Pleural SC5b-9 in Differential Diagnosis of Tuberculous, Malignant, and Other Effusions*

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A monoclonal antibody against soluble phase-terminal complement complex (SC5b-9) was used to try to differentiate pleural effusions of tuberculous vs malignant and other origin. Effusions of tuberculous origin showed a significantly higher SC5b-9 level than did plasma, suggesting activation of complement in the pleural space. All 26 patients with tuberculous effusions showed SC5b-9 levels in pleural fluid exceeding 2.0 mg/L, while 20 with malignant effusions had levels less than 2.0 mg/L. However, rheumatoid, some parapneumonic, and treated malignant effusions showed SC5b-9 levels above 2.0 mg/L. Considering a value exceeding 2.0 mg/L, the specificity and sensitivity of the SC5b-9 estimation in tuberculosi were 0.74 and 1.0, respectively. The mean values for C4d and Bb fragments of complement were significantly (p<0.05) higher in the tuberculous than in the malignant effusions. However, the values for Bb in 16 (62 percent) of the 26 patients with tuberculous or malignant effusions were in the same range. The activity of adenosine deaminase (ADA) was higher in the tuberculous than in the malignant effusions. While 18 of 26 patients with tuberculous effusions showed an ADA value exceeding 50 mU/mL, the estimated cutoff point (sensitivity = 0.69), 35 of the 36 nontuberculous effusions showed a true negative value (specificity = 0.97). A correlation between ADA and SC5b-9 values was observed in pleural effusions. These observations suggest that the estimation of SC5b-9 in pleural fluid presents a new approach to differentiating tuberculous vs malignant effusions. (Chest 1992; 102:1060-64)

PLEURAL EFFUSIONS

Pleural effusion may complicate the course of patients with a variety of local and systemic diseases. The effusion can be classified as exudative or transudative based on the elevated activity of lactate dehydrogenase (LDH) and on increase in protein content. Exudative pleural effusions may occur in patients with tuberculous, malignant, pneumonic, and other forms of pleurisy.1

Tuberculous, malignant, and bacterial pleurisy may be differentiated cytologically. Mononuclear cells usually predominate in tuberculous and malignant effusions while neutrophilic leukocytes are the major finding in bacterial pleurisy. It is difficult, however, to distinguish a case of tuberculous from malignant pleurisy by cytologic examination alone.2 Thus, a biochemical test would be useful in making the diagnosis. Adenosine deaminase activity (ADA), which is believed to be released from activated T lymphocytes,3 has been reported useful in differentiating tuberculous from other effusions.4,5 However, the test for ADA is marred by false-positive and false-negative results.6,7 A new biochemical marker was therefore sought.

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Previously reported immunologic findings in pleural effusions include a lower level of IgG in malignant vs nonmalignant effusions;8 and an increased consumption of complement in tuberculous effusions.9 However, none has been found practical for routine diagnostic use. SC5b-9 may be produced by the binding of S protein to nascent C5b-9 complexes at the C5b-7 stage of assembly in consequence of complement activation.10 We report the results of estimating SC5b-9, by means of a simple, rapid enzyme immunoassay (EIA) to differentiate tuberculous from malignant effusions.

MATERIALS AND METHODS

Patient Selection

A total of 64 patients with pleural effusions of tuberculous, malignant, parapneumonic, and immunologic origin were seen at four hospitals over a 12-month period: the Kyushu University, the Harada, the Sanshinkai-Hara, and the Shojuen Hospital. There were 34 men and 30 women aged 19 to 85 years (mean age, 60 years). Following their complete clinical and laboratory assessment, the patients were divided into five groups: (1) tuberculous (26 patients); (2) malignant untreated ("simple" effusions, 20 patients); (3) malignant effusions from treated patients ("treated" effusions, six patients); (4) parapneumonic effusions (ten patients); and (5) effusions from rheumatoid arthritis (two patients). Malignant effusions were diagnosed by the histologic demonstration of tumor cells in the effusion and/or on pleural biopsy specimens, while tuberculous effusions were diagnosed by the demonstration of mycobacteria in the effusion or biopsy specimen microscopically and/or by culture. Also considered to be compatible with tuberculous effusions was the presence of a characteristic granulomatous lesion on the biopsy specimen. Parapneumonic effusions were diagnosed on the basis of

ADA = adenosine deaminase activity; EIA = enzyme immunoassay.
than 0.6; more than 200 IU of LDH present in the pleural fluid. When the amount of SC5b-9 in pleural fluid and in plasma was estimated in a patient with an effusion of tuberculous or malignant cause, the level of SC5b-9 was consistently higher in the pleural fluid than in plasma (Fig 1).

**Estimation of SC5b-9 in Pleural Fluids with Various Cause**

All the effusions obtained from 26 patients with tuberculous origin showed a value above 2.0 mg/L, while all malignant effusions from untreated patients (simple malignant effusion, n = 20) had a value less than 2.0 mg/L (Fig 2). Routine cytologic examinations revealed that 83 percent of the tuberculous and 80 percent of the malignant effusions showed a predominance of lymphocytes, while the effusions of the remaining groups showed higher frequency of neutrophilic leukocytes. We did not find a significant relationship between cell differentiation and SC5b-9 values.

