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Cellular immune responses in the lung of hypersensitivity pneumonitis

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Bronchoalveolar lavage (BAL) of hypersensitivity pneumonitis (HP) patients is studied in the initial phases of disease. In subacute or chronic phases the pattern may be different.

In this brief report we will summarize the work that has been performed in our laboratory on cell suspensions recovered from the BAL of HP patients.

Cellular recovery from BAL of HP patients is fivefold that in controls, and mostly represented by lymphocytes [1]. Immunological evaluation demonstrated that few BAL lymphocytes express B-cell related markers, most being represented by T-lymphocytes [2]. Analysis of subsets revealed that CD8+ lymphocytes are the predominant cells retrieved, hence the CD4/CD8 ratio is extremely low (around 0.5 vs 1.8 controls). Although the % of cells bearing the proliferation associated markers (T9 and CD25 antigens) is low, a significant difference with respect to controls exists in absolute numbers. An increase of lymphocytes bearing HLA-DR determinants was also demonstrated.

number of CD56 and CD57 cells co-expressing T-cell markers is predominant over those lacking these determinants. The pattern of expression of these markers in controls is statistically different (fig. 1). Other markers strictly defining natural killer cells are lacking on the surface membrane of BAL cells [1]. Thus, the alveolitis in HP patients is mostly represented by CD3+, CD8+, CD57+, CD56+, CD16- non-major histocompatibility complex (MHC) restricted cytotoxic lymphocytes.

It is important to differentiate the pattern of BAL in HP from that in other disorders known to be associated with lymphocytosis. In sarcoidosis, tuberculosis and berylliosis the BAL lymphocytes express the helper-related phenotype. BAL lymphocytes from patients with interstitial pneumonia associated with collagen vascular diseases, silicosis, histiocytosis X, AIDS and amiodarone pneumonitis express the suppressor/cytotoxic phenotype. The presence of an alveolitis characterized by CD3+/CD8+/CD56+/CD57+/CD16- phenotype suggests HP. Introduction of more specific reagents may

Table 1. - Phenotypic analysis of cytotoxic cells recovered from the BAL of HP patients

Group	CD57		CD56		CD16		TCRβ1	
	%	ml × 10 ³	%	ml × 10 ³	%	ml × 10 ³	%	ml × 10 ³
HP patients	31.2 ±3.7	122.5 ±28.6	40.1 ±9.4	88.0 ±17.1	5.4 ±1.5	12.8 ±4.9	3.14 ±0.25	5.31 ±1.3
Controls	9.7 ±1.5	1.0 ±0.2	4.9 ±1.5	0.5 ±0.1	5.1 ±1.6	0.5 ±0.1	1.34 ±0.21	0.13 ±0.16
P	<0.001	<0.001	<0.001	<0.001	ns	<0.001	<0.001	<0.001

CD57: HNK-1; CD56: N901; CD16: NK-15; BAL: bronchoalveolar lavage; HP: hypersensitivity pneumonitis.

The pattern of reactivity with monoclonal antibodies defining cells with cytotoxic phenotype shows a significantly increased number of cells positive for HNK-1 (CD57) and NKH-1 (CD56) reagents in the lavage of HP patients with respect to controls (table 1) [1]. The

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help to differentiate some of the above disorders.

Since the lung contains a separate compartment for cytotoxic cells that react to foreign antigens, the presence of cytotoxic lymphocytes in the BAL of HP patients may fit with the pathogenesis of this disease, notably sensitization via the upper respiratory tract by extrinsic antigens.

To clarify the structures involved in recognition of putative antigens, we evaluated the distribution in BAL of T-cells expressing the membrane products of the alpha/beta or gamma/delta chain products of the T-cell receptor. We found a slightly increased % of gamma/delta positive cells. However, the absolute number of these cells in the BAL fluid, indicated a marked increase in the lung of HP patients (table 1). It has been proposed that gamma/delta T-cell receptor positive lymphocytes display non-MHC restricted cytotoxicity; this fits well with other phenotypic markers and functional studies performed on BAL HP cells reported below.

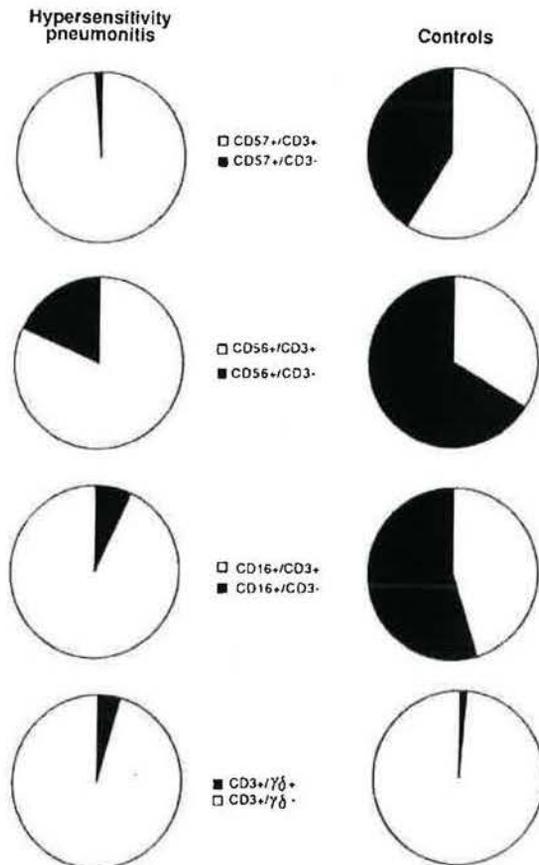


Fig. 1. - Distribution of subpopulations of cells bearing the phenotype of non-MHC restricted lymphocytes in the BAL of controls and patients with HP. Non-MHC: non-major histocompatibility complex.

