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Research Article

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Clonal Expansion of Lung V δ 1⁺ T Cells in Pulmonary Sarcoidosis

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Abstract

Sarcoidosis is a multisystem disease of unknown etiology characterized by the presence of noncaseating granulomas in involved tissues. To investigate a potential role for γ/δ T cells in the pathogenesis of pulmonary sarcoidosis, we studied lung and blood T cells from patients for preferential expression of particular γ/δ T cell receptors. An abnormally high percentage of γ/δ cells was found in the blood of some patients. However, the increased percentage did not reflect an increase in absolute number, and appeared to be secondary to a decrease in T cells expressing α/β receptors. Furthermore, as in normals, the circulating γ/δ cells in patients predominantly expressed V γ 9/V δ 2 receptors, a subset that was not enriched at the site of disease. In contrast, in the lung, an increased percentage of γ/δ cells expressing V δ 1 was found in a subset of patients. Importantly, these cells demonstrated evidence of prior activation by selectively expanding *in vitro* in the presence of interleukin 2. Furthermore, an analysis of junctional region sequences revealed their clonal nature. These clonal expansions of V δ 1⁺ cells in pulmonary sarcoidosis provide evidence for a disease process that involves specific recognition of a local antigen by T cells, and contributes new information regarding the nature of the as yet undefined antigenic stimulus. (*J. Clin. Invest.* 1993. 91:292–300.) Key words: sarcoidosis • T cell repertoire • T cell receptor • bronchoalveolar lavage • gamma/delta T cells

Introduction

Sarcoidosis is a systemic, nonmalignant disorder defined histologically by the presence of noncaseating granulomas in involved tissues (1). Especially in the lung, inflammation and chronic damage related to T cell and macrophage infiltration can lead to progressive organ dysfunction and chronic disability (1–10). The etiology of sarcoidosis is unknown. It has been postulated that the process is initiated by an exaggerated T cell response directed to an as yet unknown exogenous stimulus. The release of mediators from these activated T cells subsequently directs the accumulation of macrophages and granuloma formation (1–10). A response to a predominant antigen

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would likely lead to a situation in which T cells bearing a limited T cell receptor (TCR)¹ repertoire become activated and expanded at sites of pathology in this disease. A description of the predominant TCR variable elements expressed might provide insight into the nature of the stimulating antigen as well as an avenue for specific intervention.

Histopathologically, the granulomatous lesions of sarcoidosis resemble tissue inflammatory responses to mycobacteria (1, 11). Interestingly, profound reactivity to mycobacterial antigens is a striking feature of both murine and human T cells bearing TCR composed of γ and δ chains (12–18). In mice, after regional exposure to mycobacterial antigens, γ/δ T cells constitute a major subset of T cells at sites of antigen accumulation (12, 14). Studies have also shown marked proliferation of normal human peripheral blood γ/δ T cells in response to killed mycobacteria (16, 18). The γ/δ T cells responsible for this response reside within the subset expressing V γ 9/V δ 2 receptors (18). The second most frequent blood subset, expressing V δ 1 and a V γ other than V γ 9, appears to be nonresponsive to mycobacterial antigens. Interestingly, a recent study in patients with pulmonary sarcoidosis demonstrated an increased percentage of peripheral blood γ/δ T cells expressing V γ 9 (19, 20). However, the involvement of these cells in the lung lesions of the same patients was unclear.

In the present study, we investigated further the potential involvement of γ/δ T cells in the pathogenesis of pulmonary sarcoidosis. Although we also found patients with an increased percentage of peripheral blood V γ 9/V δ 2⁺ T cells, results indicated that this abnormality did not reflect a primary disease-related expansion of this subset nor were cells with this phenotype enriched at the site of disease. More importantly, we identified a subset of patients who demonstrate clonal expansions of activated V δ 1⁺ T cells in the lung. The data suggest that these γ/δ T cells have been specifically activated by antigen as part of the lung disease process.

Methods

Study population. The diagnosis of pulmonary sarcoidosis was established in 18 individuals using previously defined criteria (1–4). All patients underwent biopsy of either pulmonary parenchyma or mediastinal lymph nodes obtained during flexible fiberoptic bronchoscopy or mediastinoscopy. 17 patients had chest radiograph evidence of diffuse parenchymal involvement with or without hilar adenopathy, and one patient demonstrated only hilar and paratracheal lymph node enlargement. Bronchoalveolar lavage (BAL) and blood specimens were obtained while the patients were undergoing their initial diagnostic evaluation for sarcoidosis. The characteristics of the patients studied are shown in Table I. None of the patients had received corticosteroids or other immunosuppressive medications before study. As controls, 16

1. Abbreviations used in this paper: BAL, bronchoalveolar lavage; PCR, polymerase chain reaction; TCR, T cell receptor.

individuals with no history of lung disease, with normal exams, and with normal chest radiographs were evaluated for the blood studies (Table I). A separate group of 10 normal individuals similarly evaluated for lung abnormalities served as controls for the lavage studies (Table I). Informed consent was obtained from each patient and healthy volunteer, and the protocol was approved by the Institutional Review Board.

Isolation of mononuclear cells from blood and BAL. BAL was performed as previously described (21). General characteristics of the cells obtained are shown in Table I. Mononuclear cells were separated from BAL and peripheral blood by Ficoll-Hypaque density gradient centrifugation. As previously reported (21), the yield of mononuclear cells was greatly increased in bronchoalveolar lavage fluid from sarcoidosis patients ($57 \pm 7.9 \times 10^6$ cells compared with control values of $24.8 \pm 2.9 \times 10^6$ cells per lavage). Patient samples also demonstrated an increased proportion of lymphocytes ($36.9 \pm 3.6\%$ versus $17.8 \pm 3.4\%$ for normal nonsmoking controls).

Absolute numbers of T cell subsets. The absolute number of T cells and T cell subsets were calculated in blood by using the total white blood count and percentage of lymphocytes, determined by the Clinical Laboratory, and the proportion of lymphocytes positive for either CD3, CD4, CD8, or γ/δ antigens. Results are expressed as cells/mm³ of blood.

Cell culture in rIL-2. Mononuclear cells derived from BAL were washed and suspended in culture media (as described below but without fetal calf serum) at 10^6 cells/ml. Adherent cells were depleted by incubation on polystyrene culture plates (Nunc, Naperville, IL) for 15 min at 37°C. Nonadherent cells were removed, washed, and resuspended in RPMI 1640, 10% FCS, 2 mM L-glutamine, 2 mM pyruvate, 10 mM Hepes, 50 U/ml penicillin, and 50 µg/ml streptomycin (complete culture medium) plus 25 U/cc rIL-2 (Ala-125) (Amgen Biologicals, Thousand Oaks, CA). Additional IL-2 and fresh culture medium were added to the cell cultures on days 5 to 7 as required. In vitro cultures were maintained from 1 to 2 wk before harvesting for cytofluorographic analysis or RNA extraction.

Monoclonal antibodies and cytofluorographic analysis. Lymphocytes obtained from both freshly prepared samples and after culture in IL-2 were studied for cell surface receptor expression with a number of different monoclonal antibodies and cytofluorographic analysis. Antibody TCR δ 1, which recognizes the δ protein on all γ/δ T cells (22) was a generous gift from M. Brenner, Dana Farber Cancer Institute, Boston, MA. Ti γ A, which recognizes the V γ 9 epitope (23) was provided by T. Hercend (Institut Gustave-Roussy, Villejuif, France), and BB3, which is directed to the V δ 2 epitope, was a gift from E. Ciccone (Istituto Nazionale per la Ricerca Sul Cancro, Genova, Italy) (24). δ TCS-1 which recognizes V δ 1 predominantly associated with J δ 1 and the non-disulfide linked heterodimer of the γ/δ T cell receptor (25) was purchased from T Cell Sciences, Inc. (Cambridge, MA). Additional antibodies used were directed to CD3 (anti-Leu-4), CD4 (anti-Leu-3a), and CD8 (anti-Leu-2a) (all from Becton Dickinson, Mountain View, CA). Second step reagents included streptavidin-phycoerythrin (Fisher Scientific Co., Pittsburgh, PA) and a fluorescein-conjugated goat anti-mouse Ig (Tago, Inc., Burlingame, CA). Control antibodies included isotype-matched irrelevant phycoerythrin-conjugated and fluorescein-conjugated mouse myeloma antibodies (control-phycoerythrin and control FITC, Becton Dickinson). All antibodies were diluted to appropriate concentration in PBS, 5% FCS, 0.02% sodium azide, and 1 mg/ml human gamma globulin (Sigma Chemical Co., St. Louis, MO).

