CHEST

Official publication of the American College of Chest Physicians

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Chest 2005;128;690-697
DOI 10.1378/chest.128.2.690

ISSN:0012-3692

Proinflammatory Cytokines, Transforming Growth Factor-β1, and Fibrinolytic Enzymes in Loculated and Free-Flowing Pleural Exudates*

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Study objectives: **To measure tumor necrosis factor (TNF) α, interleukin (IL) 1β, and transforming growth factor (TGF) β1 in loculated and free-flowing pleural effusions caused by malignancy, tuberculosis (TB), and pneumonia and their relationship with plasminogen activator inhibitor-type 1 (PAI-1) and tissue-type plasminogen activator (tPA) and to compare the differences between loculated and free-flowing effusions.**
Design: **A prospective study.**
Patients and methods: **The effusion levels of TNF-α, IL-1β, TGF-β1, PAI-1, and tPA were measured in 29 patients with malignant effusions, 19 patients with TB, and 30 patients with parapneumonic effusions. Pleural effusions were divided into loculated and free-flowing groups by imaging studies. A group of 42 patients with loculated effusions was subdivided into primary and secondary loculation groups by chest ultrasonography.**
Results: **The median levels of TNF-α (87.0 pg/mL), IL-1β (13.8 pg/mL), TGF-β1 (10,952.9 pg/mL), PAI-1 (111.2 ng/mL), and lactate dehydrogenase (LDH) [498 IU/dL] in the loculated group were significantly higher than those in the free-flowing group (TNF-α, 15.0 pg/mL; IL-1β, 2.9 pg/mL; TGF-β1, 6,117.3 pg/mL; PAI-1, 61.5 ng/mL, and LDH, 266 IU/dL). In both the loculated and free-flowing effusions, the levels of TGF-β1 correlated positively with those of TNF-α ($r = 0.51$ and $p < 0.001$ vs $r = 0.42$ and $p < 0.05$, respectively) and IL-1β ($r = 0.52$ and $p < 0.001$ vs $r = 0.49$ and $p < 0.01$, respectively), and the values of PAI-1 correlated positively with those of TNF-α ($r = 0.59$ and $p < 0.001$ vs $r = 0.55$ and $p < 0.001$, respectively), IL-1β ($r = 0.35$ and $p < 0.05$ vs $r = 0.47$ and $p < 0.01$, respectively), and TGF-β1 ($r = 0.53$ and $p < 0.001$ vs $r = 0.58$ and $p < 0.001$, respectively). In contrast, the levels of tPA correlated negatively with those of TNF-α ($r = -0.37$, $p < 0.05$) and IL-1β ($r = -0.56$, $p < 0.001$) in loculated effusions. Twenty-seven of 42 patients with loculated effusions were classified into a secondary loculation group, which, compared with the primary loculation group, had significantly higher median levels of effusion TNF-α (119.2 vs 14.2 pg/mL, respectively; $p = 0.001$), IL-1β (33.3 vs 3.4 pg/mL, respectively; $p < 0.001$), TGF-β1 (13,152.7 vs 7746.0 pg/mL, respectively; $p = 0.041$), and PAI-1 (114.9 vs 94.1 pg/mL, respectively; $p = 0.019$).**
Conclusion: **Compared with free-flowing effusions, fibrinolytic activity was depressed in loculated effusions. A higher intensity of pleural inflammation in loculated effusions may enhance the release of TNF-α, IL-1β, and TGF-β1, which may subsequently increase the levels of PAI-1. The imbalance of PAI-1 and tPA in pleural spaces may lead to fibrin deposition and loculation of pleural effusions.**
(CHEST 2005; 128:690–697)

Key words: fibrinolysis; loculation; malignancy; pleural effusion; proinflammatory cytokines; tuberculosis

Abbreviations: IL = interleukin; IQR = interquartile range; LDH = lactate dehydrogenase; PA = plasminogen activator; PAI-1 = plasminogen activator inhibitor type 1; RPT = residual pleural thickening; TB = tuberculosis, tuberculous; TGF = transforming growth factor; TNF = tumor necrosis factor; tPA = tissue-type plasminogen activator; US = ultrasonography

