Increased Numbers of T Lymphocytes with γδ-positive Antigen Receptors in a Subgroup of Individuals with Pulmonary Sarcoidosis

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Abstract

Individuals with sarcoidosis were evaluated for preferential usage of T cells with the γδ-positive (+) type of T cell antigen receptor. Compared with normal subjects (n = 19), the group with sarcoidosis had increased numbers of CD3+ αβ-negative (−) T cells in the blood (normal, 58±12 cells/μl; sarcoid, 192±45 cells/μl, $P < 0.05$) and in the epithelial lining fluid of the lung (normal, 78±14 cells/μl; sarcoid, 240±60 cells/μl, $P < 0.04$) and a concomitant elevated number of blood and lung CD3+ γδ+ T cells, owing to a striking increase in the number of CD3+ γδ+ T cells in a subgroup (7 of 20) of sarcoid individuals. The elevated numbers of sarcoid blood γδ+ T lymphocytes were mostly TiγA+ and δTCS1−, a pattern also seen in normal individuals, consistent with the majority of γδ+ T cells expressing one γ-chain variable region, Vγ9. The observation of an increase in the total γδ+ T cell numbers in a sarcoid subgroup suggests that various specific stimuli may trigger the expansion of different T cell subpopulations within different groups of individuals with sarcoidosis. (*J. Clin. Invest.* 1990. 85:1353–1361.) sarcoidosis • T cell receptor • lung • lymphocyte

Introduction

Sarcoidosis is a chronic, nonmalignant disorder characterized by the accumulation of T lymphocytes, macrophages, and granulomata in affected organs (1, 2). The etiology of sarcoidosis is unknown, but all available evidence suggests it results from an exaggerated cellular immune response to unknown stimuli, in which activated T cells direct the accumulation of macrophages and subsequent granuloma formation (1–13).

In this context, a central question relating to the pathogenesis of sarcoidosis relates to what activates the T cells. A priori, there are three general scenarios that may be hypothesized to be responsible. First, the T cells might be randomly activated, as occurs in response to mitogens (14–17). Secondly, there may be internal activation of T cells, for example, as might occur with viral infection such as the HTLV-1 retrovirus (18). Finally, the T cells may be responding to specific classes of stimuli, i.e., one or more antigens or altered self-antigens.

To separate these possibilities, recent work has concentrated on evaluating the T cell antigen receptors (TCR)[1] of sarcoid T cells for evidence that sarcoid is associated with T cells that are similar or different. This strategy is based on the knowledge that it is the TCR that defines the uniqueness of each T cell, conferring upon that cell the ability to recognize a specific antigen (19–22). There are two types of TCR: one composed of α and β chains and another composed of γ and δ chains (23–28). The αβ-positive (+) and γδ+ TCR are expressed independently on different T cell subsets (26, 27, 29–35). In this regard, if the TCR among the T cell populations were broadly diverse (as is observed in normal subjects), it would support the concept that the activation of the T cells was nonspecific (36). Alternatively, if a large proportion of sarcoid T cells had antigen receptors that were identical, it would suggest the disease is associated with activation of a single T cell (37–41). Finally, if there were biases among the T cell populations in the use of specific TCR (or TCR using closely related elements), but with more than one dominant TCR among different subgroups of individuals with sarcoid, it would suggest the disease is initiated by specific stimuli, but with different stimuli evoking the response in different (and perhaps even the same) individual (42–46).

In the context of these considerations, there is evidence for expanded numbers of T cells with biases in the usage of elements of the β-chain of the αβ TCR in blood and/or lung of subgroups (albeit with overlap) of individuals with sarcoidosis. These observations include biases in the use of the Vβ8-variable segments (42), or in the use of the Cβ1-constant region segment (47). Furthermore, recent studies have demonstrated that lung T cells of individuals with pulmonary sarcoidosis have decreased numbers of surface αβ+ TCR compared with autologous blood T cells but exaggerated levels of TCR β-chain mRNA transcripts, consistent with the concept that the lung T cells have recently been activated through their TCR (48).

The present study has been designed to expand these concepts by asking whether there might also be some sarcoid individuals who have expanded numbers of T cells expressing the γδ TCR. To accomplish this, T cells of sarcoid individuals were compared to those of normals for the presence and the characteristics of T lymphocytes that are TCR αβ-negative (−) γδ+.

