Elevated Levels of Soluble Interleukin-2 Receptors in Tuberculous Pleural Effusions*

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The levels of soluble IL-2R were measured in pleural fluid from patients with tuberculous pleurisy. There were significantly elevated soluble IL-2R values in tuberculous pleural fluid as compared with pleural fluid of nontuberculous etiology including malignant, bacterial and transudative pleural effusions. In patients with tuberculous pleurisy, the level of soluble IL-2R in pleural fluid was markedly greater than that in serum. Furthermore, a significant positive correlation was observed between soluble IL-2R levels and adenosine deaminase levels in tuberculous pleural fluid. These findings suggest that elevated levels of pleural fluid soluble IL-2R in tuberculous pleurisy could reflect the local proliferation of activated T-cells and may be clinically useful in the diagnostic procedures for patients with pleural tuberculosis. (Chest 1990; 97:1141-43)

IL-2R = interleukin-2 receptor; ADA = adenosine deaminase activity

Since increased lymphocytes in tuberculous pleural effusions are predominantly T-lymphocytes and are highly responsive to PPD in vitro,1,2 T-cell dependent cell-mediated immunity is thought to be involved in the pathogenesis of tuberculous pleurisy.3-4 Interleukin-2 is a lymphokine which is essential to the proliferation of antigen-stimulated T-cells. The receptor for IL-2 has been identified on the surface of various cells, including activated T-cells.5 In addition, it is known that IL-2R can be released from the cell surface in a soluble form.6 Elevated serum levels of soluble IL-2R increasingly have been found in patients with adult T-cell leukemia, Hodgkin's disease, Sezary syndrome, acute and chronic lymphocytic leukemia and acquired immunodeficiency syndrome.7 In the present study, therefore, we aimed to determine if pleural fluid levels of soluble IL-2R were elevated in patients with tuberculous pleurisy, compared with patients with pleural effusions of nontuberculous etiology.

**MATERIALS AND METHODS**

Pleural fluid and peripheral blood samples were obtained at the time of closed pleural biopsy or thoracentesis in ten patients (eight men and two women) with newly diagnosed tuberculous pleurisy. The patients ranged in age from 19 to 77 years; the mean age was 49 years. All patients had a positive intradermal reaction to PPD and showed unilateral, exudative pleural effusions with a predominance of lymphocytes. A diagnosis of tuberculous pleurisy was made in six patients by the finding of typical epithelioid cell granulomas on pleural biopsy specimens in conjunction with negative clinical and laboratory data for other causes of granuloma formation.8-10 In the remaining four individuals, Mycobacterium tuberculosis was cultured from pleural fluid. All patients responded well to specific antituberculosis chemotherapy. None of the patients had evidence of malnutrition or underlying diseases.

Pleural fluid and serum also were obtained from ten patients (six men and four women) with bacterial exudates, ten patients (nine men and one woman) with primary lung cancer (five adenocarcinomas, three small cell carcinomas, two squamous cell carcinomas), and eight patients (five men and three women) with transudative effusions (seven congestive heart failures, one nephrotic syndrome). The mean ages of the three nontuberculous pleurisy patient groups were 60 years (range: 38 to 75 years), 77 years (range: 66 to 86 years) and 61 years (range: 44 to 81 years), respectively. Of the ten patients with bacterial exudates, seven had complicated and three had uncomplicated parapneumonic effusions.9 The seven patients with complicated parapneumonic effusions had purulent pleural fluid (empyema). The cultures were positive for anaerobes in six cases and for anaerobes plus Pseudomonas aeruginosa in one case. Three patients were classified as having uncomplicated parapneumonic effusions because the pleural fluid was an exudate with a predominance of polymorphonuclear leukocytes, a pH value ≥7.30, a glucose level >60 mg/dl and a LDH level <500 units/L and a negative Gram stain or culture.10 The diagnosis of malignant pleural effusions associated with lung cancer was established by finding malignant cells in pleural fluid or pleural tissue. Twenty normal volunteers (eight men and 12 women; mean age: 51 years; range: 24 to 79 years) were used as a normal control group for measurements of soluble IL-2R in serum samples.

