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Altered immunological reactivity in alveolar macrophages from patients with sarcoidosis

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Altered immunological reactivity in alveolar macraphages from patients with sarcoidosis. R.B. Gallagher, M. Guckian, A. van Breda, C. Odlum, M.X. Fitzgerald, C. Feighery.

ABSTRACT: Lung macrophages may play an important role in the pathogenesis of pulmonary sarcoidosis. In this study, the ability of pulmonary macrophages and blood monocytes from sarcoidosis patients, normal controls and disease controls to provide the accessory signal necessary for the concanavalin A-induced activation of normal blood T cells was examined. Blood monocytes from all groups supplied a significantly greater accessory signal than lung macrophages. The accessory capacity of lavage macrophages from sarcoidosis patients varied over a wide range and correlations were sought between these values and other parameters of disease activity. Whilst there was no correlation with clinical parameters, accessory function of alveolar macrophages correlated significantly with the percentage of T helper cells in bronchoalveolar lavage (BAL) fluid ($p < 0.05$) and, more closely, with the T helper:T suppressor ratio in BAL fluid ($p < 0.01$). This interrelationship between macrophage activity and the T cell infiltrate favours the probability that both cell types participate in the sarcoid disease process and raises the possibility that T cells of both helper and suppressor phenotypes contribute to the pathogenesis. *Eur Respir J. 1988, 1, 153–160.*

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Sarcoidosis is a multisystem disorder of unknown cause, characterized by chronic inflammation and granuloma formation at sites of disease activity [17]. Investigations into the disease have been largely confined to pulmonary studies, as the lung is the most frequently affected organ [6] and provides a ready source of cellular material via bronchoalveolar lavage (BAL). Analyses of BAL fluid have shown that the development of lung granulomata is secondary to a substantial inflammatory infiltrate found in the early stages of the disease [13, 14]. This infiltrate is characterized by the presence of T cells, predominantly of the helper phenotype [12] which, in several studies have been shown to be activated [18, 19, 21]. It has therefore been proposed that sarcoidosis has an immunological aetiology, with the T helper cell response ultimately resulting in granuloma formation [6].

Recently, attention has also focused on the macrophage, the other inflammatory cell type involved in the early stages of sarcoidosis. Macrophages constitute over 90% of lavage cells in the normal lung and whilst this proportion is decreased in the sarcoid lung, absolute numbers are increased [4]. Furthermore, it has been shown that a number of these cells are in an activated state [10, 11] and may therefore be directly involved in the disease process. Since macrophages can act as essential accessory cells in lymphocyte activation, the immunological competence of pulmonary macrophages in sarcoidosis is of great interest. In the experiments reported here we have measured the accessory function of BAL macrophages and blood monocytes from patients with sarcoidosis and from controls. In the assay employed, the ability of these cells to supply accessory function for the activation of T lymphocytes by the mitogen concanavalin A was measured. This assay system was chosen because allogeneic combinations of patient accessory cells and normal T cells could be used so that any abnormality in the responses could be directly attributed to accessory cell function.

Subjects and methods

Clinical details

Bronchoalveolar lavage was performed on seventeen patients in whom the diagnosis of pulmonary sarcoidosis was established by standard criteria, including biopsy. This group included nine females and eight males, mean age 40 ± 14 yr. Further clinical details are given in table I. A mixed group of non-sarcoid lung disease patients undergoing lavage were used as disease controls (table II). These included two patients with rheumatoid lung disease, two with alveolar proteinosis, one with fibrosing alveolitis and one with interstitial lung disease. The mean age was 39 ± 12 yr and the group included two females and four males.

Four normal controls, with no previous history of lung disease and normal chest X-rays also underwent

Table I. - Patient details.

