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Research Article

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Spontaneous Release of Interleukin 2 by Lung T Lymphocytes in Active Pulmonary Sarcoidosis Is Primarily from the Leu3+DR+ T Cell Subset

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Abstract

The inflammation within the lower respiratory tract of individuals with pulmonary sarcoidosis is dominated by large numbers of helper T lymphocytes that proliferate and spontaneously release interleukin 2 (IL-2). To identify the lymphocyte subpopulation that releases IL-2 in this disorder, lung lymphocytes recovered by bronchoalveolar lavage were characterized using the monoclonal antibodies Leu4 (T lymphocyte), Leu3 (helper/inducer), Leu2 (suppressor/cytotoxic), and anti-HLA-DR, and separated by panning and flow cytometry. The majority of the IL-2 spontaneously released by T cells in the sarcoid lung was contributed by the Leu3+ cell population (Leu3+ 65±23 IL-2 units released/ 10^6 cells per 24 h; Leu2+ 9±8, $P < 0.04$). Further characterization of the lung Leu3+ T cells in sarcoid demonstrated that 30±3% were expressing HLA-DR molecules on their surface compared with 6±1% in normals ($P < 0.01$). Importantly, the subpopulation of Leu3+ lung T lymphocytes expressing a high intensity of HLA-DR molecules on their surface was responsible for the majority of the release of IL-2 in the sarcoid lung (Leu3+ high-intensity DR 42±17 U/10^6 cells per 24 h, Leu3+ low-intensity DR 8±1 U/10^6 cells per 24 h; $P < 0.01$). Thus, the spontaneous release of IL-2 in the lung of sarcoid patients appears to be localized to a subset of Leu3+ high-intensity DR ("activated" lung helper/inducer) T lymphocytes. Because the sarcoid lung is characterized by markedly increased numbers of these cells, it is likely that this compartmentalized T cell population plays a major role in sustaining the exaggerated localized immune processes of this disorder.

Introduction

Pulmonary sarcoidosis is a disorder of the lower respiratory tract characterized by chronic inflammation, granuloma formation, and, in some individuals, parenchymal fibrosis (1–4). Together, these processes derange the alveoli, airways, and blood vessels, consequently impairing the ability of the lung to exchange gas in a normal fashion (5, 6). As with the other interstitial lung disorders, it is recognized that the inflammation precedes the other abnormalities that characterize this disorder (7, 8).

The inflammation of active pulmonary sarcoid is dominated by an accumulation of T helper lymphocytes in the lung parenchyma (9, 10). These T cells are thought to play a central role in the pathogenesis of sarcoidosis in two ways. First, the accumulated T cells distort the architecture of parenchyma, thus altering the intimate relationships between air and blood (11). Second, the T cell populations are activated and spontaneously release monocyte chemotactic factor and interferon-γ, mediators that recruit and activate mononuclear phagocytes, respectively, which are events that are early steps in the process of granuloma formation (12, 13).

In this context, an understanding of the pathogenesis of pulmonary sarcoid is intimately linked to understanding the processes directing the accumulation of T lymphocytes in the lower respiratory tract of individuals with active disease. Relevant to this question, it is known that the T lymphocytes recovered from the lungs of these patients are spontaneously proliferating and spontaneously releasing interleukin 2 (IL-2),[1] the T cell growth factor (14, 15). Thus, although the stimulus that initiates the process is unknown, the IL-2-releasing lung T cells are thought to be responsible for maintaining the T cell alveolitis and thus maintaining the disease in an active state.

The present study expands upon this concept by determining those T cell subsets that are responsible for the spontaneous release of IL-2 in the lower respiratory tract of these individuals. The data demonstrate that the IL-2-releasing cells are the lung Leu3+ (helper/inducer) T lymphocytes expressing a high intensity of HLA-DR antigens on their surfaces. Because this is the same subset of T lymphocytes that help in vitro proliferation of T cells in response to antigens, these observations lend credence to the concept that the T cell alveolitis of sarcoid is an exaggeration of the normal processes of T cell activation.

Methods

Study population

Normals. The control population consisted of 22 normal individuals, including 15 men and 7 women, with a mean age of 26±5 yr (all data are presented as mean±standard error of the mean). None had a history of lung disease and none had evidence of lung disease by physical examination, chest X-ray, and pulmonary function tests. All were nonsmokers; no control was taking medication at time of the study.

Patients with sarcoidosis. The diagnosis of pulmonary sarcoidosis was established in 40 individuals using previously described criteria (2), including a lung or mediastinal node biopsy. There were 16 men and 24 women with a mean age of 37±1 yr. 22 were nonsmokers, 9 were current smokers, and 9 had not smoked for >2 yr. No subject was taking medication at the time of evaluation. Evaluation of chest X-rays showed 5 were type I, 22 type II, and 12 type III (16). As a group[2] the sarcoid patients showed impaired pulmonary function characterized by reduced lung volumes (vital capacity, 71±3% predicted; total lung capacity, 72±2%

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1. *Abbreviations used in this paper:* FCS, fetal calf serum; IL-2, interleukin 2.
2. Owing to the difficulties in obtaining sufficient numbers of lung lymphocytes from the lungs of sarcoid patients, some experiments were performed on only a small portion of the total patient group. In this context, all data are presented in figures as single data points for each patient.

predicted) and diffusing capacity (DLCO 73±5% predicted) without limitation to airflow (forced expiratory volume in 1 s/forced vital capacity 103±4% predicted). The 40 individuals underwent a total of 57 evaluations by bronchoalveolar lavage.