Methods

Study population. The diagnosis of pulmonary sarcoidosis was established in 20 individuals using previously defined criteria, including intrathoracic biopsy (1, 5, 10). The average age was 36±4 yr (range 26–56)[2]; there were 7 males and 13 females; 17 were nonsmokers, and 3 were current smokers. None were receiving therapy at the time of the study or within the previous 2 mo. As is typical for such patients (1, 4, 49), the average proportion of lymphocytes recovered by bronchoalveolar lavage from the lower respiratory tract was elevated (47±5%), as

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1. *Abbreviations used in this paper:* PE, phycoerythrin; TCR, T cell antigen receptor; +/−, positive/negative.

2. All data are presented as mean±standard error of the mean and all statistical comparisons were made using the two-tailed Student's t test.

was the ratio of CD4+ (helper/inducer) to CD8+ (suppressor/cytotoxic) T cells (8.4±2.0), whereas the CD4+/CD8+ ratio for blood T lymphocytes was 1.3±0.1.

As controls, 19 normal individuals (age 34±3 yr, range 23–54, 8 males, 11 females, all nonsmokers) with no history of lung disease and with normal physical examinations, chest x-rays, and lung function tests were evaluated. Bronchoalveolar lavage of these individuals revealed that lymphocytes represented 16±2% of the recovered cells (49) with a CD4+/CD8+ ratio of 2.0±1.0, whereas in blood, the normals had a CD4+/CD8+ ratio of 1.5±0.2. As an additional control group, 10 individuals with chronic inflammatory interstitial pulmonary diseases were studied. The disorders include asbestosis (n = 4), chronic beryllium disease (n = 3), hypersensitivity pneumonitis (n = 1), histiocytosis X (n = 1), and idiopathic pulmonary fibrosis (n = 1). These individuals were not treated at the time of the evaluation or within the previous 2 mo. Each individual had findings typical for each disease (50, 51).

Source and purification of cells. Blood mononuclear cells were isolated using Ficoll-Hypaque and lung mononuclear cells were obtained by bronchoalveolar lavage (10). The mononuclear cell populations were further enriched for T cells by passage over nylon wool columns. In all cases, the resulting T cell preparations were > 85% T lymphocytes (as assessed by the pan–T monoclonal antibody anti–CD3; see below). Blood neutrophils were isolated from the cell pellets of the Ficoll-Hypaque gradients using high molecular weight dextran sedimentation. Control T lymphocyte tumor cell lines MOLT-4 (American Type Culture Collection, Rockville, MD and Jurkat (52) were grown in RPMI containing 10% fetal calf serum.

Monoclonal antibodies. The antibodies used to define T cells and T cell subtypes included anti–CD3 (pan-T cells; phycoerythrin [PE]-conjugated [Leu4, Becton, Dickinson & Co., Mountain View, CA]) (53); anti-$\alpha\beta$ TCR (identifying a nonpolymorphic determinant of the $\alpha\beta$ heterodimer of the TCR and therefore all $\alpha\beta$+ T cells, fluorescein [FITC]-conjugated [TCR1 {WT31}, Becton, Dickinson & Co.]) (54, 55); anti-TCRδ1 (recognizing the δ protein expressed on the cell surface in association with the TCR γ-chain and therefore all $\gamma\delta$+ T-cells [TCRδ1, T-Cell Sciences, Cambridge, MA]) (30); anti-δTCS1 (also recognizing the δ molecule but in a variable δ region and defining a subset of $\gamma\delta$+ T-cells [δTCS1, T-Cell Sciences]) (56); anti-TiγA (recognizing a Vγ9-encoded epitope and delineating another subset of $\gamma\delta$+ T cells [57] [TiγA, kindly provided by T. Hercend, Institut Gustave-Roussy, Villejuif, France; the TiγA antibody was identified by indirect immunofluorescence using FITC-conjugated goat anti–mouse Ig, Becton, Dickinson & Co.; see reference 58 for nomenclature of Vγ regions]). Control antibodies included isotype-matched PE-conjugated, FITC-conjugated, and unconjugated nonrelevant mouse myeloma antibodies (control-FITC, control-PE, control-Ig, Becton, Dickinson & Co.).