In contrast, five malignant effusions from six treated patients ("treated" malignant effusions) showed a

**RESULTS**

**Comparison of Levels of SC5b-9 in Plasma and Pleural Fluid**

All pleural effusions in the 64 patients examined were of an exudative nature according to one of three criteria: a ratio of pleural fluid/blood protein greater than 0.5; a ratio of pleural fluid/serum LDH greater than 0.6; more than 200 IU of LDH present in the pleural fluid. When the amount of SC5b-9 in pleural fluid and in plasma was estimated in a patient with an effusion of tuberculous or malignant cause, the level of SC5b-9 was consistently higher in the pleural fluid than in plasma (Fig 1).

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In contrast, five malignant effusions from six treated patients ("treated" malignant effusions) showed a
higher value for SC5b-9: the six specimens were obtained within one month of operation in two patients with lung cancer, and in three of four patients who were irradiated on the same side of the thorax. Three of ten patients with parapneumonic effusions showed an SC5b-9 value exceeding 2.0 mg/L. In addition, exudative effusions from two patients with rheumatoid arthritis showed an SC5b-9 value of 9.3 mg/L and 10.6 mg/L, respectively (not shown in Fig 2).

Estimation of C4d and Bb in Pleural Effusions

C4d, a marker for complement activation via the classic pathway, and Bb, that via the alternative pathway, were estimated in pleural effusions using respective EIA s. As shown in Figure 3A, the mean value for C4d (7.7 mg/ml) in the tuberculous effusions was significantly (p<0.05) higher than that (5.5 mg/ml) in the simple malignant effusions. Similarly, the mean value for Bb (6.1 mg/ml) in the former was significantly (p<0.01) higher than that (2.1 mg/ml) in the latter (Fig 3B). A total of 18 (69 percent) of 26 patients with tuberculous effusions exhibited the same range of Bb values as did those with simple malignant effusions.

Relationship between SC5b-9 and ADA

ADA is reported to be a relatively specific marker for tuberculous pleural effusions.2 Accordingly, the values of SC5b-9 were compared with those of ADA in patients with tuberculous, malignant, and parapneumonic pleural effusions. As shown in Figure 4, those with tuberculous effusions exhibited a significantly (p<0.01) higher ADA than those with malignant effusions. None of the latter showed an ADA value above 50 mg/L. While 18 of 26 patients with tuberculous effusions showed an ADA level above 50 mg/L (sensitivity = 0.69), the remaining eight had a value less than 50 (Table 1).

To investigate the relationship between ADA and...
SC5b-9 value, we plotted these two variables with respect to each other; shown in Figure 5, the SC5b-9 value correlated with ADA ($r = 0.441$, $p < 0.01$).

**DISCUSSION**

Estimation of SC5b-9 in the plasma and the pleural fluid of patients with pleurisy of tuberculous or malignant origin showed a consistently higher concentration in the latter than in the former. This observation suggests that SC5b-9 in pleural fluid is generated by the activation of complement in the pleural space, not leaked from the peripheral blood. This appears to be compatible with the physicochemical characteristics of SC5b-9, a large molecular complex (> one million dalton).10

Patients with tuberculous effusions consistently showed a high level of SC5b-9 in contrast to those with malignant effusions. All “simple” malignant effusions, ie, those obtained without manipulation or local treatment to the pleura before thoracentesis, exhibited an SC5b-9 level lower than 2.0 mg/L. In contrast, five of the six treated malignant effusions showed levels of SC5b-9 exceeding those with simple effusions. It is suggested that the high level of SC5b-9 in such treated effusions may be attributed to the activation of complement due to the operational manipulation or irradiation of the pleural space. In addition, the results that three of ten parapneumonic and both rheumatoid effusions exceeded the value of 2.0 mg/L may suggest a limitation to the specificity of elevated SC5b-9 for tuberculous pleurisy.

Considering the other indicators for complement activation, we found the C4d and Bb values to be significantly higher in the tuberculous than in the malignant effusions, as had previously been reported by Lew et al.9 However, there was a significant overlap of the values for these parameters between the two diagnostic groups, suggesting that their estimation is not useful for differentiating these effusions. Such poor discrimination of tuberculous vs malignant effusions by the initial complement breakdown products as compared with the terminal complement complex may be explained by the greater stability of SC5b-9 in biologic fluids as compared with C4d and Bb.11

Values of SC5b-9 in pleural effusions correlated with ADA despite possibly different mechanisms responsible for their elevation. While ADA is produced by activated T lymphocytes,2,3,12 SC5b-9 is generated by the activation of complement via the classic or alternative pathways.10 Since the role of cell-mediated immunity in tuberculous pleuritis is well known, this study supplies evidence for the activation of complement in association with the activated cellular immunity in this disease.

In conclusion, our observations suggest that the level of SC5b-9 in pleural effusions depends on the

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**Figure 5.** Relationship between SC5b-9 and ADA in 62 patients with tuberculous, malignant, and parapneumonic pleural effusions.
activation of complement in the pleural space; it is thus a different biochemical marker from ADA. Considering that the SC5b-9 test showed a high sensitivity while the ADA test showed a high specificity, the simultaneous estimation of these two parameters may be recommended for the differential diagnosis of tuberculous effusions.

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