Fluid loculation is common in inflammatory pleural exudates including parapneumonic effusion and tuberculous (TB) pleurisy. It is a poor prognostic factor for parapneumonic effusion or empyema, and it makes the drainage of fluid collection difficult.[1–3] Our and previous studies[4–7] indicated that fluid loculation could also occur in malignant pleural effusions, especially following repeated thoracenteses. Furthermore, secondary loculation of pleural exudates with formation of internal septa could make fluid drainage more difficult.[4] The occurrence of fluid loculation in the pleural space has been thought to be a result of fibrin deposition and subsequent adhesion between the parietal and visceral pleura.[1] Consequently, intrapleural administration of fibrinolytic agents is required for the lysis of fibrinous adhesion and to facilitate pleural fluid drainage.[8–10] Although fibrin formation and deposition have long

been held to be important in the pathogenesis of loculation of pleural effusion, the difference in regulating fibrin deposition between loculated and free-flowing pleural exudates remains speculative.[11]

In patients with exudative pleural effusions, an increase in procoagulant activity, attributable mainly to tissue factors, has been observed.[12] By and large, fibrin turnover in the pleural cavity is greatly affected by fibrinolytic activity. The formation of a key enzyme in fibrinolysis, plasmin, is based mainly on the equilibrium between plasminogen activators (PAs) and PA inhibitors (PAIs).[12] Furthermore, fibrin deposition is a hallmark of pleural inflammation,[13] which may enhance the release of proinflammatory cytokines, such as tumor necrosis factor (TNF) α and/or interleukin (IL) 1β, in pleural fluid.[14,15] These cytokines may reduce fibrinolytic activity by stimulating the release of PA inhibitor type 1 (PAI-1) and result in an imbalance between PAI-1 and tissue-type PA (tPA) in pleural cavity, which may lead to fibrin formation and deposition and subsequent loculation of pleural effusion.[14–17] However, not all pleural exudates are loculated. To our knowledge, the differences of inflammation intensity and fibrinolytic activity between loculated and free-flowing pleural exudates have never been studied.

Pleural inflammation results in fibrin deposition, which may additionally initiate a sequence of events that lead to tissue modeling and ultimate fibrosis.[18,19] Transforming growth factor (TGF) β is a multifunctional cytokine that stimulates cell proliferation and angiogenesis in the areas of inflammation.[20] High levels of TGF-β in exudative effusions with a positive correlation with those of lactate dehydrogenase (LDH), a marker of pleural inflammation, were reported,[21] and they suggest that pleural inflammation may serve as a trigger of accumulation of TGF-β in pleural spaces. Furthermore, TGF-β is a potent fibrogenic cytokine that contributes to fibrin deposition and tissue fibrosis in the pleural space via an increase of PAI-1 and extracellular matrix production.[13,22]

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This study was supported by a grant from the Taipei Medical University (TMU91-Y05-A137).
Manuscript received April 22, 2004; revision accepted January 21, 2005.

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Taken together, these findings suggest that there may be a considerable difference in the regulation of fibrinolytic activity between loculated and free-flowing pleural exudates, and the difference may be affected by proinflammatory cytokines and TGF-β. This study was conducted prospectively to evaluate the relationship among fibrinolytic enzymes (PAI-1 and tPA), proinflammatory cytokines (TNF-α and IL-1β), and TGF-β1 in exudative pleural effusions of different etiologies and to compare the differences between loculated and free-flowing effusions. In addition, the relationship between the concentrations of these parameters in pleural fluid and the development of residual pleural thickening (RPT) in patients with TB pleurisy were evaluated.

Materials and Methods

The Institutional Review Board of Taipei Medical University Hospital approved this 2-year prospective study, and informed consent was obtained from all of the patients to participate in this study. The patients who had pleural effusions of unknown causes and were admitted to Taipei Medical University Hospital for diagnostic evaluation between January 2002 and December 2003 were eligible for this study. All of the patients were subjected to routine chest radiography (frontal and lateral views), lateral decubitus view with the lesioned side down, and real-time chest ultrasonography (US). A thoracic CT scan was performed if clinically indicated. Chest US was performed by one of two physicians who were both blind to the clinical information of the patients. The sonograms were first interpreted by the examiner for determining free-flowing or loculated effusion, the presence of fibrinous strands and/or septation within pleural fluid, and associated pulmonary lesions. The sonograms were printed out and interpreted by another physician. When the two readers could not reach consensus, the case was presented to a third expert reader, and the adjudicated reading became final. In fact, no disagreement between the two readers on the determination of free-flowing or loculated effusion and on the presence of fibrin strands and/or septation was noted in this study.

With the guidance of chest US, pleural fluid was collected using a standard thoracentesis technique immediately or within 24 h after hospitalization. When pleural effusion was multiloculated, the fluid was aspirated from the largest loculus. The pleural fluid samples were mixed with 3.8% sodium citrate in a 9:1 ratio of pleural fluid to sodium citrate. The sodium citrate-mixed pleural fluid specimens were immersed in ice immediately and then centrifuged at 2,500*g* for 10 min. The cell-free supernatants of pleural fluid were frozen at −70°C immediately after centrifuge until later examinations.

