Fibronectin in exudative pleural effusions

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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 indis-
tinguishable 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 base-
ment 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 posi-
tions fibronectin apparently functions as a substrate for cell attachment and as a scaffold for cell migra-
tion 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 aureus9 and is a substrate for throm-
bin, 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

Plural fluid samples were obtained from 83 patients
admitted to hospital for the diagnostic or therapeu-
tic 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, glu-
cose, and the Waaler-Rose and latex fixation tests.
Plural fluid was also cultured for the presence of
Mycobacterium tuberculosis and analysed cyto-
logically. Breakdown of the diagnoses gave the follow-
ing groups of patients:

Group 1 (28)
Fourteen patients with a specific infectious pleural
effusion. Five patients had a pleuro-pneumonia,
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.

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723
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 (0.1-2.0 mmol/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, Uppsala, Sweden). Gelatin-Sepharose (200 μl) was incubated with 1 ml of pleural fluid overnight at room temperature in the presence of 10-10 M phenylmethyl-sulfonylfluoride 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.

Antibody to Fibronectin Serum
Fibronectin was purified from human plasma by a double step affinity procedure using non-denaturing conditions as described.13 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 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.18 The molecular weight markers used in the electrophoresis were chemically reduced purified fibronectin (MW 220 000), α2-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. 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.

Fig. 2 Concentrations of fibronectin (µg/ml) in the pleural fluid specimens. RA = rheumatoid arthritis, SLE = systemic lupus erythematosus. The horizontal lines indicate the mean values in each group.

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) µg/ml</th>
<th>% of total protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infectious and non-specific pleural effusion (group 1)</td>
<td>28</td>
<td>335 ± 104</td>
<td>0.68 ± 0.2</td>
</tr>
<tr>
<td>Tuberculosis (group 2)</td>
<td>26</td>
<td>441 ± 103†</td>
<td>0.86 ± 0.2*</td>
</tr>
<tr>
<td>Malignant disease (group 3)</td>
<td>15</td>
<td>396 ± 173</td>
<td>0.75 ± 0.3</td>
</tr>
<tr>
<td>Connective tissue disease (group 4)</td>
<td>14</td>
<td>605 ± 252†</td>
<td>1.30 ± 0.5†</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 polypeptide chain composition from the plasma form of the protein. Compared with other types of pleural effusion, the fibronectin concentration, as quantified by single radial immunodiffusion, was raised in pleural effusions caused by tuberculosis and connective tissue disease. It should be noted, however, that this method is accurate only when the molecular size of the material to be measured is the same as that of the standards as split products cause too high readings. 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 arthritis as well as in plasma of patients with certain types of rheumatic disorders. The concentration of fibronectin in pleural effusion causes inflammation or infective 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.

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 embryogenesis and in experimental granulation tissue formation. An active turnover of connective tissue components is a characteristic of connective tissue degradation and so the raised fibronectin concentrations in pleural effusions associated with connective tissue disease may be indicative of connective 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. 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.

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References

Fibronectin in pleural fluid


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