The number of alveolar macrophages bearing Class I and II molecules (in particular DQ molecules) was increased in HP patients, and the number of alveolar macrophages expressing transferrin receptors was decreased [3]. The observation that a higher number of alveolar macrophages from HP patients display Class I HLA A, B and C and Class II HLA DQ antigens is relevant to the immunopathology of HP. Since the role of Class I and II MHC antigen in the lytic function of cytotoxic/suppressor lymphocytes has recently been emphasized, their involvement in recruitment of the CD8 population is possible.

To rule out the possibility that the lung T-cells from HP patients proliferate as a clone, experiments were carried out to define the clonality of expanded populations. BAL T-lymphocytes were investigated at the molecular level by evaluating the T-cell receptor gene rearrangement. The configuration of the T-cell receptor gamma-gene region did not show monoclonal rearrangements, supporting the notion that we are dealing with a polyclonal expansion of T-cells [4]. By contrast, the evaluation of the beta-gene region of the T-cell receptor revealed faint as novel bands in BAL T-cells (unpublished observations). This pattern is not specific for HP since a similar configuration of the T-cell receptor beta-chain was found in sarcoidosis patients [5].

Using a Pokeweed Mitogen-induced B-cell differentiation assay, lung T-cells from HP patients were shown to display a suppressor *in vitro* activity [1, 6]. This finding offers major clues to the pathological pattern of HP. Evidence has been accumulating that mechanisms leading to granuloma formation are modulated by the presence of regulatory T-cells. A mononuclear cell infiltration precedes the development of granuloma, and the presence of different T-cell subsets is crucial in regulating the appearance and maintenance of granulomas, perhaps by the release of a number of lymphokines. It has been demonstrated that helper T-cells are correlated with an active granuloma formation, whereas suppressor/cytotoxic T-cells and NK cells are associated with the regression of this phenomenon. Suppressor cells may slow down granuloma formation in HP patients, hence granulomas are not as prominent as in other disorders, e.g. sarcoidosis, where alveolitis is characterized by accumulation of CD4+ lymphocytes.

A significant increase of spontaneous cytotoxicity was observed in HP patients [1]. By contrast, BAL lymphocytes from asymptomatic farmers display a cytotoxic *in vitro* function superimposable on that of controls. This difference offers an explanation for the different pathogenetic mechanisms in the two groups but must be substantiated. Attempts have been made to characterize the nature of cytotoxic cells accounting for alveolitis in patients with HP [7], and have demonstrated that different types of cytotoxic mechanisms are provided by lung cells from HP patients including NK cells, non-MHC restricted T-cytotoxic and cells and lymphokine activated killer cells.

The nature of soluble factors (interleukin-2 (IL-2) or other biological response modifiers) accounting for T-cell growth, activation and intensity of alveolitis in HP patients needs to be determined.

In a follow-up study we subdivided patients into two groups, according to their (awaited/continued) exposure to the specific antigens [8]. At first evaluation, a high number of CD8+ cells with a reversal of the CD4/CD8 ratio was seen in patients with HP. Consecutive evaluations showed a persistent increase of CD8+ cells and reversal of the CD4/CD8 ratio in patients who continued to be regularly exposed. Irrespective of group, a persistent increase of cytotoxic cells was seen [8]. Cytotoxic cells showed a persistently enhanced *in vitro*

immune function during the entire follow-up in patients who continued to be regularly exposed. Patients who continued to live in agricultural environments but without further exposure to specific antigens at work exhibited a recovery of CD4+ cells, a decrease in CD8+ cells, and an increase of CD4/CD8 ratio to the normal range six months after the first observation [8].

Immunohistological analysis, at first evaluation, showed a diffuse infiltration of lung parenchyma by CD8+ cells [2, 8]. Subsequent immunohistological observations revealed a persistent CD8+ infiltrate in the group of patients who continued to be regularly exposed, and an increase of CD4+ cells after 6 months in the lungs of subjects that were not.

These data indicate that alveolitis in HP patients is a chronic event and its intensity might be modulated by exposure to relevant antigens, amount of antigen and frequency of sensitization, thus explaining the differences of BAL lymphocytosis observed in patients who are still exposed to the specific antigens compared to those who are not. Increased cells with suppressor/cytotoxic characteristics in the lung of these patients is likely to be related to a local immunological response to the antigen. These mechanisms may be relevant in specifying the pathogenesis of HP.

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Evidence in bronchoalveolar lavage for third type immune reactions in hypersensitivity pneumonitis

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Immune complex disease and immune cellular mechanisms are thought to participate in the pathogenesis of hypersensitivity pneumonitis (HP). Data obtained from bronchoalveolar lavage (BAL) in patients with farmer's lung and relating to type III immune reactions in the lung are discussed.

Forty two patients with HP (32 men, 48.9±9.9 yrs) were studied. Only two were smokers; none had previously been treated; all had recently been exposed to the antigen (mean time lapse from last exposure: 15 days). Controls were 7 healthy nonsmoking volunteers. Diagnosis was based on standard criteria: 1) history of

exposure to HP antigens; 2) symptomatic acute episode with chills, fever, cough and breathlessness 4-8 h after exposure; 3) radiological features and/or functional patterns of interstitial lung disease; 4) evidence of antibodies against *Micropolyspora faeni*.

BAL was performed after local anaesthesia [1]. A fiberoptic bronchoscope was wedged in a segment of the right lobe or lingula and a total of 150 ml of sterile 0.9% saline (warmed to 37°C) was injected in 50 ml aliquots with immediate vacuum aspiration. BAL fluid was immediately filtered through two layers of surgical gauze and the volume measured. To separate cellular and non-cellular components, the fluid was centrifuged (800 rpm for 10 min) and washed twice with phosphate

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