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Pulmonary sarcoidosis: excess of helper T lymphocytes and T cell subset imbalance at sites of disease activity

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ABSTRACT Different lymphocyte subpopulations have been evaluated in bronchoalveolar fluid and blood obtained from six patients with active and six with inactive pulmonary sarcoidosis and from six normal subjects by means of two recently described monoclonal antibodies, 5/9 and MLR4. The percentages of OKT4 positive (helper) and OKT8 positive (suppressor) T cells were also determined. Patients with active sarcoidosis had significantly higher proportions of 5/9 positive T cells in the bronchoalveolar fluid than patients with inactive disease ($p < 0{\cdot}01$) or normal subjects ($p < 0{\cdot}001$). In contrast, the proportions of 5/9 positive blood T cells were similar in the three groups studied. Patients with active sarcoidosis had also a greater proportion proportion of MLR4 positive T lymphocytes in bronchoalveolar fluid than patients with inactive disease or normal subjects ($p < 0{\cdot}01$ for each comparison), but similar proportions of MLR4 positive blood T cells were found in each group. The ratio of 5/9 positive to MLR4 positive T cells was higher in the bronchoalveolar fluid (but not in the blood) in patients with either active or inactive sarcoidosis than in normal subjects. These observations suggest that the MLR4 negative fraction rather than the MLR4 positive fraction of the 5/9 positive T cells is preferentially expanded in the lungs of patients with pulmonary sarcoidosis and may indicate a secondary role for the MLR4 positive T cells in producing lung injury in this disorder. Comparisons of the OKT4 positive and 5/9 positive T cells showed that in patients with active disease most of the lung T lymphocytes expressed both the OKT4 and the 5/9 surface antigens, so the 5/9 monoclonal antibody may be considered a good marker of activity in this disorder. Pulmonary sarcoidosis may be characterised by the preferential expansion of helper T cell subsets at sites of disease activity.

Sarcoidosis is a generalised disorder of unknown aetiology characterised by non-caseating granulomas in the affected organs[1–5] and by polyclonal hypergammaglobulinaemia.[6–13] Recent studies on pulmonary sarcoidosis have shown that in patients with active disease (high intensity alveolitis) both granuloma formation and immunoglobulin production appear to be modulated by activated T lymphocytes.[12–15] Patients with high intensity alveolitis have increased numbers of helper T cells in the lung (as defined by OKT4 monoclonal antibody positivity) actively replicating, which spontaneously attract blood monocytes to the alveolar structures, modulate granuloma formation, and polyclonally activate B cells.[12 13 15–18] In contrast, blood T cells from such patients do not show increased proportions of the OKT4 positive fraction or release notable amounts of lymphokines or polyclonally activate B cells by comparison with the lung T cells from such patients or with the lung or blood T cells from patients with inactive disease (low intensity alveolitis). Thus active pulmonary sarcoidosis represents a model of local imbalance of the immune system, with expansion of various T lymphocyte subpopulations in the lung tissue that are not equally

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represented in the blood.

Recently two small T cell subpopulations have been defined by monoclonal antibodies. The first monoclonal antibody, 5/9, is present on about 30% of the OKT4 positive cells and reacts with only 15–20% of the peripheral T cells from normal subjects.[19] The 5/9 positive lymphocytes represent a small subset responsible for many T cell activities, such as proliferation in response to allogenic cells or to soluble antigens and helper function for the pokeweed mitogen driven B cells.[19 20] In addition, the presence of the 5/9 positive T cells is necessary to induce the 5/9 negative cells to develop cytolytic activity.[20] The second monoclonal antibody, MLR4, reacts with 3–5% of the peripheral T cells from normal subjects and defines a small fraction of the 5/9 positive cells, with helper activity for the pokeweed mitogen driven B cells[21] (also unpublished observations).

These observations suggest that the activity of the lung disease in pulmonary sarcoidosis may be mediated by a relative increase in the numbers of 5/9 positive and MLR4 positive T lymphocytes at sites of disease activity. To evaluate this hypothesis, purified T cells were isolated from the lungs and blood of patients with active pulmonary sarcoidosis and the proportions of 5/9 positive and MLR4 positive cells were determined. In addition, the percentages of OKT4 positive T cells (helper and inducer) and of OKT8 positive T cells (suppressor and cytotoxic) were also defined. A control group of patients with inactive pulmonary sarcoidosis and a group of normal subjects were also studied.