Immunofluorescence and flow cytometry. Immunofluorescence studies were carried out using a fluorescence-activated cell sorter system (FACS 440, Becton, Dickinson & Co.) with an argon laser (488 nm) as previously described (10, 42). For two-color direct immunofluorescence using FITC-conjugated monoclonal antibodies anti-$\alpha\beta$ TCR (TCR1), anti-TCRδ1, or anti-δTCS1, the cells were incubated (15 min 4°C) with 10% normal human serum, washed, and then incubated (30 min, 4°C) with either control-FITC or the FITC-linked relevant antibody; after washing, the cells were further incubated (30 min, 4°C) with the second antibody, either PE-anti–CD3 or a PE-conjugated control Ig. Cells were again washed and fixed in 1% paraformaldehyde until FACS analysis was performed. For two-color indirect immunofluorescence with the unconjugated monoclonal antibody anti-TiγA, the cells were incubated with 10% normal human sera, washed and then incubated (30 min, 4°C) with anti-TiγA (or a control nonrelevant antibody), followed by FITC anti–mouse Ig. After 30 min at 4°C, the cells were washed and incubated (15 min, 4°C) with 10% mouse serum, washed and incubated (30 min, 4°C) with PE-anti–CD3 or PE-conjugated control Ig. Cells were then washed and fixed as described above. To ensure that the numbers of cells staining positive in the two-color immunofluorescence analysis were consistent with the numbers of cells staining positive in the single-color immunofluorescence analysis, parallel wells of cells were stained by single-color immunofluorescence with FITC-conjugated anti-$\alpha\beta$ TCR, anti-TCRδ1, anti-δTCS1, control-Ig, anti-TiγA followed by FITC-Ig, and PE-conjugated anti–CD3 and control Ig. Additional control wells included cells incubated with FITC-conjugated control-Ig followed by PE-conjugated control-Ig.

Lymphocytes were discriminated from blood monocytes or alveolar macrophages based on the characteristic low forward angle and 90° light scatter profiles on FACS analysis. For two-color immunofluorescence studies, the proportions of lymphocytes staining positive for each antibody was calculated by setting four quadrants, based on control samples, dividing the contour plots for unstained cells (lower left), single stained cells: FITC-stained cells (lower right), PE-stained cells (upper left), and double-stained cells (upper right). As for single-color immunofluorescence studies, the proportion of lymphocytes staining positive for each antibody was calculated by subtracting the relevant control sample value from the values obtained using each antibody. In all cases, the controls labeled < 1% of lung and blood lymphocytes in the lymphocyte gate that was chosen.

Assessment of the absolute numbers of T lymphocytes and $\gamma\delta$+ T lymphocyte subsets in blood and epithelial lining fluid of the lung. The absolute total numbers of CD3+ T lymphocytes and of CD3+ $\gamma\delta$+ T lymphocyte subpopulations in normal and sarcoid blood were calculated using the total white blood cell count per microliter, cell differential, and the proportions of flow cytometric evaluation of CD3+ T cells and of $\alpha\beta$+, $\alpha\beta$− TCRδ1+, TiγA+, and δTCS1+ T-cells. To evaluate the absolute total numbers of CD3+ T cells and T cell subsets in normal and sarcoid lung, the amount of epithelial lining fluid recovered by bronchoalveolar lavage was quantified by the urea method (59). The numbers of CD3+ T cells and those of the CD3+ $\gamma\delta$+ T cell subsets per microliter of epithelial lining fluid of the lung were calculated from the total number and differential of cells recovered with bronchoalveolar lavage and from the data from flow cytometric evaluation of CD3+ T cells and of $\alpha\beta$+, TCRδ1+, TiγA+, and δTCS1+ T cells. All calculations were based using the proportion of CD3+ T lymphocytes as a common denominator for total $\gamma\delta$+ T cells and for $\gamma\delta$+ T cell subsets.