Analyses of pleural fluid for total leukocytes; cell differentials of leukocytes; pH value; and levels of protein, glucose, and LDH were performed in addition to cytologic and microbiological examination of pleural fluid. A pleural biopsy was performed when the results of pleural fluid analysis were suggestive of TB or malignancy.

The patients were included subsequently if pleural exudate was established in accordance with the criteria of Light et al[23] and a diagnosis of malignant, TB, or parapneumonic effusions was established by examinations of pleural fluid and/or pleural biopsy specimens. The patients were excluded from this study if they had invasive procedures directed into the pleural cavity, had chest trauma within 3 months prior to hospitalization, or had a

pleural effusion of undiagnosed cause. A diagnosis of malignant pleural effusion was established by the demonstration of malignant cells in pleural fluid and/or closed pleural biopsy specimens. A pleural effusion that resulted from pneumonia or lung abscess was defined as a parapneumonic effusion, including empyema. TB pleurisy was diagnosed by the growth of *Mycobacterium tuberculosis* from the pleural fluid or by the demonstration of granulomatous pleuritis on closed pleural biopsy specimens.

Pleural effusions were divided into loculated or free-flowing effusions by chest radiography, US, or CT scans. Loculated effusion was defined as an effusion with one or more of the following criteria: (1) failure of the effusion to gravitate to the most dependent part of the pleural space shown on lateral decubitus view of chest radiogram; (2) no significant change of the shape of fluid collection during deep breathing and/or panting on real-time chest US; (3) nondependent fluid collection or fixed fluid collections with a convex inner margin as seen on the chest CT scan. The group of patients with loculated effusions was additionally subdivided into primary and secondary loculation groups by chest US. Loculated effusion without fibrin septation within it was classified into primary loculation group. The loculated effusion divided into loculi by internal septa was classified into secondary loculation group.[4,24]

The levels of cytokines and fibrinolytic enzymes in pleural fluid were measured by the following commercially available enzyme-linked immunosorbent assay kits: (1) tPA and PAI-1 (American Diagnostica; Greenwich, CT); and (2) TNF-α, IL-1β, and TGF-β1 (R&D Systems; Minneapolis, MN).

RPT was measured as described before[15,25] and defined as a pleural thickening of ≥ 10 mm shown on chest radiographs at the completion of anti-TB chemotherapy in patients with TB pleurisy.

Nonparametric tests were used to analyze pleural fluid variables, because the variables studied were not normally distributed. Data were expressed as median, interquartile range (IQR), and 95% confidence interval for the median. Specific comparisons of continuous data between two independent groups were made using the Mann-Whitney *U* test, and Kruskal-Wallis one-way analysis of variances on ranks was used to compare the data among three independent groups. The comparisons for categoric variables between groups were examined using the χ^2 method and/or the Fisher exact test when appropriate. The correlations between variables were determined by Spearman rank correlation coefficients. Significance was defined as a p value of < 0.05, and all of the comparisons were two-tailed. Statistical analysis was performed using a statistical software package (Statistica for Windows, version 5.5; StatSoft, Inc; Tulsa, OK).

Results

Between January 2002 and December 2003, 120 patients were eligible for this study, and 42 of them were excluded due to transudative effusion (30 patients), undiagnosed effusion (8 patients), and previous chest tapping (4 patients). Finally, a total of 78 patients who met the patient selection criteria were included in this study.

Of the 78 patients, there were 46 men and 32 women with an age range from 23 to 101 years (mean age, 62 years). The etiology of pleural effusion was malignancy in 29 cases, TB in 19 cases, and pneumonia in 30 cases. The malignant tumors were lung cancer in 15 cases, colon cancer in 6 cases, breast cancer in 3 cases, hepatocellular carcinoma in 2 cases, esophageal carcinoma in 2 cases, and gastric cancer in 1 case. Loculated effusions were found in 11 of 29 patients with malignant effusions, 12 of 19 patients with TB pleurisy, and 19 of 30 patients with parapneumonic effusions.

The pleural fluid characteristics, including total leukocyte count and the values of pH, levels of glucose, LDH, and protein, and the effusion levels of cytokines, PAI-1, and tPA of different etiologies, are summarized in Table 1. Irrespective of underlying etiologies, loculated exudates had significantly lower values for pH and glucose and significantly higher values of LDH than did free-flowing effusions. The values of PAI-1, TNF-α, IL-1β, and TGF-β1 were significantly higher in loculated than in free-flowing effusions. There was no significant difference in effusion tPA between loculated and free-flowing effusions. Of note, in parapneumonic effusions, the total leukocyte count was significantly higher in loculated than in free-flowing effusions (Table 1).