Patient	Sex	Age yrs	X-Ray stage	Disease duration yrs	Treatment	Smoker
1	F	69	2	6	-	-
2	F	46	1	2	-	-
3	F	30	2	0.5	-	-
4	M	34	2	1	+(1)	-
5	F	49	2	20	-	-
6	M	45	3	4	-	-
7	M	33	3	1	-	-
8	F	26	2	0.5	-	+
9	M	41	3	1	+(2)	-
10	M	21	3	4	-	-
11	M	46	3	1	-	-
12	M	64	1	0.5	-	-
13	F	17	2	2	-	-
14	F	46	3	2	-	-
15	F	30	2	0.5	-	+
16	M	43	3	5	-	-
17	F	46	3	0.5	-	-

(1) Prednisolone 25mg/day, (2) prednisolone 40mg/day.

Table II. - Disease controls.

Patients	Sex	Age	Treatment	Smoker	Clinical details
1	M	28	-	-	Alveolar proteinosis
2	M	32	(1)	-	Fibrosing alveolitis
3	M	54	-	-	Rheumatoid lung
4	F	46	-	-	ILD
5	F	26	-	-	Alveolar proteinosis
6	M	49	(2)	-	Rheumatoid lung

(1) Prednisolone 30mg/day. (2) Azathioprine 100mg/day. ILD: interstitial lung disease.

bronchoalveolar lavage. All were male and non-smokers, mean age 31 ± 6 yr.

Processing of bronchoalveolar lavage fluid

The volume of fluid recovered was recorded and, after removing mucus by straining through sterile surgical cotton gauze, the total cell count was determined. The cells were washed twice in phosphate buffer saline (PBS) and aliquoted for monoclonal antibody analysis and cell separation.

Monoclonal antibody staining

Cell preparations were assessed by immunofluorescence using monoclonal antibodies against total T lymphocytes (Leu 4, Becton-Dickinson), T helper and T suppressor subsets (Leu 3a and Leu 2a), NK cells (Leu 7) and HLA-DR positive cells (anti-HLA-DR, Becton-Dickinson). Alveolar macrophages and blood monocyte populations were also examined using Mo2 (Coulter), an antibody which detects cells of the monocyte/macrophage lineage.

Preparation of alveolar macrophages

Cells from BAL were washed and resuspended in RPMI 1640 medium (Gibco) containing 10% foetal calf serum (FCS) (Seralab), at a concentration of 5×10^6/ml. These were incubated in plastic tissue culture bottles for an hour at 37°C following which nonadherent cells were washed off and adherent cells

detached using a rubber policeman. This adherent population, which was always >95% Mo2-positive, was exposed to 2500 Rads irradiation to prevent proliferation in the subsequent culture, and kept on ice until use.

Preparation of monocytes

Blood was collected into heparinized tubes, diluted ×2 using Hanks Balanced salt solution (HBSS) (Gibco) and layered onto Ficoll-hypaque density separation medium (Pharmacia). After centrifugation mononuclear cells were collected, washed and separated into adherent and nonadherent populations and irradiated as described above, the adherent population being used as a source of monocytes.

Preparation of T lymphocytes

The nonadherent population obtained by plastic adherence of peripheral blood mononuclear cells (see above) was applied sequentially to two nylon wool columns which were incubated for 45 min at 37°C. Cells eluted from the second column were used as the T cell preparation and were always found to be >95% OKT3-positive.

Accessory cell function assay

2×10^5 T cells per well were cultured in the presence or absence of 5×10^4 accessory cells (alveolar macrophages or blood monocytes) with or without concanavalin A (Con A) at a final concentration of 1μg/ml in 200 μl. In a preliminary series of experiments this ratio of T cells to accessory cells (4:1) was found to be optimal and the concentration of Con A used (1μg/ml) found to give the best discrimination between the groups of accessory cells studied.

Tritiated thymidine (3H-TdR) was added for the final 18 hours of the 72 hour culture, after which time the cells were harvested and lymphocyte proliferation assessed by liquid scintillation counting. Triplicate cultures of each preparation were performed.

Results and statistics

To minimize the effect of inter-experimental variation, the results of proliferation assays are, where indicated, presented as percentages of the value obtained using control blood monocytes. Thus in every experiment, allogeneic control blood monocytes were included and the value obtained taken as 100%. In figures showing comparisons between groups of data, the statistical analyses used were the Wilcoxon rank test and Spearman's rank correlation.

