

Lymphocyte subsets in peripheral blood and pleural fluid

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ABSTRACT: We have examined the distribution of B and T lymphocytes, T-cells with helper/inducer (T4+) or suppressor/cytotoxic (T8+) phenotypes and a subset of cells with natural killer (NK) activity and positive for the Leu 7 (HNK-1) surface antigen in peripheral blood and in lymphocyte-rich pleural effusions of patients with tuberculosis or malignancies (mesothelioma and lung cancer with pleural metastasis). In individual patients, the percentages of T lymphocytes were uniformly higher in pleural effusions than in peripheral blood; however, lower percentages of B lymphocytes and cells positive for the Leu 7 antigen were present in pleural fluids. The analysis of T-cell subpopulations demonstrated a selective enrichment of T lymphocytes with helper/inducer phenotype in pleural effusions, while the percentages of T-cells with suppressor/cytotoxic phenotype were similar in pleural fluid and peripheral blood. These results indicate that in lymphocytic pleural effusions the main lymphoid cell population is represented by T lymphocytes with helper/inducer phenotype, regardless of whether the effusion is due to tuberculosis or malignancies such as mesothelioma or lung cancer.

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Lymphocytic pleurisy is commonly observed in tuberculous effusions and in approximately 30% of neoplastic effusions secondary to mesothelioma or pleural metastasis [1, 2]. Several groups have analysed pleural effusions of different aetiology, looking for humoral or cellular components of the immune reactions; these studies have been carried out mainly for diagnostic and/or prognostic purposes. In this regard KAY *et al.* [3] have reported reduced levels of IgG in neoplastic pleural effusions, in comparison to non-malignant exudates. Furthermore several authors [4, 5] have observed increased levels of immune complexes in neoplastic effusions.

As far as the distribution of T and B lymphocytes in pleural effusions is concerned, PETERSSON *et al.* [1], MOISAN *et al.* [6] and DOMAGALA *et al.* [7] have reported increased percentages of T lymphocytes in tuberculous or neoplastic lymphocytic pleural effusions, compared to the values observed in blood samples. More recently several authors [8-10] have examined T-cell subsets in pleural fluid of patients with a variety of pleural diseases (tuberculosis, malignancies, connective tissue diseases, congestive heart failure) and showed that T-cells express mainly the helper/inducer phenotype.

In this report, we have investigated the distribution of B and T lymphocytes, T-cell subsets with helper/inducer (T4+) or suppressor/cytotoxic (T8+) phenotypes [11], and lymphoid cells with natural killer activity and expressing the Leu 7 (or HNK-1) surface antigen [12] in peripheral blood and pleural fluid of groups of patients with lymphocyte-rich

pleural effusions of tuberculous or neoplastic (mesothelioma or lung carcinoma) aetiology. We aimed to characterize phenotypically the lymphocyte subsets in pleural fluid and to test the hypothesis that distinct antigenic stimulations might elicit immune responses mediated by different lymphocyte subpopulations at pleural level.

Materials and methods

Patients

We have examined twenty-two consecutive patients (fifteen males and seven females; age range 17-86 yr), who were admitted for pleural effusions of unknown aetiology. Diagnostic procedures included physico-chemical analysis of effusion samples, cytological examination of cells from pleural effusions or bronchial brushings and washings during bronchoscopy, histology of pleural or bronchial biopsy, skin tests for delayed hypersensitivity to Purified Protein Derivative (PPD) and bacteriological analysis.

The diagnosis of mesothelioma was confirmed in six patients (mean age 62 yr, range 52-86 yr) by histology of pleural biopsy; in three patients neoplastic cells were also detected by cytology of pleural effusion. The diagnosis of lung carcinoma (not small-cell) was based on histology of bronchial biopsy and cytology of pleural fluid cells in six patients and on cytology only in three additional patients. The mean age of these patients was 55 yr (range 35-75 yr). In the group of seven patients with tuberculous pleurisy (mean age 22 yr, range 17-28 yr), the diagnosis was

made by detection of *Mycobacterium tuberculosis* in pleural fluid cultures in four patients and by histology of pleural biopsy showing granulomatous inflammation with caseation necrosis in two patients; in an additional patient the diagnosis was based on a positive intradermal reaction to PPD and a favourable clinical response to specific antitubercular therapy.