Because the pleural fluid characteristics, except for total leukocyte count and cell differentials of leukocytes, and effusion levels of cytokines and fibrinolytic enzymes in both loculated and free-flowing effusions caused by different etiologies showed no significant difference (Table 1), 42 patients with loculated effusions of various etiologies were pooled in the loculated group, and the remaining 36 patients were classified as being in the free-flowing group. Comparisons of pleural fluid characteristics and the effusion levels of cytokines and fibrinolytic enzymes between the loculated and free-flowing groups are shown in Table 2 and Figure 1. The loculated group had significantly lower values of pH and glucose, and significantly higher values of LDH than did the free-flowing group. The median levels of PAI-1, TNF-α, IL-1β, and TGF-β1 were significantly higher in the loculated group than in free-flowing group. No significant difference in the effusion levels of tPA was found between the two groups (Table 2, Fig 1).

In the loculated group, the levels of effusion tPA correlated negatively with those of TNF-α, IL-1β, and LDH, and the values of effusion PAI-1 and the PAI-1/tPA ratios correlated positively with those of TNF-α, IL-1β, TGF-β1, and LDH (Table 3). In the free-flowing group, the levels of effusion PAI-1 and the PAI-1/tPA ratios were positively correlated with those of TNF-α, IL-1β, and TGF-β1 (Table 3). There was no significant correlation between PAI-1 and tPA in both the loculated group ($r = 0.06$, $p > 0.05$) and the free-flowing group ($r = 0.10$, $p > 0.05$).

The relation of effusion levels of TGF-β1, proinflammatory cytokines, and LDH in the loculated and

Table 1—*Pleural Fluid Characteristics and Effusion Levels of Cytokines and Fibrinolytic Enzymes Between Loculated and Free-Flowing Pleural Effusions Caused by Malignancy, TB, and Pneumonia

Effusion Variables	Malignant			Tuberculous			Parapneumonic		
	L (n = 11)	F (n = 18)	p Value	L (n = 12)	F (n = 7)	p Value	L (n = 19)	F (n = 11)	p Value
pH value	7.25 (0.15; 7.21–7.38)	7.36 (0.11; 7.25–7.42)	0.041	7.32 (0.11; 7.22–7.35)	7.39 (0.02; 7.35–7.42)	0.023	7.20 (0.43; 6.87–7.30)	7.41 (0.01; 7.41–7.46)	0.0002
Glucose, mg/dL	81 (83; 40–133)	125 (47; 90–141)	0.034	85 (33; 71–100)	142 (32; 99–161)	0.011	65 (99; 32–157)	123 (55; 100–210)	0.036
Protein, g/dL	3.5 (1.0; 1.9–4.3)	4.1 (1.3; 3.0–4.4)	0.356	4.7 (1.2; 3.2–5.2)	5.2 (1.1; 3.8–6.4)	0.383	4.2 (1.5; 2.7–4.8)	3.7 (1.8; 2.6–4.9)	0.339
LDH, IU/dL	310 (5,207; 136–5,394)	271 (262; 172–380)	0.043	445 (433; 178–723)	210 (133; 94–307)	0.025	1,238 (1,638; 258–1,938)	164 (260; 62–358)	0.019
Total leukocytes, cells/μL	330 (334; 117–586)	829 (1,714; 378–1,530)	0.076	1,760 (2,903; 963–4,410)	1,437 (1,280; 244–7,344)	0.494	9,660 (12,725; 288–14,175)	580 (5,344; 118–6,000)	0.029
PAI-1, ng/mL	108.4 (20.5; 88.0–111.2)	49.5 (38.7; 18.0–68.2)	0.001	118.1 (99.0; 111.1–213.0)	103.7 (22.8; 24.4–114.5)	0.017	104.0 (132.7; 33.5–168.0)	63.5 (96.4; 3.0–104.1)	0.027
tPA, ng/mL	26.1 (22.6; 9.9–34.2)	17.7 (13.6; 8.1–22.3)	0.388	15.9 (14.3; 5.2–22.7)	19.4 (20.4; 1.0–62.7)	0.701	12.7 (16.2; 3.2–17.0)	15.0 (13.9; 1.5–25.9)	0.180
TNF-α, pg/mL	61.0 (200.1; 11.6–213.0)	12.9 (6.4; 11.8–18.2)	0.024	95.8 (112.8; 40.1–153.3)	32.0 (35.2; 12.9–83.5)	0.035	51.2 (122.3; 14.2–132.4)	16.8 (5.5; 11.8–20.0)	0.036
IL-1β, pg/mL	11.4 (13.0; 2.5–33.5)	2.3 (2.2; 2.2–4.1)	0.011	17.2 (28.1; 2.8–33.2)	4.4 (2.0; 2.3–25.9)	0.043	31.7 (84.1; 8.1–92.2)	3.0 (4.8; 2.2–7.3)	0.003
TGF-β1, pg/mL	7,702.4 (8,459.5; 4,328.6–13,663.0)	5,308.6 (4,255.7; 2,056.7–6,362.8)	0.038	13,692.2 (5,514.5; 10,952.9–16,605.3)	9,546.9 (6,449.0; 4,567.0–13,452.7)	0.035	9,826.7 (9,496.5; 5,903.7–15,400.2)	7,173.3 (5,141.6; 1,887.9–8,464.6)	0.045

