Antimycobacterial Antibodies in Pleural Effusions*

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We devised a dot blot assay to evaluate the IgG and IgA response to P32, a recently isolated antigen specific to mycobacteria. Pleural fluids and the corresponding sera were tested, obtained from five patients with pleural tuberculosis proven by direct examination and/or culture and from 14 patients with pleural effusions of other origins. We measured the total IgG and IgA levels in all samples and determined the anti-P32 titer after adjusting IgG and IgA respectively to the same levels in all samples. Those pleural fluids and sera from tuberculous patients contained a higher proportion of anti-P32 antibodies than samples obtained from nontuberculous control subjects; in those patients, the proportion of anti-P32 antibodies was generally higher in pleural effusion fluid than in serum. (Chest 1990; 97:88-90)

Pleural tuberculosis is often difficult to diagnose because it is a paucibacillary disease. Mycobacteria are rarely seen on direct examination of pleural fluid and pleural biopsy specimens and cultures are positive in less than 50 percent. The diagnosis is assessed by histologic examination of pleural biopsy samples which reveals granulomas.1 As in other limited tuberculous involvements, serum antimycobacterial antibody levels are frequently low.2

Recent reports demonstrated the accumulation of T-lymphocytes reacting to mycobacterial components in pleural fluids.3,4 We tested the reactivity of the IgA and the IgG antibodies present in both pleural effusion and the corresponding serum of tuberculous and nontuberculous patients. We used a dot blot assay5 and the recently purified mycobacterial specific P32 antigen, identified as the 85A component of the heterogenous 85 complex in the BCG reference system.6

Pleural and serum total IgG and IgA concentrations were adjusted to the same concentrations as we previously adjusted them for the study of antimycobacterial antibodies levels in cerebrospinal fluids.7

MATERIAL AND METHODS

Patients

Tuberculous Pleural Effusions (n = 5): Diagnosis was assessed in two cases by histologic examination of pleural biopsy specimens and confirmed by culture (d,e), in two others by culture of pleural fluid (b,c). In the last case, pleural fluid stained with auramin showed acid-fast bacilli and the diagnosis was confirmed by culture (this patient also had pulmonary cavitary lesions)(a).

Nontuberculous Pleural Effusions (n = 14): Three patients presented with a pleural transudation associated with a cardiac failure, nine with a neoplastic pleural involvement, one with a pleural reaction secondary to a pulmonary embolism, and one to a pneumonia. Mycobacterial cultures were performed in all cases and remained negative.

Protein concentrations were determined using a protein assay kit and IgG and IgA concentrations were evaluated by immunonephelometry.

Preparation of P32: This purified 32 kilodalton protein antigen was prepared from zinc deficient Sauton culture filtrate of Mycobacterium bovis strain 1173P2 as described previously.8

Dot Blot assay:4 We performed this assay for the serodiagnosis of tuberculous disease. It gives the same results as the better known ELISA. A reflectance densitometer is used to quantify the peroxidase stain on the immunoblots. The titer of each sample was defined as the reciprocal of the highest dilution giving a reflectance measurement higher than a predetermined limit (0.2 reflectance unit).

RESULTS

Total proteins, IgG and IgA concentrations are shown in Table 1. Each pleural fluid or serum sample was further diluted to the same IgG (0.5 g/L) or IgA (1 g/L) concentration before assaying specific antimycobacterial reactivities.

Results are shown in Figure 1. Pleural fluids and sera of tuberculous patients contained a higher proportion of anti-P32 antibodies than in nontuberculous patients. In tuberculous patients, the proportion

Table 1—Total IgG and IgA Concentrations (g/L) in Serum (S) and Pleural Fluid (P) (Mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>IgG</th>
<th>IgA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S</td>
<td>P</td>
</tr>
<tr>
<td>Tuberculous</td>
<td>12.5±5.3</td>
<td>7.2±2.4</td>
</tr>
<tr>
<td>patients (n = 5)</td>
<td>4.2±2.1</td>
<td>3.1±1.3</td>
</tr>
<tr>
<td>Nontuberculous</td>
<td>9.3±2.2</td>
<td>5.5±2.6</td>
</tr>
<tr>
<td>patients (n = 14)</td>
<td>3.5±1.6</td>
<td>2.3±1.1</td>
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</tbody>
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specific antibodies IgA or IgG were higher in pleural effusion than in serum in four of five cases leading to a better discrimination of this pathologic condition.

**DISCUSSION**

The optical density method is commonly used to express ELISA data in the field of mycobacterial disease, we used a similar technique (reflectance densitometry) to express immunoblotting data.

In this work, we tried to demonstrate the interest in adjusting immunoglobulin concentrations before testing the samples. Moreover, we think that titration of specific antibodies allows better evaluation of the response than comparisons of results obtained using a single dilution of serum samples.

Anti-P32 IgG and IgA were found in the serum of the nontuberculous patients, but the titers measured were lower than those observed in tuberculous patients. The higher titer was noted in the patient who presented with severe associated pulmonary involvement.

We previously reported an anti-P32 serum humoral response in IgG and IgA classes which enabled us to discriminate between tuberculous patients and nontuberculous subjects. The higher levels were measured in patients with severe disease. As in all previous studies concerning serologic studies in mycobacterial infections, specific antibodies were detected in uninfected subjects' serum, but levels were lower than those measured using the PPD as antigen suggesting that the P32 was more specific of mycobacteria.

In fact, P32, the 85A component of the heterogenous 85 complex in the BCG reference system, also elicits a detectable cellular response in the infected patients. The P32 seems to be restricted to mycobacterial species and more precisely to *Mycobacterium tuberculosis*, *Mycobacterium bovis*, *Mycobacterium kansasii*, and *Mycobacterium avium*.

Only a few studies have been performed until now in the field of local humoral antimycobacterial immunity in tuberculous diseases. The determination of the presence of specific antibodies has been essentially used in an attempt to establish the diagnosis of tuberculous meningitis. We demonstrated recently the interest of comparing local and systemic humoral response in order to diagnose such a mycobacterial meningeal involvement.

Fujiwara and Tsuyuguchi and Rossi et al. studied the reactivity of pleural cells and demonstrated the local accumulation of specific T-lymphocytes suggesting a compartmentalization of the pleural spaces. Only Shim et al. studied the pleural antibody reactivity without comparing serologic and local humoral responses.

In our study, total serum immunoglobulin levels varied from one patient to another and were higher in tuberculous patients than in the others. More important individual variations were observed at the local

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**FIGURE 1.** Antimycobacterial IgG and IgA titers in sera (S) and respective pleural fluids (PL) after adjusting the total IgG concentrations to 0.5 g/L and IgA to 1 g/L. Small closed circles are nontuberculous patients; large closed circles are tuberculous patients.
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