Occurrence of matrix metalloproteinases and tissue inhibitors of metalloproteinases in tuberculous pleuritis


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Summary  Objective: Matrix metalloproteinases (MMP) and tissue inhibitors of metalloproteinases (TIMP) have been found in high concentrations in pleural effusions. Because MMP and TIMP may play a part in the causation of the fibrosis seen in tuberculous (TB) pleuritis their occurrence was examined.

Design: Pleural effusion fluid and plasma concentrations of MMP-1, MMP-2, MMP-3, MMP-8, MMP-9, TIMP-1 and TIMP-2 were determined by ELISA in 21 patients with TB pleuritis. To adjust for the total protein content, respective ratios were calculated. Activities of MMP-2 and MMP-9 were measured by gelatine zymography and the MMP-9/MMP-2 ratios calculated. Pleural effusions and plasma of 15 patients with congestive heart failure (CHF) and plasma of 15 healthy persons (CON) served as controls.

Results: Immunoreactive pleural fluid concentrations of MMP-1, MMP-2, MMP-8, and MMP-9 were higher in TB compared to CHF, but plasma concentrations were not different between the groups. TB pleural fluid concentrations of MMP-1, MMP-2, TIMP-1, and TIMP-2 were higher compared to TB plasma. MMP-3 was found in trace amounts only. The MMP-9/total protein ratios in pleural fluid were higher in TB compared to CHF (0.4492 ± 0.1633 vs 0.0364 ± 0.0145, P < 0.005) but the TIMP-1 ratios were lower (139.0 ± 28.7 vs 517.8 ± 183.7, P < 0.0005). In TB pleural fluid vs TB plasma, the respective MMP-1, MMP-2, TIMP-1, and TIMP-2 ratios were increased (0.46 ± 0.10 vs 0.17 ± 0.02; 25.2 ± 2.8 vs 4.2 ± 0.9; 139.0 ± 28.7 vs 27.8 ± 8.2; 0.67 ± 0.13 vs 0.18 ± 0.04, P < 0.0005 each). Gelatine zymography demonstrated MMP-2 and MMP-9 bands of different brightness in TB effusions but in CHF effusions the MMP-9 band was barely visible. The MMP-9/MMP-2 effusion ratios were therefore higher in TB compared to CHF (0.46 ± 0.15 vs 0.05 ± 0.04, P < 0.0005).

Conclusion: Compartmentalized MMP-1, MMP-2, TIMP-1, and TIMP-2 and, compared to CHF, a surplus of MMP-1, MMP-2, MMP-8, and MMP-9 in the pleural space obviously contribute to the fibrotic reactions in TB pleuritis. © 2001 Harcourt Publishers Ltd.

INTRODUCTION

Tuberculous (TB) infection is characterized by the formation of granulomata with fibrinoid or caseous necrosis as a manifestation of cell-mediated immunity against M. tuberculosis. TB pleuritis may resolve spontaneously suggesting that local cell-mediated immune defense mechanisms effectively control the infection, but if left untreated may return as active tuberculosis elsewhere in the body.1,2 Delayed hypersensitivity is considered to play
a major role in the pathogenesis of TB pleuritis. The rupture of a subpleural caseous focus into the pleural space is thought to allow TB protein to enter the pleural compartment and to generate a hypersensitivity reaction responsible for most of the clinical manifestations.\(^3,4\) TB pleuritis is characterized by compartmentalized accumulation of inflammatory cells and pro-inflammatory cytokines in the pleural space.\(^5\)\(^\text{--}\)\(^8\) Furthermore, a disturbed balance of procoagulant and fibrinolytic factors contributes to the fibrotic reactions in the pleural space observed during the course of the disease.\(^9\)\(^\text{--}\)\(^11\)