Bronchoalveolar lavage

Lung mononuclear cells were obtained by bronchoalveolar lavage as previously described (17). Briefly, 100 ml of sterile saline in five 20-ml aliquots were infused through a fiberoptic bronchoscope (Olympus BF-2, Olympus Corp. of America, New Hyde Park, NY) into each of three lobes of the lower respiratory tract and epithelial lining fluid was recovered by gentle suction. The cells recovered were washed three times in RPMI-1640 (Biofluids, Rockville, MD) before being used. Cell differential counts were determined as previously described (18) on Millipore filter preparations (Millipore Corp., Bedford, MA) stained with hematoxylin and eosin.

Blood mononuclear cells

Autologous blood mononuclear cells were obtained by venipuncture prior to the lavage procedure. Mononuclear cells were separated by Ficoll-Hypaque centrifugation (19). Thereafter, the cells were handled in a manner identical to the lung cells.

Monoclonal antibodies

Fluorescein, biotin, or phycoerythrin-conjugated mouse monoclonal antibodies Leu4 (T lymphocytes [20]), Leu3 (helper/inducer T-cell subset [21]), Leu2 (suppressor/cytotoxic T-cell subset [21]) and the anti-HLA-DR mouse monoclonal antibody L243 (HLA-DR framework [22]) were from Becton, Dickinson & Co. (Sunnyvale, CA). The monoclonal antibody anti-Tac (IL-2 receptor [23]) was a gift of W. Leonard (National Cancer Institute, Bethesda, MD). Fluorescein-conjugated mouse monoclonal IgG_1 and IgG_2 and phycoerythrin-conjugated mouse monoclonal IgG_1 controls were also from Becton, Dickinson & Co. Mouse monoclonal RPC5 (IgG_2) was from Bionetics (Kensington, MD). Monoclonal antibodies anti-Tac and RPC5 were purified by ammonium sulfate precipitation and protein A chromatography (Pharmacia Fine Chemicals, Piscataway, NJ) and subsequently conjugated with biotin using 0.1 mg/ml of biotin *N*-hydroxysuccimide ester (Calbiochem-Behring Corp., LaJolla, CA), 1 mg/ml purified antibody in 10% dimethyl sulfoxide, 0.1 M bicarbonate buffer). Biotin-conjugated RPC5 was used as a control.

Two-color immunofluorescence

Because of the broad spectrum autofluorescence of human alveolar macrophages causing possible artifacts in flow cytometric analysis of unfractionated cells recovered by bronchoalveolar lavage, all quantitative analysis of one- and two-color monoclonal antibody binding to the surface of lymphocytes was carried out by a combination of phase-contrast and immunofluorescence microscopy as previously described (24). Briefly, cells (0.5×10^6) were aliquoted into V-shaped wells (96 wells/plate; Flow Laboratories, McLean, VA) and incubated with 50 μl of "blocking medium" containing 0.1 mg/ml human IgG (Sigma Chemical Co., St. Louis, MO) and 0.1 mg/ml deoxyribonuclease (Sigma Chemical Co.) in phosphate-buffered saline (PBS; M. A. Bioproducts, Walkersville, MD) to prevent nonspecific staining of dead cells and of Fc receptor-bearing cells. After centrifugation and decanting the "blocking medium," cells were incubated for 30 min with optimal concentrations of the two primary monoclonal antibodies (fluorescein or biotin-conjugated) in 50 μl of "staining medium" containing 1% bovine serum albumin (Sigma Chemical Co.) and 0.2% sodium azide (Sigma Chemical Co.) in PBS. Cells were washed three times with "staining medium" and incubated 20 min with 1 μg of rhodamine-conjugated avidine in 50 μl of "staining medium." Cells were then washed three times with the same medium, fixed for 15 min in 10% buffered formalin, washed two times with "staining medium," and stored in 50% glycerol-PBS until examined. The proportion of cells reacting with each monoclonal antibody was determined by examining the cells in suspension by means of the combination of phase-contrast microscopy and epifluorescence at × 600 magnification using a Standard 16 microscope fitted with a 100-W mercury light source, epifluorescence condenser, and fluorescein and rhodamine filter sets (Carl Zeiss, Inc., New York).

Separation of lung lymphocyte subpopulations

Two methods were used to separate lung lymphocytes into subpopulations identified by monoclonal antibodies: "panning" and flow cytometry.

The "panning" technique (25) was carried out to separate lung Leu3+ and Leu2+ T lymphocytes by positive selection of the Leu2+ cells. Positive selection for the Leu2+ cells (rather than negative selection with the Leu3 antibody) with the Leu2 antibody was chosen for two reasons: (*a*) this approach has proven excellent in selecting small proportions of cells (25); and (*b*) the Leu2 antibody does not alter the function of the positively selected cells as the T8 antibody (26). Lung T lymphocytes, obtained by adherent cell depletion through nylon wool columns (13), were incubated on ice for 30 min with purified Leu2 monoclonal antibody (0.5 $\mu g/10^6$ cells) in Hanks' balanced salt solution (HBSS; M. A. Bioproducts) containing 5% fetal calf serum (FCS; Biofluids) and washed twice with the same medium. Cells were then incubated at 4°C in 100 × 15 or 60 × 15-mm petri dishes (Falcon Labware, Oxford, CA) coated with 20 μg/ml of affinity-purified goat antimouse IgG (Cappel Laboratories, Cochranville, PA). Leu3+ cells were gently washed off (negative selection) the dishes with cold HBSS-5% FCS, and Leu2+ cells were obtained (positive selection) by scraping the plates with a rubber policeman. After washing twice with HBSS-5% FCS, cells were resuspended in culture medium. The purity of the separated populations was assessed by immunofluorescence with fluorescein-conjugated Leu2 and Leu3 antibodies. Viability, determined by trypan blue exclusion, was always >85%.

