PHA and PPD Reactivity of Lymphocytes in Pleural Effusions*

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The functional properties of lymphocytes in pleural fluid were studied in 23 patients admitted to the hospital for the diagnostic evaluation of a unilateral or bilateral pleural effusion. The in vitro reactivities of the patients' pleural fluid lymphocytes and peripheral blood lymphocytes to phytohemagglutinin (PHA) and to purified protein derivative (PPD) were compared. In most patients with pleural effusion, the function of pleural fluid lymphocytes was intact and comparable to that of lymphocytes from peripheral blood. The PHA- and PPD-stimulated ³H-thymidine incorporation by peripheral blood lymphocytes did not differ significantly from that by pleural fluid lymphocytes in any of the patient groups. Similarly, the comparison of PHA- and PPD-stimulated ³H-thymidine uptake by peripheral blood or pleural fluid lymphocytes from patients with pleural effusions of various causes revealed no significant differences. In vitro reactivity to PPD by peripheral blood and pleural fluid lymphocytes correlated positively with the in vivo intradermal reactivity to PPD.

Characterization of lymphocytes by their surface markers has shown that the number of T lymphocytes is significantly higher in pleural fluid than in peripheral blood in patients with tuberculous pleurisy and that the number of B lymphocytes is significantly lower in pleural fluid than in peripheral blood in patients with tuberculous, malignant, or nonspecific pleural effusion.¹ A ratio of pleural fluid to peripheral blood T lymphocytes greater than one suggests that the pleural effusion is due to malignancy, tuberculosis, or to a connective tissue disease.² Moreover, T lymphocytes have been reported to be significantly more numerous in a variety of human exudates than in peripheral blood.³

To learn more about the functional properties of the lymphocytes that migrate into pleural effusions, we studied the in vitro response of lymphocytes from the peripheral blood and pleural fluid of the same patient after culturing these cells in the presence of the nonspecific mitogen, phytohemagglutinin (PHA), and the specific antigen, purified protein derivative (PPD). We also compared the in vitro results obtained with PPD with the patient's in vivo intradermal reactivity to this antigen.

Materials and Methods

The series consisted of 23 adult patients admitted to the

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Department of Pulmonary Diseases, Mjölby Hospital, for diagnostic evaluation of a unilateral or bilateral pleural effusion. The final etiologic diagnosis was based on clinical, radiologic, and laboratory findings. Total and differential blood cell counts, total protein level, glucose level, antigen-nuclear factor, concentrations of complement components C3 and C4, as well as lysozyme activity, were determined in the pleural fluid of all patients. Rheumatoid factor was sought in all pleural fluids by the Waaler-Rose and latex fixation tests. All pleural fluids were also studied cytologically, and samples were cultured to investigate the presence of Mycobacterium tuberculosis.

Based on the final diagnosis, the patients were divided into the following four groups:

1. Nine patients (mean age, 47 years; range, 23 to 70 years) had a tuberculous pleural effusion. In seven, the diagnosis was based on a positive pleural biopsy result or on a positive culture of M tuberculosis in pleural fluid or sputum, and in two it was based on clinical findings and a favorable response to specific antituberculosis therapy.

2. Four patients (mean age, 51 years; range, 24 to 73 years) had a malignant pleural effusion; two had metastatic breast carcinoma, and two had malignant mesothelioma.

3. Four patients (mean age, 46 years; range, 18 to 67 years) had a pleural effusion owing to a connective tissue disease. One had rheumatoid arthritis, and three had systemic lupus erythematosus (SLE) as defined according to the criteria of the American Rheumatism Association.

4. Six patients (mean age, 60 years; range, 24 to 80 years) had a nonspecific pleural effusion.

No patient had received antimicrobial, corticosteroid, or cytotoxic drugs before admission to the hospital.

Nineteen healthy laboratory personnel and patients treated for minor complaints (mean age, 35 years; range, 23 to 50 years) served as the control group for the in vitro assays performed on lymphocytes from peripheral blood.

Blood and pleural fluid samples were collected on the same day. Pleural fluid was obtained by intercostal needle puncture, after which a pleural biopsy was performed according to Abrams.⁴ Peripheral blood and pleural fluid, 20 ml each, were collected into sterile, pyrogen-free syringes (Juntan Terumo Co. Ltd., Tokyo), which contained 1,500

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units of heparin (Medica, Finland). All samples were processed within 24 hours of the puncture.

White blood cell counts were performed in a Coulter counter. Differential leukocyte counts were performed from blood and pleural fluid smears, in which 200 cells were counted.