In parallel experiments equal numbers of cells were analyzed by either single color or two color immunofluorescence as described (26). For single color immunofluorescence, cells were stained directly with FITC-conjugated TCR δ 1 or δ TCS-1 or incubated with unconjugated Ti γ A or BB3 followed by staining with FITC-goat anti-mouse Ig. For two color immunofluorescence, cells were incubated with biotinylated TCR δ 1 (20 min 37°C) followed by incubation with streptavidin-phycoerythrin and either FITC-anti-CD4 or FITC-anti-CD8. Lympho-

cytes from freshly prepared samples were distinguished by their characteristic forward angle and 90° light-scatter pattern. When analyzing lymphocytes obtained after culture in IL2, forward angle and 90° light-scatter patterns were used to gate on the large blastic lymphocytes which are easily distinguished from small resting lymphocytes (26). In these samples, blasted cells were the predominant viable population. Fluorescence intensity was analyzed with an Epics Profile Cytofluorograph (Coulter Corp., Hialeah, FL).

Amplification of TCR γ/δ cDNA with the polymerase chain reaction (PCR). Total cellular RNA was extracted from the various mononuclear cell preparations as described (27). cDNA synthesis was performed as described using 2 µg of RNA per 20 µl reaction volume (28). 25 U of placental ribonuclease inhibitor (Amersham Corp., Arlington Heights, IL), 200 U of Moloney virus reverse transcriptase (Bethesda Research Laboratories, Gaithersburg, MD), and 50 ng of random hexamers (Pharmacia, Uppsala, Sweden) were included in each reaction. After 1 h at 37°C the reaction mixture was heated at 95°C for 2 min and 1 µl was added directly per each 50 µl of PCR reaction mixture.

PCR was performed using a Perkin-Elmer-Cetus DNA Thermal Cycler and TAQ polymerase as described (Perkin-Elmer Cetus Instruments, Norwalk, CT) (28). Both 5' and 3' oligonucleotide primers were present at 0.3 µM. All primers were taken from published sequences (29–36). Sequences of the 5' V δ 1 and 5' V γ subgroup primers were as follows: V δ 1: TACTCAAGCCCAGTCATCAGTATCC; V γ I: TACCTACACCAGGAGGGGAAG; V γ II: GGCAGTGT-CAGAAAGGAATC; V γ III: TCGACGCAGCATGGGTAAGAC; and V γ IV: GATTGCTCAGGTGGGAAGACT. The four 5' V γ primers are specific for the four subgroups of the human T cell receptor gamma chain (32–36). The sequence of the most 3' C δ "outer primer" was 5'-CCAAGCTTGACAGCATTGTACTTCC-3' and that for the 3' C γ primers was 5'-CTGTCTTTATTGGAGGAAAGATAAT-3'. The 3' C γ primer hybridizes to both C γ 1 and C γ 2 (35, 36). Each PCR cycle consisted of incubations at 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min. Each V γ primer was used in an independent PCR in conjunction with the C γ primer and analyzed in parallel with the independently run V δ 1 PCR product. The PCR cycle number for the cDNA derived from the IL-2-stimulated lavage mononuclear cultures was chosen to ensure logarithmic generation of the V δ 1 PCR product.

V δ 1 transcripts derived from the IL-2 stimulated BAL mononuclear cells were amplified using 40 cycles of PCR with the 5' V δ 1 primer and 3' C δ outer primer. In the analysis of V δ 1 transcripts from fresh blood and lavage mononuclear cells, two sequential PCR amplifications were employed to increase the specificity of PCR products amplified, given the low percentage of V δ 1⁺ cells in freshly isolated populations. The first PCR (24 cycles) used the 5' V δ 1 primer and 3' C δ outer primer. 4 µl of the 100 µl PCR reaction was transferred to a second 100-µl PCR reaction mixture containing the 5' V δ 1 primer and an internal C δ primer (5'-TATATCCTTGGGGTAGAATTC-3') (31), and amplification was continued for 30 cycles.

Cloning of PCR products and DNA sequencing. The V δ 1 PCR products were ligated into the PCR 1000 TA cloning Vector as specified by the manufacturer (Invitrogen, San Diego, CA). The ligation product was transformed into the INVaF' competent *Escherichia coli* cells (Invitrogen) and plaques containing appropriate inserts were isolated and chosen randomly for sequencing. DNA sequencing was performed on double stranded plasmid DNA by the dideoxy method using Sequenase (United States Biochemical Corp., Cleveland, OH).

Results

Increased percentage of peripheral blood γ/δ cells in sarcoidosis: evidence for lack of involvement in disease process. The study population consisted of 18 newly diagnosed and untreated patients with pulmonary sarcoidosis. As shown in Table I, these patients demonstrated a decrease in the percentage of peripheral blood mononuclear cells expressing CD3 com-

Table I. Characteristics of Patients Studied and Analysis of Cell Subsets in Blood and BAL

Characteristic	Sarcoidosis patients <i>n</i> = 18	Controls-blood <i>n</i> = 16	Controls-BAL <i>n</i> = 10
Age	35±2.1	33±0.9	27±1.8
Gender (M/F)	12/6	11/5	6/4
Smoking History (CS/FS/NS)*	5/1/12	4/0/12	0/0/10
Peripheral Blood Cells			
Absolute Lymphocyte ct. (×10 ⁻⁶ /mm ³)	1409±118 [‡]	2518±190	
% CD3 ⁺ (Absolute No.)	59.9±2.4 (873±103) [‡]	70.4±2.3 (1795±172)	
% of CD3 ⁺ expressing CD4 (Absolute No.)	58.6±3.1 (506±71) [‡]	60.2±2.4 (1082±125)	
% of CD3 ⁺ expressing CD8 (Absolute No.)	34.9±1.9 (291±39) [‡]	36.4±1.9 (655±63)	
Bronchoalveolar Lavage Fluid (BAL) Cells			
% Recovery	53±2.4		71±2.7
Total WBC yield (×10 ⁶)	56.9±7.9		24.8±2.9
% Lymphocytes	36.9±3.6		17.8±3.4
% Macrophages	59.7±3.6		80.6±3.5
% CD3 ⁺	75.5±4.3		66.7±9.9 [§]
% of CD3 ⁺ expressing CD4	74.9±4.5		44.0±10.8 [§]
% of CD3 ⁺ expressing CD8	23.5±4.9		47.0±9.7 [§]

Data are expressed as the mean±SE. * The data are presented as the number of patients or controls who were chronic smokers (CS), former smokers, stopping at least one year before evaluation, (FS), and never smokers (NS). ‡ Absolute numbers for patients are significantly different compared to controls, *P* < 0.001. § Only five of the ten controls were analyzed for these cell surface markers.

pared with age- and sex-matched normal controls (*P* < 0.001 by Student's *t* test). This was associated with a significant drop in absolute lymphocyte count, resulting in an even more marked decrease in absolute number of total T cells. On average, patients with sarcoidosis had less than half the number of circulating CD3⁺ T cells compared with normal controls. The decrease in CD3⁺ cells appeared to be mostly secondary to a decrease in TCR α/β⁺ cells, with proportional decreases in both the CD4 and CD8 subsets (Table I).