*Values given as median (IQR; 95% confidence interval), unless otherwise noted. The effusion levels of fibrinolytic enzymes and cytokines in both loculated and free-flowing effusions caused by different etiologies showed no significant differences. F = free-flowing; L = loculated.

free-flow groups is also shown in Table 3. In the loculated group, the effusion levels of TGF-β1 correlated positively with those of TNF-α, IL-1β, and LDH. In the free-flowing group, the effusion levels of TGF-β1 were significantly related to those of TNF-α and IL-1β.

According to the findings of the chest US, 15 of 42 patients (36%) with loculated effusions were classified into the primary loculation group because of the absence of fibrin septation in effusions. The remaining 27 patients (64%) with fibrin septation within the effusions were classified into the secondary loculation group. The pleural fluid characteristics and effusion levels of cytokines and fibrinolytic enzymes in the primary and secondary loculated groups are summarized in Table 4. No significant difference in the pleural fluid characteristics was observed between the two groups except for significantly lower values of glucose and higher levels of LDH observed in the secondary loculated effusions. The levels of effusion PAI-1, TNF-α, IL-1β, and TGF-β1 were significantly higher in the secondary than in the primary loculation group. No significant difference in the effusion tPA level was found between the two groups.

In patients with TB pleurisy, RPT was observed in 5 of 19 patients (26.3%) after completion of anti-TB chemotherapy, and loculated effusions were found initially in all 5 of the patients with RPT. However, there was no significant difference in the incidence of RPT between the loculated and free-flowing groups (5 of 12 vs 0 of 7, respectively; $p = 0.27$). Significantly higher levels of TNF-α, IL-1β, TGF-β1, and PAI-1 in effusions and significantly lower values of tPA in effusions were found in patients with RPT than in those without RPT (Table 5).

Discussion

The present study demonstrated that the levels of TNF-α, IL-1β, TGF-β1, and PAI-1 were significantly higher in loculated effusions than in free-flowing effusions caused by malignancy, TB, or pneumonia. In both the loculated and free-flowing effusions, the levels of TGF-β1 correlated positively with those of TNF-α and IL-1β, and the values of PAI-1 and the PAI-1/tPA ratio correlated positively with those of TNF-α, IL-1β, and TGF-β1. In contrast, the levels of tPA correlated negatively with those of TNF-α and IL-1β in loculated effusions. The secondary loculation of pleural effusions was found in 27 of 42 patients with loculated effusions. The levels of TNF-α, IL-1β, TGF-β1, and PAI-1 were significantly higher in the secondary than in the primary loculated effusions. RPT was found in 5 of

Table 2—*Pleural Fluid Characteristics and Effusion Levels of Cytokines and Fibrinolytic Enzymes Between Loculated and Free-Flowing Effusions

Effusion Variables	Loculated (n = 42)	Free-Flowing (n = 36)	p Value
pH value	7.29 (0.17; 7.21–7.35)	7.40 (0.06; 7.36–7.41)	< 0.0001
Glucose, mg/dL	77 (58; 71–108)	125 (51; 115–141)	0.0001
Protein, g/dL	4.2 (1.7; 3.6–4.8)	4.1 (1.2; 3.5–4.3)	0.532
LDH, IU/dL	498 (1360; 300–944)	266 (215; 156–320)	0.004
PAI-1, ng/mL	111.2 (75.6; 101.5–115.0)	61.5 (78.5; 30.6–90.2)	< 0.0001
tPA, ng/mL	16.1 (17;5; 9.9–20.5)	17.6 (14.2; 13.5–20.7)	0.714
TNF-α, pg/mL	87.0 (134.9; 30.8–120.3)	15.0 (7.1; 12.9–18.8)	< 0.0001
IL-1β, pg/mL	13.8 (48.5; 10.1–33.5)	2.9 (2.2; 2.3–4.3)	< 0.0001
TGF-β1, pg/mL	10,952.9 (9,480.1; 8,322.3–13,453.0)	6,117.3 (5,946.9; 4,002.6–7,173.3)	0.0001

*Values given as median (IQR; 95% confidence interval), unless otherwise noted.