Southern blot analysis. Southern blot analysis was used to evaluate blood T lymphocyte DNA for the presence of possible biased use of rearrangements of the γ gene of the TCR. DNA was recovered by standard techniques, digested (6 μg) with the restriction enzymes BamHI, EcoRI, KpnI, in appropriate buffers, analyzed by fractionating by size on 0.8% agarose gels, transferred to nitrocellulose filters (60), and hybridized to a ^{32}P-labeled Jγ probe, a 700-bp HindIII-EcoRI fragment derived from clone M13H60 (kindly provided by T. H. Rabbits, Medical Research Council, Cambridge, United Kingdom). This Jγ probe contains the Jγ1.3 germline segment and cross hybridizes with Jγ2.3 but not with other Jγ segments (see reference 61 for nomenclature of the Jγ regions). In addition, filters were rehybridized to a ^{32}P-labeled Vγ9 probe, a 700-bp PstI-HindIII fragment from clone λ SHV7 (kindly provided by T. H. Rabbitts and M.-P. Lefranc, Medical Research Council, Cambridge). This probe contains the Vγ9 region (62) which hybridizes only to the single copy Vγ9 gene. Conditions for hybridization and washing were as described by Lefranc et al. (63).

To evaluate the lower limit of detection of γ-gene rearrangements, mixing experiments were performed by diluting germline DNA from blood neutrophils with DNA from the Jurkat T cell tumor cell line, a T cell line known to have rearrangements of the γ genes (58). Southern analysis was carried out for DNA mixtures in which the Jurkat DNA represented 50%, 30%, 10%, 5%, 3%, 2%, and 1%, respectively, of the total DNA content, using the conditions described above.

Results

CD3+ $\alpha\beta$− T cells in sarcoidosis. Evaluation of blood T lymphocytes from individuals with pulmonary sarcoidosis demon-

strated, for some of these individuals, a striking increase in the proportions of CD3+ $\alpha\beta-$ T cells compared with normal individuals. Using two-color direct immunofluorescence with the anti-$\alpha\beta$ TCR and anti-CD3 monoclonal antibodies, flow cytometric analysis demonstrated that, although normal subjects had few blood T cells that were CD3+ $\alpha\beta-$ (Fig. 1, *A–D;* Fig. 2), a subgroup of sarcoid individuals had markedly increased proportions of blood CD3+ $\alpha\beta-$ T-cells (Fig. 1, *E–H;* Fig. 2). On the average, although normal individuals had 5±1% (range 0.4–8.5%) of CD3+ blood T cells that were CD3+ $\alpha\beta-$, sarcoid individuals had 17±4% (range 3–59%) of blood CD3+ $\alpha\beta-$ T cells (Fig. 2, $P < 0.01$). Using 16% (i.e., > 5 SD above the mean of the normal control group) as the highest level for the proportions of blood CD3+ $\alpha\beta-$ T cells in normal individuals (33–35), a subgroup of 7 of 20 (35%) sarcoid individuals had proportions of blood CD3+ $\alpha\beta-$ T cells above this level (range 27–59%, see Fig. 2). Even more strikingly, 2 of 20 (10%) of the sarcoid individuals had more CD3+ $\alpha\beta-$ T lymphocytes than CD3+ $\alpha\beta+$ T cells, i.e., CD3+ blood T cells that did not express a surface $\alpha\beta+$ TCR heterodimer represented more than half of the total blood CD3+ T cells (Fig. 1 *H;* Fig. 2). The seven individuals with increased numbers of CD3+ $\alpha\beta-$ T cells did not differ from those with normal numbers of these T

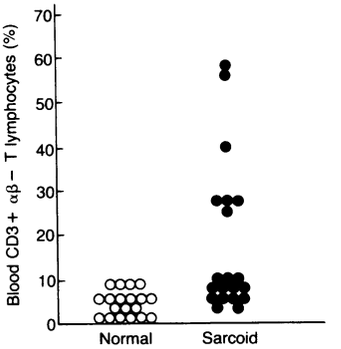

Figure 2. Proportion of blood CD3+ $\alpha\beta-$ T lymphocytes in normal subjects and individuals with sarcoidosis. The data was generated using dual-color direct immunofluorescence and flow cytometric analysis as described in the legend of Fig. 1.

cells in age, sex, length of time since diagnosis of disease, bronchoalveolar cell count and differential, or extrapulmonary involvement by sarcoidosis.