The percentage of freshly isolated peripheral blood cells expressing γ/δ TCR was initially analyzed using indirect immu-

nofluorescence and cytofluorographic analysis. Examples of the staining patterns observed are shown in Fig. 1. In the example shown, 10.1% of the lymphocytes express a marker recognized by monoclonal antibody TCRδ1 which is present on all γ/δ expressing cells (22). Using this antibody, percentages of γ/δ cells were compared in patients and controls (Fig. 2 A), and a modest increase in the mean value for patients was noted. Four patients demonstrated percentages > 20% whereas the highest control value was 15%. However, when absolute numbers of γ/δ cells were compared, no difference was observed between patients and controls (Fig. 2 B). Thus, overall, the

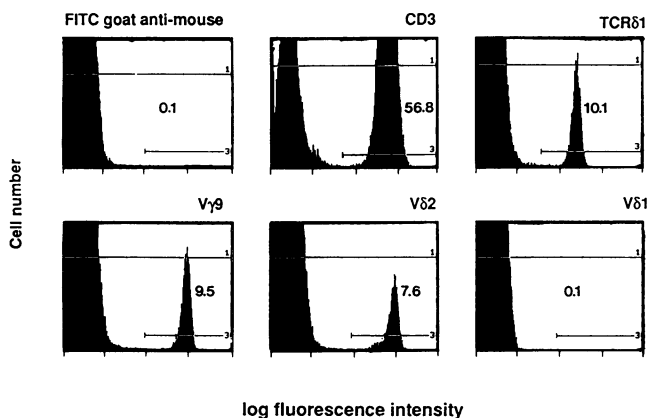


Figure 1. Cytofluorographic analysis of blood T cell subsets from a patient with sarcoidosis. The second-step for each analysis was an FITC-conjugated goat-anti-mouse Ig reagent, shown as a control in the top left-hand histogram. 2 × 10⁴ lymphocytes were generally analyzed for each cell surface marker, and the percentage of positively staining lymphocytes in each histogram is printed to the right of the positive peak.

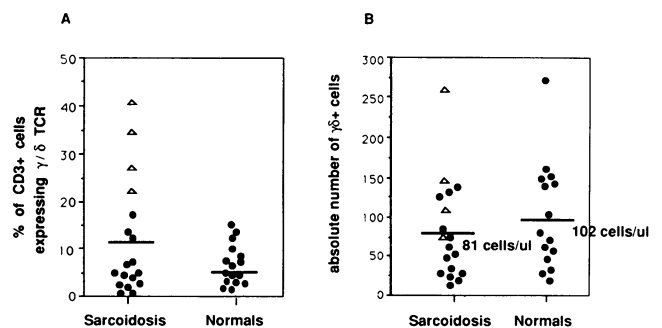


Figure 2. (A) Percentage of peripheral blood CD3⁺ T cells that express γ/δ TCR in patients with sarcoidosis and normal individuals. Data were calculated from separate analyses using mAb directed to CD3 and TCRδ1 (see Fig. 1). (B) Absolute number of γ/δ cells per microliter in the peripheral blood of patients with sarcoidosis and normals. The data were calculated from the total white blood cell count, percent lymphocytes on the differential analysis, and the proportion of lymphocytes staining positive with mAb TCRδ1. In the patient population, the open triangles represent the four patients with the highest percentages of γ/δ cells as shown in A. The lines represent the mean levels for each group.

results suggest that the increased percentage of blood γ/δ cells is not primary but rather secondary to the TCR α/β^+ cell lymphopenia.

Using monoclonal antibodies directed to particular γ/δ V gene-encoded products (i.e., V γ 9, V δ 2, and V δ 1), we also determined whether there may be selective alterations in the γ/δ peripheral blood repertoire in sarcoidosis patients. As shown in Figs. 1 and 3, in the majority of patients, including those with the highest percentages of total γ/δ cells, most γ/δ cells express V γ 9 and V δ 2. However, as demonstrated previously by others (37), the largest subset of peripheral blood γ/δ cells in normal adult individuals also bears V γ 9/V δ 2 and there was little evidence for a disease-related expansion of this subset in our patient population (Fig. 3). It should be noted that the subset expressing V δ 1 was also similar in both patients and controls, although in several patients, this subset was barely detectable in peripheral blood (Figs. 1 and 3).

Skewing of V δ 1⁺ but not V γ 9/V δ 2⁺ cells in BAL vs. peripheral blood cells of individual patients. The percentage of γ/δ cells as a component of the total T cell pool was also compared in freshly isolated blood and BAL cells of individual patients (Fig. 4 A). In most patients, only a small percentage of CD3⁺ cells in BAL expressed γ/δ TCR, including those with an elevated percentage of circulating γ/δ (i.e., V γ 9/V δ 2⁺) T cells. Thus, γ/δ cells in general appeared to be excluded from the BAL T cell pool or to be diluted by the influx of CD4⁺ and less so by CD8⁺ T cells (see Table I). One clear exception to the above pattern was noted for patient SW who demonstrated 2 and 13% γ/δ cells in blood and BAL, respectively. An analysis of γ/δ V gene expression (Fig. 4 B) further showed that most of the γ/δ cells in this patient's lung expressed V δ 1 whereas only a small component of the blood γ/δ cells were V δ 1⁺. Five other patients with an evaluable percentage of total γ/δ cells (> 3% of total T cells) were also analyzed in terms of γ/δ subsets, and subset percentages were compared in blood and BAL. Nearly all of these patients showed an increase in the component of γ/δ cells expressing V δ 1 receptors at the expense of the V γ 9/V δ 2 subset (Fig. 4 B). It should also be emphasized that al-

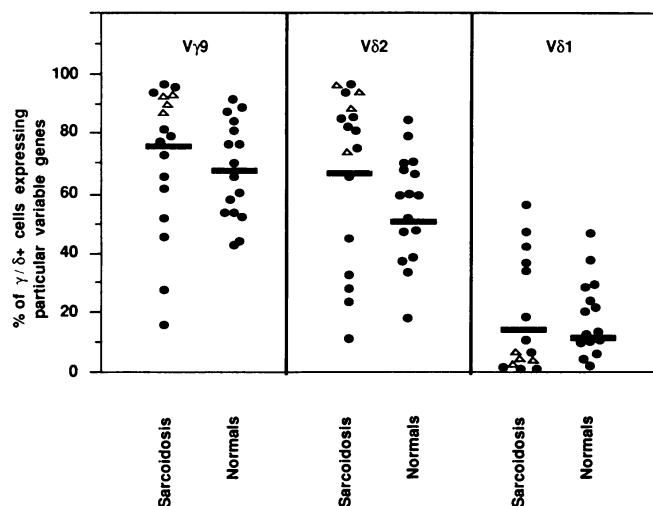


Figure 3. Percentage of blood γ/δ cells expressing different variable genes as determined by immunofluorescence staining and cytofluorographic analysis. The monoclonal antibodies used are described in the Methods, and examples of the staining patterns are shown in Fig. 1. The mean for each group is represented by a line.

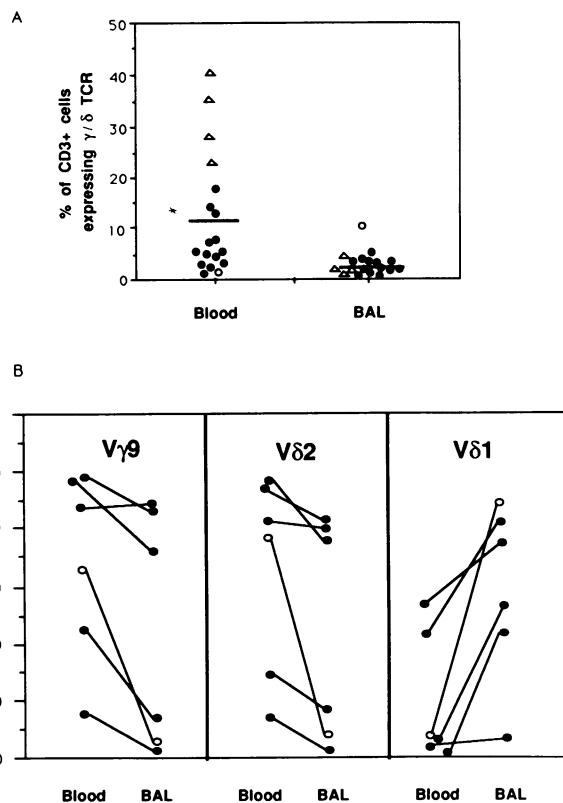


Figure 4. (A) Percentage of T cells expressing γ/δ TCR in blood and BAL fluid of patients with sarcoidosis. The open triangles represent data from patients with > 20% of blood T cells expressing γ/δ TCR (see Fig. 2 A). The open circle represents data from patient SW, who was the only patient with a higher percentage in BAL compared with blood. The lines represent the mean levels for each group, and the asterisk indicates that the mean levels for blood and BAL are significantly different, $P < 0.01$. (B) Percentage of blood and BAL γ/δ cells expressing particular variable genes. Data are presented for patients who had a percentage of γ/δ cells in BAL allowing this analysis, i.e., > 3% of BAL T cells expressing γ/δ . Samples from two patients were not evaluable for V gene expression. Solid lines connect data for blood and BAL for the same individual. The open circle represents data for patient SW, described in A. The monoclonal antibodies used and staining patterns are described in Fig. 1.

though the percentage of lung γ/δ cells is relatively low, the yield of T lymphocytes from the BAL of these patients is much greater than that obtainable from controls ($16.5 \pm 5.6 \times 10^4$ T cells per ml of lavage from these patients vs. $2.9 \pm 1.1 \times 10^4$ T cells from controls in this study ($n = 5$) vs. $1.2 \pm 0.3 \times 10^4$ T cells from controls in a previous study [21]).