Matrix metalloproteinases (MMP), a family of zinc dependent endoproteinases, and their endogenous inhibitors, the tissue inhibitors of metalloproteinases (TIMP), are involved not only in normal tissue remodeling during growth and differentiation, but also in numerous pathophysiological processes including infection and immunologically mediated diseases.\(^12\)\(^\text{--}\)\(^13\) MMP are implicated in a range of pulmonary diseases characterized by alterations in alveolar structure or abnormal healing responses, including emphysema, adult respiratory distress syndrome, interstitial fibrosis, granulomatous disease, lung cancer, asthma, and pleural diseases.\(^14\)\(^\text{--}\)\(^16\) Recent reports have investigated the role of MMP and TIMP in pleural effusions of different origin suggesting that these enzymes and their antagonists play a role in homeostasis in the pleural space and some of them seem to be induced in specific disease states.\(^17\)\(^\text{--}\)\(^19\)

TB pleuritis sometimes results in massive fibrosis, in contrast to pleural effusions in congestive heart failure (CHF), where substantial fibrotic reactions are not observed. As MMP and TIMP contribute to this reaction this study aimed to characterize the occurrence of MMP and TIMP in TB pleural effusions in comparison to CHF effusions. Moreover, as higher concentrations of MMP and TIMP in the pleural vs the plasma compartment have been reported in effusions of exudative type\(^17\) we believe that an increase in pleural fluid would be expected in TB pleuritis.

METHODS

Collection of pleural fluid

Patients with TB pleuritis from the Prince of Wales Hospital, the Haven of Hope Hospital, both Hong Kong, and the University Hospital of Leipzig, Germany, were consecutively entered into the study before initiation of treatment. There were no risk factors or clinical evidence of HIV infection and none of the patients had received immunosuppressive medication. The diagnosis of TB was based on histological evidence of granulomatous pleuritis in pleural biopsies in 10 cases, microbiological proof in effusion fluid or sputum in six cases, and on response to antituberculosis therapy in eight cases. The diagnosis of CHF was based on clinical and radiological findings, the transudative nature of the effusion, and exclusion of other etiologies. The study had been approved by the ethical committees of each institution. Informed consent was obtained from each patient. Pleural fluids were collected during the first diagnostic or therapeutic thoracentesis. Following withdrawal, the pleural fluid was placed in tubes containing EDTA to a final concentration of 1 mg/ml. A separate portion was placed in plain tubes for determination of lactate dehydrogenase (LDH) and total protein. The supernatants and cell pellets were separated by centrifugation (600 \(\times\) g, 30 min, 4°C). The total cell, white cell and differential cell counts (May-Grünwald-Giemsa stain) were determined by counting at least 200 cells by light microscopy. Blood was withdrawn from a peripheral vein and collected in tubes containing EDTA (final concentration 1 mg/ml) or in plain tubes. The plasma or serum was separated from the cell pellet by centrifugation. The LDH and total protein concentrations were determined in the effusion supernatant and in serum by automated standard methods. Plasma, serum, and effusion supernatants were aliquotted and stored at –70°C for later analysis.

Enzyme linked immunosorbent assay (ELISA)

Immunoreactive levels of MMP-1 (interstitial collagenase), MMP-2 (72-kDa-gelatinase, gelatinase A), MMP-3 (stromelysin-1), MMP-8 (neutrophil collagenase), MMP-9 (92-kDa-gelatinase, gelatinase B), TIMP-1 and TIMP-2 were determined in duplicate in effusion fluid and plasma samples utilizing commercially available ELISA assays (Biotrack\textsuperscript{TM}, Amersham Pharmacia Biotech, Freiburg, FRG). The assays measure the following (ranges): MMP-1: proMMP-1, MMP-1, and MMP-1/TIMP complexes (6.25–100 ng/ml); MMP-2: proMMP-2 and proMMP-2/TIMP-2 complexes (1.5–24 ng/ml); MMP-3: proMMP-3, MMP-3, and MMP-3/TIMP-2 complexes (3.75–120 ng/ml); MMP-8: proMMP-8, MMP-8, and MMP-8/TIMP complexes (0.25–4 ng/ml); MMP-9: proMMP-9 and proMMP-9/TIMP complexes (4–128 ng/ml); TIMP-1: TIMP-1 and MMP/TIMP-1 complexes (3.13–50 ng/ml); TIMP-2: TIMP-2 and MMP/TIMP-2 complexes (8–128 ng/ml). Undiluted samples were used for measurement of MMP-1, MMP-3, and TIMP-2. Samples were diluted 1 : 50 for MMP-2, 1 : 10 for MMP-8 and MMP-9, and 1 : 100 for TIMP-1.

