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Analysis of CD4-positive T cell subpopulation in sarcoidosis

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SUMMARY

Double-labelling immunofluorescence analysis within the $CD4^+$ cell subset was carried out in 27 bronchoalveolar lavage fluids and 11 peripheral blood samples of sarcoidosis patients with anti-TQ1, anti-2H4 and anti-4B4 monoclonal antibodies. Helper/inducer $CD4^+TQ1^-/4B4^+$ cells were strongly increased in the lung and slightly, but significantly, decreased in the blood of sarcoidosis patients with respect to normal controls. No differences were found in the number of both lung and blood $CD4^+2H4^+$ cells between sarcoidosis patients and controls. The findings are further evidence for a compartmentalization of T cell subsets in sarcoidosis.

Keywords sarcoidosis T lymphocytes immune system

INTRODUCTION

Pulmonary sarcoidosis is a systemic disorder of unknown etiology characterized by the presence of granulomas in the lung. Although granulomas are the hallmark of this disease, recent investigations have shown that the first lesion which occurs in the lung is an inflammatory process of the alveolar structures (Crystal, 1981). Studies using bronchoalveolar lavage (BAL), a valuable tool for characterizing the alveolitis of sarcoidosis (Reynolds, 1987), have demonstrated that such an alveolitis mainly consists of T lymphocytes and macrophages (Thomas & Hunninghake, 1987). Despite the increased numbers of both these cell populations, T lymphocytes are increased to a far greater degree. T lymphocytes seem to modulate sarcoidosis activity, since most of lung T cells in patients with active disease belong to the $CD4^+$ cell subset (Hunninghake & Crystal, 1981), that mainly exerts a helper activity (Reinherz & Schlossman, 1980). However, a functional heterogeneity within the $CD4^+$ cell population has recently been demonstrated. A monoclonal antibody (MoAb), termed 2H4, has been used to subdivide $CD4^+$ lymphocyte populations into a helper/inducer subset ($CD4^+2H4^-$) and an inducer of suppression ($CD4^+2H4^+$) for pokeweed mitogen (PWM)-driven and antigen-driven immunoglobulin production (Morimoto et al., 1985a). A monoclonal antibody anti-TQ1 has been utilized to show further that a major inducer of help is the $CD4^+2H4^-TQ1^-$ lymphocyte subset (Reinherz *et al.*, 1982). More recently, helper/inducer $CD4^+$ T cells have been more accurately identified by using anti-4B4 MoAb (Morimoto et al., 1985b). Thus, attempts to define the pulmonary $CD4^+$ cells in sarcoidosis are valuable in characterizing the specific cell subse involved in alveolitis, and useful for understanding the immun process that may lead to the lung damage.

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MATERIALS AND METHODS

Patients and normal volunteers

The study included 27 sarcoidosis patients (nine men, 1 women) with a mean age of 47 years (range, 24–73). Fiv subjects with idiopathic pulmonary fibrosis (two men, thre women; mean age: 46 years, range 30–49) and six norma subjects (three men, three women; mean age: 42 years, range 24 55) acted as the two control groups. Informed consent wa obtained from all participants. The diagnosis of sarcoidosis wa based on consistent clinical features confirmed by laborator findings. In all cases, material obtained from lungs, lymp nodes, liver or bone marrow contained non-caseating epithe lioid-cell granulomas, with no evidence of inorganic materia known to cause granulomatous diseases. Gallium-67 scans wer not done.

Preparation of cell suspension

Mononuclear cells were obtained from heparinized periphera blood (PB) samples and BAL fluid collected after local anaes thesia. A total of 150 ml sterile 0·9% saline was injected in 50 m aliquots via a fibreoptic bronchoscope. Each aliquot wa immediately aspirated and filtered through surgical gauzes an the volume measured. After determination of the total reco vered cells, the lavage fluid was centrifuged at 450 *g* for 10 min at 4°C. The cell pellet was resuspended in Hanks' balanced sal solution (Gibco, Grand Island, NY, USA) without Ca^{2+} an Mg^{2+}. Cytocentrifuged slides were prepared and stained wit May–Grünwald–Giemsa to determine cell morphology. Th mononuclear cells from both PB and BAL were separated b

Table 1. Sarcoidosis patient characteristics according to the radiological pulmonary picture

Patients*	Age (years)	Race	Sex	Symptom duration (months)	Extrathoracic sarcoidosis	Smoke	Drug therapy	BAL total cells ($\times 10^6$)	BAL total CD4+ cells ($\times 10^6$)
Stage I $n=17$	44·2±11†	caucasians	5M, 12F	12·1±15	1U, 1AR, 3SS	none	none	20·9±10	4·3±5
Stage II $n=4$	50·7±16	caucasians	2M, 2F	4·7±1	1AR	none	none	21·8±15	4·2±4
Stage III $n=6$	52·2±11	caucasians	2M, 4F	13·8±9	1AR, 1SS	none	none	22·7±10	4·7±3

* I Hilar adenopathy alone II Hilar adenopathy and parenchimal infiltrates III Parenchimal fibrosis alone.
† Mean±s.d.
M Male; F Female U Uveitis; AR Arthritis SS Skin sarcoidosis.