Results

Characterization of cell populations recovered by lavage

The results of monoclonal antibody studies on the lavage samples from sarcoidosis patients, disease control patients and normal volunteers are presented in tables III, IV and V. As can be seen from table III, the percentage of T cells found in the lavage of sarcoidosis patients varied greatly, such that the arbitrary division of patients into low and high intensity alveolitis, as used by other authors [1, 20] was deemed inappropriate.

Table III. - Analysis of T cell component of BAL cells in sarcoidosis patients.

Patient	Sex	%T cells	% T helper	% T suppressor	Th:Ts ratio
1	F	20	11	9	1.2
2	F	12	10	2	5.0
3	F	20	16	4	4.0
4	M	37	34	4	8.0
5	F	20	15	6	2.6
6	M	17	9	7	1.5
7	M	58	22	36	0.6
8	F	3	1	2	0.7
9	M	5	3	3	1.0
10	M	45	39	7	6.0
11	M	43	17	28	0.6
12	M	27	23	8	3.0
13	F	73	55	14	4.0
14	F	36	29	12	2.0
15	F	3	1	3	0.3
16	M	41	31	10	3.0
17	F	8	3	5	0.5

The results shown are the percentage of lavage cells reacting positively with Leu 4 (total T cells), Leu 3a (T helper cells) and Leu 2a (T suppressor cells).

Table IV. - Analysis of lavage T cells from disease controls

Patient	Sex	% T cells	% T helper	% T suppressor	Th:Ts ratio
1	M	10	7	3	2.4
2	M	28	10	18	0.5
3	F	7	5	2	2.0
4	F	16	8	8	1.0
5	M	26	5	21	0.2
6	M	10	3	7	0.5

The results shown are the percentage of lavage cells reacting positively with Leu 4 (total T cells), Leu 3a (T helper cells) and Leu 2a (T suppressor cells).

Table V. - Analysis of lavage T cells from normal controls

	Sex	% T cells	% T helper	% T suppressor	Th:Ts ratio
1	M	6	4	2	2.0
2	M	17	8	9	1.0
3	M	10	6	4	1.5
4	M	14	11	3	3.5

The results shown are the percentage of lavage cells reacting positively with Leu 4 (total T cells), Leu 3a (T helper cells) and Leu 2a (T suppressor cells).

Demonstration of the necessity for accessory cells in the response to concanavalin A

Figure 1 gives the results of a representative experiment, showing the uptake of tritiated thymidine by purified T cells cultured with concanavalin A in the presence and absence of BAL macrophages. This figure demonstrates that T cells will not proliferate in the absence of accessory cells and shows that BAL macrophages can supply accessory function. The level of response recorded when the two cell types were cultured together in the absence of concanavalin A was found to be very low throughout these experiments.

Accessory function of alveolar macrophages and blood monocytes in sarcoidosis

The accessory function of alveolar macrophages and blood monocytes was assessed by their ability to support T lymphocyte proliferation in the presence of Con A. In figure 2, the results of these assays are represented as percentages of the response obtained using normal blood monocytes, the accessory function of which was taken as 100% in each individual experiment. In the three groups studied, namely sarcoidosis patients, disease controls and normal controls, blood monocytes provide a greater level of help than lung macrophages from each of these groups ($p < 0.01$). Alveolar macrophages from sarcoidosis patients provided $78 \pm 41\%$ of the help provided by normal blood monocytes while disease and normal control alveolar macrophages provided only 47 ± 27 and $56 \pm 15\%$ respectively. The number of samples in the two control groups ($n = 4$ and $n = 6$) were too small for statistical analysis. When the results from the two normal groups were pooled and compared to the sarcoidosis group the differences did not reach significance ($p < 0.09$), reflecting the very wide range of responses measured in the sarcoidosis group (13–156%).