Expansion of BAL V δ 1⁺ cells after culture in IL2. The above evidence for enrichment of V δ 1⁺ cells in the BAL lymphocyte population of some patients suggested that these cells may be activated and could possibly be further expanded in the presence of IL2. BAL mononuclear cells were therefore cultured in the presence of IL2 for one to two weeks and the percentage of γ/δ cells was determined. Surprisingly, in nearly all samples, the majority of viable cells were blastic, as determined by forward and 90° light-scatter cytofluorographic patterns, after this culture period (data not shown). Fig. 5 shows that in three patients, including patient SW with the highest initial level of γ/δ cells, the percentage of γ/δ cells clearly

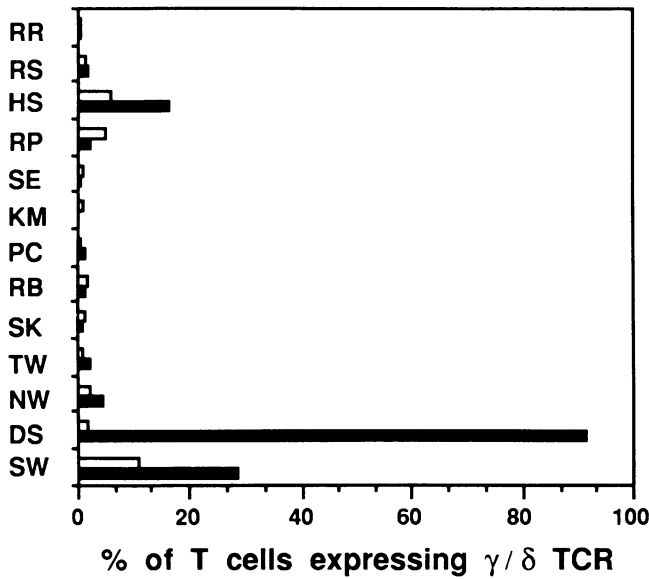


Figure 5. Expression of γ/δ TCR before (\square) (day 0) and after (\blacksquare) culture in IL2. The data are presented as percentage of total T lymphocytes expressing γ/δ TCR as determined by immunofluorescent staining with mAb TCR δ 1 and cytofluorographic analysis (see Fig. 1). For unstimulated BAL cells analyzed before culture, scatter gates were set on the lymphocyte peak as determined by forward angle and 90° light-scatter patterns. After culture in medium supplemented with IL2, forward angle and 90° light-scatter patterns were used to gate on the large blastic cells, which predominated and were easily distinguished from small lymphocytes. In two samples not shown, growth was minimal in vitro and sufficient blasts were not available for analysis. Samples from three other patients were not cultured.

increased after culture. Despite a low initial level, cells from patient DS were almost entirely γ/δ^+ after culture in IL2. Furthermore, the γ/δ cells that expanded in culture were almost entirely $V\delta 1^+$ and $V\gamma 9^-$ (Fig. 6). A subset of proliferating $V\delta 1^+$ cells in both patients expressed a low density of CD8.

T cells from BAL of normal individuals: low percentage of γ/δ cells and absence of expansion after culture in IL2. The above results prompted us to determine whether the BAL lymphocyte population from normal individuals contains an expanded or activated subset of γ/δ cells. As shown in Table I, the yield of total cells and especially lymphocytes is much lower from normals compared to sarcoidosis patients. Of the 10 controls studied, five mononuclear cell samples were analyzed immediately after isolation for γ/δ expression. Table II shows that very few of the T cells in these normal samples expressed γ/δ receptors. The percentage of $V\delta 1^+$ cells was also at the limits of detection in each case.

Mononuclear cells from each control were depleted of adherent cells and cultured in IL-2 in an identical fashion as described above for patients' cells. Over a 1–2-wk culture period, none of these normal samples demonstrated blasting that was characteristic of nearly all of the sarcoidosis cultures. Cytofluorographic patterns confirmed the absence of T cell blasts in these cultures. Furthermore, as shown in Table II, the viable lymphocytes remaining in these cultures did not contain an increased percentage of γ/δ cells or $V\delta 1^+$ cells.

V γ gene expression and junctional diversity of BAL $V\delta 1^+$ cells. The selective enrichment of activated $V\delta 1^+$ cells in the BAL of certain sarcoidosis patients suggested that these cells may be responding and proliferating in response to local antigen. It was also possible that these cells were somehow being

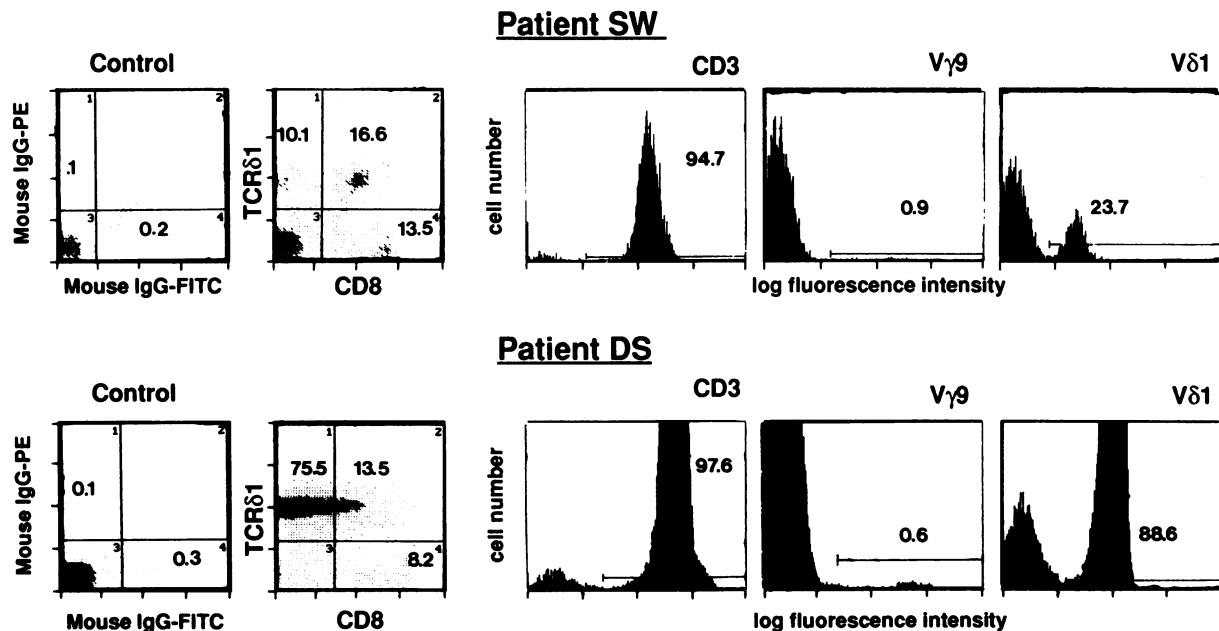


Figure 6. Percentage of cells expressing $V\delta 1$ and $V\gamma 9$ after culture in media containing IL2. Data are presented for two patients who demonstrated the highest percentages of γ/δ cells after culture. Monoclonal antibodies used are described in the Methods. Forward angle and 90 light-scatter pattern were used to gate on the large blastic population which was easily distinguished from small lymphocytes and which constituted the majority of viable cells in both experiments. For two color cytofluorographic analysis, 2×10^4 cells were analyzed. The percentage of positive cells are indicated in each histogram. In both experiments, nearly all of the γ/δ cells expressed $V\delta 1$ gene segments, whether or not these cells were $CD8^+$. In experiments not shown, $< 1\%$ of the γ/δ cells co-expressed $CD4$.