Methods

STUDY POPULATIONS

Patients with sarcoidosis A diagnosis of sarcoidosis was established in 12 untreated patients on the basis of the following criteria: (1) transbronchial biopsy showing non-caseating epithelioid cell granulomas in the lung parenchyma and coexisting morphological features compatible with sarcoidosis; (2) no evidence of mycobacterial, fungal, or parasitic infection; (3) no history of exposure to inorganic or organic materials known to cause granulomatous lung disease. The 12 patients with sarcoidosis (three men and nine women) were divided into two groups according to the activity of the lung disease. Activity was assessed from the percentages of T lymphocytes in bronchoalveolar lavage fluid and from gallium 67 (^{67}Ga) lung scans. Patients were considered to have high intensity alveolitis if they had 28% or more T lymphocytes in bronchoalveolar lavage fluid and a positive ^{67}Ga lung scan, and to have low intensity alveolitis if they had less than 28% T lymphocytes in the lavage fluid and a negative ^{67}Ga lung scan.[8 13 22–25] On the basis of these criteria six patients had high intensity alveolitis and six patients low intensity alveolitis. The above criteria were used to characterise the activity of the lung disease, since recent studies have showed that most untreated patients with high intensity alveolitis have functional deterioration within six months, whereas most patients with low intensity alveolitis remain in a stable condition.[13 22–24] The two groups of patients could not be distinguished on the basis of various clinical and physiological characteristics (table 1).

Normal controls Six normal non-smokers (four men and two women with a mean age of 30 (SD 7) years) were used as controls. The results of pulmonary function tests and chest radiographs were within normal limits in all six subjects (table 1).

PREPARATION OF INFLAMMATORY AND IMMUNE EFFECTOR CELLS

Cells were obtained from the lower respiratory tract by bronchoalveolar lavage, which was performed in both patients and control subjects, a total of 100 ml of 0·9% sterile saline solution being used.[13–18 25] The cells were separated by centrifugation and resuspended in Hank's balanced salt solution (HBSS) without Ca^{++} or Mg^{++} at the desired cell density (10×10^6 cells/ml). A small portion was taken for determination of cell numbers, viability, and differential counting.[13–18]

Heparinised venous blood was obtained immediately before the lavage procedure. Differential cell counts were carried out. Mononuclear cells were isolated by Hypaque-Ficoll centrifugation.[26]

Table 1 *Clinical and physiological characteristics (means with standard errors in parentheses) of normal controls and patients with pulmonary sarcoidosis and either high intensity or low intensity alveolitis*

Groups	*No*	*Age (y)*	*Sex (M:F)*	*Duration of disease (months)*	*Radiographic stage*	*Vital capacity*	*Total lung capacity*	*Diffusing capacity*
						% of predicted normal values		
Normal controls	6	30(7)	4:2			98(3)	100(6)	94(10)
Sarcoidosis								
Low intensity alveolitis	6	35(8)	2:4	26(9)	3–2–1	74(8)	77(10)	76(8)
High intensity alveolitis	6	31(6)	1:5	19(8)	2–3–1	75(7)	78(8)	71(6)

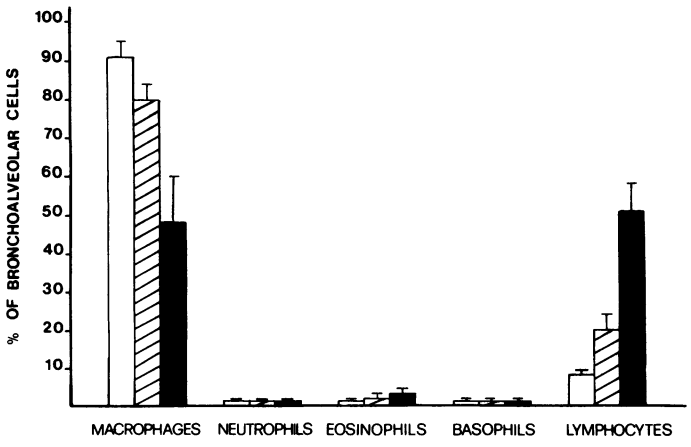

Fig 1 *Cellular composition of bronchoalveolar lavage fluid of normal subjects (□) and patients with sarcoidosis and either low intensity (▨) or high intensity (■) alveolitis. The data represent the means and standard errors of the results for six normal subjects, six patients with sarcoidosis and high intensity alveolitis, and six patients with sarcoidosis and low intensity alveolitis.*

