Fibronectin in exudative pleural effusions

MATTI KLOCKARS,* TOM PETTERSSON,* TAPIO VARTIO,†‡ HENRIK RISKA,§ ANTTI VAHERI†

From the *Fourth Department of Medicine, Helsinki University Central Hospital, the †Department of Virology and ‡Department of Pathology, University of Helsinki, Helsinki, and §Mjöbolsta Hospital, Mjöbolsta, Finland

SUMMARY Fibronectin is a glycoprotein found in body fluids, loose connective tissue matrix and in basement membranes. Fibronectin in pleural effusion was found to be immunologically indistinguishable from the plasma form, as shown by double-diffusion analysis. Fibronectin isolated from pleural fluid by affinity chromatography on gelatin-Sepharose had a polypeptide pattern similar to that of plasma fibronectin in SDS-polyacrylamide gel electrophoresis. In 28 patients with infectious or non-specific pleural effusion fibronectin concentrations in pleural fluid were 335 ± 104 μg/ml (mean ± SD), in 15 patients with malignant disease the concentrations were 369 ± 173 μg/ml and in 26 patients with tuberculosis 441 ± 103 μg/ml. The highest concentrations, 605 ± 252 μg/ml, of fibronectin in pleural fluid were detected in 14 patients with connective tissue diseases. The results suggest that increased fibronectin concentrations reflect the presence of a pleurisy due to connective tissue disease or tuberculosis rather than other infectious or malignant disease.

Fibronectin is a high molecular weight glycoprotein found in body fluids and connective tissue and it occurs as both an insoluble and a soluble form.1–3 Insoluble fibronectin is found associated with basement membranes and in interstitial connective tissue matrix4 as well as in the pericellular matrix formed around cultured adherent cells such as endothelial cells, fibroblastic cells and hepatocytes. In these positions fibronectin apparently functions as a substrate for cell attachment and as a scaffold for cell migration and movement.1–3 The soluble circulating form of fibronectin was described over 30 years ago as “cold-insoluble globulin” as it coprecipitated with fibrin in the cold.5 The concentration of fibronectin in normal human plasma is about 300–500 μg/ml.6 Soluble fibronectin also binds to collagen,7 heparin,8 Staphylococcus aureus8 and is a substrate for thrombin, plasmin and plasma transglutaminase.1,3

The presence of high concentrations of fibronectin in rheumatoid synovial fluid, an inflammatory exudate,10 and the involvement of fibronectin during the early stages of tissue repair11 prompted us to investigate the diagnostic significance of fibronectin determinations of exudative pleural effusions.

Patients material and methods

PLEURAL FLUID SAMPLES

Pleural fluid samples were obtained from 83 patients admitted to hospital for the diagnostic or therapeutic evaluation of a unilateral or bilateral exudative (protein concentration > 30 g/l) pleural effusion. The cause of the pleural effusion was determined from clinical, laboratory, and radiological findings. Studies performed on all samples included total and differential cell counts, tests for total protein, glucose, and the Waaler-Rose and latex fixation tests. Pleural fluid was also cultured for the presence of Mycobacterium tuberculosis and analysed cytologically. Breakdown of the diagnoses gave the following groups of patients:

Group I (28)

Fourteen patients with a specific infectious pleural effusion. Five patients had a pleuropneumonia, eight patients had a bacteriologically verified pleural empyema, and one patient had a pleurisy associated with a Coxsackie B 5 pneumonitis. Another 14 patients had a non-specific, probably infectious pleural effusion. This type of diagnosis of pleural effusion was based on the exclusion of any other type of specific pleurisy.
Group 2 (26)
Twenty-six patients had tuberculous pleurisy. In 18 patients this diagnosis was based on either a positive culture for *M tuberculosis* or a pleural biopsy compatible with active tuberculosis. In eight patients the diagnosis of tuberculous pleurisy was most confident on the basis of clinical data and a positive response to tuberculostatic treatment.

Group 3 (15)
Fifteen patients had a malignant pleural effusion. Four had a pulmonary adenocarcinoma, one an epidermal bronchial carcinoma, one a microcellular carcinoma, four had a metastatic adenocarcinoma, four had a malignant mesothelioma and one a malignant lymphoma.

