuchsinophilia which contrasted with the intense red colour of the collagen of the core of the villi. With the picropolychrome stain the nodules could often be seen to be composed of fine blue-staining fibres which contrasted with the coarse red mature collagen fibres of the lamina propria.

Fig. 8 Synovial biopsy from case of osteoarthritis showing presence of 'micro nodules' in thickened synovium. A: Stained by MSB method. Area marked F stained bright red (as for fibrin) but micronodules X and Y and surrounding fibrillar network stained blue (as for 'procollagen') in original. B: Corresponding field in adjacent section from same block after treatment with antifibronectin. Note bright fluorescence of area staining as fibrin (F), of micronodules (X and Y) and weaker positive reaction of surrounding network but negative reaction of cell nuclei. ×630.

Fig. 9 Synovial biopsy from same case of osteoarthritis as in Fig. 8 showing another area of synovium containing 'micronodules'. A: Stained by MSB. In the original the micronodules stained pale blue at the periphery (as for procollagen) and dark blue at the centres (as for mature collagen). B: Corresponding field in adjacent section from same block after treatment with antifibronectin. Note specific fluorescence at periphery of micronodules but negative reaction of centres.
Such appearances were considered to be consistent with an intermediate stage of organisation of nodular fibrin/fibronectin deposits (incorporated into the superficial lining of villi) and their replacement by freshly synthesised collagen but before the transformation of the latter into mature collagen. To study this further the immunoperoxidase technique was applied to the biopsies and also to selected specimens of rice bodies.

Distribution by immunoperoxidase technique. The application of this technique to the biopsy material available as frozen sections gave essentially the same picture for the codistribution of fibronectin and FRA as had been seen using immunofluorescence. But in those instances where material from the same patients was available fixed in cold ethanol or formalin the application of the immunoperoxidase technique provided better cytological detail and allowed additional observations.

For example, occasionally in sections tangential to the surface of a villus the brown reaction product identifying the presence of the peroxidase-labelled antifibronectin antibody could be seen, not only on the cytoplasm of some synovial cells but also on cytoplasmic processes arising from such cells (Fig. 10). This technique also confirmed the codistribution of fibronectin and FRA in the irregular extracellular masses in or on villi (Fig. 11) and allowed the direct observation of the negative reactions to these antisera of plasma cells and lymphoid cells in the lamina propria of villi.

The immunoperoxidase technique (applied as the PAP complex method) was also used to examine 'rice-bodies.' The loose granular debris so designated is heterogeneous detritus, consisting of fragments of synovium, cartilage, bone, and also of particles which are composed initially of fibrin but which,
with time, show varying degrees of organisation and conversion to fibrous tissue. For the present purpose specimens were selected from 12 patients known from a separate study to show various stages of this progression from fibrinous to fibrous rice body. Immunohistological localisation of fibronectin and of FRA was contrasted with the results obtained with conventional histological staining methods.

Some rice bodies appeared to consist entirely of relatively freshly formed fibrin. These stained uniformly bright red with the MSB stain and purple with PTAH but failed to react for collagen in staining yellow with van Gieson's fluid and with the picropolychrome method. Rice bodies of this kind showed uniform and identical staining for fibronectin or FRA (Fig. 12).

Fig. 12 Rice bodies stained by MSB method. The irregularly shaped rice bodies in the upper portion of the field stained uniformly bright red in the original (as fibrin). The large rice body in the lower half of the field stained red on its periphery but deep blue (as mature collagen) in the central portion. B: Corresponding field in adjacent section from same block after treatment with rabbit antihuman fibrinogen and peroxidase-labelled sheep anti-rabbit IgG. Note uniform reactivity of upper rice bodies and of periphery of lower rice body but negative reaction of central area of latter. Identical distribution obtained with antifibronectin (see also Fig. 13 A, B, and C). ×125.