Table 2. BAL characteristics of sarcoidosis (SC) patients idiopathic pulmonary fibrosis (IPF) and normal controls (NC) (mean±s.d.)

	Vol. recov. (% infused)	Total lymph. ($\times 10^6$)	Lymph.%	CD3+%	CD4+%	CD8+%
SC (27)	51·8±10	6·1±5*	25·7±7	85·0±8†	66·4±14	22·1±11
IPF (5)	50·7±11	0·6±0·5	5·0±3	78·2±5	56·4±4	23·9±4
NC (6)	51·0±12	1·2±0·4	10·3±3	77·6±4	55·2±4	24·8±5

* $P<0·001$.
† $P<0·05$.

centrifugation on Ficoll–Hypaque density gradient, washed and resuspended in RPMI-1640 (Gibco) containing 10% fetal calf serum. After depletion of alveolar macrophages by plastic adhesion at 37°C in a 5% CO_2 atmosphere, lymphoid cells (>97%) were adjusted to a concentration of 1×10^6 cells/ml.

Identification of T cell subsets

T lymphocytes and T cell subsets were identified by indirect immunofluorescence technique using 10 μl of OKT3 (anti-CD3), OKT4 (anti-CD4) and OKT8 (anti-CD8) MoAb (Ortho, Raritan, NJ, USA) (Reinherz & Schlossman, 1980). Ten microlitres of a fluoresceine-conjugated goat anti-mouse IgG antiserum (Kallestad, Chaska, MA, USA) was employed as second labelled antibody for each sample. The percentage of fluorescent cells was determined on at least 200 cells with a Leitz Dialux microscope, using phase contrast to evaluate the lymphoid morphology. Two-colour immunofluorescence studies were performed with MoAb TQ1 (Coulter Electronics Ltd, Luton, UK), 2H4 and 4B4 (kindly supplied by Dr. Schlossman, Boston, MA, USA) to dissecate the CD4+ subpopulation. Appropriate isotype-specific goat anti-mouse IgG antisera conjugated with fluoresceine (FITC) or tetramethyl-rodamine sothiocianate (RITC) were used as developing reagents for each MoAb (Southern Biotechnology Ass., Birmingham, Al.) (Gerli *et al.*, 1986). Specificity of the isotype-specific goat anti-mouse fluorochrome-conjugated antibodies was proven by the fact that they never cross-reacted with non-corresponding isotype MoAb. Moreover, negative controls for indirect staining with each MoAb were obtained by using mouse MoAb unreactive with human determinants, followed by fluorochrome-labelled goat anti-mouse antibody, or by using the latter reagent alone.

Table 3. PB lymphocyte characteristics of sarcoidosis patients (SC), idiopathic pulmonary fibrosis (IPF) and normal controls (NC) (mean±s.d.)

	Lymph./mm³	CD3+%	CD4+%	CD8+%
SC (11)	1882±355	77·4±7·3	50·8±4·2	28·3±5·3
IPF (5)	2083±261	78·5±5·9	54·6±4·3	26·4±3·1
NC (6)	2117±225	79·7±5·4	53·8±4·1	27·3±2·2

Statistical analysis

The unpaired Student's *t*-test was adopted for sample analysis. Values of *P* below 0·05 were considered significant.

RESULTS

Patients were classified into stages I (17 patients), II (four patients) and III (six patients), according to the radiological pulmonary pattern. Table 1 shows the relationship between radiological findings and clinical and BAL data of the patients. In particular, the three radiologically distinct groups of patients manifest no difference in either total cells or the number of CD4+ cells recovered in BAL. As shown in Table 2, there was no significant difference in the percentage of BAL fluid recovered from sarcoidosis patients and controls. Analysis of bronchoalveolar cells revealed that patients had increased numbers of T lymphocytes. In ten patients the CD3+ percentage was greater than 28%, which, according to previously proposed criteria (Hunninghake & Crystal, 1981), suggests the presence of high intensity alveolitis. There were no significant differences in the CD4+ and CD8+ percentages.

Table 4. Absolute percentages of $CD4^+TQ1^-$, $CD4^+2H4^+$ and $CD4^+4B4^+$ cells in BAL and PB of sarcoidosis patients (SC), idiopathic pulmonary fibrosis (IPF) and normal controls (NC) (mean ± s.d.)

		CD4+		
		TQ1−	2H4+	4B4+
BAL	SC (27)	43·1 ± 22·9*	18·2 ± 6·0	47·6 ± 16·9*
	IPF (5)	14·0 ± 2·0	22·1 ± 1·9	24·2 ± 1·6
	NC (6)	13·2 ± 1·9	23·0 ± 1·5	23·3 ± 1·9
PB	SC (11)	8·2 ± 9·6*	23·1 ± 2·7	21·8 ± 1·5*
	IPF (5)	15·1 ± 2·1	21·9 ± 1·8	17·9 ± 1·7
	NC (6)	14·2 ± 2·3	22·0 ± 1·5	18·2 ± 1·7

* $P < 0{\cdot}001$.

