# T Lymphocyte Motility toward IL-1 in Patients with Interstitial Lung Diseases

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## Synopsis

T Lymphocyte alveolitis is considered to be a prerequisite for the formation of epithelioid cell granuloma in sarcoidosis and for the subsequent inflammatory processes in interstitial lung diseases with lung lymphocytosis. To investigate whether T cell accumulation in the lungs is dependent on the recruitment of blood T cells into the lungs, we examined the T cell motility toward IL-1, which has been reported as a T cell chemoattractant, by using a microchemotactic assay. The number of blood T cells migrating toward IL-1 significantly ($p<0.001$) increased when compared with that toward medium. Inferring from the findings that the copresence or pre-incubation of IL-1 with T cells inhibits T cell motility, we confirmed IL-1 as a T cell chemoattactant. In minute amounts of IL-1 (0.000025pg/ml), the number of T cells migrating toward IL-1 significantly increased in sarcoidosis ($n=19$) and interstitial lung diseases (ILD) with lung lymphocytosis ($n=5$), compared to healthy subjects ($n=17$). A raised T cell motility toward IL-1 could be shown in both blood and BALF T cells in sarcoidosis. Conclusively, raised T cell motility toward IL-1 in an in vitro chemotactic assay might contribute to the accumulation of T cells in the lung of patients with sarcoidosis and other ILD.

## Introduction

Pulmonary sarcoidosis is characterized by T lymphocyte alveolitis and epithelioid cell granuloma formation[4,12]. T lymphocyte alveolitis is considered to be a prerequisite for the formation of granuloma. T cell which comprise alveolitis are activated and produce lymphokines which commit granuloma formation and then surround it[7]. Some of other interstitial lung diseases also show lung lymphocytosis, which is considered to play an important role in the development of inflammatory processes in the lungs. The mechanisms of lung lymphocytosis have not yet been elucidated completely. One possible resolution could be an in situ T cell proliferation which depends on a localized production of IL-2[19]. However, a substantial IL-2 activity in the culture supernatant of T cells in patients with pulmonary sarcoidosis was not detectable[14,15]. Another possibility could be an increased T cell recruitment toward the lung. However, no reports have demonstrated whether T cell recruitment into the lungs occurs as the result of blood T cell activation or an increased production of T cell chemoattractants in the

lungs. On the other hand a minute amount of IL-1 is proven to be a T cell chemoattractant in in vitro studies[8,11]. Though we could not detect a significant increase of IL-1 activity released from alveolar macrophages (AM) in patients with sarcoidosis, a low but substantial IL-1 activity could be detected in both sarcoidosis and healthy subjects under non-stimulated conditions[14,16].

In this study, we tried to examine the role of IL-1 as a T cell chemoattractant and the possibility of an increased T cell recruitment into lungs of patients with ILD.

## Materials and Methods

*Study Population*

Pulmonary Sarcoidosis : Among 19 sarcoidosis patients, 12 cases were diagnosed by transbronchial lung biopsy (TBLB). Further, all of them were diagnosed clinically (chest X ray findings, elevated sACE and BALF lymphocytosis). The population consisted of 14 males and 5 females. They were all non-smokers with a mean age of 37.0±2.5 years.

Other Interstitial Lung Diseases (ILD) : Five cases were selected : idiopathic pulmonary fibrosis (IPF : n=2), interstitial pneumoniae associated with RA (IP-RA : n=1), bronchiolitis obliterans with organizing pneumonia (BOOP : n=1), and mixed connective tissue disease (MCTD : n=1). Three cases were males and 2 were females with the mean age of 55.5±2.3. All cases were non current smokers, which were diagnosed by open lung biopsy. No corticosteroids were given to them. The lavage study showed lung lymphocytosis in all cases (Table 1).

Table 1. BALF CELLS FINDINGS OF THE STUDY GROUPS

| | Number | BALF | | | | BLOOD | |
|---|---|---|---|---|---|---|---|
| | | cells ($\times 10^5$/ml) | Lymphocytes (%) | $E^+_{37}$ cells (%) | $CD4^+/CD8^+$ (ratio) | $E^+_{37}$ cells (%) | $CD4^+/CD8^+$ (ratio) |
| $SAR_{(NCS)}$ | 19 | 1.79±2.27@** | 28.3±5.7* | 26.5±5.5** | 3.89±0.82 | 20.0±3.4 | 1.53±0.21 |
| $IP_{(NCS)}$ | 5 | 1.59±0.32** | 17.3±11.6* | 26.4±12.8** | 0.58±0.1** | 17.7±8.0 | 1.76±0.4 |
| $H_{(NCS)}$ | 17 | 0.53±0.09 | 11.1±1.67 | 3.7±0.41 | 3.19±0.49 | 16.4±2.18 | 1.90±0.23 |