Fig. 13 A: Rice bodies stained by MSB method. The structure in the upper right hand corner and the surface of the structure in the lower portion of the field showed patchy areas of bright red staining but mainly stained greyish blue (as condensed fibrin or procollagen) in the original. The central portion of the lower rice body stained deep blue (as mature collagen). B: For FRA by PAP technique; C: For fibronectin by PAP technique. Note codistribution of FRA and fibronectin in areas staining as condensed fibrin or procollagen. ×135.
Fibronectin in rheumatism

Other rice bodies showed partial organisation and fibrosis. The process of fibrosis often seemed to begin in the core of the rice body which showed the staining reactions with van Gieson's fluid (deep pink), the MSB stain (blue), and picropolychrome (dark red) characteristic of mature collagen. The peripheral portions of such partially organised rice bodies showed variable reactions to these conventional stains. In some cases the outer layers of the rice body gave the staining reactions of fresh fibrin (as detailed above and see Fig. 12). In other instances these staining reactions were distributed patchily, while the remainder of the outer rim of the rice body showed staining reactions of an intermediate character resembling those given by older condensed fibrin or freshly formed collagen. Rice bodies thus partially organised showed a codistribution of fibronectin and FRA in areas reacting clearly as fresh histological 'fibrin'; similar codistribution of fibronectin and FRA in the intermediate areas; but entirely negative reactions for both fibronectin and FRA in areas composed of histologically characteristic 'mature collagen' (Figs 12 and 13).

In organised rice bodies in which elastic fibres and reticulin were demonstrable by conventional stains the distribution of fibronectin was not identical with that of elastin but was similar to that of reticulin (Fig. 14 A and B). A further study, with consecutive sections of similar material, is in progress to examine in more detail the relation between the distribution of fibronectin and reticulin.

Discussion

The experiments described above confirm that fibronectin is immunologically distinct from fibrinogen and establish that it is produced by synovial cells. This is not surprising, since it is known that, when explants of synovial tissue are grown in tissue culture, some of the cells show many of the functional characteristics of fibroblasts, which have long been known to synthesise this protein. Our observations in human skin fibroblasts on the initial localisation of fibronectin within the cytoplasm of these cells, its later appearance as a membrane protein, and its final distribution as an extracellular or pericellular fibrillar network, are very similar to those made by Yamada in chick fibroblasts.

In confirmation of a previous study made in human rheumatic disease, we have not found any significant alteration of the plasma level of fibronectin in typical and uncomplicated cases of rheumatoid arthritis. On the other hand the mean level of fibronectin in synovial fluid in RA was about twice that found in the plasma. It has been suggested by Ruoslahti and Vaheri that plasma fibronectin may

Fig. 14  A: Central portion of rice body stained by elastic—van Gieson's stain. On original, area X stained red (as collagen), area Y pink (as procollagen), and remainder of tissue yellow (as condensed fibrin). B: Adjacent section stained for reticulin. C: Section from same block treated with peroxidase-labelled antifibronectin. Immunospecific reaction product (brown) present in original in distribution similar to that of procollagen (areas Y of Fig. 14 A). ×54.
actually originate as the secretory product of fibroblasts and other connective tissue cells. If this is indeed the case, one would expect plasma levels to be affected only by very widespread and severe involvement of the connective tissue throughout the body.

In contrast the raised levels in synovial fluid from rheumatoid joints might possibly be accounted for by a restricted and localised increase in production of the protein actually at the site of the articular disease. Certainly the increased immunohistological localisation of fibronectin in the inflamed and hypertrophic synovium of arthritic, as opposed to control, joints would be consistent with this suggestion. In this sense the synovial fluid level of fibronectin may possibly serve as a better index of disease activity in a particular joint than more indirect tests, such as the erythrocyte sedimentation rate or the levels of acute phase proteins which are produced by the liver, and which presumably respond only to more generalised stimuli.

In normal human tissues Stenman and Vaheri have proposed a structural role for fibronectin in positioning and anchoring cells by securing the adhesion of cells to one another and to underlying substrata. This is because they found fibronectin to be distributed in health largely in the basement membranes underlying epithelial cells in many organs, as pericellular arrays, or as a loose meshwork in connective and areolar tissues. This, indeed, conforms in general with the distribution we have found of fibronectin in normal synovium. However, in inflamed membranes we observed fibronectin also to be codistributed with fibrin deposits. Initially this was seen as irregular masses of material on the surface of occasional villi. It has been suggested that this apparently specific adsorption of fibronectin on fibrin is due to the cross-linking of fibronectin to fibrin by the transglutaminase of plasma known as fibrin stabilising factor (factor XIII) during coagulation.