Slight lymphopenia was present in peripheral blood of sarcoidosis patients, but relative $CD3^+$, $CD4^+$ and $CD8^+$ percentages were similar in patients and controls (Table 3). Two-colour immunofluorescence studies revealed a marked increase in sarcoid BAL and a less marked, but statistically significant, decrease in sarcoid PB of $CD4^+TQ1^-$ and $CD4^+4B4^+$ percentages (Table 4). Percentages of $CD4^+2H4^+$ cells in patients' BAL and PB were similar to those of the controls. $CD4^+TQ1^-$ cell number was much higher in the ten patients with high intensity alveolitis (mean ± s.d. $103 \times 10^3 \pm 54$ cells/ml) than in the 17 patients with low intensity alveolitis ($9 \times 10^3 \pm 7$ cells/ml).

DISCUSSION

Although granulomas are the characteristic pathological feature in sarcoidosis, there is evidence that the initial lesion in the lung is an alveolitis (Crystal *et al.*, 1981; Thomas & Hunninghake, 1987). Macrophages and T cells are the prominent cell populations during inflammation of the alveolar structures, but active lung disease is mainly characterized by a marked increase in the number of the functionally heterogeneous $CD4^+$ cell subset (Huninghake & Crystal, 1981). The present results confirmed that sarcoidosis patients have a much higher number of T lymphocytes in their BAL fluid than controls. The finding that the overall $CD4^+$ cell percentage showed no significant decrease was not unexpected, since not all patients manifested signs of active disease (Rossi *et al.*, 1986). Not surprisingly, analysis of lung $CD4^+$ cells revealed that most co-expressed 4B4 and lacked the TQ1 antigen. The latter antigen distinguishes two subsets of $CD4^+$ inducer cells with distinct repertoires. Both $CD4^+TQ1^+$ and $CD4^+TQ1^-$ cells proliferate equally well to soluble antigens and alloantigens, but more importantly, only $CD4^+$ cells lacking the TQ1 epitope can provide a good signal to facilitate B cell differentiation in a pokeweed mitogen-driven system (Reinherz *et al.*, 1982). In the same way, $CD4^+4B4^+$ cells seem to represent a subset with good helper activity for B cell immunoglobulin secretion, but with poor inducer function for $CD8^+$ suppressor cells. In addition, the $CD4^+4B4^+$ cell subpopulation proliferates relatively poorly to concanavalin A, but well to tetanus toxoid and mumps antigen (Morimoto *et al.*, 1985b). These functional characteristics are consistent with the observation that T cells from lungs of sarcoidosis patients are actively proliferating and spontaneously secreting factors that stimulate B cells to differentiate into immunoglobulin-secreting cells. Furthermore, these findings may account, at least in part, for the described defective mitogenic response of sarcoid lung T cells to concanavalin A (Hudspith *et al.*, 1987). Despite the fact that our patients and controls had a similar number of blood $CD4^+$ cells, patient $CD4^+TQ1^-$ and $CD4^+4B4^+$ circulating cells were decreased. This suggests that helper/inducer T lymphocytes are preferentially drawn from peripheral blood to disease activity sites in sarcoidosis. This phenomenon was particularly evident in active disease patients, but we were unable to establish whether such a reduction in $CD4^+TQ1^-$/$4B4^+$ blood cells was associated with the known anergy of sarcoidosis or not. It is worth noting that the percentages of patients' $CD4^+2H4^+$ cells, a T cell subset with suppressor/inducer activity (Morimoto *et al.*, 1985a), did not differ greatly from that of controls in either lungs or peripheral blood, thereby confirming that T cells with helper/inducer activity are the main lymphoid cell population involved in regulating the immune response in this disorder. Recent data suggested that assessment of BAL lymphocyte count from sarcoidosis patients has scant clinical utility in predicting the evolution of the disease (Buchalter *et al.*, 1986). Other investigators proposed that more specific determination of BAL lymphocyte characteristics such as phase of cell-cycle, membrane markers specific for T cell subsets or activated T lymphocytes, in both lung and extrathoracic sarcoidosis may be better predictors of outcome and response to treatment than total lymphocytes alone (Cordier, Brune & Revillord, 1986; Semenzato *et al.*, 1986; Wallaert *et al.*, 1986). In particular, it has been proposed that analysis of lung $CD4^+$ cells would provide the most useful clinical tool for assessing and perhaps monitoring disease activity in sarcoidosis (Keogh *et al.*, 1983; Bauer *et al.*, 1985; Israel-Biet, Venet & Chrétien, 1986; Costabel *et al.*, 1986). The present data, however, point to the preferential expansion of a particular $CD4^+$ cell subset at the sites where inflammation develops. Although, at the moment, follow-up is too short to firmly establish the clinical predictive value of these findings, our data seem to lead to the conclusion that more accurate evaluation of the T cell phenotype in BAL fluid is required to establish the usefulness of BAL analysis in sarcoidosis, and to further improve our knowledge of the role played by cellular immunity in sustaining sarcoid alveolitis.

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