Flow cytometry (27) was used to separate Leu3+ and Leu2+ lung lymphocytes and Leu3+ lung lymphocytes into "high-intensity DR" and "low-intensity DR" subpopulations. Lung T lymphocytes, obtained from bronchoalveolar lavage cells depleted of adherent cells through nylon wool columns, were incubated for 30 min on ice at a concentration of 10^7/ml in 5-ml round-bottomed tubes (Falcon Labware) with 20 μg/ml of fluorescein-conjugated Leu3 and phycoerythrin-conjugated L243 (anti-HLA-DR) in "sheath medium" (HBSS without phenol red [M. A. Bioproducts], 100 U/ml penicillin, 100 μg/ml streptomycin [M. A. Bioproducts], and 1% FCS [Biofluids]). In some experiments, lung T lymphocytes were reacted with fluorescein-conjugated Leu3 and phycoerythrin-conjugated Leu2 to separate Leu3+ and Leu2+ lung lymphocytes. Cells were then washed two times, resuspended in "sheath medium" and separated using a FACS II (Becton, Dickinson & Co., FACS Systems, Sunnyvale, CA) operated using an argon laser at the wavelength of 488 nm to excite fluorescein and phycoerythrin. Forward angle light scatter was used to discriminate viable lymphocytes from dead cells and the small numbers of mononuclear phagocytes present (27). Sorted cells were collected in RPMI-1640 without phenol red (M. A. Bioproducts) on ice and reanalyzed through the FACS II to assess viability, purity, and HLA-DR fluorescence intensity of the sorted cell populations. After centrifugation, cells were resuspended in culture medium for use. Viability, determined by forward angle light scatter, was always >95%.

Quantification of IL-2 release

IL-2 release by lung T cells was assessed as previously described (14). Briefly, lung mononuclear cells or purified lymphocytes were cultured for 24 h in a 5% CO_2 humidified incubator at 37°C, in RPMI-1640 (M. A. Bioproducts) containing 1% FCS, 0.2 mM glutamine (M. A. Bioproducts), 100 U/ml penicillin, and 100 μg/ml streptomycin at a concentration of 10^6 cells/ml in 24-well plates (Costar, Cambridge, MA). As a control, parallel cultures were incubated with 5 μg/ml concanavalin A (Sigma Chemical Co.). At the end of the culture period the supernatants were collected by centrifugation and stored at −20°C until assayed for IL-2 activity by their ability to stimulate the incorporation of [^{3}H]thymidine (Amersham International, Amersham, UK) by IL-2-dependent CT6 murine T-lymphocytes. IL-2 activity was expressed as units/10^6 cells per 24 h as based on Probit analysis in comparison with an international standard, (Biological Response Modifier Program reference

reagent human IL-2, Biological Resources Branch, National Cancer Institute, Frederick Cancer Research Facility, Frederick, MD).

Data analysis

Statistical comparisons of IL-2 release by lung lymphocyte subpopulations were made using the two-tailed Student's t test on the logarithm of the IL-2 units plus one. Logarithm transformation was made to reduce the inequality of the variance (28). All other comparisons were made using the two-tailed Student's t test.

Results

Lymphocytes present in the lower respiratory tract of sarcoid patients. Comparison of the inflammatory cell populations recovered from individuals with sarcoidosis and normals demonstrated that the alveolitis of pulmonary sarcoid is characterized by large numbers of Leu3+ T lymphocytes expressing HLA-DR (Table I). Bronchoalveolar lavage was performed without difficulties in both groups, and there were no significant differences in the percentage of lavage fluid recovered from patients and normal controls ($P > 0.2$; Table I). Consistent with previous observations, more cells were removed from the sarcoid patients in the study ($P < 0.01$) and the proportions of inflammatory cells recovered that were lymphocytes was significantly elevated in sarcoid patients compared to normals ($P < 0.01$; Table I). Evaluation of T cell proportions with the Leu4 monoclonal antibody showed that most of lung lymphocytes recovered by lavage of sarcoid patients (92±3%) as well as in normals (87±5%) were T cells. However, because the lymphocytes comprised such a large proportion of the total inflammatory cell population in sarcoid, the lungs of sarcoid patients contained more than two-fold more T-cells (Table I; sarcoid 47±3% of the cells recovered by lavage, normals 18±2%, $P < 0.01$). Strikingly, evaluation of the subsets of T cells showed that the absolute increase of T lymphocyte numbers in sarcoid patients was almost entirely accounted for by marked expansion in the number of Leu3+ T lymphocytes. In this context, whereas 9±1% of all cells recovered from normals were Leu3+Leu4+ lymphocytes, fourfold greater numbers (37±3%) of Leu3+Leu4+ lymphocytes were recovered from the patients with sarcoid (Table I; $P < 0.01$). In marked contrast, Leu2+Leu4+ lymphocytes accounted for only 10±1% of the cells recovered by lavage in sarcoid patients, a proportion similar to that of normals (7±1%; Table I; $P > 0.1$).

Table I. Characteristics of Inflammatory Cells Recovered from the Lower Respiratory Tract of Individuals with Sarcoidosis Compared with Normals

Parameters	Normal	Sarcoidosis
Individuals evaluated (*n*)	22	40
Evaluations (*n*)	22	57
Lavage fluid recovered (%)	47±3	57±2
Total number of cells recovered ($\times 10^7$)	38±4	78±6*
Cell differential (*% cells recovered*)		
Lymphocytes	21±2	48±2*
Neutrophils	1±1	2±1
Eosinophils	1±1	1±1
Alveolar macrophages	76±4	48±3*
Subtypes of lymphocytes (*% cells recovered*)		
Leu4+	18±2	47±3*
Leu3+	9±1	37±3*
Leu2+	7±1	10±1
Expression of surface antigens associated with activation		
Tac+ (*% 3+ cells*)	8±1	6±1
Tac+ (*% 2+ cells*)	1±1	1±1
DR+ (*% 3+ cells*)	6±1	30±3*
DR+ (*% 2+ cells*)	6±1	22±3*
Spontaneous release of IL-2 (*units/10^6 cells per 24 h*)	0	35±5*

* Significantly different from normals at $P < 0.01$ (two-tailed Student's t test).