Gelatine zymography

For enzymographic analysis of MMP-2 and MMP-9 in polyacrylamide gels the method of Tyagi et al. was applied with few modifications.\(^20\) It follows the Laemmli
procedure for separation of proteins in polyacrylamide gels in the presence of sodium dodecyl-sulphate (SDS)\textsuperscript{21} with gelatine incorporated into the gel at a concentration of 1 mg/ml (Sigma type A, 300 Bloom; Sigma, Deisenhofen, FRG). Of the 21 TB patients 21 pleural effusion and 15 plasma samples and of the 15 CHF patients 13 effusion and nine plasma samples were available for gelatin zymography. The samples were diluted 5-fold with water and the dilutions were mixed with three volumes of collagenase sample buffer (200 mM Tris/HCl, pH 8.0 containing 10% SDS, 4% sucrose and 1 mg/ml bromophenol blue) immediately prior to loading 5 μl of the final mixture in duplicate onto the gels. Separation was achieved at 150 V at room temperature. SDS was removed by washing the gels in 2.5% (w/v) Triton X-100 under continuous shaking for 30 min at room temperature with one change of the washing solution. The gels were then incubated overnight in developing buffer (50 mM Tris/Cl, pH 8.0, containing 8 mM CaCl\textsubscript{2}, 1 μM ZnCl\textsubscript{2} and 0.02% NaN\textsubscript{3}) at 37 °C. The reaction was stopped by transferring the gels into methanolic acid-water (5 : 10 : 85). Afterwards the gels were stained with Coomassie Brillant Blue G-250 (0.25% in stopping solution). Finally, the gels were destained in 10% acetic acid. For molecular weight calibration the calibration proteins of the combithek (Boehringer Mannheim, FRG) were separated in one lane of the electrophoresis gel. After staining/destaining their corresponding bands were visible as superimposed dark blue lines on the blue gel enabling the correlation of lysis bands with molecular weights. Furthermore, purified MMP-2 and MMP-9 (Boehringer Mannheim, FRG) were run in parallel with some plasma and pleural fluid samples, giving additional confidence for identification of MMP-2 and MMP-9 derived lytic zones. As by this method standardized individual gelatinolytic activities cannot be determined and to minimize the risk of intersample variation gelatinolytic activities were estimated by densitometric scanning using the video documentation system E.A.S.Y. plus (Herolab, Wiesloch, FRG) applying the spot method of this system. The intensities of the lytic zones corresponding to MMP-2 and MMP-9 were obtained as arbitrary units and the MMP-9/MMP-2 ratios were calculated allowing comparison of individual samples.\textsuperscript{22}

Statistics

Data are presented as mean ± standard error of mean (SEM). Statistical analysis was performed using the SPSS\textsuperscript{TM} 8.0 software (SPSS Scientific, Erkrath, FRG). As the data were not normally distributed Wilcoxon signed-rank-tests were performed for paired samples and Mann-Whitney U-tests for independent samples. For comparisons of the three independent groups of blood samples the Kruskal-Wallis one way analysis of variance of ranks (ANOVA) was performed. An overall P-value ≤ 0.05 and to exclude by chance significances for multiple comparisons a P-value ≤ 0.0025 for individual pairs was considered significant (Bonferroni-adjustment). To test for correlations of pairs of variables the Spearman rank order correlation method was applied.

RESULTS

Patients

Effusion fluid and plasma of 21 patients with TB pleuritis (five women, 16 men, age 51 ± 5 years) and 15 patients with CHF (six women, nine men, age 72 ± 3 years), and plasma of 15 healthy blood donors (five women, 10 men, age 37 ± 2 years) were examined.