19 patients (26.3%) with TB pleurisy. The values of effusion TNF-α, IL-1β, PAI-1, and especially TGF-β1 were significantly higher, and the levels of tPA were significantly lower in TB pleurisy patients with RPT.

Fluid loculation is a common clinical problem and a poor prognostic factor in patients with exudative pleural effusions.[1–7] In an animal model of acute pleural injury, lower pH and glucose levels in effusions were associated with the formation of pleural adhesion.[26] Himelman and Callen[2] reported significantly lower values of pH and glucose, higher levels of LDH, and larger effusion size in patients with loculated effusions compared with those with nonloculated effusions. These findings suggest that pleural inflammation may play an important role in the development of loculation of exudative pleural effusions.

Fibrin deposition is a hallmark of pleural inflammation, and the turnover of pleural fibrin was regulated by fibrinolytic activity.[13] In *in vitro* studies,[13,16] TNF-α and IL-1β were shown to have an effect on the release of PAI-1 by human mesothelial cells, and a synergetic effect exerted by these two cytokines was observed. Furthermore, the levels of TNF-α were reported to be significantly higher in TB effusions than in malignant and transudative effusions,[15,27–29] and they correlated positively with those of PAI-1.[15] Taken together, these studies indicate that pleural inflammation may reduce fibrinolytic

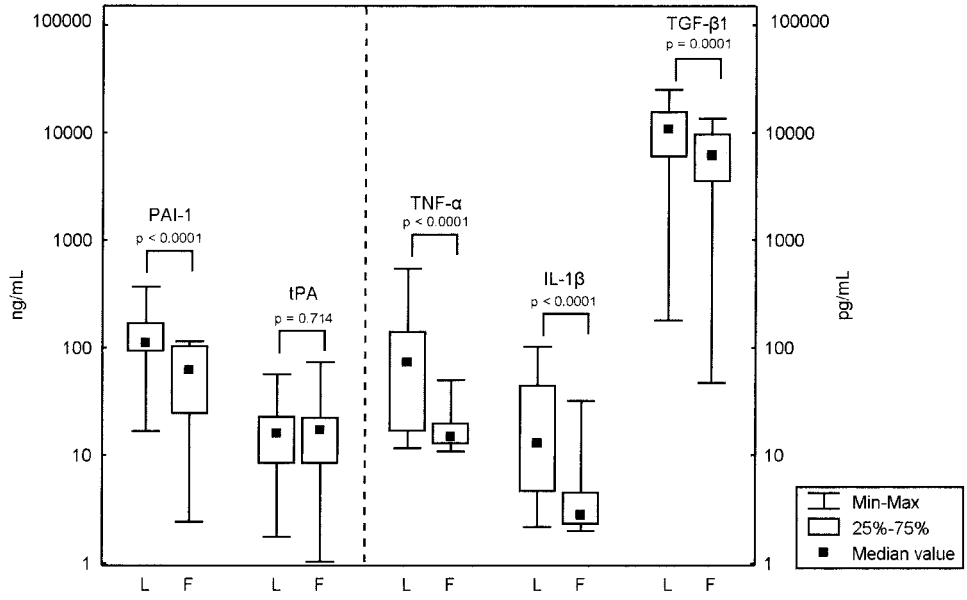

FIGURE 1. Box-and-whisker plots for the effusion levels of cytokines and fibrinolytic enzymes in loculated and free-flowing effusions. The box plots are based on median, quartile, and extreme values. The box represents the IQR that contains 50% of values. The whiskers are lines that extend from the box to the highest and lowest values. L = loculated group (n = 42); F = free-flowing group (n = 36).