In contrast to the lung T lymphocytes of patients with sarcoid, the blood helper T cells from the same individuals were decreased in number. In normals, the proportion of blood lymphocytes that were Leu4+Leu3+ was 38±2% while, in contrast, in sarcoid patients it was 27±2% ($P < 0.01$). Thus, there was a striking compartmentalization of the Leu3+ T cells in patients with sarcoid, with a marked accumulation in the lung, i.e., at the site of disease.

Spontaneous release of IL-2 by lung T lymphocytes of patients with sarcoidosis. Consistent with prior studies (14), the lung mononuclear cells recovered from the study population with sarcoidosis spontaneously released IL-2 (Table I). Whereas no IL-2 could be detected in the supernatants of cells cultured from the lungs of normal individuals, an average of 35±5 U of IL-2 spontaneously were released/10^6 cells per 24 h ($P < 0.01$). Thus, the inflammatory cell populations in the sarcoid lung differed from the normal lung in two ways: (*a*) There were more Leu3+ T cells in the sarcoid lung; and (*b*) the T cells in the sarcoid lung, but not the normal lung, were spontaneously releasing IL-2. This is not to say, however, that normal T lymphocytes were not capable of releasing IL-2; when normal lung mononuclear cells were incubated with concanavalin A (5 μg/ml) for 24 h, amounts of IL-2 comparable to that spontaneously released in the sarcoid lung were readily detected (data not shown). Thus, normal lung lymphocytes were capable of responding to an activation signal by releasing the T cell growth factor but were different than sarcoid lung T cells in that they are not already activated "in vivo" to do so. The sarcoid lung T cell population was also different from the autologous blood lymphocyte population: in none of the patients did blood T cells spontaneously release detectable amounts of IL-2. However, as with normal blood T-cells, when stimulated in vitro, the sarcoid blood T cells readily released IL-2.

Separation of lung Leu3+ from Leu2+ T cell populations recovered from the lung of sarcoid patients demonstrated that the majority of the IL-2 was being spontaneously released by the lung Leu3+ T-lymphocytes (Fig. 1). The purity of the isolated Leu3+ lung T cell population from the sarcoid lung was of 94±2% with a contamination of Leu2+ T cells of <5% and a macrophage contamination <10%. For the lung Leu2+ T cell population, the purity was 87±7% with a contamination of Leu3+ T cells of <5% and macrophages <10%. Using these preparations, the IL-2 activity present in supernatants harvested from unstimulated sarcoid lung Leu3+ T-lymphocyte cultures was of 65±23 U/10^6 cells per 24 h (3.83±0.60 log transformed units). In contrast, only 8±8 U/10^6 cells per 24 h (1.29±0.77 log transformed units) were detected in supernatants from unstimulated sarcoid lung Leu2+ T lymphocyte cultures ($P < 0.04$).

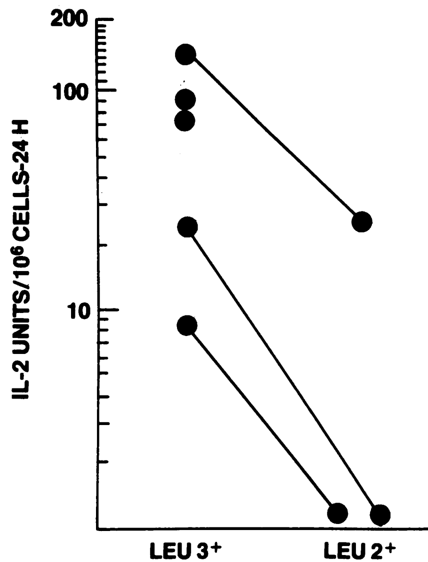

Figure 1. Spontaneous release of IL-2 by Leu3+ (helper/inducer) and Leu2+ (suppressor/cytotoxic) T lymphocytes recovered from the lungs of patients with pulmonary sarcoidosis. The lung Leu3+ and Leu2+ T lymphocyte populations recovered by lavage were separated by panning or flow cytometry and supernatants from 24-h unstimulated cultures were assessed for IL-2 activity expressed as units/10^6 cells per 24 h.

Expression of HLA-DR molecules on the surface of lung Leu3+ cells in sarcoidosis. Consistent with the concept that the lung T cells in sarcoidosis are "activated," two-color immunofluorescence analysis demonstrated that strikingly increased proportions of lung Leu3+ T lymphocytes in the sarcoid lung expressed HLA-DR molecules (Table I). In these individuals, the proportion of Leu3+ cells that reacted with the anti-HLA-DR monoclonal antibody was markedly higher (30±3% of Leu3+ cells) than the proportion of lung Leu3+ lymphocytes in normals that were DR+ (6±1% of Leu3+ cells, $P < 0.01$ compared to sarcoid). In the sarcoid lung Leu2+ T-cells also expressed surface HLA-DR (22±3% of Leu2 cells; Table I). However, owing to the significantly lower numbers of Leu2+ cells in the lower respiratory tract of these patients, the burden of Leu2+DR+ cells in the sarcoid lung (3±1% of cells recovered) was significantly lower than that of Leu3+DR+ cells (13±1% of cells recovered; $P < 0.001$ compared to Leu2). In addition to HLA-DR, lung 3+ T cells in sarcoidosis also expressed the IL-2 receptor. Although 6±1% of Leu3+ cells were Tac+ (Table I), only 1±1% of Leu2+ T cells were Tac+. The proportions of Leu3+ cells that were Tac positive were not significantly different in sarcoid from normal (8±2% of Leu3+, $P > 0.1$ compared with sarcoid). However, because of the much larger numbers of lung Leu3+ cells in sarcoidosis, the density of Leu3+Tac+ cells in the sarcoid lung is greater than normal (sarcoid 2.1±0.3%, normal 0.8±0.1% of total cells recovered; $P < 0.01$ compared with sarcoid). Sarcoid patients also had increased numbers of T cells expressing activation antigens in the blood. In contrast to normals, an increased proportion of sarcoid blood Leu3+ T cells expressed DR (14±1% of Leu3+ cells, normal 1±1%; $P < 0.01$) and the IL-2 receptor (10±2% of Leu3+ cells, normal 1±1%, $P < 0.01$). However, the proportions of Leu3 cells that express DR in sarcoid blood were significantly less than in the lungs of autologous patients (lung 30±3% of Leu3+, blood 14±1%, $P < 0.01$). Furthermore, although sarcoid blood Leu3+ T cells did express activation antigens, this was not accompanied by spontaneous release of IL-2.