@ : Mean±SE. SAR: Active sarcoidosis. NCS: Non-current smokers. IP: Interstitial pneumoniae. H: Healthy. * Significant to $H_{NCS}$ ($p<0.05$) ** Significant to $H_{NCS}$ ($p<0.01$)

Healthy Controls : Seventeen healthy subjects with a mean age of 41.2±2.6 years included 5 males and 12 females. All healthy subjects had a normal physical examination, laboratory test, chest X-ray film and pulmonary function test.

*Purification of BALF and blood T cells*

BALF and peripheral blood (PB) T cells were prepared according to the method of Nagai et at[14]. Rosette forming T cells were further purified by passing on a washed nylon wool column and incubated for 1 hour at 37°C. Nonadherent cells were eluted from the column with warm RPMI 1640 supplemented with 10% fetal calf serum (FCS). Subsequently the rosette forming T cells were washed twice with minimal essential medium (MEM) and counted.

*Preparation of AM culture supernatants*

Non-rosette formimg cells ($1x10^6$/ml) were further purified by plastic adhesion at 37°C for 1 hour. After the incubation, the BALF AM were washed with warmed MEM to remove non-adherent cells. RPMI 1640 supplemented with 15% FCS was added to AM which had adhered on the bottom of the culture plates (24 well tissue culture plate : Falcon 3047, Oxnard, USA), and AM was cultured with or without lipopolysaccharide : LPS (B E coli 055 : B5, Difco, Detroit, USA) for 24 hours at 37°C under 5% $CO_2$. Then, AM sup was drawn off and stored at -20°C until use.

*Lymphocyte chemotaxis assay*

Purified T cells were incubated for 18 hours at 37°C under 5% $CO_2$ and recounted before use in the assay. The migration of T cells was measured, using 48 blind well micro-chemotaxis chambers (Neuroprobe Inc, Bethesda, USA). An 8$\mu$m nitrocellulose filter separated the upper compartment from the lower compartment. Twenty-five microliter of T cells ($2x10^6$ cells/ml) suspended in RPMI 1640 (medium) were placed in the upper compartment of a chamber. The lower one was filled with 25$\mu$l of the medium alone or various concentrations (2500 pg/ml to 0.000025 pg/ml) of standard IL-1 (2500 pg/ml, Genzyme, Boston, USA) . The chambers were incubated for 3 hours at 37°C in 5% $CO_2$. Then, the filters were fixed with methanol and stained with haemotoxylin[2]. The membrane was made transparent with xylene and the cells migrating through the filters could be examined microscopically. Using a x40 objective, the distance from the focal plane at the basement of the filter to the focal plane of the fastest migrated cells (the cells must be at least 3 in number, along one focal plane) was measured in micrometers, using the fine focus. At the same time, the number of the fastest migrated cells in that focal plane was counted[22].

Triplicated wells were used for each experiment and three high power fields were examined for each well.

*Lymphocyte chemotactic assay in the presence of AM sup*

The AM culture sup (100 times dilution) was used instead of IL-1. To determine whether the present chemotactic activity is IL-1 or not, we pre-incubated the sup with 25 units of anti-human IL-1$\beta$ monoclonal antibody (mouse, $IgG_1$ : 500 units, Cistron, Biotech, NJ, USA) for 30 minutes and thereupon a micro-chemotactic assay was done. As control, we pre-incubated the sup with 5$\mu$g/ml of anti-human IgG anti-body (mouse, Tago, Burlingame CA, USA).

*Statistics*

All data were expressed as the mean$\pm$SE. Statistical significance was assessed by a two-tailed students' t-test. A p value less than 0.05 was considered significant.

## Results

*The effect of the pre-incubated purified blood T cells before a chemotactic assay*

The motility of purified T cells toward IL-1 was assayed, before or after 18 hours incubation. The results showed no difference in the number of migrated T cells toward both medium and IL-1 in the lower compartments between, before (medium : 65.0$\pm$0.9, IL-1 : 73.7$\pm$1.3 (n=3)) or after incubation (medium : 63.0$\pm$5.2, IL-1 : 69.3$\pm$1.9 (n=7)). Chemotactic assays were usually done after 18 hours incubation.