Consistent with the knowledge that pulmonary sarcoidosis is associated with changes in the numbers and ratios of various T cell subsets in both blood and lung, the numbers of CD3+ $\alpha\beta-$ T cells were increased in both blood and lung of individuals with sarcoidosis (Fig. 3). In this regard, sarcoid individuals showed an increase in total numbers of CD3+ $\alpha\beta-$ blood T

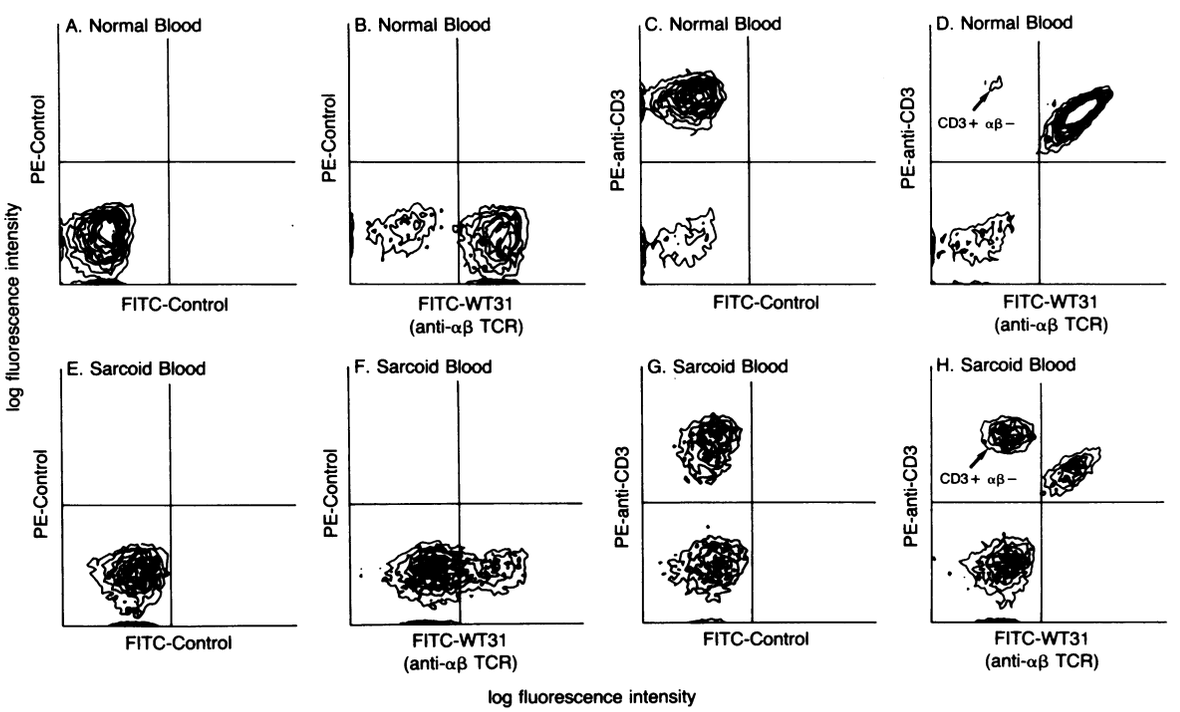

Figure 1. Example of the analysis of blood T cells of individuals with sarcoidosis for the presence or absence of the $\alpha\beta$ TCR heterodimer. Blood T lymphocytes of a normal individual (*A–D*) and an individual with sarcoidosis (*E–H*) were evaluated with the monoclonal antibodies PE conjugated anti-CD3 (pan-T cell) and fluorescein (FITC)-conjugated anti-$\alpha\beta$ heterodimer of the TCR, using flow cytometry. Controls included cells incubated with isotype-matched, nonrelevant mouse myeloma antibodies conjugated with FITC followed by further incubation with nonrelevant mouse myeloma antibodies conjugated with PE, cells incubated with anti-$\alpha\beta$ TCR antibodies FITC-conjugated followed by control antibodies PE-conjugated, and cells incubated with control antibodies FITC-conjugated followed by anti-CD3 antibodies PE-conjugated (see Methods). The gate was set on lymphocytes as determined by forward-angle and 90° light scatter. *Ordinate:* log fluorescence intensity for PE. *Abscissa:* log fluorescence intensity for FITC. Contour plots were divided into quadrants based on control samples to identify unstained cells (*lower left*), single stained cells: FITC-staining cells (*lower right*), PE-staining cells (*upper left*), and dual fluorescence-stained cells (*upper right*). Arrows in *D* and *H* indicate CD3+ $\alpha\beta-$ T cells (5.2% of total CD3+ T cells in the normal individual, 56% of total CD3+ T cells in the sarcoid individual). Note that in this example the sarcoid individual had more CD3− lymphocytes than the normal individual. This did not represent a consistent finding among all sarcoid individuals, but may represent a relative increase in CD3− natural killer-like cells in this individual (see references 79–81).