When blood monocytes were used as accessory cells no differences were found amongst the three groups, sarcoidosis patients supplying $103 \pm 29\%$ and disease controls $111 \pm 25\%$ of the normal blood monocyte levels (fig. 3).

Correlation between accessory function and alveolitis

Correlations were sought between the accessory function of lavage macrophages and the degree and nature of the pulmonary T cell infiltrate. A striking correlation ($p < 0.01$) was found between the T

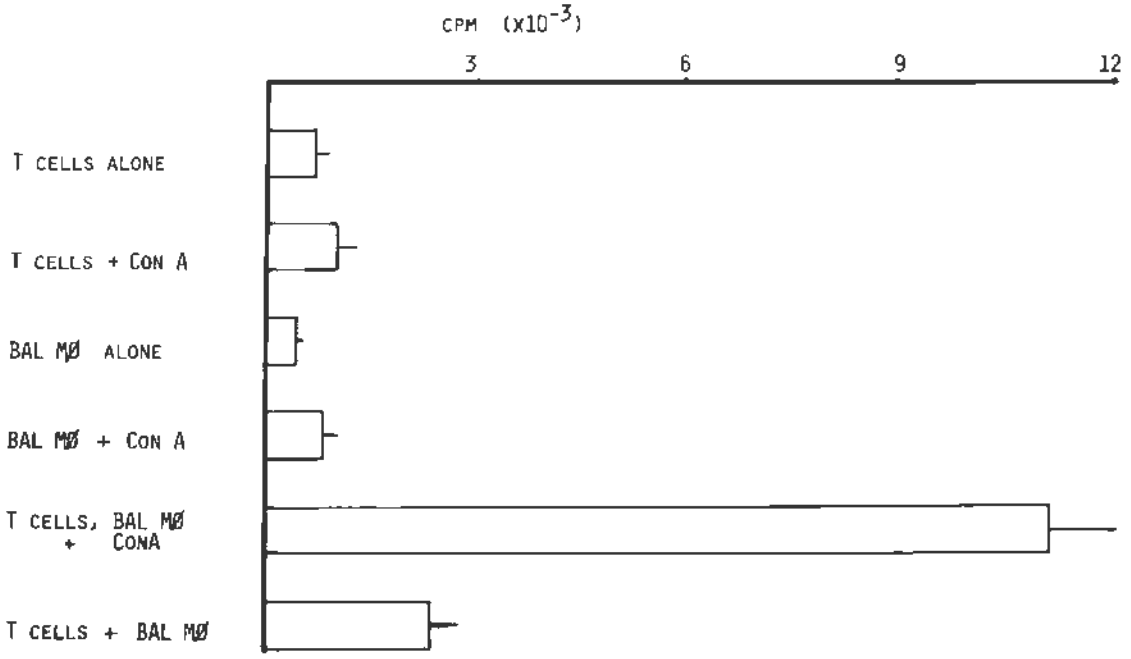

Fig. 1. BAL macrophages can act as accessory cells in the T cell response to Con A. Results shown are the mean ± SD for uptake of 3HTdR included during the final 18 h of a 72 h incubation at 37°C. These results are from a normal control.