Spontaneous release of IL-2 by Leu3+ high-intensity DR lung T lymphocytes in sarcoidosis. To evaluate whether the release of IL-2 was confined to a subset of sarcoid lung Leu3+ cells, we arbitrarily divided the study population into those with <18% of lung Leu3+ expressing DR and those with >18% lung Leu3+ cells expressing DR and compared this with the amount of IL-2 spontaneously released by the whole population of recovered cells ([Fig. 2; spontaneous IL-2 release data from Table I divided into group "Leu3+DR+ ≤ 18% Leu3+" and "Leu3+DR+ > 18% Leu3+"; the value of 18% was chosen for the groups because 18% represents the highest proportion of lung Leu3+DR+ ([3 SD above the mean] of normal individuals). The group with lung Leu3+ cells > 18% released significantly more IL-2 (41±6 U/10^6 per 24 h) than those with ≤18% lung Leu3+DR+ cells (8±6 U/10^6 cells) (Fig. 2; $P < 0.05$), suggesting that the Leu3+ cell population expressing DR may contain the functionally activated cells compartmentalized in the sarcoid lung that were releasing IL-2.

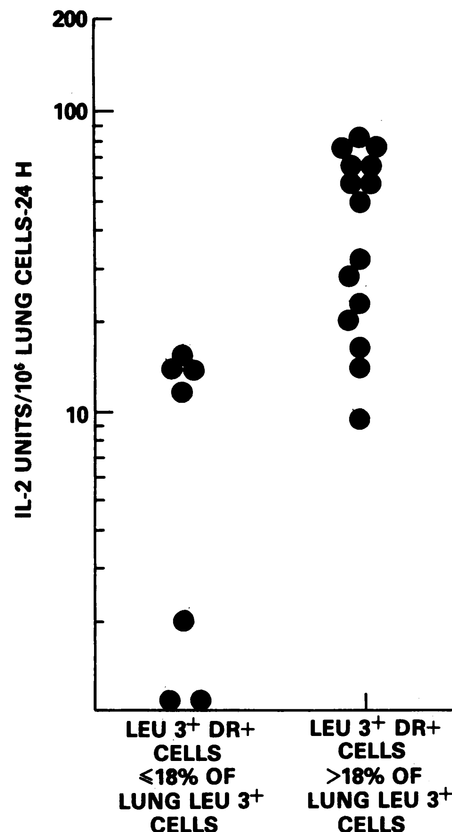

Figure 2. Comparison of the spontaneous release of IL-2 by lung lymphocytes of individuals with sarcoidosis with an exaggerated proportion of lung Leu3+ lymphocytes that are DR+ to individuals with sarcoidosis with proportions of lung Leu3+ lymphocytes that are DR+ that are within the normal range. The data shown represent the IL-2 release data in Table I grouped according to the relative proportion of lung Leu3+ lymphocytes that also expressed DR as assessed by double-staining immunofluorescence. The dividing point of 18% for the % of Leu3+ cells that were DR+ was chosen because this is the value corresponding to 3 SD above the mean proportion of DR+ cells among the population of Leu3+ lung lymphocytes of normal individuals.

Consistent with the fact that IL-2 was being spontaneously released in the lungs of those individuals with large numbers of Leu3+DR+ lymphocytes in the lower respiratory tract, the proportion of Leu3+DR+ T cells in the sarcoid lung correlated with the total proportions of lung lymphocytes recovered (those individuals with Leu3+DR+ > 18% had lymphocytes representing 57±3% of cells recovered; those with Leu3+DR+ ≤ 18% had lymphocytes representing 35±3%, $P < 0.01$). Furthermore, the Leu3+DR+ > 18% group had a Leu3+ to Leu2+ ratio of 10±2 to 1 whereas the Leu3+DR+ ≤ 18% group had a Leu3+ to Leu2+ ratio of 3±1 to 1 ($P < 0.01$). The expansion of Leu3+DR+ lung T cell proportions in the sarcoid lung was also found in individuals with worse pulmonary function; the vital capacity and diffusing capacity (DLCO) were significantly lower in patients with proportions of Leu3+DR+ cells > 18% than those with ≤18% ($P < 0.01$). To directly determine whether the Leu3+ T lymphocytes that express the activation antigen HLA-DR in the lower respiratory tract of sarcoid patients were the functionally activated T cells that spontaneously release IL-2, the spontaneous release of IL-2 was evaluated in Leu3+ high-intensity DR and Leu3+ low-intensity DR sarcoid lung T lymphocytes separated by flow cytometry. The separated cells obtained were highly purified Leu3+ lymphocytes with an insignificant contamination of other cell types (Fig. 3, *E–F*). Analysis with the flow cytometer demonstrated that both Leu3+ high-intensity DR and Leu3+ low-intensity DR subpopulations contained 98±1 Leu3+ viable lymphocytes. Flow cytometric evaluation of the separated lung lymphocyte Leu3+ population demonstrated that HLA-DR proteins were expressed on the cell surfaces in varying intensity, consistent with known observations of activated T-cells (29–32). In this context, the Leu3+DR+ population was observed as a skewed peak of DR intensity (Figs. 3 *A–C*), which was separated into a high-intensity DR and low-intensity DR population by setting appropriate windows (Fig. 3 *D*). Analysis of the sorted populations demonstrated that the mean fluorescence channel of the high-intensity DR population was 21±1 while the mean fluorescence channel of the low-intensity DR population was 13±1 ($P < 0.01$). Evaluation of the size distribution of the high-intensity DR and low-intensity DR lung Leu3+ lymphocytes by forward angle light scatter showed no significant difference ($P > 0.05$), consistent with the concept that size alone could not explain the different intensity of DR expression on the sorted populations.