General characteristics of pleural fluids

Although the number of total cells was higher in TB vs CHF effusions (31 026 ± 22 770 vs 3892 ± 1764/ml) it did not reach statistical significance due to wide variation of results. The number of white blood cells was higher in TB vs CHF effusions of (3150 ± 884 vs 319 ± 113/ml; P < 0.0005), with higher amounts of lymphocytes (81 ± 6 vs 52 ± 17%; P = 0.04), but lower amounts of monocytes (8 ± 2 vs 57 ± 18% monocytes; P < 0.005). The pleural fluid concentrations of total protein in TB were 49.2 ± 2.5 g/l vs 27.8 ± 3.7 g/l in CHF (P < 0.0005), respective concentrations of LDH were 10.6 ± 3.1 vs 2.6 ± 0.4 mmol/l*s (P < 0.0005) identifying TB effusions as exudative (total protein/effusion/serum > 0.5 and LDH\textsubscript{effusion}/serum > 0.6) and CHF effusions as transudative (respective ratios < 0.5 and < 0.6).\textsuperscript{23}

ELISA

Immunoreactive levels of MMP and TIMP were detected by ELISA in pleural effusion fluid and plasma of patients, and in plasma of healthy persons in different concentrations. To allow comparison in relation to the total protein content MMP/- and TIMP/total protein ratios in pleural fluid and blood were calculated. Highest absolute values were found for MMP-2 and TIMP-1. As MMP-3 was only detectable in trace amounts, defined as values at the lower detection limit (range: 0–18.8 ng/ml), only part of the samples were examined and no statistical comparisons were performed. MMP pleural fluid concentrations were higher in TB when compared to CHF: MMP-1: 21.7 ± 3.1 vs 8.3 ± 1.0 ng/ml (P < 0.0005), MMP-2: 1233 ± 169 vs 662 ± 81 ng/ml (P = 0.002), MMP-8: 42.7 ± 11.9 vs 3.8 ± 0.8 ng/ml (P < 0.0005), and MMP-9: 36.5 ± 17.0 vs 0.9 ± 0.3 ng/ml (P < 0.0005). TIMP pleural
Table 1  MMP/− and TIMP/total protein ratios in pleural fluid and plasma of study populations

<table>
<thead>
<tr>
<th>Parameter/Total protein</th>
<th>Pleural fluid</th>
<th>Plasma</th>
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<tbody>
<tr>
<td></td>
<td>TB</td>
<td>CHF</td>
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<tr>
<td>MMP-1/total protein</td>
<td>0.46 ± 0.10</td>
<td>0.30 ± 0.06</td>
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<tr>
<td>MMP-2/total protein</td>
<td>25.2 ± 2.8</td>
<td>25.7 ± 2.8</td>
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<tr>
<td>MMP-8/total protein</td>
<td>0.81 ± 0.23</td>
<td>0.20 ± 0.05</td>
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<tr>
<td>MMP-9/total protein</td>
<td>0.45 ± 0.16*</td>
<td>0.04 ± 0.01</td>
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<tr>
<td>TIMP-1/total protein</td>
<td>139.0 ± 28.7***</td>
<td>517.8 ± 183.7</td>
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<tr>
<td>TIMP-2/total protein</td>
<td>0.67 ± 0.13</td>
<td>1.29 ± 0.32</td>
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Data are mean ± standard error of mean (SEM). TB = tuberculosis; CHF = congestive heart failure. *TB vs CHF pleural fluid: *P < 0.005; **P < 0.0005; †Plasma vs pleural fluid; ‡P < 0.005; ††P < 0.0005.