IDENTIFICATION AND ISOLATION OF LYMPHOCYTE SUBPOPULATIONS

T lymphocytes were identified by their ability to form rosettes with neuraminidase treated sheep red blood cells (N-SRBC) at 4°C.[26] Purified T lymphocytes were obtained by rosetting the mononuclear cell suspensions from blood and lavage twice with N-SRBC at 4°C followed by Hypaque-Ficoll centrifugation.[15–18] The final T cell suspensions contained more than 98% rosette forming cells.

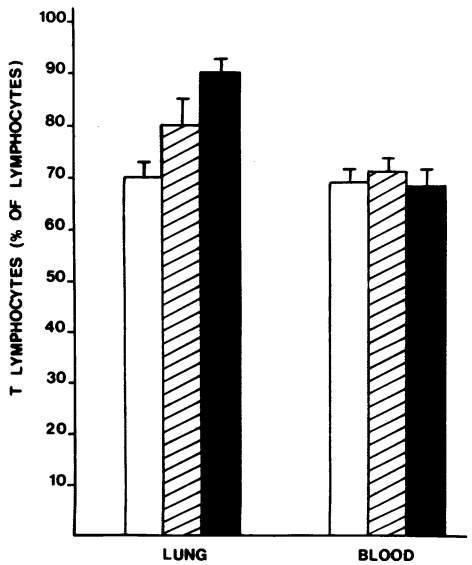

Fig 2 *Proportions of T lymphocytes in the bronchoalveolar fluid and in blood of normal subjects (□) and patients with sarcoidosis and either low intensity (▨) or high intensity (■) alveolitis. The data represent the means and standard errors of the results for six normal subjects, six patients with sarcoidosis and high intensity alveolitis, and six patients with sarcoidosis and low intensity alveolitis.*

IDENTIFICATION OF T CELL SUBSETS

T lymphocyte suspension from lung and blood were tested with murine monoclonal antibodies 5/9 and MLR4, which have previously been shown to be specific for subsets of human T cells.[19–21] In addition, the OKT4 and OKT8 monoclonal antibodies (Ortho Diagnostic System, Milan) were used.[27–29] Five microlitres of the appropriate dilution of the monoclonal antibody 5/9, MLR4, OKT4, or OKT8 (50 μg per ml) was added to 50 μl of the T cell suspensions (5×10^6 cells per ml) at 4°C for 30 minutes. The cells were then washed three times and 50 μl of an appropriate dilution of a fluorescein conjugated $F(ab')_2$ goat antimouse immunoglobulin reagent were added to the cell suspensions at 4°C for 30 minutes. The cells were then washed three times and then examined by fluorescence microscopy by a Leitz Dialux microscope. At least 300 cells were counted in every preparation.

ANALYSIS OF DATA

All data are presented as means with standard errors in parentheses; comparisons were made with the Mann-Whitney U test.

Results

Bronchoalveolar lavage was performed without difficulty or complications in normal controls and in patients with sarcoidosis. Visualisation of the

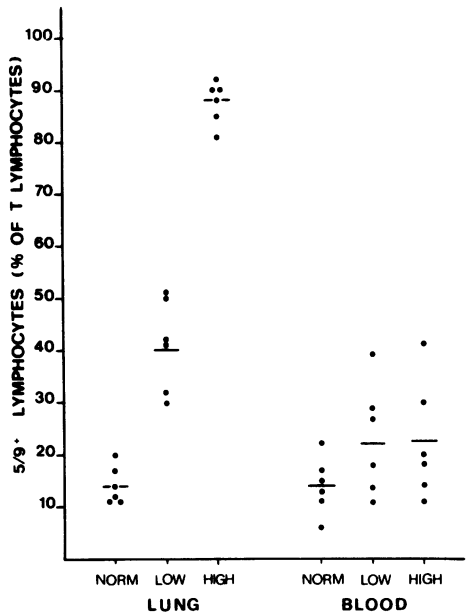

Fig 3 *Proportions of 5/9 positive T lymphocytes in the bronchoalveolar fluid and blood of normal subjects and patients with sarcoidosis and either high or low intensity alveolitis.*