Table 3—*Correlation Among Proinflammatory Cytokines, TGF-β1, Fibrinolytic Enzymes, and LDH in Loculated and Free-Flowing Pleural Effusion*

Variables	tPA	PAI-1	PAI-1/tPA	TGF-β1
Loculated effusion (n = 42)				
TNF-α	− 0.37*	0.59‡	0.58‡	0.51‡
IL-1β	− 0.56‡	0.35*	0.64‡	0.52‡
TGF-β1	0.01	0.53‡	0.31*	
LDH	− 0.41†	0.35*	0.56‡	0.39*
Free-flowing effusion (n = 36)				
TNF-α	− 0.18	0.55‡	0.53‡	0.42*
IL-1β	− 0.10	0.47†	0.36*	0.49†
TGF-β1	0.08	0.58‡	0.33*	
LDH	− 0.14	− 0.03	0.09	0.13

*Correlation is statistically significant at the level of 0.05.
†Correlation is statistically significant at the level of 0.01.
‡Correlation is statistically significant at the level of 0.001.

activity in pleural spaces via the enhanced release of proinflammatory cytokines, such as TNF-α and IL-1β. However, the effects of these cytokines on loculation of pleural exudates have not, to our knowledge, been studied previously.

Pleural inflammation may induce the production of TGF-β, a potent fibrogenic cytokine, in the pleural cavity.[30] In exudative pleural effusions, the level of LDH was highly correlated with that of TGF-β.[21] The proinflammatory cytokine IL-1β has been shown to stimulate the expression of TGF-β[31] and is thought to be involved in fibrin deposition of pleural effusion, because TGF-β could stimulate the secretion of PAI-1 by human pleural mesothelial cells.[13] These findings indicate that pleural inflammation may induce the local release of TGF-β and proinflammatory cytokines and may subsequently enhance the release of PAI-1. The imbalance of PAI-1 and tPA may lead to formation and deposition of fibrin in pleural spaces and loculation of pleural effusions.

In this study, we have demonstrated that the values of pH and glucose were significantly lower, and the levels of LDH were significantly higher in loculated effusions than in free-flowing effusions caused by malignancy, TB, and pneumonia (Tables 1, 2). Loculated effusions had significantly higher levels of TNF-α, IL-1β, TGF-β1, and PAI-1 than did free-flowing effusions (Tables 1, 2, and Fig 1). In addition, we observed that in both loculated and free-flowing effusions, the levels of TNF-α, IL-1β, and TGF-β1 were correlated positively with those of PAI-1 and the PAI-1/tPA ratio. However, the levels of tPA correlated negatively with those of TNF-α and IL-1β in loculated effusions (Table 3). Furthermore, the effusion levels of TGF-β1 correlated positively with those of TNF-α, IL-1β, and LDH in loculated effusions (Table 3). These results strongly suggest that the intensity of pleural inflammation was greater in loculated effusions than in free-flowing effusions, which may subsequently reduce fibrinolytic activity via TNF-α, IL-1β, and TGF-β1, leading to the formation and deposition of fibrin in pleural spaces and subsequent fluid loculation.

We also examined the differences in levels of LDH, cytokines, and fibrinolytic enzymes between primary and secondary loculated effusions (Table 4). The results indicated that the levels of LDH, PAI-1, TNF-α, IL-1β, and TGF-β1 were significantly higher in secondary effusions than in primary loculated effusions. These findings additionally supported the concept that a higher intensity of pleural inflammation may enhance the release of proinflammatory and fibrogenic cytokines with a resultant increase in PAI-1. The imbalance in PAI-1 and tPA may lead to the formation and deposition of fibrin, and the subsequent fluid loculation and septation of pleural effusions.

In patients with TB pleurisy, RPT occurred in 5 of 19 patients (26.3%) after the completion of anti-TB chemotherapy. Although RPT occurred exclusively in patients with loculated effusion, there was no

Table 4—*Pleural Fluid Characteristics and Effusion Levels of Cytokines and Fibrinolytic Enzymes Between Primary and Secondary Loculated Pleural Effusions**

Effusion Variables	Primary Effusion (n = 15)	Secondary Effusion (n = 27)	p Value
pH value	7.30 (0.16; 7.12–7.36)	7.27 (0.20; 7.18–7.30)	0.619
Glucose, mg/dL	105 (76; 74–167)	73 (53; 55–109)	0.014
Protein, g/dL	3.9 (1.5; 3.3–5.0)	4.2 (1.8; 3.7–5.0)	0.977
LDH, IU/dL	300 (258; 155–462)	724 (1631; 328–1544)	0.047
PAI-1, ng/mL	94.1 (44.9; 23.0–101.5)	114.8 (103.8; 108.4–168.0)	0.019
tPA, ng/mL	15.9 (16.0; 8.7–25.9)	16.8 (16.4; 8.3–21.0)	0.918
TNF-α, pg/mL	14.2 (48.4; 12.4–61.0)	119.2 (144.9; 40.5–138.8)	0.001
IL-1β, pg/mL	3.4 (7.2; 2.4–9.6)	33.3 (75.3; 13.8–53.1)	< 0.001
TGF-β1, pg/mL	7,746.0 (8,823.0; 4,328.6–13,288.0)	13,152.7 (7,894.7; 8,827.0–13,692.2)	0.041

*Values given as median (IQR; 95% confidence interval), unless otherwise noted.