*Number of blood T cells migrating toward various concentrations of IL-1*

T cells from healthy nonsmokers (HNS) were put into the upper compartment, whereas various dilutions of IL-1 ($10^{-1}$ to $10^{-8}$ dilution were loaded into the lower comparment of a micro-chemotactic chamber. As a result a chemotactic migration of T cells toward IL-1 was detected in all concentrations of IL-1 comparing with the number of migrated T cells toward medium (Fig. 1). However, no significant difference in the number of the fastest migrating cells

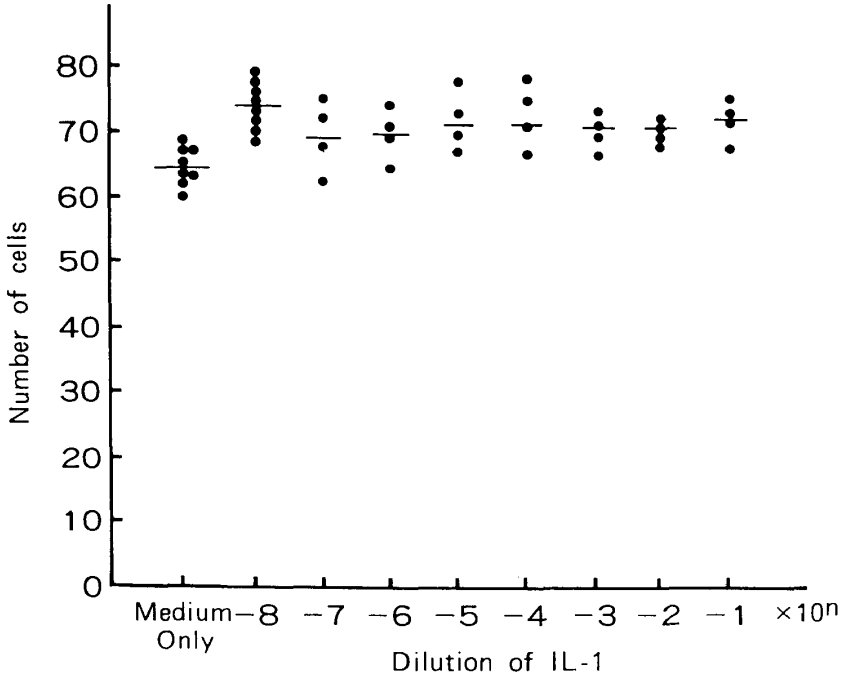


**Fig. 1.** The number of blood T cells migrating toward various concentrations of IL-1 in healthy subjects. Standard IL-1 was diluted by medium and the number of the fastest migrated cells toward IL-1 was counted.

toward various concentrations of IL-1 could be shown. Consequently, we selected $10^{-8}$ dilution (0.000025 pg/ml) of IL-1 as a test concentration for further experiments.

*Number of T cells migrating toward IL-1 in healthy subjects, sarcoidosis patients and other interstitial lung diseases*

The number of blood T cells migrating toward IL-1 ($10^{-8}$ dilution )($p<0.001$) increased significantly, compared with the number of cells migrating toward medium alone in all the examined groups (Fig. 2). A significant increase in the number of the fastest migrating blood T cells toward IL-1 could be detected in patients with sarcoidosis ($p<0.001$) and other ILD ($p<0.001$), compared to those in healthy subjects (HNS). No significant difference in the number of fastest migrating blood T cells could be show between sarcoidosis and other ILD (Fig. 2).

Furthermore a significant increase in the number of the fastest migrating BALF T cells could be found, whereas no difference in the number was detected between blood and BALF T cells (Fig. 2).

*Migration inhibition of blood T cells in the copresence of IL-1*

Both blood T cells and various concentrations of IL-1 were mixed in the upper compartment of a micro-chemotaxis chamber. In the lower compartment, medium instead of IL-1 was added. The distance of migrated blood T cells was measured. When blood T cells were placed in the upper compartments with IL-1 ($10^{-2}$ to $10^{-8}$ dilution: 25 pg/ml to 0.000025pg/ml), the motility of T cells was significantly ($p<0.001$) inhibited compared to that toward medium in the