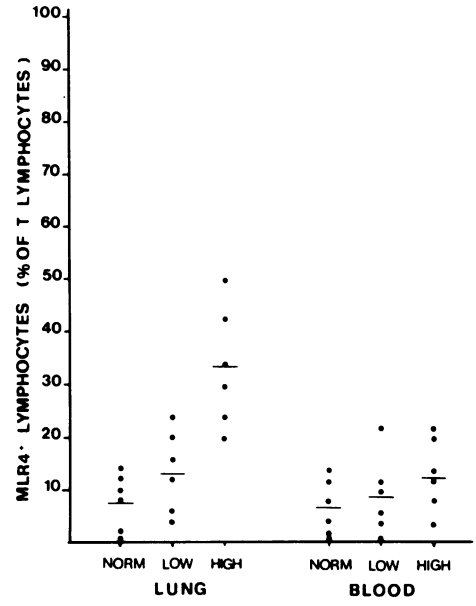

Fig 4 *Proportions of MLR4 positive T lymphocytes in the bronchoalveolar fluid and blood of normal subjects and patients with sarcoidosis and either high or low intensity alveolitis.*

tracheobronchial tree before lavage showed normal airways in all subjects with no evidence of bronchitis. Apart from the occasional isolation of organisms from the oral flora, the recovered fluid was sterile in all cases. The total amount of fluid recovered was similar in the three groups of subjects (54 (7) ml, 52 (6) ml, and 51 (8) ml respectively; $p > 0{\cdot}2$). The bronchoalveolar cell suspensions from normal subjects contained 10 (2) $\times$ 10^6 cells and comprised 92% (3)% alveolar macrophages, 1·0% (0·5%) neutrophils or eosinophils, and 6% (2%) lymphocytes (fig 1). The total numbers of bronchoalveolar cells were about twofold higher in patients with sarcoidosis and low intensity alveolitis and about fourfold higher in patients with sarcoidosis and high intensity alveolitis. An increased proportion of lymphocytes was seen in both high intensity and low intensity alveolitis (53% (8%) and 18% (4%) respectively (fig 1)). The proportions of cells from lavage fluid that were T lymphocytes were significantly higher in patients with high intensity alveolitis than in patients with low intensity alveolitis or in control subjects ($p < 0{\cdot}05$ for each comparison (fig 2)). In contrast, the proportions of T cells in the blood were similar in the three groups of patients ($p > 0{\cdot}2$).

In normal controls there was no significant difference in the proportions of bronchoalveolar or blood T cells that were 5/9 positive (13% (2%) and 13% (3%) respectively; $p > 0{\cdot}2$ (fig 3)). In contrast, patients with sarcoidosis had significantly higher proportions of 5/9 positive T cells in the bronchoalveolar fluid than in the blood, the difference between bronchoalveolar fluid and blood being greater for patients with high intensity alveolitis (87% (2%) and 20% (4%) respectively; $p < 0{\cdot}001$) than for patients with low intensity alveolitis (39% (4%) and 22% (4%) respectively; $p < 0{\cdot}02$). The proportion of 5/9 positive T cells was higher in the bronchoalveolar fluid from patients with sarcoidosis than in that from normal controls ($p < 0{\cdot}001$ for each comparison) and patients with high intensity alveolitis had significantly higher proportions of 5/9 positive T cells in the bronchoalveolar fluid than patients with low intensity alveolitis ($p < 0{\cdot}01$). The proportions of 5/9 positive T cells in blood were by contrast similar in normal subjects and all patients with sarcoidosis with either high or low intensity alveolitis ($p > 0{\cdot}2$ for each comparison).