Samples of pleural fluid and serum were centrifuged at 2,500 rpm for 10 min to remove cell pellets. Soluble IL-2R was measured with a sandwich enzyme immunoassay available as the CellFree Interleukin-2 Receptor Test Kit (T-cell Science, Inc, Cambridge, MA).11 In brief, a mouse monoclonal antibody to human IL-2R was first adsorbed onto a polystyrene microtiter well. A patient's pleural fluid supernatant, serum, or standard was added to the antibody-coated well. A second horseradish peroxidase-conjugated murine

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This study was supported in part by a grant from the Osaka Foundation for Promotion of Clinical Immunology.
Manuscript received October 28, 1988; revision accepted October 23, 1989.
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CHEST / 97 / 5 / MAY 1990 1141

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monoclonal antibody to human IL-2R was then used to bind a second epitope on the molecule captured by the first antibody. After washing, a substrate solution was added to the well. The reaction was terminated by addition of a stop solution and then the absorbance at 490 nm was measured. Levels of IL-2R are expressed in units per milliliter. Simultaneous determinations of soluble IL-2R in pleural fluid and serum were performed. The ADA also was measured with AD test Maruho (Maruho, Osaka, Japan) as previously described. 14

RESULTS

A comparison of tuberculous and nontuberculous pleural fluid samples for soluble IL-2R concentrations is depicted in Figure 1. The value for soluble IL-2R in tuberculous pleural fluid samples was 8,651 ± 2,779 (mean ± SD) units/ml as compared with 1,469 ± 490 units/ml, 2,528 ± 831 units/ml and 422 ± 209 units/ml for bacterial, malignant and transudative pleural fluid samples, respectively. The difference was shown to be highly significant by Student's t test. Both samples of bacterial and malignant pleural fluid also had greater values of soluble IL-2R than transudate pleural fluid samples. In patients with tuberculous pleurisy, the mean concentration of soluble IL-2R in pleural fluid was markedly higher than that in serum (1,704 ± 1,942 units/ml). The concentrations of soluble IL-2R in serum samples from nontuberculous patients were 672 ± 380 units/ml for patients with bacterial effusions, 765 ± 413 units/ml for patients with malignant effusions and 749 ± 367 units/ml for patients with transudative effusions. There were no significant differences in serum levels of soluble IL-2R between patients with tuberculous pleurisy and patients with nontuberculous pleural effusions. No significant differences were observed in serum levels of soluble IL-2R among the groups of patients with nontuberculous pleural effusions. All groups with pleural effusions had higher values of serum soluble IL-2R than the normal control group (261 ± 69 units/ml; p < 0.05).

Shown in Figure 2 are individual soluble IL-2R values plotted as a function of ADA levels for tuberculous pleural effusions. The resulting correlation coefficient was highly significant, indicating a strong positive relationship between these two parameters (linear regression coefficient: r = 0.7748, p < 0.01). However, there was no significant correlation between them in patients with nontuberculous pleural effusions (data not shown).

DISCUSSION

We detected strikingly elevated soluble IL-2R values in tuberculous pleural fluid samples as compared to bacterial, malignant and transudative pleural fluid samples, respectively. Furthermore, the levels of pleu-
ral soluble IL-2R in patients with tuberculous pleurisy were significantly increased as compared to serum values of the same subjects, other pleurisy patients and normal control subjects.

Recent studies have shown that T-cells, B-cells and monocytes/macrophages release soluble IL-2R following in vitro activation. However, the amount of soluble IL-2R produced by in vitro stimulated B-cells and monocytes/macrophages is trivial compared to T-cell production of soluble IL-2R. The levels of soluble IL-2R could be related to T-cell activation. Tuberculous pleural effusions also have been shown to contain great numbers of PPD-reactive T-cells. Thus, our results indicate that the elevated soluble IL-2R values in tuberculous pleural fluid could represent the accumulation and the proliferation of mycobacterial products-activated T-cells at sites of disease activity. However, the exact function of soluble IL-2R remains to be clarified.

In addition, we found that there was a positive correlation between soluble IL-2R and ADA levels in tuberculous pleural effusions. Previous reports have demonstrated that elevated levels of pleural ADA reflect the local proliferation of activated T-cells and may indeed be of use in the differential diagnosis of tuberculous and nontuberculous pleural effusions.

In summary, the present study suggests that soluble IL-2R concentrations are markedly elevated in tuberculous pleural effusions and that measurements of pleural soluble IL-2R may be clinically useful in the diagnostic procedures for patients with pleural tuberculous.

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