The lymphocytes were separated by density gradient centrifugation on Ficoll-Hypaque according to Böyum, washed twice in phosphate-buffered saline solution (Orion Diagnostica, Helsinki, Finland), adjusted to 1.5 x 10⁶ cells per microtiter plate well (Sterlin, Teddington, Great Britain), and then cultured in 0.15 ml of RPMI-1640 (Orion Diagnostica) supplemented with L-glutamine, 2 percent heat-inactivated fetal bovine serum (Microbiological Associates, Walkersville, Md.), and 50 units/ml of penicillin and 50 µg/ml of streptomycin. Thereafter, optimal concentrations of either PHA, 10 µg per well (Phytohemagglutinin P, Difco Laboratories, Detroit) or PPD, 10 µg per well (Statens serum-institut, Copenhagen) were added. Cells exposed to PHA were cultured for three days, and those exposed to PPD for six days. The cultures were incubated at 37°C in an atmosphere of 2 percent CO₂ and 100 percent humidity. Twenty-four hours before the cells were harvested, 0.5 µCi of 3H-thymidine (methyl 3H-thymidine, specific activity 5 Ci/m mole; The Radiochemical Centre, Amersham, England) was added to each well.

The contents of the wells were passed through a microfilter with a semiautomatic harvester. The filters were then placed in vials containing scintillation fluid (liquifluor diluted with toluene to a volume of 1,000 ml + 100 ml of Protosol; New England Nuclear, Boston) and incorporated radioactivity was measured with a Wallac-LKB 8100 liquid scintillation counter. Reported radioactivity, expressed as counts per minute, is the mean radioactivity of triplicate cultures.

Each batch of cultured peripheral blood and pleural fluid lymphocytes contained cultures to which no mitogen had been added and in which spontaneous incorporation of 3H-thymidine was measured. Peripheral blood lymphocytes from healthy subjects were cultured in an identical manner, both with and without PHA and PPD, as a control.

Intradermal reactivity to 2 TU of PPD was evaluated in 22 patients. The test was regarded as positive when an induration with a diameter greater than 7 mm developed within 72 hours.

Statistical analyses were performed with the Student's t test.

RESULTS

In no patient group or culture period did the spontaneous incorporation of 3H-thymidine by peripheral blood lymphocytes differ significantly from that of pleural fluid lymphocytes (Table 1). Neither was there any significant difference between the spontaneous incorporation of 3H-thymidine by peripheral blood lymphocytes in the control subjects and the patients with pleural effusions.

Reactivity to PHA and PPD by peripheral blood lymphocytes from the patients with pleural effusion and the healthy control subjects showed no significant difference.

In most patients with pleural effusion, the reactivity of pleural fluid lymphocytes to PHA and

| Table 1—Spontaneous Incorporation of 3H-Thymidine After Culture Without Mitogen for 3 and for 6 Days* |
|--------------------------------------------------|--------------------------|--------------------------|--------------------------|
| Diagnosis                                         | No. of Subjects | Peripheral Blood | Pleural Fluid |
| Tuberculosis                                      | 3-day culture        | 423 ± 104            | 1,940 ± 1,080       |
|                                                  | 6-day culture        | 813 ± 288            | 1,790 ± 1,140       |
| Malignant disease                                | 3-day culture        | 410 ± 130            | 642 ± 100           |
|                                                  | 6-day culture        | 490 ± 214            | 1,050 ± 198         |
| Connective tissue disease                        | 3-day culture        | 419 ± 213            | 299 ± 57            |
|                                                  | 6-day culture        | 845 ± 437            | 1,020 ± 588         |
| Non-specific pleural effusion                    | 3-day culture        | 398 ± 122            | 768 ± 375           |
|                                                  | 6-day culture        | 389 ± 119            | 1,280 ± 515         |
| Healthy control subjects                         | 3-day culture        | 320 ± 54             | ...                 |
|                                                  | 6-day culture        | 450 ± 82             | ...                 |

*Results are presented as counts per minute ± SEM.
† Cultures from one patient omitted for technical reasons.

PPD was intact and comparable to the reactivity of peripheral blood lymphocytes to these same antigens. There was no significant difference between the PHA- or PPD-stimulated 3H-thymidine incorporation by peripheral blood lymphocytes and pleural fluid lymphocytes in any of the patient groups (Table 2). Comparison of PHA- and PPD-stimulated 3H-thymidine incorporation by peripheral blood lymphocytes to PHA and PPD (Table 2) showed no significant difference.