Group 4 (14)
Five patients had classical rheumatoid arthritis with positive rheumatoid factor in the blood and pleural fluid and a low pleural fluid glucose concentration (0–1.2 μmol/l); three patients had systemic lupus erythematosus (SLE). Six further patients had an undefined connective tissue disease. In addition to the exclusion of any specific cause for the pleural effusion this was defined as a chronic (duration more than one month) bilateral pleural effusion which did not respond to antibiotic or tuberculostatic treatment and which was associated with parenchymal abnormalities and a decreased alveolar diffusion capacity. In all six patients the pleural effusion disappeared rapidly after corticosteroid treatment.

Pleural fluid was taken into EDTA and clarified by centrifugation at room temperature to sediment cells. The samples were stored at -20°C until assayed.

Isolation of Fibronectin from Pleural Fluid
Fibronectin of the pleural fluid was isolated according to the method of Engvall and Ruoslahti by affinity chromatography on gelatin (Type I, Sigma, St Louis, MO) coupled to Sepharose 4 B particles (Pharmacia, Uppala, Sweden). Gelatin-Sepharose (200 μl) was incubated with 1 ml of pleural fluid overnight at room temperature in the presence of 10^-4 M phenylmethyl-sulphonylfluoride and 0.02% sodium azide. After incubation the gelatin-Sepharose was centrifuged and washed twice with phosphate-buffered saline (PBS). Finally, the gelatin-Sepharose pellet was dissolved in 400 μl of Laemmli's sample buffer containing 4% sodium dodecyl sulphate (SDS), pH 6.8, with or without 10% -mercaptoethanol. The samples were incubated in a boiling water bath for 3 min and analysed by polyacrylamide gel electrophoresis. A control sample of fibronectin from normal human plasma was similarly prepared and analysed.

Anti-fibronectin Serum
Fibronectin was purified from human plasma by a double step affinity procedure using non-denaturing conditions as described. The purity of the antigen used for immunisation of rabbits was verified by SDS-polyacrylamide gel electrophoresis, in which a single polypeptide band was detected. The anti-fibronectin serum gave a single precipitation line against normal human plasma.

Fibronectin and Protein Concentrations
The concentration of fibronectin in pleural fluid and plasma was measured by a single radial immunodiffusion method in 0.8% agarose using a sample volume of 7 μl and purified plasma fibronectin and normal human plasma as standards. Protein concentrations were determined by the Biuret technique.

Immunodiffusion Analysis
The tests were made in 1.0% agarose plates according to the double diffusion method of Ouchterlony.

Polyacrylamide Gel Electrophoresis
Polyacrylamide gel electrophoresis was performed in the presence of SDS according to the method of Laemmli using vertical slab gels. The acrylamide concentration was 3.3% in the spacer gel and 5% in the separating gel. After electrophoresis the gels were fixed in 10% acetic acid and stained with Coomassie brilliant blue R-250 according to Fairbanks et al. The molecular weight markers used in the electrophoresis were chemically reduced purified fibronectin (MW 220 000), α-,macroglobulin (MW 170 000), phosphorylase a (MW 94 000), human serum albumin (MW 68 000) and ovalbumin (MW 43 000).

Statistical Analysis
Differences were tested for significance by Student's t test.

Results
Identification of Fibronectin in Pleural Fluid
Fibronectin was found in all pleural fluid specimens studied. Pleural fluid fibronectin showed immunological identity with fibronectin of human plasma in double-diffusion tests (not shown). In order to compare the physicochemical characteristics of fibronectin from pleural fluid and plasma, fibronectin was isolated by a single-step purification procedure, affinity chromatography on gelatin-
Fibronectin in pleural fluid

Fig. 1 Analysis of proteins in plasma and pleural fluid and of fibronectins purified from them. SDS-polyacrylamide gel electrophoresis under non-reducing (A) and reducing (B) conditions. The tracks show total proteins of normal plasma (tracks 1) and pleural fluid (2), 2-5 μl each. Tracks 3-4 show gelatin-Sepharose-purified fibronectin from these two samples. Apparent molecular weights are indicated on the right.

Sepharose.13-15 Gelatin-bound plasma and pleural fluid proteins were analysed by SDS-polyacrylamide slab gel electrophoresis. Protein staining revealed single polypeptide bands with an apparent molecular weight of about 440 000 daltons, the fibronectin from pleural fluid comigrating with plasma fibronectin (Fig. 1A). After reduction of the disulphide bonds single polypeptide bands were seen in the 220 000 dalton molecular weight region (Fig. 1B), indicating that the pleural fluid fibronectin, as well as plasma fibronectin, is a disulphide-bonded dimer. Identical results were obtained from the samples of the four patients studied.