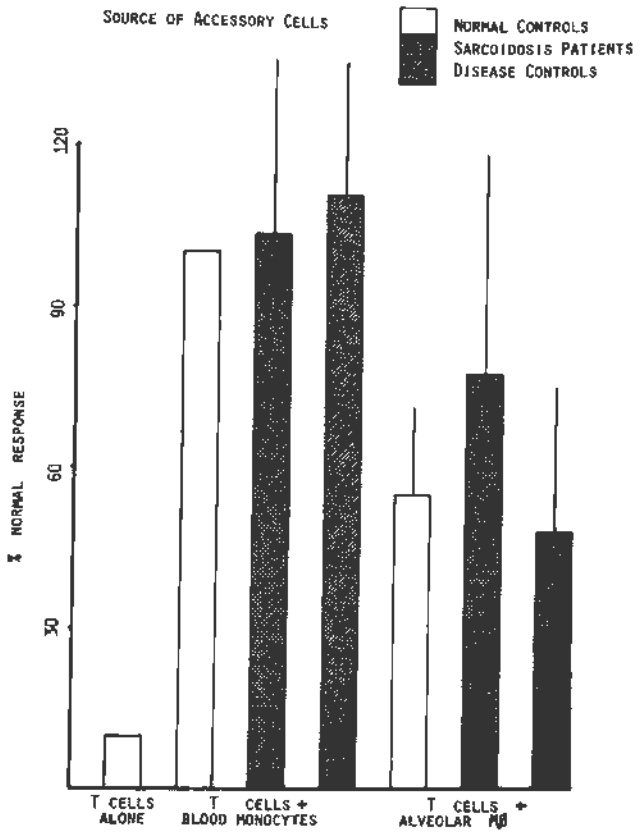

Fig. 2. Accessory function of blood monocytes and alveolar macrophages from sarcoidosis patients and controls. This figure shows the proliferation of T cells in the presence of Con A (1μg/ml) and in the presence and absence of accessory cells. Results are presented as percentage of the response induced using normal peripheral blood monocytes (taken as 100%). Results are mean ± SD of triplicate cultures.

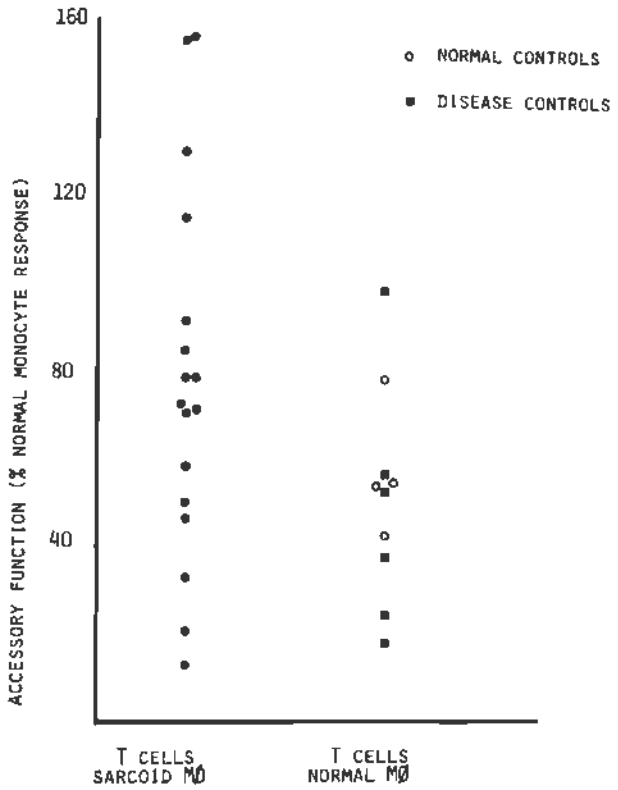

Fig. 3. This figure shows accessory function of BAL macrophages from individual sarcoidosis patients and controls as measured by the ability to induce uptake of 3HTdR in T cells cultured with Con A. Results expressed as in figure 2.

helper:T suppressor ratio of lavage T cells and accessory function of macrophages from the same source. This information is depicted in figure 4. A significant correlation ($p<0.05$) was also found between the percentage of helper T cells in the alveolitis and accessory function (fig. 5). It is of interest that accessory function did not significantly correlate with the severity of the alveolitis as measured either in absolute numbers of T cells or in percentage of T cells present in lavage fluid. Also no significant correlations were found between accessory function and clinical parameters, namely X-ray staging and duration of disease (results not shown).

Discussion

In this study the role played by alveolar macrophages in the development of pulmonary sarcoidosis was examined. This was done by measuring the capacity of these cells to provide the accessory signal for the proliferative response of normal blood T cells to Con A. The ability of macrophages from sarcoidosis patients to supply accessory signals was shown to be very variable (13–156% of the help provided by normal peripheral blood monocytes). It is of interest that the level of help correlated significantly with the percentage of T cells found in lavage fluid and even more closely with the T helper:T suppressor cell ratio in the fluid. In contrast, the level of help provided by both normal and disease controls fell within a narrower range ($56\pm15\%$ and $47\pm27\%$ respectively) and accessory function did not correlate with pulmonary T cells.