Table II. Analysis of BAL Lymphocytes from Normal Individuals

	Before culture		After culture*	
	Mean±SE	Range	Mean±SE	Range
	n = 5		n = 5	
Percentage of lymphocytes expressing:				
CD3	66.7±9.9	(33-91)	75.7±6.8	(52-91)
γ/δ	1.5±0.3	(0.3-2.1)	2.6±0.3	(1.8-3.3)
Vδ1	<0.5	(<0.5-0.7)	<0.5	(all <0.5)

* Cells were analyzed after 1-2 wk of culture. In contrast to sarcoidosis samples, a viable blast population containing T cells was not apparent and therefore gates were set to include the resting lymphocyte population remaining.

nonspecifically recruited and activated in the lung. We therefore analyzed other variable elements of the TCR to determine if there was evidence of oligoclonality and therefore antigen-driven expansion. Vγ gene expression was analyzed by PCR only in the IL2 expanded BAL cells of patients SW and DS, since Vδ1+ cells occupied a major portion of these populations. In each patient, the predominantly expressed Vγ was a member of group 1, which includes Vγ2, Vγ3, Vγ4, Vγ5, and Vγ8 (data not shown). In patient DS, Vγ expression was con-

finied to subgroup 1; additionally, in patient SW, usage also included subgroup III (Vγ10) receptors (data not shown). Consistent with data shown in Fig. 6, Vγ9 receptor expression was barely detectable. Thus, overall, we observed a limited expression of Vγ genes in both of these populations of Vδ1+ cells.

PCR-amplified Vδ gene segments were also cloned and then analyzed for sequence diversity in the junctional region. Sequences of the different clones analyzed, compared to germline, are shown in Fig. 7. Analysis of the freshly isolated Vδ1 population in patient SW showed that 15 of 16 sequences were identical. The one nucleotide difference in one clone may reflect a sequencing error. An additional 10 clones were sequenced from the IL2 expanded population and 9 of 10 were identical. Not surprisingly, this sequence was identical to that obtained from the Vδ1 population prior to culture. cDNA clones were analyzed from patient DS only after IL2 expansion, and again 9 of the 10 sequences were identical. Importantly, the clonal populations in patients SW and DS expressed different junctional sequences. Peripheral blood lymphocytes from patient SW were also analyzed for similar Vδ1 clones as found in the lung. Of 14 Vδ1 sequences identified, none were identical to the expanded lung clone, although a set of four sequences were identical to each other (data not shown). It should be emphasized that this patient had barely detectable levels of circulating Vδ1+ T cells (<0.2% of total lymphocytes), and unlike the other samples studied, an extended number of PCR amplification cycles were required to obtain enough DNA for ligation and cloning.

Sample	# of Sequences Analyzed	# of Sequences	Vδ1	N	Dδ1	N	Dδ2	N	Dδ3	N	Jδ
Germline			CTTGGGGAAC.....GAAATAGT.....				CCTTCTAC.....		ACTGGGGATACG.....		ACACCGATAAACT (Jδ1)
Patient SW (BAL/Before culture)	16	15	CTTGGGGAAC•AATGA•GAA.....				TGGGGAT.....		CCGAGG.....		ACCGATAAACT (Jδ1)
		1	CTTGGGGAAC•AATGA•GAA.....				TGGGGAT.....		CCGAGG.....		CCGATAAACT "
Patient SW (BAL/IL2-expanded)	10	9	CTTGGGGAAC•AATGA•GAA.....				TGGGGAT.....		CCGAGG.....		ACCGATAAACT (Jδ1)
		1	CTTGGG.....		CCTA.....	CCTTCC.....	CCTTTT.....	ACTGGGGATACG•CGATGCACCCAC•			ACCGATAAACT "
Patient DS (BAL/IL2-expanded)	11	10	CTTGGGG.....		ACCGG.....	CCTAC.....	CTGGGG.....	CTTTGGGC.....			TAAACT (Jδ1)
		1	CTTGGGGAA•TTTTTT•AATA.....		A.....	CCT.....	AAACGTGGG•CTGGGGATAC•	TGGGACTCGG•			GACAGCACAACT (Jδ2)
Control 1 PBL	10	1	CTTGGGGAAC.....		CCTGG.....	CCTTCTAC.....	TACGCGT•ACTGGGGGA.....	AAGTCCCCTGT•			ACACCGATAAACT (Jδ1)
		1	CTTGGG.....		ACCGGA.....	TAC.....	ACACT.....	ACTGGGG.....			GCTTTAGC•TCCTGGGACACC (Jδ3)
		1	CTTGGGGAA•GAGGG•TAG•				CTGGGG.....	TTGTGCA.....			ACACCGATAAACT (Jδ1)
		1	CTTGGGGAAC.....		CCTGG.....	CCTTCTAC.....	A.....	ACTGGGGATA.....			GGTCA.....CGATAAACT "
		1	CTTGGGGA.....		TA•CCACGCCG•		GGGGAT.....	CATCCTT.....			TAAACT "
		1	CTTGGGGAA.....		GG•	TTCTAC.....	T.....	ACTGGGGATA.....			GGTCA.....CGATAAACT "
		1	CTTGGGG.....		CCATTCCGGGG•	CCTTCTAC.....	ATGTTG•	GGGGAT.....			GCT.....ACCGATAAACT "
		1	CTTGGGGAA•TTTTTT•AATA.....			TC.....	TAAACGTGGG•CTGGGGATAC•	TGGGACTCG•			GACAGCACAACTC (Jδ2)
		1	CTTGGGGAAC.....		CTG.....		CTGG.....	AGAAC.....			ACCGATAAACT (Jδ1)
		1	CTTGGGGAAC.....		CTGT.....		TGGG.....	AGAAC.....			ACCAATAAACT "
Control 2 PBL	11	4	CTTGGGGA.....		CGT.....	TTCTAC.....	TCAA.....	GGGGATAC.....			AGAAGGTGG.....CT (Jδ1)
		1	CTTGGGGA.....		CGGGG.....	CCTAC.....	TTAA.....	GGGGATA.....			GAGTAGGTGG.....CT "
		1	CTTGGGGAA.....		ATGGGGCAGTTAG•			TGGGGG.....			TT.....TAAACT "
		1	CTTGGGGAAC.....		T.....			ACTGGGG.....			CTGGT.....ACACCGATAAACT "
		1	CTT.....		TGT.....	TTT.....	AACAGGGGG•CTGGGGAT.....	GATCA.....			ACCGATAAACT "
		1	CTTGGGGA.....		GATA.....		ACTGGGG.....	CGCCTGT.....			ACACCGATAAACT "
		1	CTTGGGGAAC•AAAGGG•GAAA.....		GTGC.....	CTTCTA.....	ATGA.....	GATACG•CGGAGCTGTGGT•			CGATAAACT "
		1	CTTGG.....		AAAACA.....	AGT.....	GAGTCCAC.....	CCT.....			CCT•ACTGGGGGA.....CTGTGG.....TAAACT "

Figure 7. Analysis of junctional sequences from BAL and peripheral blood Vδ1+ cells. The number of cDNA clones sequenced and the number with the same sequence are shown. Sequences shown include 3' region of Vδ1, N regions, the three diversity segments (Dδ), and the 5' region of the Jδ element (Jδ) with the specific Jδ element used listed to the right of each sequence. The Vδ1 germline sequences and orientation were taken from references 29-31.

PCR-amplified V δ 1 cDNA from the blood of two normal individuals was cloned and sequenced in a manner similar to that used for patient lung samples. In one example, none of 10 sequences were identical whereas in the other, only 4 of 11 sequences were the same. The choice of this latter individual may have influenced this result since this control individual persistently expresses a high percentage of γ/δ and V δ 1⁺ T cells in peripheral blood.