Cell analysis of peripheral blood and pleural fluid samples

Differential counts of white blood cells and of pleural fluid cells were obtained by microscopical examination of cytological preparations stained with May-Grunwald-Giemsa. The effusions were considered as 'lymphocytic' when lymphocytes represented 60% or more of the cells in the cytological preparations.

Surface marker analysis was performed on mononuclear cell suspensions isolated from samples of venous blood (20 ml) and pleural effusion (30–50 ml) by centrifugation over a Ficoll-Hypaque density gradient [13]. T lymphocytes were identified by their ability to form rosettes with neuraminidase-treated sheep red blood cells as described by WEINER *et al.* [14]. B lymphocytes positive for surface immunoglobulins were detected by direct immunofluorescence using fluorescein-conjugated goat antibodies to human immunoglobulins (from Litton Bionetics Inc., Kensington, MD, USA). The percentages of T-cells expressing the T4 (helper/inducer phenotype) or T8 (suppressor/cytotoxic phenotype) antigens and of lymphoid cells with natural killer activity and positive for the Leu 7 (HNK-1) antigen were evaluated by indirect immunofluorescence. Briefly, the cells were incubated with 10 μ l of OKT4, OKT8 (from Ortho Diagnostics) and anti-Leu 7 (from Becton Dickinson) murine monoclonal antibodies; after 30 min incubation at 4°C and two washes with cold phosphate buffered saline (PBS)+0.2 NaNO₃, the cells were incubated with 10 μ l of fluorescein-conjugated goat antibodies to mouse immunoglobulins, washed twice with cold PBS, centrifuged onto glass slides by a Shandon Cytospin and examined with a Leitz Dialux 20 microscope equipped for epi-illumination fluorescence and phase contrast.

Statistical analysis

The Student's t-test was used to evaluate the statistical significance of the differences between the values obtained in the groups of patients.

Results

Differential counts of nucleated cells have demonstrated that the percentages of lymphocytes are significantly higher ($p < 0.001$) in pleural fluid than in peripheral blood of patients with tuberculous or neoplastic pleurisy (data not shown).

The percentages of T lymphocytes forming rosettes

with sheep red blood cells were significantly higher in the pleural fluid of patients with lung carcinoma ($p < 0.05$) and tuberculosis ($p < 0.001$) than in peripheral blood (table 1). Looking at the T-cell subsets, a significant increase of T-cells expressing the T4 antigen was observed in pleural effusions of patients with mesothelioma ($p < 0.05$), lung carcinoma ($p < 0.01$) and tuberculosis ($p < 0.001$) compared to the values in peripheral blood, whilst no difference was observed in the percentages of T-cells positive for the T8 antigen. Conversely, the percentages of lymphoid cells with natural killer activity and bearing the Leu 7 antigen and B lymphocytes were significantly lower in pleural fluid.

Finally in the group of patients with tuberculosis the percentages of Leu 7-positive cells in the peripheral blood were significantly lower than the values observed in patients with mesothelioma ($p < 0.001$) or lung cancer ($p < 0.05$).

Discussion

Our results confirm previous reports that indicated a relative increase of T-cells in pleural effusions of patients with tuberculosis or neoplastic diseases in comparison to the values observed in peripheral blood [1, 6, 7, 15, 16]. The analysis of T-cell subsets phenotypically distinguishable by murine monoclonal antibodies and with opposite immunoregulatory functions [11] have demonstrated that the increase of the helper/inducer T-cells bearing the T4 antigen accounts for the T lymphocyte enrichment in pleural fluid of the groups of patients examined. No significant difference was observed within the three groups of patients regarding the percentages of T lymphocytes and T-cell subsets. Our observations are in close agreement with recent reports from other authors. GINNS *et al.* [17] have reported similar data in sixteen patients with lung carcinoma; GUPTA [18] has also reported an increase in the percentage of T-cells bearing 'helper' phenotype (IgM-Fc receptor) in the pleural effusion of a patient with histiocytic lymphoma. More recently KOCHMAN *et al.* [8], GHOSH *et al.* [9] and BERGROTH *et al.* [10] have observed increased percentages of T lymphocytes with helper/inducer phenotype in pleural fluid *versus* peripheral blood in patients with a variety of diseases. These findings suggest that the immune reactivity of pleurae towards different antigenic stimulations (*i.e.* mycobacterial or neoplastic antigens) elicits a cellular response that recruits T-cells with helper/inducer phenotype regardless of the nature of the antigens. There is a possibility of an antigen-induced replication of T4+ cells at local level. However, BERGROTH *et al.* [10] have shown that in tuberculous pleural effusions only a fraction of pleural fluid lymphocytes is activated and that most of the mononuclear cells in pleural fluid are resting cells.