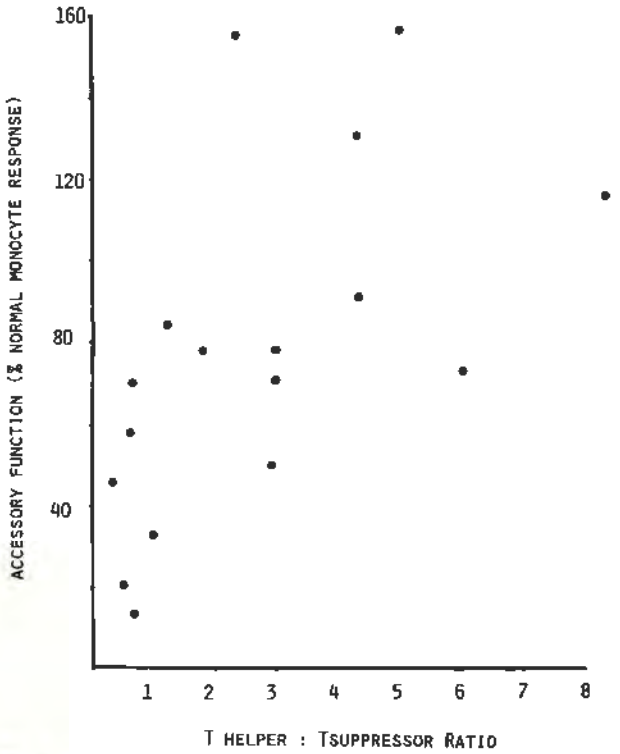

Fig. 4. This figure shows the Th:Ts ratio of BAL T cells plotted against accessory capacity of BAL macrophages (from sarcoidosis patients) assessed by their ability to induce proliferation of T cells cultured with Con A ($r=0.71$, $p<0.01$).

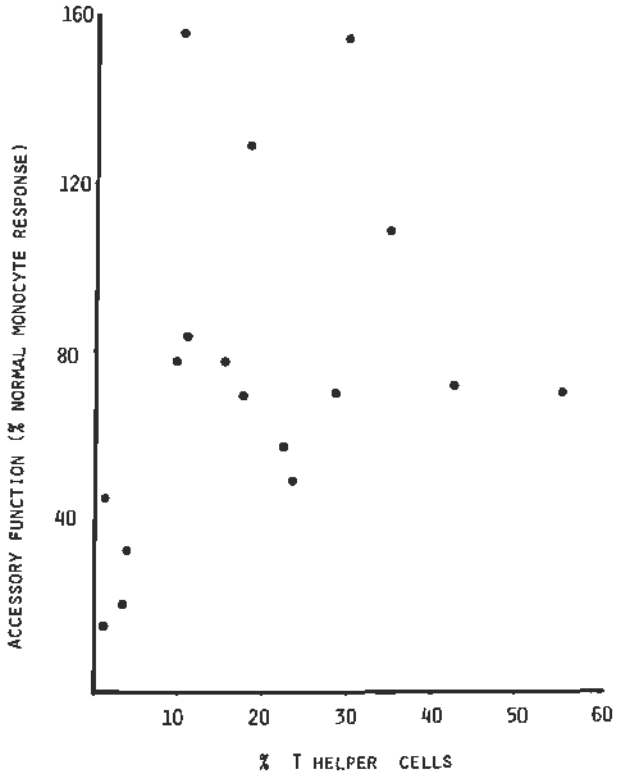

Fig. 5. This figure shows the percentage of T helper cells in lavage populations plotted against accessory capacity of BAL macrophages (from sarcoidosis patients) assessed by their ability to induce proliferation of T cells cultured with Con A ($r=0.51$, $p<0.05$).