Discussion

Pulmonary sarcoidosis is characterized by a marked accumulation of T cells in the lungs, and these cells appear to be critically involved in the disease process (1–10). Results presented herein indicate that the lung T cell repertoire is not a passive reflection of T cells in the blood. Indeed, the blood findings alone appeared to be misleading in terms of identifying disease-related T cells. Thus, in concert with a prior report (19), we also noted that some patients with sarcoidosis have an elevated proportion of circulating T cells bearing γ/δ TCR. The elevated percentage, however, did not reflect a true increase in number, but was apparently secondary to a proportional reduction in α/β ⁺ (CD4⁺ and CD8⁺) cells. Even in one patient in whom 40% of blood T cells expressed γ/δ receptors, the absolute number of γ/δ cells was within the normal range for age-matched controls. These data are consistent with prior studies of peripheral blood cells in sarcoidosis showing an absolute lymphopenia resulting from a reduction in CD4⁺ T cells (3, 7, 38, 39). The blood γ/δ cells in patients also did not appear to be qualitatively different from those in normals. Most expressed V γ 9/V δ 2 receptors, and in contrast to a previous report (20), we found that certain junctional regions of V γ 9 were not restricted in patients compared to normals, including the patients with the highest percentage of γ/δ cells in blood (data not shown). Therefore, circulating γ/δ cells in patients lacked evidence of selective expansion or oligoclonality.

In contrast, lung T cells obtained by BAL were distinctly different from those found in the blood of the same patients and controls. We found that the V γ 9/V δ 2 subset is selectively excluded from the lung T cell pool or is diluted out by the large influx of predominantly CD4⁺, α/β ⁺ cells. Other investigators have also failed to demonstrate an increase in the proportion of total γ/δ cells in the lungs and in other involved tissues of patients with sarcoidosis (19, 40). More importantly, we noted that in patients with detectable lung γ/δ cells, a higher proportion expressed V δ 1 segments compared with blood cells. For example, in the patient with the highest percentage of pulmonary γ/δ cells (12% of T cells), 90% were V δ 1⁺. By comparison, only 2% of blood T cells from the same patient expressed γ/δ , of which the great majority were V γ 9/V δ 2⁺. In all of the control lavage samples studied, the percentage of V δ 1⁺ cells was extremely low. Thus, the absolute yield of V δ 1⁺ cells from a subset of patients is at least 20-fold greater than average control yields.

To assess whether the lung γ/δ cells in sarcoidosis patients were selectively activated, BAL mononuclear cells were placed into short-term culture with IL2. After culture, most of the viable lymphocytes appeared blastic based on forward and side-scatter cytofluorographic parameters, and in two of these samples, > 25% of the T cells expressed γ/δ TCRs. Results obtained for the one patient with an increase in V δ 1⁺ cells

before culture supports the validity of using IL2 to expand *in vivo* activated cells, since the proportion of V δ 1⁺ T cells increased by over twofold in this sample. Of note, V γ 9⁺/V δ 2⁺ cells were not selectively expanded with IL2 in any of the BAL cell cultures. Furthermore, a significant percentage of blasted cells was not observed in any of the IL2 cultures with control BAL cells, and residual cells in these cultures showed no increases in the percentage of γ/δ cells. Consistent with prior reports describing activated CD4⁺ lymphocytes in pulmonary sarcoidosis (1–4, 6–8, 10), 12 of 13 cultures with patients' cells were dominated by CD4⁺ α/β cells. Usage of beta chain variable regions (V β s) was markedly limited in a number of these patients (Forrester, J. M., Y. Wang, J. Loveless, B. Malissen, T. King, L. Newman, and B. L. Kotzin, manuscript in preparation), including a few with expansion of V β 8⁺ cells as previously reported (41). Analysis of V β repertoire before and after culture with IL2 has provided additional validity for this approach.

Although V δ 1 preferentially associates with V γ 2 or V γ 4 of the V γ I subgroup, the potential repertoire of this population is extensive based on junctional variability (29–31, 42, 43). To determine whether the V δ 1⁺ cells from patients' lungs represented oligoclonal expansions, we sequenced random cDNA clones encoding V δ 1 gene segments. In the one patient who demonstrated an increased percentage of V δ 1⁺ cells before culture, 15 of 16 sequences were identical. Thus, nearly 10% of the entire lung T cell population obtained at lavage was composed of one γ/δ clone. Clearly, this suggests activation by a localized (lung) antigen. Furthermore, this was the predominant γ/δ clone that appeared to be expanded by culturing with IL2, since 9 of 10 V δ 1 sequences from this cell preparation were identical to each other and the same as that of the unmanipulated population. None of the V δ 1 sequences from the blood of this patient matched the dominant lung clone. Strikingly, in another patient, nearly all of the T cells that expanded in IL2 expressed γ/δ TCRs, and these cells were composed essentially of one V δ 1⁺ clone. The dominant clones for the two patients studied were distinct. Evidence for clonal T cell expansions in the lung was not an artifact introduced during PCR amplification of V δ 1 cDNA. Using peripheral blood V δ 1⁺ cells from a normal individual as a source of cDNA, 10 different sequences were found. A second individual demonstrated 7 of 11 distinct sequences. None of the sequences matched those found in the patients.

The presence of activated V δ 1-bearing clones in the lungs of some patients raised the question of whether these cells might arise from a resident lung γ/δ population. Studies in murine models have shown, for example, that the majority of intraepithelial lymphocytes express γ/δ TCRs, and in addition, particular families of V γ and V δ genes associate with epithelial tissues such as skin (44), gut (45), reproductive tract (46), and lung (14). In humans, γ/δ cells are virtually absent in epithelial tissues such as skin, but have been found to be localized to gut epithelium (43, 47–49). Interestingly, these human intestinal intraepithelial lymphocytes preferentially express V δ 1 (43, 49). In contrast, we were only able to detect an extremely small percentage of γ/δ or V δ 1⁺ cells in the lavage of normal individuals. TCR usage within this small population was not determined. It should be pointed out that our conclusions regarding antigen selection of γ/δ TCR repertoire would be altered if the activated V δ 1⁺ cells were derived from a lung population that expressed little to no TCR diversity. Consider-

ing the complexity of the junctional regions analyzed, we believe that this possibility is very unlikely.

Immune responses to certain human infectious agents, such as mycobacteria and leishmania, are also characterized by the formation of granulomatous lesions (11, 17, 50). It is interesting that γ/δ cells are prominent in the T cell response to these organisms, especially early after infection. For example, immunohistochemical staining of skin lesions associated with acute delayed-type hypersensitivity reactions to either *Mycobacterium leprae* or leishmania demonstrated marked infiltration with γ/δ cells, while more mature lesions did not (17). Relevant to the current study, the vast majority of γ/δ cells in the epidermis of *M. leprae*-induced skin lesions expressed V δ 1 gene segments (50). Furthermore, analysis of junctional sequences demonstrated oligoclonality suggesting local selection by antigen. In contrast, it seems unlikely that the clonally expanded V δ 1⁺ cells in pulmonary sarcoidosis are related to the high frequency response of peripheral blood γ/δ cells to mycobacterial antigens and certain heat-shock proteins (15, 16, 51). This response appears to be dependent on the V γ 9/V δ 2 subset, which was virtually absent from the sarcoid lungs. Furthermore, the remarkably clonal nature of the V δ 1 expansions precludes a local driving force that is relatively independent of junctional diversity, such as that driven by bacterial or host heat-shock proteins. It seems unlikely that the local antigen could be a γ/δ -specific superantigen.

It remains unclear why γ/δ T cell expansions were only observed in a subset of patients. It should also be emphasized that CD4⁺ T cells predominated in nearly all samples, both before and after culture in IL2. At present, no functional attribute unique to the γ/δ subset (vs. α/β subsets) has been described (43). Especially in relation to studies of infectious diseases discussed above, we wondered whether duration of disease might be a critical variable (43, 50). The patients in the present study were newly diagnosed and unfortunately, in pulmonary sarcoidosis, disease duration before clinical presentation is difficult to evaluate due to its often asymptomatic or insidious onset. In the group of patients in this study, no association between the duration of symptoms and presence of γ/δ cells was apparent. We also found no association between γ/δ infiltration and the severity of pulmonary disease or presence and extent of extra-pulmonary disease. The irregular association of γ/δ cells with CD4⁺ T cell populations has also been noted in lymphoid infiltrations that characterize other autoimmune diseases. For example, in rheumatoid arthritis, synovial T cell clones have been noted to express both γ/δ and α/β T cell receptors (51–55). In a series of patients with polymyositis/dermatomyositis, infiltrating T cells in the muscles from one patient predominantly expressed γ/δ receptors whereas α/β ⁺ (CD4⁺ and CD8⁺) cells predominated in the others (56). Finally, recent studies suggest that an expanded population of γ/δ (V δ 1⁺) cells are present in the cerebrospinal fluid of a subset of patients with early multiple sclerosis, but that the dominant T cell population in nearly all patients expresses α/β receptors and CD4 (Shimonkevitz, R. P., C. Colburn, J. Burham, R. Murray, and B. L. Kotzin, unpublished observations).