fluid concentrations were not significantly different in TB compared to CHF: TIMP-1: 6325 ± 1379 vs 10 502 ± 1861 ng/ml and TIMP-2: 33.7 ± 7.5 vs 32.1 ± 7.1 ng/ml. When adjusted to total protein only the MMP-9 ratio was significantly higher in pleural fluid in TB compared to CHF but the TIMP-1 ratio was lower (Table 1). MMP and TIMP plasma concentrations in all study groups were not significantly different. Plasma concentrations in healthy controls were: MMP-1: 13.9 ± 0.6 ng/ml; MMP-2: 316 ± 18 ng/ml; MMP-8: 11.7 ± 2.2 ng/ml; MMP-9: 176 ± 4.0 ng/ml; TIMP-1: 703 ± 80 ng/ml; and TIMP-2: 7.7 ± 1.8 ng/ml. However, TB pleural fluid concentrations of MMP-1, MMP-2, TIMP-1, and TIMP-2 were significantly higher compared to TB plasma (21.7 ± 3.1 vs 11.5 ± 1.2 ng/ml, P = 0.005; 1233 ± 169 vs 283 ± 60 ng/ml, P < 0.0005; 6325 ± 1379 vs 1693 ± 401 ng/ml, P = 0.003; 33.7 ± 7.5 vs 12.3 ± 2.5 ng/ml, P = 0.001). When adjusted to total protein the increase of MMP-1, MMP-2, TIMP-1, and TIMP-2 ratios in TB pleural fluid vs TB plasma was even more significant (Table 1). CHF pleural fluid concentrations were higher compared to CHF plasma for TIMP-1 only (10 502 ± 1861 vs 1595 ± 304, P < 0.005), but lower for MMP-8 and MMP-9 (3.8 ± 0.8 vs 16.0 ± 4.8 ng/ml, P = 0.005; 0.9 ± 0.3 vs 64.0 ± 167 ng/ml, P = 0.001). When adjusted to total protein, higher ratios were found in CHF pleural fluid vs CHF plasma for MMP-1, MMP-2, TIMP-1, and TIMP-2, but a lower ratio for MMP-9 (Table 1).

Gelatine zymography

MMP-2 and MMP-9 were detectable as separate lytic bands migrating at molecular weights of approximately 90 kDa and 70 kDa respectively (Fig. 1A–D). The intensities of the lytic bands correspond to the enzyme activity. The MMP-9/MMP-2 ratios obtained by densitometric scanning are depicted in Figure 2. The identity of MMP-2 and MMP-9 was demonstrated by comparison with the purified enzymes which both gave complex patterns with several lytic zones visible (Fig. 1D). In MMP-2 the major band corresponds to proMMP-2 and its active form is visible as the faster moving band close to it (Fig. 1D, lane 1). It must be noted, however, that the active form was observed without prior activation step having been performed. This active form was hardly observed in patient or control samples but if detected at all then only in TB and never exceeding the amount of an estimated 5% of the proform. In the case of purified MMP-9 only the zymogen was detected at the appropriate position (Fig. 1D, lane 7). Very often lytic bands with a molecular weight around 130 kDa were observed especially in TB effusions. The position of these bands varied (Fig. 1A: patient 1/lane 2 compared to patient 2/lane 4) and at times even represented the most prominent of all lytic bands (Fig. 1A: patient 4/lane 8). It has been shown that these bands correspond to proMMP-9 bound to a neutrophil gelatinase-associated lipocalin.

In TB effusions distinct patterns for individual patients were found, whereas in CHF effusions more uniform patterns dominated. In TB effusions MMP-2 and MMP-9 were always detectable with bands of different brightness and size reflecting a wide potential activity range (Fig. 1A–B). In contrast, in CHF effusions the MMP-9 band was barely visible or even missing. As a consequence individual MMP-9/MMP-2 effusion ratios were higher in TB varying over a wide range, in contrast to CHF with very low ratios and little variation (P < 0.0005) (Fig. 2). In TB plasma the intensities of the lytic bands of both enzymes differed substantially (Fig. 1A). In CHF, however, mainly the MMP-9 band varied in contrast to more consistent MMP-2 bands (Fig. 1B). There was a significant difference of plasma MMP-9/MMP-2 ratios in TB vs healthy persons (0.39 ± 0.10 vs 0.59 ± 0.04, P = 0.001) but not vs CHF. Comparison of MMP-9/MMP-2 effusion vs plasma ratios did not reach statistical significance for neither TB nor CHF (Fig. 2).