The immunological competence of pulmonary macrophages is currently the subject of some controversy. We have found lavage macrophages to be significantly poorer accessory cells than peripheral blood monocytes. This is in agreement with the findings of some groups [22] and at odds with those of others who have shown BAL macrophages to be superior to monocytes [8, 9] or to be completely immunologically inert [15, 16]. Such contradictions must result from the variety of materials and methods employed and it is not known which, if any, of these conditions reflect the physiological situation.

It has previously been suggested by VENET *et al.* [22] that sarcoid pulmonary macrophages display enhanced immunological function. However, as autologous BAL macrophages and blood T lymphocytes were used in this study, the increased response to antigen could not be conclusively assigned either to enhanced antigen presentation or to altered T cell responsiveness. Further evidence of increased reactivity of BAL macrophages in sarcoidosis is given by reports of large quantities of interleukin 1 being constitutively produced, and increased levels of HLA-DR expression by these cells [3]. In this study we have shown that macrophage function is also intimately

associated with the lymphocyte alveolitis. None of these alterations in cellular reactivity are mirrored in the peripheral blood monocytes of sarcoidosis patients which provide an accessory signal equivalent to that of control responses to mitogen (this study) and antigen [22].

Our finding of a very close correlation between macrophage accessory function and T helper:T suppressor ratio (Th:Ts) suggests that T cells of both phenotypes contribute to the disease process. In support of this, the subgroup of sarcoidosis patients with a Th:Ts ratio of 1 or less ($n=6$) have an accessory capacity of $40\pm21\%$ whilst for the subgroup with Th:Ts greater than 1 the accessory capacity is 98 ± 35 ($n=11$). This difference is highly significant ($p<0.01$) and may reflect a role for both T cell subclasses. The finding is very interesting in light of recent suggestions that the T helper:T suppressor ratio is an important prognostic indicator of pulmonary sarcoidosis [5], although this has been challenged by other studies [2, 7].

Although the triggering factor(s) have not yet been identified, all the ingredients necessary to mount an active immune response are present in the lower respiratory tract in the early stages of sarcoidosis. Once triggered, the following sequence of events could be postulated: macrophages present antigen (from the triggering agent) to T cells, resulting in their activation; these T cells in turn produce factors which further enhance macrophage function; with progression an increasing spiral of cell activation would result. If these mechanisms are operative in sarcoidosis it would suggest that, rather than being a disorder of T helper cells, the cellular events in the lung follow the typical pattern of a normal immune defence to an extrinsic agent. In the majority of patients a localized, self-limiting immune reaction takes place. In others, a more progressive disease develops with continuing participation of activated helper cells and enhanced accessory function; T suppressor cells may play a role in down-regulating the immune response.

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RÉSUMÉ: Les macrophages alvéolaires pourraient jouer un rôle important dans la pathogénie de la sarcoidose pulmonaire. Dans cette étude nous avons examiné le pouvoir des macrophages alvéolaires et des monocytes sanguins, provenant de malades

sarcoidosiques et de sujets contrôlés sains et malades, de fournir les signaux accessoires, nécessaires à l'activation par la Concaviline-A des cellules T sanguines normales. Dans tous les groupes les monocytes sanguins fournissent des signaux accessoires plus grands que les macrophages alvéolaires. Le pouvoir des macrophages alvéolaires des sujets sarcoidosiques voire largement de sorte que des corrélations furent recherchées entre ces valeurs et d'autres indices d'activité de la maladie. Alors qu'il n'y a aucune corrélation avec les données cliniques, le pouvoir des macrophages alvéolaires corrélait avec le pourcentage de celluels T auxiliaires dans le liquide de lavage ($p < 0.5$) et de façon plus étroite avec le rapport cellules T auxiliaires/cellules T suppressives ($p < 0.01$). Cette interrelation activité macrophagique-infiltration à cellules T suggère que les deux types cellulaires sont impliqués dans le processus sarcoidosique et que les cellules T suppressives aussi bien que les T auxiliaires pourraient contribuer à la pathogénie de la sarcoidose.