Analysis of the spontaneous release of IL-2 by these sorted populations demonstrated, strikingly, that almost all the IL-2 activity spontaneously released was produced by the Leu3+ high-

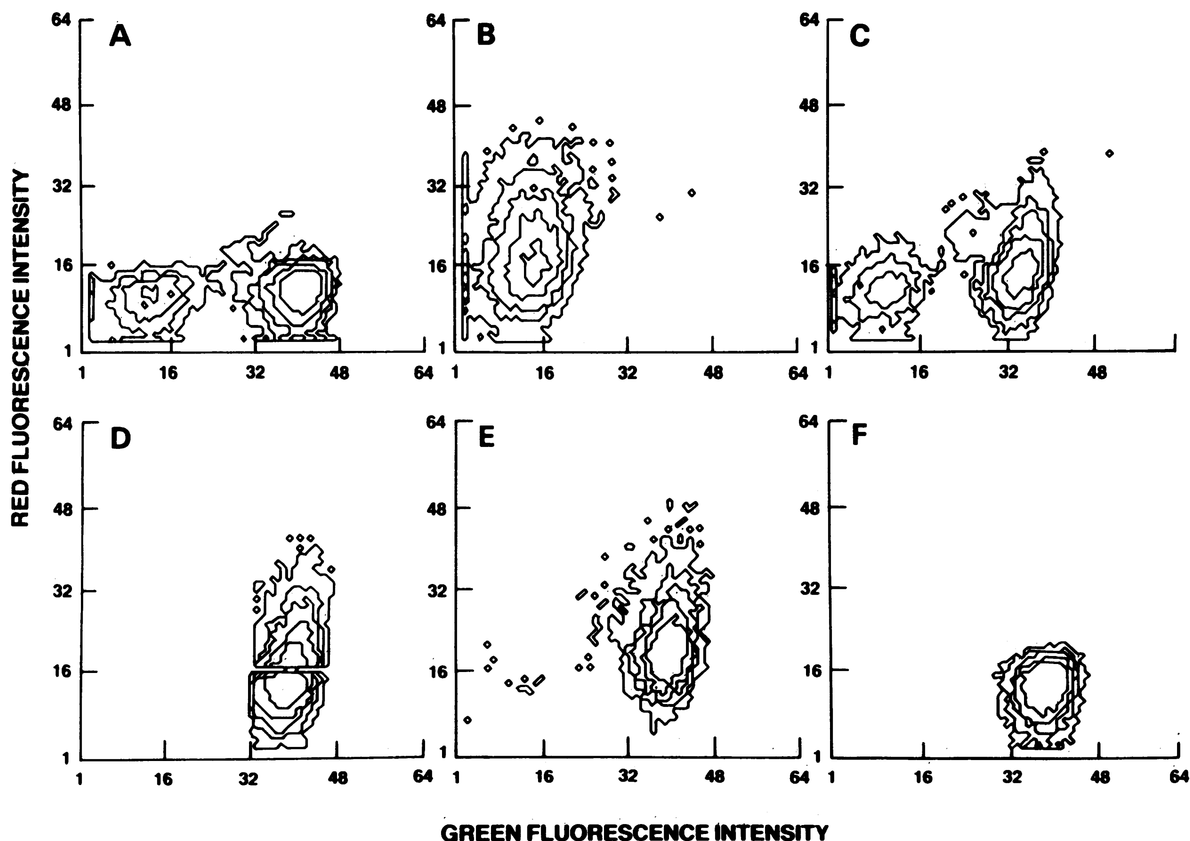

Figure 3. Separation of Leu3+ high-intensity HLA-DR and Leu3+ low-intensity HLA-DR lung lymphocytes from sarcoid patients by flow cytometry. The data are presented as two-parameter contour maps. Macrophage-depleted lung lymphocytes reacted with Leu3-fluorescein (green) (*A*) and anti-HLA-DR-phycoerythrin (red) (*B*) were analyzed with a FACS II flow cytometer to determine the windows for Leu3 and anti-HLA-DR-positive cells. Cells stained with Leu3 in combination with anti-HLA-DR (*C*) were then analyzed to gate between the Leu3+ low-intensity DR and Leu3+ high-intensity DR subpopulations (*D*), which were sorted and reanalyzed through the flow cytometer to assess the proportions of high-intensity DR cells present within the two sorted Leu3+ high-intensity DR (*E*) and Leu3+ low-intensity DR (*F*) subpopulations.

intensity DR subpopulation (Fig. 4). Of six of seven patients evaluated, Leu3+ high-intensity DR lymphocytes spontaneously released significant amounts of IL-2, whereas the autologous Leu3+ low-intensity DR lymphocytes released none or negligible amounts. Furthermore, even taking into account the one patient in whom the Leu3+ high-intensity DR and Leu3+ low-intensity DR lung lymphocytes populations were releasing comparable amounts of IL-2, on the average, for all of the individuals evaluated, the Leu3+ high-intensity DR cells were releasing fivefold more IL-2 (high-intensity 42±17 U/10^6 cells [3.30±0.56 log-transformed units]; low-intensity DR 8±1 U/10^6 cells [0.75±0.61 log-transformed units]; $P < 0.01$). Consistent with these observations, as a control, negative selection of the Leu3+ low-intensity DR population obtained using an anti-HLA-DR antibody and complement demonstrated that IL-2 release was abolished. Thus, the expression of a high intensity of HLA-DR surface molecules characterized the subpopulation of functionally activated Leu3+ T lymphocytes in the sarcoid lung that were spontaneously releasing the T cell growth factor. These observations are consistent with the concept that the accumulation and increased density of functionally activated Leu3+HLA-DR+ lung T lymphocytes in sarcoidosis may play a major role in expanding and maintaining the alveolitis by promoting proliferation and expansion of lymphocyte numbers through the augmentation of IL-2 concentration in the alveolar milieu.