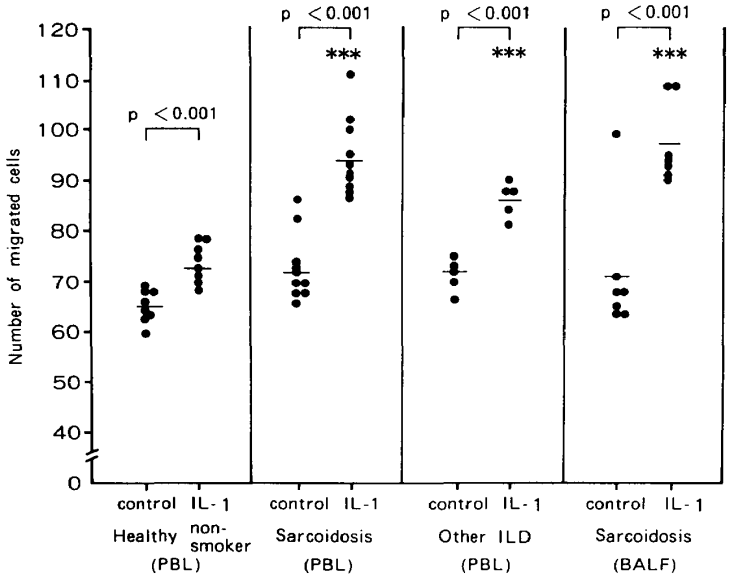


**Fig. 2.** The number of T cells migrating toward $10^{-8}$ diluted standard IL-1 . The number of the fastest migrating T cells toward IL-1 and the migration toward medium alone (negative control) were compared. (8 cases of HNS, 10 cases of active sarcoidosis and 5 cases of other ILD)
* * *Significant to HNS ($P<0.001$)

absence of IL-1 in the upper chamber (Fig. 3). Inhibition of migration was not statistically different in various concentrations of IL-1 in the upper chamber. Pre-incubated blood T cells with diluted IL-1 ($10^{-8}$) for 18 hours at 37°C under 5% $CO_2$ also significantly ($p<0.001$) inhibited the migration of T cells (Fig. 3).

*Blood T cell motility toward AM sup*

LPS stimulated and non-stimulated AM sup (100 times dilution) from HNS or patients with sarcoidosis were used as test samples instead of IL-1. There was no difference in the number of healthy T cells migrating toward AM sup between healthy subjects and sarcoidosis cases. Similarly, no difference in the number of migrating healthy T cells was observed when non-stimulated and LPS (10$\mu$g/ml) stimulated AM sup were compared. The number of T cells migrating toward AM sup was similar when T cells obtained from HNS were compared to T cells obtained from sarcoidosis (Fig. 4).

The number of cells migrating toward AM sup did not change when AM sup was pretreated with 25 units/ml of anti-IL-1$\beta$ monoclonal antibody. (Fig. 5).

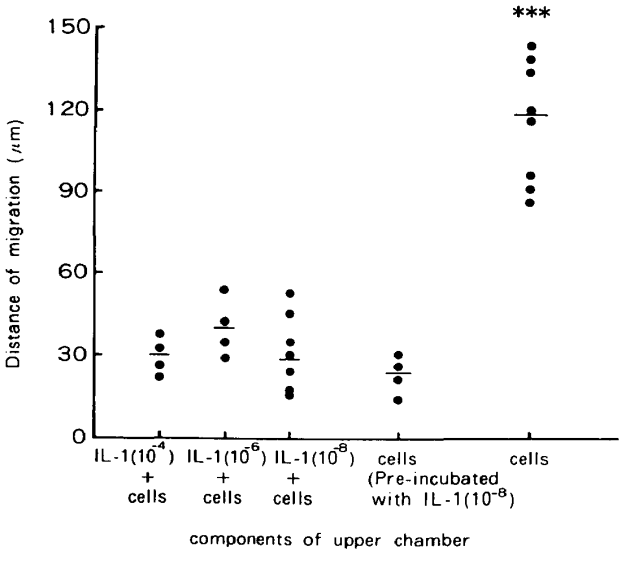


**Fig. 3.** Distance of migration ($\mu$m) of the blood T cells toward medium in HNS related to various concentrations of standard IL-1 ($10^{-4}$ to $10^{-8}$) and T cells pre-incubated with standard IL-1 ($10^{-8}$) for 18 hours.
* * *Significant to IL-1 ($10^{-8}$) +cells ($p<0.001$)

## Discussion

T lymphocyte alveolitis, which is found in sarcoidosis and other ILD is considered to play an important role in the processes of their pathogenesis. BALF T cells in patients with pulmonary sarcoidosis and other ILD were activated as shown in Table 1. The mechanism of T cell activation are obscure, but there is a possibility that lung macrophages are responsible[7]. Lung macrophages might have a responsibility for maintaining T lymphocyte alveolitis by functioning as accessory cells for T cell proliferation in situ[14].