In normal subjects the proportion of MLR4 positive T cells was similar in the bronchoalveolar fluid to that in the blood (5% (2%) and 3% (1%) respectively; $p > 0{\cdot}2$ (fig 4)). The same was true for patients with sarcoidosis and low intensity alveolitis (7% (3%) and 5% (2%) respectively; $p > 0{\cdot}2$). Furthermore, there was no significant difference between the proportion of MLR4 positive T cells in the bronchoalveolar fluid in normal subjects and

Table 2 *T lymphocyte subpopulations in the lungs and in the blood of normal controls and patients with pulmonary sarcoidosis and either high intensity or low intensity alveolitis*

Groups	*No*	*Lung*				*Blood*			
		5/9	*MLR4*	*OKT4*	*OKT8*	*5/9*	*MLR4*	*OKT4*	*OKT8*
		Mean (SEM) of % of T lymphocytes							
Normal controls	6	13(2)	5(2)	49(3)	28(3)	13(3)	3(1)	51(3)	24(2)
Sarcoidosis									
Low intensity alveolitis	6	39(4)	7(3)	55(4)	37(3)	22(4)	5(2)	47(4)	39(4)
High intensity alveolitis	6	87(2)	17(3)	86(5)	14(2)	20(4)	6(2)	35(3)	40(5)

patients with low intensity alveolitis ($p > 0{\cdot}2$); the proportion of MLR4 positive T cells in blood were also similar in these two groups ($p > 0{\cdot}2$). In contrast, patients with sarcoidosis and high intensity alveolitis were found to have a higher proportion of MLR4 positive T cells in the bronchoalveolar lavage fluid than in the blood (17% (3%) and 6% (2%) respectively; $p < 0{\cdot}01$) and a higher proportion of MLR4 positive T cells in the bronchoalveolar fluid than patients with low intensity alveolitis or normal controls ($p < 0{\cdot}01$ for both comparisons). The proportion of MLR4 positive T cells in the blood was similar in all three groups ($p > 0{\cdot}2$). These studies suggest that the ratio of 5/9 positive to MLR4 positive cells is altered in the lungs but not in the blood of patients with sarcoidosis compared with normal subjects. In bronchoalveolar fluid of patients with sarcoidosis and high intensity alveolitis the mean ratio of 5/9 positive to MLR4 positive cells was 5·1:1. This ratio was similar to that in patients with sarcoidosis and low intensity alveolitis (5·5:1; $p > 0{\cdot}2$) and both ratios were higher than that in the lungs of normal subjects (2·7:1; $p < 0{\cdot}01$). In contrast, the ratio of 5/9 positive to MLR4 positive cells was similar in the blood of the three groups ($p > 0{\cdot}2$). Since the MLR4 positive T cells represent a fraction of the 5/9 positive T cells, these observations suggest that the 5/9 positive MLR4 negative T lymphocyte subset is preferentially expanded in the lungs of patients with pulmonary sarcoidosis compared with the MLR4 positive T cell subset.

Studies using the standard OKT4 and OKT8 monoclonal antibodies showed, as previously reported by other investigators, that compared with the normal and with inactive sarcoidosis active sarcoidosis is characterised by an increased proportion of OKT4 positive T cells in bronchoalveolar fluid ($p < 0{\cdot}001$ for each comparison), a reduced proportion of OKT4 positive T cells in the blood ($p < 0{\cdot}05$ for each comparison), and a reduced proportion of OKT8 positive T cells in bronchoalveolar fluid ($p < 0{\cdot}01$ for each comparison (table 2)). In contrast, compared with the normal and with active sarcoidosis inactive sarcoidosis is associated with an increased proportion of OKT8 positive T cells in both bronchoalveolar fluid and blood ($p < 0{\cdot}01$ for all comparisons).

Comparisons between the percentages of T cell subsets with helper functions defined by OKT4, 5/9, and MLR4 monoclonal antibodies showed that in patients with high intensity alveolitis about 90% of the T cells in bronchoalveolar fluid expressed both OKT4 and 5/9 surface antigens. In contrast, in patients with low intensity alveolitis lymphocytes expressing both OKT4 and 5/9 antigens represented about 35% of bronchoalveolar fluid cells, while in normal controls about 10% of bronchoalveolar fluid cells expressed both antigens. These data suggest that in pulmonary sarcoidosis the expansion of the OKT4 positive helper T cells at sites of disease activity is associated with a preferential proliferation of the 5/9 positive T cell fraction.