In summary, this report identifies a subgroup of patients with pulmonary sarcoidosis who have clonal expansions of lung V δ 1⁺ cells. These results support the hypothesis that the inflammatory process is perpetuated by a selective, in situ expansion of antigen-specific T cells. The limited junctional diversity of V δ 1⁺/V δ 9⁻ cells in sarcoidosis is consistent with

conventional antigen recognition rather than a superantigenic response (26). Further analysis of proposed ligands for lines and clones derived from inflamed tissue may yield insight into their function in sarcoidosis. In this regard, it is interesting that 70–90% of patients with sarcoidosis demonstrate a granulomatous cutaneous reaction to Kveim reagent, a complex antigen derived from the spleen of patients with sarcoidosis (57). Regardless of specificity it should be emphasized that activated T cell populations in pulmonary sarcoidosis are readily obtainable by bronchoalveolar lavage and perhaps short-term culture. Once identified by receptor expression, these cells are potentially amenable to specific therapy.

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References

1. Thomas, P. D., and G. W. Hunninghake. 1987. Current concepts of the pathogenesis of sarcoidosis. *Am. Rev. Respir. Dis.* 135:747–760.
2. Hunninghake, G. W., J. E. Gadek, R. C. Young, O. Kawanami, V. J. Ferrans, and R. G. Crystal. 1980. Maintenance of granuloma formation in pulmonary sarcoidosis by T lymphocytes within the lung. *N. Engl. J. Med.* 302:594–598.
3. Hunninghake, G. W., and R. G. Crystal. 1981. Pulmonary sarcoidosis: a disorder mediated by excess helper T-lymphocyte activity at sites of disease activity. *N. Engl. J. Med.* 305:429–434.
4. Hunninghake, G. W., G. N. Bedell, D. C. Zavala, M. Monick, and M. Brady. 1983. Role of interleukin-2 release by lung T-cells in active pulmonary sarcoidosis. *Am. Rev. Respir. Dis.* 128:634–638.
5. Vanet, A., A. J. Hance, C. Saltini, B. W. S. Robinson, and R. G. Crystal. 1985. Enhanced alveolar macrophage-mediated antigen induced T lymphocyte proliferation in sarcoidosis. *J. Clin. Invest.* 75:293–301.
6. Robinson, B. W. S., T. L. McLemore, and R. G. Crystal. 1985. Gamma interferon is spontaneously released by alveolar macrophages and lung T lymphocytes in patients with pulmonary sarcoidosis. *J. Clin. Invest.* 75:1488–1495.
7. Costabel, U., K. J. Bross, K. H. Ruhle, G. W. Lohr, and H. Matthys. 1985. Ia-like antigens on T-cells and their subpopulations in pulmonary sarcoidosis and in hypersensitivity pneumonitis: analysis of bronchoalveolar and blood lymphocytes. *Am. Rev. Respir. Dis.* 131:337–342.
8. Saltini, C., J. R. Spurzem, J. J. Lee, P. Pinkston, and R. G. Crystal. 1986. Spontaneous release of interleukin 2 by lung T lymphocytes in active pulmonary sarcoidosis is primarily from the Leu3⁺DR⁺ T cell subset. *J. Clin. Invest.* 77:1962–1970.
9. Moseley, P. L., C. Hemken, M. Monick, K. Nugent, and G. W. Hunninghake. 1986. Interferon and growth factor activity for human lung fibroblasts: release from bronchoalveolar cells from patients with active sarcoidosis. *Chest.* 89:657–662.
10. Konishi, K., D. R. Moller, C. Saltini, M. Kirby, and R. G. Crystal. 1988. Spontaneous expression of the interleukin 2 receptor gene and presence of functional interleukin 2 receptors on T lymphocytes in the blood of individuals with active pulmonary sarcoidosis. *J. Clin. Invest.* 82:775–781.
11. Siltzbach, L. E. 1968. Sarcoidosis and mycobacteria. *Am. Rev. Respir. Dis.* 97:1–8.
12. Janis, E. M., S. H. E. Kaufmann, R. H. Schwartz, and D. M. Pardoll. 1989. Activation of $\gamma\delta$ T cells in the primary immune response to mycobacterium tuberculosis. *Science (Wash. DC)*. 244:713–716.
13. O'Brien, R. L., M. P. Happ, A. Dallas, E. Palmer, R. Kubo, and W. K. Born. 1989. Stimulation of a major subset of lymphocytes expressing T cell receptor $\gamma\delta$ by an antigen derived from mycobacterium tuberculosis. *Cell.* 57:667–674.
14. Augustin, A., R. T. Kubo, and G. K. Sim. 1989. Resident pulmonary lymphocytes expressing the γ/δ T-cell receptor. *Nature (Lond.)*. 340:239–241.
15. Haregewoin, A., G. Soman, R. C. Hom, and R. W. Finberg. 1989. Human $\gamma\delta$ ⁺ T cells respond to mycobacterial heat-shock protein. *Nature (Lond.)*. 340:309–312.
16. Kabelitz, D., A. Bender, S. Schondelmaier, B. Schoel, and S. H. Kauf-