CONCENTRATION OF FIBRONECTIN IN PLEURAL EFFUSIONS

The concentrations of fibronectin in the specimens of pleural fluid are shown in the Table and Fig. 2.

Compared with the acute infectious pleural effusions the concentration of fibronectin was significantly raised in the effusion fluid of patients with tuberculosis or connective tissue disease. The mean concentration of fibronectin was also significantly higher in the connective tissue disease group compared with the tuberculosis group. The highest concentrations of fibronectin were seen in patients with rheumatoid pleurisy. In malignant pleural effusions the concentrations of fibronectin were not increased over effusions associated with infections.

The concentration of fibronectin in pleural effusions did not correlate significantly with the protein concentration nor with the total number of lymphocytes or polymorphonuclear neutrophils in the pleural fluid (not shown). Neither was the fibronectin concentration influenced by the number of eosinophils in pleural fluid. No relation was observed between the duration of subjective symptoms of the pleural effusion and fibronectin concentrations.

Concentrations of fibronectin in pleural fluid of four groups of patients with pleural effusions

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of patients</th>
<th>Fibronectin in pleural fluid (mean ± SD)</th>
<th>µg/ml</th>
<th>% of total protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infectious and non-specific pleural effusion</td>
<td>28</td>
<td>335 ± 104</td>
<td>0-68 ± 0-2</td>
<td></td>
</tr>
<tr>
<td>(group 1)</td>
<td></td>
<td>441 ± 103†</td>
<td>0-86 ± 0-2*</td>
<td></td>
</tr>
<tr>
<td>Tuberculosis (group 2)</td>
<td>26</td>
<td>396 ± 173</td>
<td>0-75 ± 0-3</td>
<td></td>
</tr>
<tr>
<td>Malignant disease (group 3)</td>
<td>15</td>
<td>605 ± 252†</td>
<td>1-30 ± 0-5†</td>
<td></td>
</tr>
<tr>
<td>Connective tissue disease (group 4)</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Compared with infectious and non-specific pleural effusion group (group 1) the differences between the means are significant at the levels *p < 0-005 and †p < 0-001.
Discussion

The present results show that pleural fluid contains fibronectin indistinguishable both immunologically and in polyacrylamide gel electrophoresis of fibronectins isolated using gelatin-Sepharose we found no evidence for fragmentation of fibronectin in pleural fluid. Obviously, the possibility remains that both in plasma and pleural fluid fibronectin fragments not binding to gelatin may be present. Increased concentrations of fibronectin have been found in joint exudates of rheumatoid arthritis10 as well as in plasma of patients with certain types of rheumatic disorders.19 The concentration of fibronectin in pleural effusion fluid caused by infection or malignant disease was about the same as in normal human plasma—that is, 330–350 μg/mL. Normal concentrations of fibronectin in plasma in rheumatoid arthritis have been demonstrated previously.10 The source of pleural fluid fibronectin is not known. However, in tuberculous pleurisy as well as in pleural effusions associated with connective tissue disease, it seems possible that in addition to probable exudation from plasma, fibronectin may be in part produced locally by activated connective tissue cells.

Fibronectin is known to be particularly abundant in newly formed connective tissue both during embryogenesis20 and in experimental granulation tissue formation.11 An active turnover of connective tissue components is a characteristic of connective tissue disease21 and so the raised fibronectin concentrations in pleural effusions associated with connective tissue disease may be indicative of tissue repair induced by the enhanced connective tissue degradation. The fibronectin produced by activated macrophages has also been shown to be chemotactic to fibroblasts and thus possibly serves as an inflammatory mediator that can recruit fibroblasts to an area of damaged tissue.22 Moreover, fibronectin may serve as a defence factor as it has been suggested that it acts as a non-specific opsonin which permits cells of the mononuclear phagocyte system to remove damaged tissue fragments from the circulation.23 24

References

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Requests for reprints to: Dr Matti Klockars, Fourth Department of Medicine, Unioninkatu 38, 00170 Helsinki 17, Finland.
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M Klockars, T Pettersson, T Vartio, H Riska and A Vaheri

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