To determine whether gelatine zymography MMP-9/MMP-2 ratios are comparable to results obtained by
Fig. 1 Zymographic detection of MMP-2 and MMP-9 in plasma and pleural effusion fluids. Typical zymographic patterns of four patients with (A) tuberculous pleuritis (TB), (B) congestive heart failure (CHF), and (C) eight healthy control persons (CON) are shown. Lanes 1, 3, 5, 7 in (A), (B), and all lanes in (C) represent plasma, lanes 2, 4, 6, 8 in (A) and (B) corresponding effusion fluids. (D) The position of MMP-2 is identified by a parallel run of purified proMMP-2 (70 kDa) in lane 1 with the major band corresponding to proMMP-2 (arrow) and its active form close to it (open arrow). The position of MMP-9 is identified by a parallel run of purified proMMP-9 (90 kDa) in lane 7. Lanes 2 and 3 show plasma and effusion fluid of a CHF patient, lane 4 plasma of a healthy control, lanes 5 and 6 plasma and effusion fluid of a TB patient.

ELISA respective ratios were calculated. A tendency for positive correlation for zymography vs ELISA ratios could be shown for plasma samples of all study groups combined ($r = 0.317; P = 0.052$) and significantly for all pleural effusion samples combined ($r = 0.480; P = 0.005$).

DISCUSSION

This study demonstrates the occurrence of MMP and their specific inhibitors TIMP, in TB pleuritis. High concentrations of interstitial collagenase (MMP-1), gelatinase A (MMP-2), and of TIMP-1 and TIMP-2 were found in TB pleural fluid compared to plasma demonstrating their compartmentalization at the site of active disease. Furthermore, interstitial collagenase (MMP-1) and neutrophil collagenase (MMP-8), gelatinase A (MMP-2) and gelatinase B (MMP-9) were found in higher amounts in TB pleural effusion fluids in comparison to CHF pleural fluids. This surplus of MMP and TIMP obviously contributes to the fibrotic reactions observed in TB pleuritis. Although differences of MMP and TIMP plasma concentrations between TB, CHF, and healthy controls were observed they did not reach statistical significance, due to wide variation of individual values, albeit smallest in healthy control persons.

An explanation for high concentrations of MMP-1, MMP-2, TIMP-1, and TIMP-2 in the pleural compartment in TB pleuritis compared to plasma would be their increased production and secretion by local cells. In TB pleuritis compartmentalization in the pleural space has been reported for cytokines like tumor necrosis factor-$\alpha$ (TNF-$\alpha$), interferon-$\gamma$ (IFN-$\gamma$), interleukin (IL)-6, IL-8, or subpopulation of lymphocytes.$^{5-8,25}$ The local interaction of cells and cytokines possibly contributes to MMP and TIMP accumulation. IL-6, for example, augmented TIMP production of connective tissue cells and alveolar macrophages.$^{26,27}$ IL-1-$\alpha$ enhanced MMP-3, MMP-9, and TIMP-1 but not MMP-2 production in trabecular explant cultures.$^{28}$ IL-6 enhanced TIMP and augmented IL-1 induced production of MMP-1, MMP-3, and TIMP in rheumatoid synovial fibroblasts.$^{29}$ Subsets of lymphocytes produced...
MMP-9 and TIMP-1 being further augmented or downregulated by cytokines.\textsuperscript{30} Passive diffusion of MMP and TIMP from the pleural space into the systemic circulation followed by plasmatic inhibitor neutralization might add to a surplus in the pleural compartment. On the other hand, TB associated inflammation with increased pleural permeability might facilitate passive diffusion of MMP and TIMP from the blood into the pleural space. Most striking was the observation that in TB pleuritis MMP-8 and MMP-9 were present in comparable amounts in pleural fluids and plasma, whereas the opposite was true for CHF. In CHF MMP-8 and MMP-9 were almost absent in pleural fluids compared to plasma, although, when adjusted to the total protein content, the difference remained significant for MMP-9 only. This study confirmed this observation for MMP-9 by gelatine zymography.