Discussion

Pulmonary sarcoidosis is characterized by the accumulation of large numbers of T lymphocytes in the lower respiratory tract (9, 10). Unlike resting T cells, these sarcoid lung T cells are actively releasing IL-2, the T cell growth factor (14, 15), and are spontaneously proliferating (14, 15, 33, 34)—observations that led to the concept that lung T cells play a central role in maintaining the T cell alveolitis of sarcoidosis. The present study expands this concept by demonstrating that it is the lung Leu3+ (helper/inducer) T lymphocytes that are expressing a high intensity of HLA-DR molecules that are spontaneously releasing IL-2 and thus driving the persistent lung T cell inflammation that characterizes this disorder. Together with the knowledge that 30±3% of all lymphocytes in the sarcoid lung are Leu3+DR+ T cells, this suggests that active pulmonary sarcoid represents a markedly exaggerated immune response compartmentalized to the lower respiratory tract.

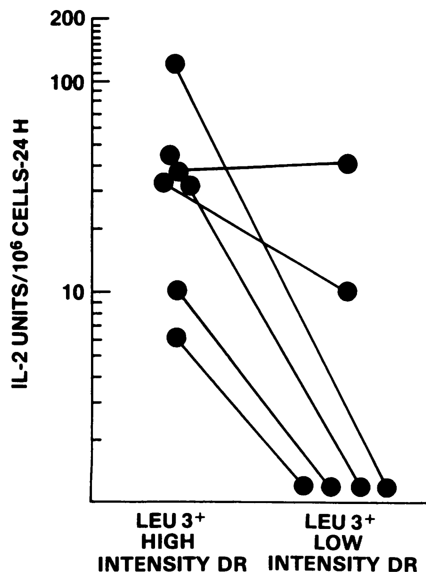

Figure 4. Spontaneous release of IL-2 by Leu3+ high-intensity DR and Leu3+ low-intensity DR lung lymphocytes from patients with sarcoidosis. Supernatants from unstimulated cultures (24 h, 37°C) of purified Leu3+ high-intensity DR and Leu3+ low-intensity DR lung lymphocytes were assayed for IL-2 activity expressed as units released/10^6 cells per 24 h.

T lymphocyte accumulation in the sarcoid lung. Pulmonary sarcoidosis is characterized by a marked accumulation of helper/inducer T cells in the lower respiratory tract, a remarkable example of how the immune system can compartmentalize within the body (35–39). The observations discussed here are consistent with our prior observations using single-monoclonal antibody analysis demonstrating the accumulation of OKT4+ (helper/inducer) T lymphocytes in the sarcoid lung, despite normal or reduced numbers of OKT8+ (suppressor/cytotoxic) lung T lymphocytes and relatively normal numbers of OKT4+ and OKT8+ T lymphocytes in blood (35). The accumulation of Leu3+ T cells in the lung in sarcoidosis has two profound consequences to the affected individual.

First, and perhaps most importantly, the T cells and mononuclear phagocytes recruited by the T cells take up space and, in doing so, distort the alveolar, bronchiolar, and vascular walls such that the normal spatial relationships among air and blood are disturbed, thus disorganizing the intimate patterns of air and blood flow that are required for normal gas exchange.

Second, lung T lymphocytes in sarcoidosis are activated in vivo to release monocyte chemotactic factor (12), a 12,500-D mediator that attracts blood monocytes along a concentration gradient (40), and interferon-γ (13), a 20,000–25,000-D protein (41) that can contribute to the activation of mononuclear phagocytes (42). Together, these mediators likely play an important role in the local aggregation of mononuclear phagocytes in the lung parenchyma into granulomata, space-occupying lesions that, if present in critical regions of the alveolar, bronchiolar, and/or vascular walls, contribute to altering lung function.

Role of IL-2 in the accumulation of lung T lymphocytes in sarcoidosis. T lymphocytes recovered from the lungs of patients with sarcoidosis are spontaneously releasing IL-2, suggesting that these cells are the driving force for the T cell proliferation ongoing in the lower respiratory tract, and hence for the chronic accumulation of lung T cells that characterize individuals with active disease. Several lines of evidence support this conclusion.

(*a*) Sarcoid lung lymphocytes proliferate at an increased rate compared with normal lung lymphocytes (14, 15, 33, 34).

(*b*) The release of IL-2 by sarcoid T cells is compartmentalized in that it is confined to sites of disease (e.g., lung) and is not observed in blood (14, 15).

(*c*) There is an increase in the total numbers of Leu3+Tac+ (IL-2 receptor) T cells in the lungs of patients with sarcoid (43). Thus, not only is there IL-2 released in the lower respiratory tract in increased amounts, but there are Leu3+ T-cells present that are capable of responding to this growth signal.

(*d*) When patients with active sarcoid are treated with corticosteroids, a drug that suppresses the expression of the IL-2 gene (44), the spontaneous release of IL-2 by lung T cells is suppressed as is their enhanced proliferation (45). Subsequently,

the proportion of lung Leu3+ T cells decreases and the number of lung lymphocytes returns to normal (37, 45, 46).