- mann. 1990. A large fraction of human peripheral blood γ/δ^+ T cells is activated by mycobacterium tuberculosis but not by its 65-kD heat shock protein. *J. Exp. Med.* 171:667-679.
17. Modlin, R. L., C. Pirmez, F. M. Hofman, V. Torigian, K. Uyemura, T. H. Rea, B. R. Bloom, and M. B. Brenner. 1989. Lymphocytes bearing antigen-specific gamma/delta T-cell receptors in human infectious disease lesions. *Nature (Lond.)*. 339:544-547.
18. Kabelitz, D., A. Bender, T. Prospero, S. Wesselborg, O. Janssen, and K. Pechhold. 1991. The primary response of human gamma/delta⁺ T-cells to mycobacterium tuberculosis is restricted to V gamma 9-bearing cells. *J. Exp. Med.* 173:1331-1338.
19. Balbi, B., D. R. Moller, M. Kirby, K. J. Holroyd, and R. G. Crystal. 1990. Increased numbers of T lymphocytes with $\gamma\delta$ -positive antigen receptors in a subgroup of individuals with pulmonary sarcoidosis. *J. Clin. Invest.* 85:1353-1361.
20. Tamura, N., K. J. Holroyd, T. Banks, M. Kirby, H. Okayama, and R. G. Crystal. 1990. Diversity in junctional sequences associated with the common human V γ 9 and V δ 2 gene segments in normal blood and lung compared with the limited diversity in a granulomatous disease. *J. Exp. Med.* 172:169-181.
21. The BAL Cooperative Group Steering Committee. 1990. Bronchoalveolar lavage constituents in healthy individuals, idiopathic pulmonary fibrosis, and selected comparison groups. *Am. Rev. Respir. Dis.* 141 (Suppl.):s168-s202.
22. Band, H., F. Hochstenbach, J. McLean, S. Hata, M. S. Krangel, and M. B. Brenner. 1987. Immunochemical proof that a novel rearranging gene encodes the T cell receptor δ subunit. *Science (Wash. DC)*. 238:682-684.
23. Jitsukawa, S., F. Faure, M. Lipinski, F. Triebel, and T. Hercend. 1987. A novel subset of human lymphocytes with a T cell receptor- γ complex. *J. Exp. Med.* 166:1192-1197.
24. Ciccone, E., S. Ferrini, C. Bottino, O. Viale, I. Prigione, G. Pantaleo, G. Tambussi, A. Moretta, and L. Moretta. 1988. A monoclonal antibody specific for a common determinant of the human T cell receptor γ/δ directly activates CD3⁺/WT31⁻ lymphocytes to express their functional program(s). *J. Exp. Med.* 168:1-11.
25. Wu, Y. J., W. T. Tian, R. M. Snider, C. Rittershaus, P. Rogers, L. LaManna, and S. H. Ip. 1988. Signal transduction of γ/δ T cell antigen receptor with a novel mitogenic anti- δ antibody. *J. Immunol.* 141:1476-1479.
26. Kappler, J., B. Kotzin, L. Herron, E. W. Gelfand, R. D. Bigler, A. Boylston, S. Carrel, D. N. Posnett, Y. Choi, and P. Marrack. 1989. V β -specific stimulation of human T cells by staphylococcal toxins. *Science (Wash. DC)*. 244:811-813.
27. Chomczynski, P., and N. Sacchi. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* 162:156-159.
28. Choi, Y., B. L. Kotzin, L. Herron, J. Callahan, P. Marrack, and J. Kappler. 1989. Interaction of staphylococcus aureus toxin "superantigens" with human T cells. *Proc. Natl. Acad. Sci. USA.* 86:8941-8945.
29. Loh, E. Y., S. Cwirila, A. T. Serafini, J. H. Phillips, and L. L. Lanier. 1988. Human T-cell-receptor δ chain: genomic organization, diversity, and expression in populations of cells. *Proc. Natl. Acad. Sci. USA.* 85:9714-9718.
30. Loh, E. Y., L. L. Lanier, C. W. Turck, D. R. Littman, M. M. Davis, Y.-H. Chien, and A. Weiss. 1987. Identification and sequence of a fourth human T cell antigen receptor chain. *Nature (Lond.)*. 330:569-572.
31. Takiyama, Y., D. Tkachuk, E. Michalopoulos, E. Champagne, J. Reimann, M. Minden, and T. W. Mak. 1988. Sequence and organization of the diversity, joining, and constant region genes of the human T-cell δ -chain locus. *Proc. Natl. Acad. Sci. USA.* 85:6097-6101.
32. Lefranc, M.-P., A. Forster, R. Baer, M. A. Stinson, and T. H. Rabbitts. 1986. Diversity and rearrangement of the human T cell rearranging γ genes: nine germ-line variable genes belonging to two subgroups. *Cell.* 45:237-246.
33. Lefranc, M.-P., A. Forster, and T. H. Rabbitts. 1986. Rearrangement of two distinct T-cell γ -chain variable-region genes in human DNA. *Nature (Lond.)*. 319:420-422.
34. Huck, S., P. Dariavach, and M.-P. Lefranc. 1988. Variable region genes in the human T-cell rearranging gamma (TCR) locus: V-J junction and homology with the mouse genes. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:719-726.
35. Lefranc, M.-P., A. Forster, and T. H. Rabbitts. 1986. Genetic polymorphism and exon changes of the constant regions of the human T-cell rearranging gene γ . *Proc. Natl. Acad. Sci. USA.* 83:9596-9600.
36. Krangel, M. S., H. Band, S. Hata, J. McLean, and M. B. Brenner. 1987. Structurally divergent human T cell receptor γ proteins encoded by distinct C γ genes. *Science (Wash. DC)*. 237:64-67.
37. Parker, C. M., V. Groh, H. Band, S. A. Porcelli, C. Morita, M. Fabbri, D. Glass, J. L. Strominger, and M. B. Brenner. 1990. Evidence for extrathymic changes in the T cell receptor γ/δ repertoire. *J. Exp. Med.* 171:1597-1612.
38. Hedros, E., G. Holm, and D. Pettersson. 1974. Lymphocyte subpopulations in sarcoidosis. *Clin. Exp. Immunol.* 17:219-226.
39. Daniele, R. P., J. H. Dauber, and M. D. Rossman. 1980. Immunologic abnormalities in sarcoidosis. *Ann. Intern. Med.* 92:406-416.
40. Tazi, A., I. Fajac, P. Soler, D. Valeyre, J. P. Battesti, and A. J. Hance. 1991. Gamma/Delta T-lymphocytes are not increased in number in granulomatous lesions of patients with tuberculosis or sarcoidosis. *Am. Rev. Respir. Dis.* 144:1373-1375.
41. Moller, D. R., K. Konishi, M. Kirby, B. Balbi, and R. G. Crystal. 1988. Bias toward use of a specific T cell receptor β -chain variable region in a subgroup of individuals with sarcoidosis. *J. Clin. Invest.* 82:1183-1191.
42. Moretta, L., E. Ciccone, S. Ferrini, P. G. Pellicci, M. C. Mingari, J. Zerromski, C. Bottino, C. Grossi, and A. Moretta. 1991. Molecular and cellular analysis of human T lymphocytes expressing $\gamma\delta$ T-cell receptor. *Immunol. Rev.* 120:117-135.
43. Porcelli, S., M. B. Brenner, and H. Band. 1991. Biology of the human $\gamma\delta$ T-cell receptor. *Immunol. Rev.* 29:137-182.
44. Asarnow, D. M., W. A. Kuziel, M. Bonyhadi, R. E. Tigelaar, P. W. Tucker, and J. P. Allison. 1988. Limited diversity of $\gamma\delta$ antigen receptor genes of Thy-1⁺ dendritic epidermal cells. *Cell.* 55:837-847.
45. Goodman, T., and L. Lefrancois. 1988. Expression of the $\gamma\delta$ T-cell receptor on intestinal CD8⁺ intraepithelial lymphocytes. *Nature (Lond.)*. 333:855-858.
46. Itohara, S., A. G. Farr, J. J. Lafaille, M. Bonneville, Y. Takagaki, W. Haas, and S. Tonegawa. 1990. Homing of a $\gamma\delta$ thymocyte subset with homogeneous T-cell receptors to mucosal epithelia. *Nature (Lond.)*. 343:754-757.
47. Groh, V., S. Porcelli, M. Fabbri, L. L. Lanier, L. J. Picker, T. Anderson, R. A. Warnke, A. K. Bhan, J. L. Strominger, and M. B. Brenner. 1989. Human lymphocytes bearing T cell receptor γ/δ are phenotypically diverse and evenly distributed throughout the lymphoid system. *J. Exp. Med.* 169:1277-1294.
48. Bucy, R. P., C. N. Chen, and M. D. Cooper. 1989. Tissue localization and CD8 accessory molecule expression of $\gamma\delta$ cells in humans. *J. Immunol.* 142:3045-3049.
49. Spencer, J., P. G. Isaacson, T. C. Diss, and T. T. MacDonald. 1989. Expression of disulfide-linked and non-disulfide-linked forms of the T cell receptor γ/δ heterodimer in human intestinal intraepithelial lymphocytes. *Eur. J. Immunol.* 19:1335-1338.
50. Uyemura, K., R. J. Deans, H. Band, J. Ohmen, G. Panchamoorthy, C. T. Morita, T. H. Rea, and R. L. Modlin. 1991. Evidence for clonal selection of γ/δ T cells in response to a human pathogen. *J. Exp. Med.* 174:683-692.
51. Brennan, F. M., M. Londei, A. M. Jackson, T. Hercend, M. B. Brenner, R. N. Maini, and M. Feldmann. 1988. T cells expressing $\gamma\delta$ chain receptors in rheumatoid arthritis. *J. Autoimmun.* 1:319-326.
52. Holoshitz, J., F. Koning, J. E. Coligan, J. DeBruyn, and S. Strober. 1989. Isolation of CD4⁻, CD8⁻ mycobacteria-reactive T lymphocyte clones from rheumatoid arthritis synovial fluid. *Nature (Lond.)*. 339:226-229.
53. Sioud, M., O. Forre, and J. B. Natvig. 1991. T cell receptor δ diversity of freshly isolated T lymphocytes in rheumatoid synovitis. *Eur. J. Immunol.* 21:239-241.
54. Meliconi, R., C. Pitzalis, G. H. Kingsley, and G. S. Panayi. 1991. γ/δ T cells and their subpopulations in blood and synovial fluid from rheumatoid arthritis and spondyloarthritis. *Clin. Immunol. Immunopathol.* 59:165-172.
55. Paliard, X., S. G. West, J. A. Lafferty, J. R. Clements, J. W. Kappler, P. Marrack, and B. L. Kotzin. 1991. Evidence for the effects of a superantigen in rheumatoid arthritis. *Science (Wash. DC)*. 253:325-329.
56. Hohfeld, R., A. G. Engel, K. Ii, and M. C. Harper. 1991. Polymyositis mediated by T lymphocytes that express the γ/δ receptor. *N. Engl. J. Med.* 324:877-882.
57. Siltzbach, L. E., D. G. James, E. Neville, J. Turiaf, J. P. Battesti, O. P. Sharma, Y. Hosoda, R. Mikami, and M. Odaka. 1974. Course and prognosis of sarcoidosis around the world. *Am. J. Med.* 57:847-852.