Our observations indicate that lower percentages of B lymphocytes and cells positive for the Leu 7 antigen are detected in pleural effusions, compared to

Table 1. - Distribution of lymphocyte subpopulations in patients with lymphocyte-rich pleural effusions

Aetiology of pleural effusion	Percentages of cells expressing surface markers (mean values±SD)									
	peripheral blood					pleural fluid				
	E rosette	T4 antigen	T8 antigen	Leu 7 antigen	surface immunoglobulins	E rosette	T4 antigen	T8 antigen	Leu 7 antigen	surface immunoglobulins
Mesothelioma n=6	49.4 ±24.3	24.7 ¹ ±10.0	16.8 ±8.6	30.7 ³ ±7.6	13.0 ² ±4.7	74.8 ±14.0	52.0 ¹ ±21.0	18.2 ±7.3	5.9 ³ ±3.0	3.7 ² ±1.7
Lung carcinoma n=9	67.4 ¹ ±11.3	38.6 ² ±13.9	26.2 ±8.9	20.4 ² ±9.0	10.5 ² ±3.4	80.4 ¹ ±8.1	60.6 ² ±8.9	20.1 ±7.6	5.5 ² ±3.3	3.1 ² ±1.7
Tuberculosis n=7	62.3 ³ ±8.3	35.7 ³ ±11.5	21.6 ±10.0	11.3 ³ ±1.4	14.7 ³ ±6.0	83.0 ³ ±8.2	59.9 ³ ±6.6	22.6 ±7.5	3.0 ³ ±1.7	2.8 ³ ±1.0

Statistical significance of the differences between the values observed in pleural fluid versus peripheral blood: ¹ p<0.05; ² p<0.01; ³ p<0.001. The percentages of Leu 7-positive cells in the peripheral blood of patients with tuberculosis are significantly lower than the values observed in the peripheral blood of patients with mesothelioma (p<0.001) and with lung carcinoma (p<0.05).

peripheral blood. This finding suggests that humoral immunity and natural killer activity do not play a relevant role in the development of pleural immune reactivity towards antigenic stimuli. Indeed, the observation of low percentages of cells positive for the Leu 7 antigen in pleural effusions agrees with the reduced cytotoxic activity observed by several authors in pleural or ascitic fluid [19, 20].

Patients with tuberculosis have presented lower percentages of circulating Leu 7-positive cells compared to patients with mesothelioma or lung cancer. As the percentage of cells bearing Leu 7 antigen increases with age [12], the different ages of the three groups of patients is the most likely explanation for the lower levels of Leu 7-positive cells found in patients with tuberculosis.

In summary, our results indicate that an enrichment of T lymphocytes with helper/inducer phenotype at pleural level is a common feature in patients with lymphocyte-rich pleural effusions, regardless of the aetiology (infectious versus neoplastic) of the pleurisy.

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RÉSUMÉ: Nous avons examiné la distribution des lymphocytes B et T, des cellules T à phénotypes stimulateur/déclencheur (T4+) ou suppresseur/cytotoxique (T8+) et d'une sous-série de cellules tueuses naturelles (NT) et positives pour l'antigène de surface Leu 7 (HNK-1) dans le sang périphéral et dans les effusions pleurales riches en lymphocytes chez les patients atteints de tuberculose ou de malignité (mésothéliome et carcinome pulmonaire avec métastase pleurale). Dans chaque cas particulier, les pourcentages de lymphocytes T étaient constamment plus élevés dans les effusions pleurales que dans le sang périphéral; en revanche, des pourcentages inférieurs de lymphocytes B et de cellules positives pour l'antigène Leu 7 ont été constatés dans le liquide pleural. L'analyse des sous-populations de cellules T a démontré un enrichissement sélectif des lymphocytes T à phénotype stimulateur/déclencheur dans les effusions pleurales, tandis que les pourcentages des cellules T à phénotype suppresseur/cytotoxique étaient similaires dans le liquide pleural et dans le sang périphéral. Ces résultats indiquent que dans les effusions lymphocytiques pleurales, la population principale de cellules lymphoïdes est composée de lymphocytes T à phénotype stimulateur/déclencheur, que l'effusion soit due ou non à la tuberculose ou à des malignités telles le mésothéliome ou le carcinome pulmonaire.