| Table 2—Incorporation of 3H-Thymidine After Culture for 3 Days in the Presence of Phytohemagglutinin (PHA) and After Culture for 6 Days in the Presence of Purified Protein Derivative (PPD)* |
|--------------------------------------------------|--------------------------|--------------------------|--------------------------|
| Diagnosis                                         | No. of Subjects | PHA-Stimulated Culture | PPD-Stimulated Culture |
| Tuberculosis                                      | Peripheral blood        | 33,000 ± 9,040          | 4,500 ± 1,380           |
|                                                  | Pleural fluid           | 31,700 ± 13,800         | 3,780 ± 1,500           |
| Malignant disease                                | Peripheral blood        | 20,700 ± 1,940          | 11,600 ± 7,740          |
|                                                  | Pleural fluid           | 23,600 ± 10,100         | 4,650 ± 2,880           |
| Connective tissue disease                        | Peripheral blood        | 13,900 ± 7,970          | 2,800 ± 1,940           |
|                                                  | Pleural fluid           | 10,200 ± 3,940          | 4,510 ± 2,190           |
| Non-specific pleural effusion                    | Peripheral blood        | 33,400 ± 9,200          | 1,790 ± 651†            |
|                                                  | Pleural fluid           | 17,400 ± 7,160          | 1,400 ± 609†            |
| Healthy control subjects                         | Peripheral blood        | 22,100 ± 3,500          | 3,040 ± 602             |

*Results are presented as net counts per minute (cpm; i.e., the cpm in the stimulated culture minus the cpm in the unstimulated culture) ± SEM.
†N = 5.

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al blood or pleural fluid lymphocytes from patients with pleural effusions owing to various causes revealed no significant difference.

Reactivity to PHA and PPD varied widely within each patient group. Surprisingly, there was almost no reactivity of pleural fluid lymphocytes to PPD in four of the nine patients with tuberculous pleurisy. At the time of the assays, these patients with hyporeactive pleural fluid lymphocytes had all had pleuritic symptoms for two weeks or less. The records of four of the five patients with tuberculous pleural effusion, whose pleural fluid lymphocytes reacted normally to PPD, showed that their pleuritic symptoms had persisted for six weeks or longer.

Figure 1 shows that the in vitro incorporation of 
3H-thymidine by PPD-stimulated peripheral blood and pleural fluid lymphocytes from the 22 patients studied correlated positively with the diameter of the induration provoked by the intradermal PPD test. The correlation, however, was not statistically significant.

**DISCUSSION**

Most pleural effusions in which lymphocytes predominate are due to tuberculosis or malignant disease. However, the results of one study (lacking patients with tuberculous pleurisy) showed that total and differential leukocyte counts in pleural fluid were not disease-specific and, hence, of no value in the differential diagnosis of the origin of the effusion.

Except in the most extreme forms of immuno-deficiency, the counting of T and B lymphocytes in the peripheral blood of patients with different diseases has not proved profitable in clinical practice. That T lymphocytes predominate in human exudates has been thought to reflect a local accumulation due to specific immunologic stimuli or the migration of recirculating T lymphocytes alone from the blood to these fluids.

In tuberculous pleurisy, T lymphocytes are significantly more numerous in pleural fluid than in peripheral blood. Moreover, in patients with cancer and SLE, the mean percentage of T lymphocytes is also significantly greater in pleural fluid than in peripheral blood.

Pleural fluid lymphocytes respond with blast transformation and DNA synthesis when stimulated by PHA or concanavalin A. In patients with tuberculous or malignant pleurisy, the lymphocytes in pleural fluid may be more active than those in peripheral blood. Recently, Schapira et al observed that the function of T lymphocytes in pleural fluid was normal in patients with metastatic malignant pulmonary neoplasms judged by their reactivity to PHA, to PPD, and in a mixed lymphocyte culture.

In this study, lymphocyte reactivity to stimulation by PHA and PPD showed that in most patients with pleural effusion the function of pleural fluid lymphocytes was intact and comparable to that of lymphocytes from peripheral blood. Whereas the number of T lymphocytes is increased in tuberculous pleural effusion, we observed no enhanced in vitro stimulation of these cells by PHA or PPD. In some patients with tuberculous pleurisy of short duration, the pleural fluid lymphocytes scarcely responded to PPD. This suggests that in tuberculous pleurisy the pleural fluid lymphocytes pass through various stages of in vivo activation, each stage possibly having distinct in vitro characteristics.

After density gradient centrifugation of pleural fluids, we have observed that the interphase con-
tains mostly lymphocytes, but also variable amounts of macrophages and mesothelial cells, and in the case of malignant effusions, often tumor cells (unpublished observations). Lymphocyte activity could be affected by the presence of the other cells or by mediators released by them. In our series, lymphocyte predominance (range 54.5 to 100 percent) occurred in 20 of the 23 pleural fluid samples. Studies concerning the macrophages in pleural fluid are presently being undertaken.

In conclusion, the results of this study show that assessment of peripheral blood and pleural fluid lymphocyte activation by PHA and by PPD yields no additional information to that obtained by conventional clinical and laboratory procedures used in the differential diagnosis of pleural effusions. For a more complete understanding of the role of lymphocytes in pleural effusions, further studies are needed on T lymphocyte subpopulations, on additional specific features of lymphocyte activation, and on the role of the macrophages in the effusion.

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