T cell activation can be evaluated by various parameters including the increase of hot rosette cells, the expression of HLA-DR antigen and their capacity of producing lymphokines. T cells could be highly activated locally in the lung under the effect of cytokines or some

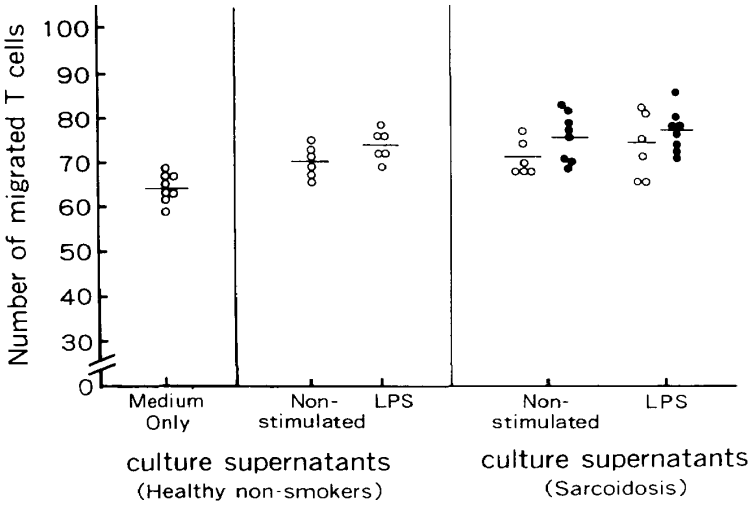


**Fig. 4.** The number of blood T cells migrating toward LPS (10$\mu$g/ml) stimulated BALF sup. Open circles express T cells obtained from HNS and closed circles those from sarcoidosis.

biologically active materials after their accumulation into the lungs.

T cell accumulation in the lung can be induced by chemoattractants such as IL-1 and IL-2[8,11]. One of the lymphokines, lymphocyte chemoattractant factor (LCF), has been isolated but not yet identified in clinical sarcoidosis[1]. All chemoattractants to T cells were only identified in healthy subjects, non of them were identified in sarcoidosis. In general it is very difficult to isolate a significant chemoattractant to T cells from tissue extracts or lavage fluid/culture supernatants.

An established T cell chemoattractant, however, can be used as a tool for evaluating T cell motility. As for chemotactic movement of cells depends on the interaction between a chemotactic factor and a specific receptor to the chemotactic factor on the referred cells. Therefore, the degree of expression of a specific receptor can determine the cell motility.

As expected, activated T cells show an enhanced motility toward T cell chemoattractants in patients with ILD, including sarcoidosis. The results show that the motility of both BALF and blood T cells in patients with ILD, including sarcoidosis increased compared with that in healty subjects (HNS). Moreover, IL-1 was confirmed to be a T cell chemoattractant.

Furthermore a statistically significant difference in the number of the fastest migrating T

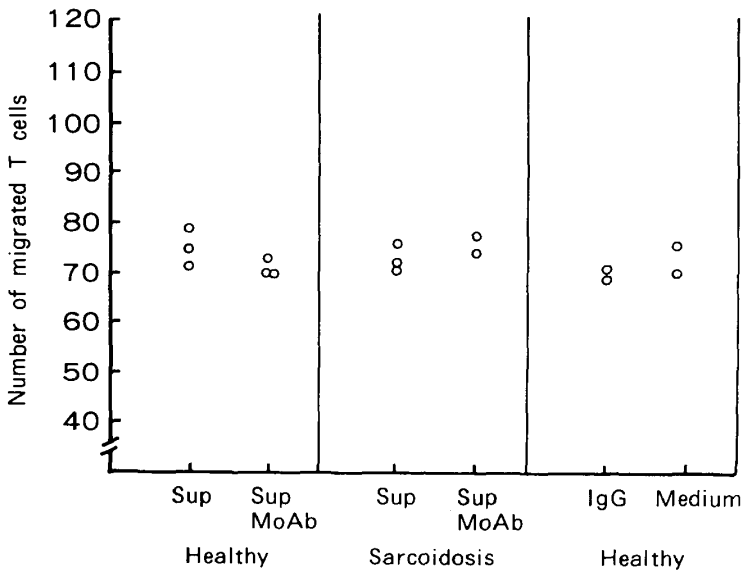


**Fig. 5.** The number of blood T cells migrating toward hundred fold dilute AM sup, AM sup pre-incubated with 25 units of anti-human IL-1$\beta$ monoclonal antibody and AM sup pre-incubated with 5$\mu$g/ml of anti-human IgG antibody in HNS and sarcoidosis cases.

cells toward various concentrations of IL-1 could not be observed. This is in contrary to an earlier report[8], in which migration of T cells toward IL-1 was detected only in $10^{-5}$ and $10^{-8}$ dilutions of IL-1. The disparity in observation might be due to the usage of different method in each assay.