Discussion

Pulmonary sarcoidosis is a disease characterised by a heightened cellular immune response within the lung. Patients with high intensity alveolitis have large numbers of T lymphocytes in the alveolar structures, actively replicating and spontaneously secreting a variety of lymphokines.[12–18] These lymphokines appear to stimulate the clonal increase of responsive T lymphocytes, to attract monocytes (the building blocks of granulomas) to the lung, and to modulate granuloma formation.[4 13–18 24 25] In addition, patients with high intensity alveolitis appear to have a heightened humoral immune response manifested by increased amounts of circulating antibodies with activity towards multiple antigens.[4–11] This polyclonal response may be mediated by the lung T cells, which stimulate B lymphocytes to produce immunoglobulins.[4–12] In contrast, lung T lymphocytes from patients with low intensity alveolitis seem neither to secrete lymphokines spontaneously nor to polyclonally activate B cells.[4 12–18] These immune abnormalities in patients with high intensity alveolitis are associated with an excess of helper OKT4 positive T cells at sites of disease activity.[17]

We have shown that, firstly, patients with sarcoidosis and either high or low intensity alveolitis have increased proportions of 5/9 positive helper T lymphocytes in the lung compared with blood of the same patients and with the lungs and blood of normal subjects; secondly, the expansion of the 5/9 positive T cells in the lungs of patients with active sarcoidosis is associated with an increased proportion of MLR4 positive T cells; and, thirdly, the ratio of 5/9 positive to MLR4 positive T cells is higher in the bronchoalveolar fluid of patients with sarcoidosis than in blood of the same patients or in the bronchoalveolar fluid or the blood of normal subjects. Although associated with normal percentages of OKT4 positive T cells, the increased proportion of 5/9 positive lymphocyte in the lungs of patients with low intensity alveolitis suggests that inactive pulmonary sarcoidosis is also characterised by expansion of helper T cell subsets in the alveolar structures. This contrasts with the findings of previous studies.[12 15 17 18] Since the total numbers of lung lymphocytes, the percentages of lung T lymphocytes, and the percentages of 5/9 positive lung T lymphocytes are all higher in patients with sarcoidosis and high intensity alveolitis, the total number of 5/9 positive helper T cells in the lungs must have been very much greater than in patients with low intensity alveolitis. These observations suggest that in patients with sarcoidosis and low intensity alveolitis the 5/9 positive helper T cells in the lungs, although present in greater numbers than in the blood of the same patients or the lungs and blood of normal controls, may still be too few to produce significant quantities of lymphokines. Alternatively, since inactive disease is associated with increased proportions of OKT8 positive T cells, it is possible that these patients generate sufficient suppressor cell activity in the lungs to modulate helper T cell functions.

The role of hypergammaglobulinaemia in sarcoidosis is not clear since it is not known whether antibodies participate in granuloma formation or represent a "byproduct" of T lymphocyte activation. The presence, however, in patients with high intensity alveolitis of increased numbers of MLR4 positive lung T lymphocytes (the T cell subpopulation able in vitro to stimulate B cells) supports the idea that in pulmonary sarcoidosis immunoglobulin production is modulated by activated T lymphocytes at sites of disease activity.

Interestingly, our study has shown that the ratio of 5/9 positive to MLR4 positive T lymphocytes in the lungs of patients with sarcoidosis is similar in active and inactive disease and is higher than in normal subjects. These data suggest that in pulmonary sarcoidosis the MLR4 negative fraction of the 5/9 positive T cells (the subset of inducer T lymphocytes able to stimulate the cellular immune response[19–21]) is more expanded than the MLR4 positive fraction. Thus it is possible that, compared with the 5/9 positive MLR4 negative T cells, the MLR4 positive T cells play a secondary role in producing lung injury in this disorder. This hypothesis is supported by recent studies showing that in patients with Hashimoto thyroiditis, where self-antigen stimulation results in high titres of specific antibodies, the MLR4 positive subset is considerably expanded and the ratio of 5/9 positive to MLR4 positive T cells is lower than in normal subjects.[30] Furthermore, examination of the results obtained with the OKT4 and the 5/9 monoclonal antibodies indicates that in the lungs of patients with active pulmonary sarcoidosis most of the OKT4 positive helper T cells express the 5/9 surface antigen and therefore that the 5/9 monoclonal antibody may be considered a good marker of activity in this disorder.

This study shows that pulmonary sarcoidosis is characterised by differential expansion of various T lymphocyte subsets with helper functions at sites of disease activity and suggests that the use of surface markers, specific for T cell subpopulations, may be a useful tool in the evaluation of the pathogenetic mechanisms concerned in interstitial lung disorders.

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