(*e*) In addition to driving T cells to proliferate (47), IL-2 has been shown to be chemotactic for OKT4+ (helper/inducer) T cells expressing the IL-2 receptor, but not OKT8+ (suppressor/cytotoxic) T cells (48). Thus, it is conceivable that the release of IL-2 in the lower respiratory tract may attract Leu3+Tac+ T cells as they pass through the pulmonary capillaries.

Cells responsible for the release of IL-2 in the sarcoid lung. Although it is recognized that all T cell subtypes have the capacity to release IL-2 (49–51), the present study demonstrates that the spontaneous release of IL-2 by T cells in the lung of sarcoid patients is due primarily to the Leu3+ T cell subset. Furthermore, fractionation of these Leu3+ T cells into those that are expressing HLA-DR at high intensity and those that are expressing DR at low intensity demonstrates that the majority of the IL-2 released in the sarcoid lung is by the Leu3+ high-intensity DR T cell subset.

The observation that the spontaneous release of IL-2 in sarcoid is confined to a specific subpopulation is consistent with the in vitro observations that: (*a*) soluble protein antigens, allogenic cells, and autologous mixed leukocyte reaction induce OKT4+, but not OKT8+, T cells to release IL-2 (50, 52); (*b*) mitogen activated OKT8+ human T cells can produce IL-2 but do so to a lesser extent than OKT4+ T cells (49); (*c*) in the mouse system, Lyt2− (helper) T cells release more IL-2 than do Lyt2+ (suppressor/cytotoxic) T cells (53), consistent with the observation that only a fraction of murine Lyt-2+ T cell clones release IL-2 after mitogenic stimulation (51); (*d*) in leprosy granulomas, the cells with IL-2 in the cytoplasm (presumably the IL-2 producers) are Leu4+Leu3+ and Leu4+Leu2− (54).

Furthermore, the fact that the Leu3+DR+ T cell subset is releasing IL-2 in the sarcoid lung supports the concept that the expression of DR molecules may identify an activated population of helper T cells that are releasing IL-2. Consistent with this concept, Reinherz et al. (55) have shown that in vitro activation of T-cells with tetanus toxoid results only in the OKT4+DR+ subset releasing "lymphocyte mitogenic factor."

Implications for the pathogenesis of sarcoidosis. Put in the context of a variety of studies relating to T cell activation and proliferation, the fact that there are large numbers of Leu4+Leu3+ high-intensity DR lymphocytes in the sarcoid lung, and that this cell subtype is spontaneously releasing IL-2 implies several things about the pathogenesis of this disease.

First, the presence of larger numbers of Leu3+DR+ T lymphocytes in the lung compared to the blood (24, 56) suggests that the stimulus for their activation (30–32) is compartmentalized to the lung (and presumably at other sites of disease). Consistent with this concept, it is known that, whereas at least some DR antigens are expressed on all T cells (29), the expression of DR greatly increases after in vitro activation with mitogens, soluble protein antigens, or alloantigens (30–32), i.e., if the systemic stimulus was present in the blood, it would be expected that more blood Leu3+ T cells would also express DR. For example, in vivo studies with systemic tetanus toxoid reimmunization have shown that a significant proportion of blood T cells are transiently DR+ (57). The concept that the immune response may compartmentalize at the site of the activation stimulus is consistent with the observations of the compartmental accumulation of T cells in the lung in berylliosis (58, 59) and hypersensitivity pneumonitis (56), T cells in the lesions of leprosy (39, 54), activated T cells in the cerebrospinal fluid of individuals with multiple sclerosis (60), in synovial fluid of patients with rheumatoid arthritis (61), and the salivary glands of patients with Sjogren's syndrome (62).

Secondly, although all T cells are capable of proliferating, the fact that only Leu3+ T cells accumulate in the sarcoid lung is consistent with the concept that the localized stimulus that activates the T cells is specific for the Leu3+ subset. In this regard, because activated Leu3+ and Leu2+ cells can proliferate in response to IL-2 (52, 63, 64), the knowledge that there is enhanced amount of IL-2 in the sarcoid lung, but accumulation of only Leu3+ cells, suggests that the stimulus that activates these cells is Leu3+ specific. In support of this concept, several studies have documented that certain classes of antigens are helper/inducer subset-specific. Interestingly, in berylliosis as well, the intradermal injection of beryllium salts determines a selective accumulation of Leu3+ cells surrounding newly formed granulomas (65), consistent with in vitro observations of Meuer et al. (50), that responses to certain classes of antigens may be T cell type-specific. Interestingly, the fact that sarcoid patients respond to intradermal injection of the Kveim–Siltzbach antigen (a suspension of an extract of sarcoid spleen) by producing granulomas surrounded by Leu3+ T cells (66) is consistent with concept that such suspensions may contain a class of antigens that specifically activate the Leu3+ T cell subtype.

Thirdly, the observations in the sarcoid lung are also consistent with the hypothesis that the disease is associated with a loss of the normal suppressor processes such that normal antigenic stimuli are not normally dampened, thus permitting an overexuberant helper/inducer T cell response (67).

Finally, it is conceivable that sarcoid represents an infectious disorder in which the causative agent is trophic for Leu4+Leu3+ lymphocytes, causing them to proliferate and release IL-2. In this regard, whereas HTLV-I and HTLV-III retrovirus infections are models for T cell-specific accumulation or loss, respectively (68), studies in sarcoid lung T cells have failed to reveal reverse transcriptase or specific evidence of HTLV-I (69), the closest retrovirus model for sarcoid.

Independent of the inciting mechanism, because IL-2 promotes the growth of activated lymphocytes, and because the sarcoid lung is dominated by Leu4+Leu3+ high-intensity DR lymphocytes, it is reasonable to conclude that the Leu4+Leu3+ high-intensity DR IL-2-releasing cells play a central role in the persistent accumulation of large numbers of T lymphocytes in the lower respiratory tract.

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