Besides, we confirmed that the used concentration of IL-1 ($10^{-8}$) could not induce a substantial T cell proliferation. Moreover, this showed a true chemotactic activity and not a chemokinetic response from the finding of the copresence or pre-incubation with IL-1 inhibited the T cell motility.

By using similar concentrations of IL-1 ($10^{-8}$), we found that the number of blood T cells migrating toward IL-1 significantly increased in both patients with sarcoidosis and IP associated with collagen vascular disease (IP-CVD), compared to healthy subjects (HNS). Next we also demonstrated an increased BALF T cells motility in patients with sarcoidosis. We suspect that a T cell traffic has two directions : one is the movement into the lung, and another is that from the lung into the circulation. Though T cell movement might occur spontaneously in a in vitro system, as shown in the absence of IL-1 in the lower chamber, a specific chemotactic factor to T cells is required to detect a significant difference in T cell motility, which is related to the presence of a lung disease. According to our results, IL-1 is an effective T cell chemoattractant, which can detect a significant increased T cell chemotactic motility in both sarcoidosis and other ILD.

We did not examine whether an increase of T cell motility toward IL-1 is in parallel with the expression of the IL-1 receptor on activated T cells. We evaluated the increase of T cell motility toward IL-1 in ILD as one expression of T cell activation which is different from the expression of hot rosette cells. It is unknown whether this induced activation by IL-1 occurs in vivo. IL-1 activities in the AM sup without in vitro stimulation were not significantly higher in sarcoidosis and other ILD than in HNS[5,16]. However, a minute amount of IL-1 can function as

a T cell chemoattractant. Thus, we tried to detect a difference of T cell motility toward culture supernatants between sarcoidosis and HNS. The result showed no difference and even preincubation with anti-IL-1$\beta$ monoclonal antibody could not detect any difference. Furthermore, an increase of blood T cell motility toward IL-1 in sarcoidosis could not be demonstrated, compared to those toward culture supernatants. We have already reported that IL-1 inhibitory factors were significantly higher in AM sup[14,16]. Further study demonstrated that the IL-1 inhibitory factor was not an anti-IL-1 antibody and not one of the prostaglandins (Nagai et al., unpublished data). If IL-1 inhibitory factor binds to the IL-1 receptor[9], it is difficult to evaluate the effect of anti-IL-1$\beta$ monoclonal antibody. Namely, IL-1 in the sup can not show T cell chemotactic activity, as the IL-1 receptors on T cells might be occupied by IL-1 inhibitory factor. This possibility remains to be examined.

T cell activation and their accumulation in the lung can easily be found in patients with sarcoidosis and other ILD as shown in Table 1. One possible mechanism is that T cells are activated by unknown factors at extrathoracic sites, tend to accumulate primarily into the lungs. We have two evidences, acquired in in vivo studies: one is that subclinical T lymphocytosis could be found in patients with a collagen vascular disease showing normal chest radiography[21] and another is that lung lymphocytosis was shown Crohn's disease[20] and the presence of extrathoracic lesions causes higher lung lymphocytosis in patients with sarcoidosis[21]. One in vivo animal study pointed out that accumulation of T cells in the lung occurred always significantly compared to other organs, independent of the sensitization route in mice[10]. This phenomenon can be understood from the following points of view: the production of chemotactic factors to T cells in the lung is significantly highter than in other organs, or activated T cells are expanded and migrate into the lungs by unknown mechanisms related to the presence of allergic diseases. Thus the activation and accumulation of T cells in lung might be concerted events, in which lung macrophages and activated T cell in both thoracic and extrathoracic sites play important roles in patients with sarcoidosis and other ILD.

An increased motility of BALF T cells toward IL-1 in patients with sarcoidosis may reflect an induced expression of the IL-1 receptor on T cells by locally produced IL-1, however, the significance of T cell traffic from the lung to the circulation remains to be established in interstitial lung diseases with lung lymphocytosis.

Conclusively, increased T cell motility toward IL-1, as detected by in vitro chemotactic assays, might contribute to the accumulation of T cells in the lung in sarcoidosis and other ILD with lung lymphocytosis.

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