Determination of MMP-2 and MMP-9 by gelatine zymography in exudative pleural effusions has been reported before,\textsuperscript{17}–\textsuperscript{19} but not in effusions of TB origin. In this study the intensities of MMP-2 and MMP-9 bands differed substantially in TB effusions, whereas MMP-2 bands varied little and MMP-9 bands were almost absent in CHF effusions. This resulted in a dramatic difference of MMP-9/MMP-2 ratios in TB vs CHF effusions. In pulmonary TB\textsuperscript{31} M. tuberculosis and its major cell antigenic component lipoarabinomannan stimulate the release of MMP-9 and upregulate the expression of genes for MMP-1 and MMP-9 in macrophages.\textsuperscript{31} In this way M. tuberculosis contributes to tissue destruction by stimulating macrophages to release MMP and by stimulating the release of IL-1\textbeta and TNF-\alpha which in turn induce fibroblasts to release MMP.\textsuperscript{31} The presence of high amounts of mononuclear cells, including macrophages, in TB effusion fluid may therefore, together with tissue macrophages and fibroblasts in the pleura, contribute to cell induced production of MMP-9 leading to high amounts of MMP-9 in pleural fluid as evidenced by this study. Another study examined pleural effusions of transudative, paraneoplastic, and parainfectious type and found MMP-1 by immunoblotting in equal amounts and MMP-8 in trace amounts correlating with the number of neutrophils.\textsuperscript{19} The same study reports high amounts of MMP-9 in parainfectious and in paraneoplastic effusions and low amounts in CHF effusions.\textsuperscript{19} In contrast, we report higher amounts of MMP-1 and MMP-8 determined by ELISA in TB effusions compared to CHF but confirming a similar observation with regards to MMP-9. A further study showed that human pleural and peritoneal mesothelial cells express mRNA of MMP-1, MMP-2, MMP-9, and TIMP which could be upregulated except for MMP-2 by phorbol myristate acetate.\textsuperscript{32} Of particular interest was their observation that epithelial cells displayed a more matrix-degradative phenotype with increased MMP-9 and decreased TIMP expression than their fibroblastoid counterparts.\textsuperscript{32} This observation suggests that mesothelial cells are capable to upregulate MMP-9 production following serosal injury whereby MMP-2 seems constitutively expressed.\textsuperscript{32}

Our observation by gelatine zymography that lytic bands representing active forms of MMP-2 and -9 were exclusively found in purified protein samples but neither in effusion fluid or plasma samples can be explained by the fact that active forms are immediately neutralized in vivo by specific or unspecific inhibitors or other proteolytic enzymes. This contributes to explain the difference in MMP concentrations when heparin plasma, EDTA plasma, or serum of the same patient were measured by ELISA.\textsuperscript{33}

The observations with regards to TIMP remain conflicting. This study demonstrated lower immunoreactive TIMP-1 concentrations in TB effusions but comparable TIMP-2 concentrations in TB and CHF effusions. In contrast, another study found equal amounts of TIMP-1 by immunoblotting in transudates and exudates but no TIMP-2 in transudates.\textsuperscript{19} The production of TIMP is thought to be enhanced by a range of cytokines for example in stromal cells.\textsuperscript{16} TIMP-1 and TIMP-2 inhibit the active form of all MMP, binding in a non-covalent manner to form a 1 : 1 complex. The inhibitory action of TIMP seems to be important in the process of activation as well as in the subsequent regulation of enzymatic matrix degradation.\textsuperscript{34} Activation of MMP also involves the plasminogen activator cascade. Plasmin, once generated, can activate stromelysin and gelatinase B (MMP-9), plasmin and stromelysin in turn can activate collagenase (MMP-1). This process is regulated by \alpha_2-antiplasmin (\alpha_2AP) and by TIMP.\textsuperscript{34} Therefore, the interaction between MMP, TIMP, and fibrinolytic factors obviously contributes to the fibrotic reactions observed in TB pleuritis. Whether similar observations could be made in other pleural effusions with a tendency to cause fibrosis, such as parapneumonic effusions, should be further investigated.

In summary, MMP-1, MMP-2, TIMP-1, and TIMP-2 are found in high concentrations in the pleural space in TB pleuritis demonstrating their compartmentalization at the site of active disease. In TB pleuritis pleural fluid concentrations of MMP-1, MMP-2, MMP-8, and MMP-9 are higher in comparison to CHF effusion fluid. This surplus of MMP and TIMP together with their close interaction with constituents of the coagulatory/fibrinolytic cascades obviously contributes to the fibrotic reactions following pleural injury in TB pleuritis.

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