In extracellular fibrinous deposits on villi, and in rice bodies, undergoing early organisation with alteration of some of the histochemical characteristics of the fibrin, but as yet without evidence of complete replacement by collagen, there was persistence of the codistribution of fibronectin and FRA. But in areas of mature collagen formation (as assessed histochemically) fibronectin was usually no longer detectable. There was also no evidence that the distribution of fibronectin is related to areas where elastic tissue is present. By contrast there was a broad similarity (but not a complete identity) of distribution of fibronectin and of 'reticulin' (in the histological sense of argyrophilic fibrils) in rice bodies undergoing the later stages of organisation (see Figs. 14 A–C), as Stenman and Vaheri also noted in other normal tissues and as Linder et al. have observed in certain pathological conditions.

In cultures of fibroblasts it has been noted that fibronectin and 'procollagens' are often codistributed, as we have now observed in diseased synovium, while fibronectin produced by epidermal cells serves to attach such cells to collagenous substrates. On the other hand the absence of fibronectin in areas of mature collagen formation but its presence in areas where histologically defined 'reticulin' is present in vivo is less easily accounted for on the basis of in-vitro observations.

Histologically defined 'reticulin' is known to contain certain noncollagenous glycoproteins, of which it is presumed that fibronectin is one. Isolated 'reticulin' from some tissues contains only type IV collagen, whereas that derived from immature tissues, such as embryonic skin or wound healing tissue, correlates with the distribution of type III collagen.

From these biochemical data and from the immunohistological distribution of fibronectin one might be led to expect that, if the latter reflects fibronectin distribution in the 'reticulins' of connective tissues, fibronectin would show in vitro preferential or selective affinity for type III (wound healing) or type IV (basement membrane) collagens. However, in fact fibronectin has been shown to bind in vitro to the native, and perhaps even more strongly to the denatured, forms of all types of collagen. The underlying biochemical basis of the above histological observations is thus not at present apparent.