Table 5—*Effusion Levels of Cytokines and Fibrinolytic Enzymes in Tuberculous Pleurisy Patients With or Without Development of RPT

Effusion Variables	RPT (+) [n = 5]	RPT (−) [n = 14]	p Value
PAI-1, ng/mL	199.0 (99.0; 103.0–234.0)	113.7(16.2; 90.2–118.1)	0.045
tPA, ng/mL	8.4 (7.6; 1.8–20.5)	20.9 (19.2; 8.3–28.7)	0.033
TNF-α, pg/mL	120.3 (28.2; 40.1–332.0)	49.3 (80.8; 14.5–95.8)	0.021
IL-1β, pg/mL	33.2 (8.4; 5.1–38.2)	4.5 (14.4; 2.5–17.2)	0.016
TGF-β1, pg/mL	16,868.5 (1,963.4; 13,242.8–19,059.6)	11,021.9 (5,020.0; 6,493.2–13,453.0)	0.004

*Values of median (IQR; 95% confidence interval) are given unless otherwise noted. RPT (+) = with residual pleural thickening; RPT (-) = without residual pleural thickening.

significant difference in the incidence of RPT between the loculated and free-flow groups. Previous studies[15,25] have indicated that higher levels of TNF-α, IL-1β, and PAI-1 in effusions and lower values of tPA in effusions were important indicators for the development of RPT in patients with TB pleurisy. In agreement with previous studies, we found that the effusion levels of TNF-α, IL-1β, and PAI-1 were significantly higher, and the effusion values of tPA were significantly lower in TB pleurisy patients with RPT (Table 5). In addition, the pleural levels of TGF-β1 were significantly higher in those with RPT. To our knowledge, the relation between effusion TGF-β and the development of pleural thickening in TB pleurisy has never been investigated. Our results suggest that TGF-β may play a role in the development of pleural fibrosis in TB pleurisy.

What are the clinical implications of the present study? It is believed that the loculation of pleural effusion or fibrin deposition in pleural effusion is a hallmark of pleural inflammation. The presence of fibrin septation within loculated effusions suggests that an increase of pleural inflammation and a decrease of fibrinolytic activity occur in pleural effusions. As a consequence, more aggressive therapeutic modalities, like early intrapleural administration of fibrinolytic agents or early thoracentesis, may be indicated in patients with loculated and fibrinous parapneumonic effusions or TB pleurisy to facilitate pleural fluid drainage and to prevent subsequent pleural adhesion/fibrothorax or RPT. By contrast, as suggested in our previous study,[7] the presence of fibrin or loculation of pleural effusion may be of considerable value in predicting the success of the subsequent pleurodesis if needed in patients with malignant pleural effusions.

There are some limitations of the present study. The power of the test in subgroup comparison was < 0.80, except for PAI-1 and TNF-α in malignant effusions and TGF-β1 in TB pleurisy (Table 1). The power of the test will increase up to ≥ 0.80 to detect a significant difference if we double the case number in each subgroup. However, the power of the test was > 0.80 to detect a significant difference when all of the patients were considered and divided into loculated and free-flow groups irrespective of the underlying entities (Table 2). Additional studies with larger population are needed to verify these issues.

In conclusion, compared with free-flowing pleural effusions, pleural inflammation was increased in loculated pleural effusions. A higher intensity of pleural inflammation in loculated effusions may enhance the release of TNF-α, IL-1β, and TGF-β1, which may subsequently increase the release of PAI-1 in the pleural cavity. The imbalance of PAI-1 and tPA in pleural spaces may lead to the formation and deposition of fibrin, which may subsequently result in fluid loculation and the septation of pleural effusions or in the development of pleural thickening in patients with TB pleurisy.

ACKNOWLEDGMENT: We thank Dr. Benjamin Ing-Tiau Kuo of the Laboratory of Epidemiology and Biostatistics, Department of Medical Research and Education, Taipei Veterans General Hospital, for statistical help.

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Proinflammatory Cytokines, Transforming Growth Factor-β1, and Fibrinolytic Enzymes in Loculated and Free-Flowing Pleural Exudates *

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Chest 2005;128; 690-697
DOI 10.1378/chest.128.2.690