Nevertheless, it is clear that fibronectin probably has an important structural and functional role in the organisation and repair of inflamed intraarticular structures. In our hands the distribution of fibronectin has been distinctive in actively involved rheumatoid joints as compared with most joints affected by osteoarthritis. However, in long-standing arthritis showing evidence of intraarticular fibrosis, whether this was associated with RA or OA, certain broad similarities were seen in areas undergoing organisation. This is probably because it is now coming to be recognised that there are commonly inflammatory and healing components in long-standing OA. It has been suggested that fibronectin may also be concerned with the disposal of fibrin, collagen, and their degradation products by the reticuloendothelial system. It is therefore possible that the local production of fibronectin within affected joints may influence either the resolution or the continuance of arthritis, depending on the amounts synthesised by the stimulated synovial tissue.
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References
1 Eddsall J T, Gilbert G A, Scheraga H A. The non-clotting
component of the human plasma fraction I–I (‘cold
2 Yamada K M, Olden K. Fibronectins—adhesive glyco-
proteins of cell surface and blood. Nature 1978; 275:
179–84.
3 Engvall E, Ruoslahti E. Binding of soluble forms of
fibroblast surface protein, fibronectin, to collagen. Int J
4 Ruoslahti, E, Vaheeri A. Novel human serum protein from
5 American Rheumatism Association. Diagnostic criteria
for rheumatoid arthritis. 1958 revision. Ann Rheum Dis
1959; 18: 49–53.
6 Williamson N, Popert A J, Williamson T J, El-Badawy
S A, Wainwright A C. Fibron deposition in rheumatoid
joints and the use of intra-articular urokinase. In
preparation.
7 Dessau W, Adelmann B C, Timpl R, Martin G R.
Identification of the sites in collagen α-chains binding
serum anti-gelatin factor (cold insoluble globulin).
8 Soothill J F. Estimation of eight serum proteins by a gel
9 Nairn R C. Fluorescent Protein Tracing. 3rd ed. London:
Livingstone, 1969.
10 Nakane P K, Kawai A. Peroxidase-labelled antibody.
11 Sternberger L A, Hardy P H, Cuculis J J, Meyer H G.
The unlabelled antibody enzyme method of immuno-
chemistry. Preparation and properties of soluble antigen-
antibody complex (horseradish peroxidase-antihorseradish
peroxidase) and its use in identification of sirohaeataces.
12 Walton K W, Williamson N. Histological and
immunofluorescent studies on the evolution of the human
13 Saint-Marie G. A paraffin embedding technique for
studies employing immunofluorescence. J Histochem
14 Miller P J. An elastin stain. Med Lab Technol 1971; 28:
148–9.
15 Gordon H, Sweet H H. A simple method for the silver
16 Lendrum A C, Fraser D S, Slidders W, Henderson R.
Studies on the character and staining of fibrin. J Clin
17 Herovici C. Le picropolychrome. Technique de colora-
tion histologique destine a l’etude du tissu conjonctif,
normal et pathologique. Rev Fr Etud Clin Biol 1963; 8:
88–9.
18 Mancini G, Carbonara A O, Heremans J F. Immuno-
chemical quantitation of antigens by single radial immuno-
19 Scheidegger J J. Une micro-methode de l’immuno-
20 Clarke H G M, Freeman T. Quantitative immuno-
21 Laurell C B. Quantitative estimation of proteins by
electrophoresis in agarose gel containing antibodies. Anal
22 Mosesson M W. Structure of human plasma cold-
insoluble globulin and the mechanism of its precipitation
in the cold with heparin or fibrinogen-fibrogen complexes.
23 Ruoslahti E, Vaheeri A. Interaction of soluble fibroblast
surface antigen with fibrinogen and fibrin. Identity with
24 Engvall, E, Ruoslahti E, Miller E J. Affinity of fibro-
nectin to collagens of different genetic types and to
25 Yamada K M. Immunological characterisation of a
major transformation-sensitive fibroblast cell surface
glycoprotein. Localisation, redistribution and role in
26 Fyran O, Munthe E, Solum N O. Studies on cold
insoluble globulin. I Concentrations in citrated plasma
27 Stenman S, Vaheeri A. Distribution of a major connective
tissue protein, fibronectin, in normal human tissues.
28 Mosher D F. Cross-linking of plasma and cellular fibro-
nectin by plasma transglutaminase. Ann N Y Acad Sci
1978; 312: 38–42.
29 Miller E, Stenman S, Lehto V P, Vaheeri A. Distribution
of fibronectin in human tissues and relationship to other
312: 151–9.
30 Bornstein P, Ash J F. Cell surface-associated structural
proteins in connective tissue cells. Proc Natl Acad Sci
31 Vaheeri A, Alitalo K, Hedman K, Kesi-Oja J, Kurfkenen
M, Wartiovaara J, Fibronectin and the pericellular
matrix of normal and transformed adherent cells. Ann
32 Murray J C, Stingl G, Kleinman H K, Martin G R,
Katz S I. Epidermal cells adhere preferentially to type IV
33 Pras M, Glynn L E. Isolation of a non-collagenous
reticulin component and its primary characterisation.
34 Pras M, Johnson G D, Holborow E J, Glynn L E. Anti-
genic properties of a non-collagenous reticulin com-
ponent of normal connective tissue. Immunology 1974;
27: 469–78.
35 Nowack H, Gay S, Wick G, Becker U, Timpl R. Prepara-
tion and use in immunohistology of antibodies specific
for type I and type III collagen and pro-collagen. J
36 Jilek F, Hörmann H. Cold insoluble globulin (fibro-
nectin) IV. Affinity to soluble collagen of various types.
37 Saba T M, Blumenstock F A, Weber P, Kaplan J E.
Physiologic role of cold insoluble globulin in systemic
host defense: Implications of its characterisation as the
opsonic α2-surface-binding glycoprotein. Ann N Y Acad
38 Hopper K E, Adelmann B C, Gentner G, Gay S. Recogni-
tion by guinea-pig peritoneal exudate cells of con-
formationally different states of the collagen molecule.