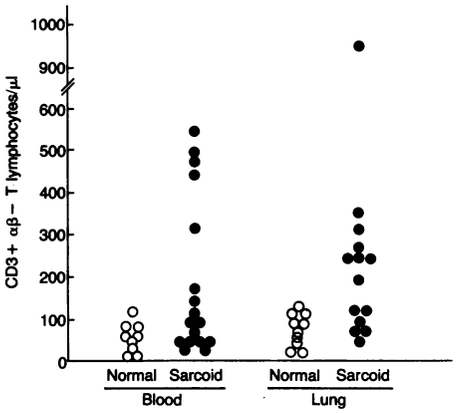

Figure 3. Numbers of CD3+ $\alpha\beta-$ T lymphocytes of blood and lung epithelial lining fluid of normals and individual with sarcoidosis. The data were generated from the total and differential white blood cell count and the total and differential count of lung cells recovered with bronchoalveolar lavage and the proportions of flow cytometric evaluation of CD3+ and $\alpha\beta+$ T cells. The blood data is expressed per microliter of blood and the lung data per microliter of epithelial lining fluid removed by bronchoalveolar lavage (see Methods).

cells as compared with normal individuals (normal, 58±12 cells/μl; sarcoid, 192±45 cells/μl; $P < 0.05$) i.e., in sarcoidosis there is an expansion in the total number of circulating blood CD3+ $\alpha\beta-$ T cells. Furthermore, evaluation of lung T lymphocytes showed that, whereas in normal subjects CD3+ $\alpha\beta-$ T cells were present in the lung in low numbers (similar to normal blood, $P > 0.2$), sarcoid individuals demonstrated an absolute increase also in the numbers of lung CD3+ $\alpha\beta-$ T cells (normal, 78±14 cells/μl epithelial lining fluid; sarcoid, 240±60 cell/μl; $P < 0.04$). In addition, of the seven sarcoid individuals of the subgroup with elevated proportions of blood CD3+ $\alpha\beta-$ T cells, five had also increased numbers of lung CD3+ $\alpha\beta-$ T cells, i.e., the bias for elevated numbers of CD3+ $\alpha\beta-$ T cells was present in both the blood and lung compartments. Interestingly, whereas in sarcoid blood the increased numbers of CD3+ $\alpha\beta-$ T cells were associated with numbers of CD3+ $\alpha\beta+$ T cells that were similar compared to normals ($P > 0.05$, Table I), the exaggerated numbers of CD3+ $\alpha\beta-$ T cells in sarcoid lung were associated with a marked increase in the numbers of lung CD3+ $\alpha\beta+$ T cells ($P < 0.01$, Table I); i.e., although in sarcoidosis CD3+ $\alpha\beta-$ T cells were increased in numbers in both blood and lung compartments, other T cell populations compartmentalized in a different fashion.

To investigate whether the bias for $\alpha\beta-$ T cells was sarcoid disease specific, we evaluated a group of 10 patients with a variety of chronic inflammatory pulmonary disorders (see Methods). In this group there was no evidence for an expansion of CD3+ $\alpha\beta-$ T lymphocytes, either in blood or in lung. The proportions of blood CD3+ $\alpha\beta-$ T cells in blood were 5±1% (range 1–10%) and in lung 4±2% (range 2–8%) of total CD3+ T lymphocytes ($P > 0.2$ with normals each comparison, data not shown).

Chronicity of the presence of increased numbers of CD3+ $\alpha\beta-$ T cells in sarcoid. To evaluate whether the increase in CD3+ $\alpha\beta-$ T cells in sarcoidosis is a short-lived perturbation of the immunologic regulatory system or a chronic abnormality, five individuals in the subgroup of sarcoid patients who had elevated proportions of blood CD3+ $\alpha\beta-$ T cells were evaluated several times over the period of a year. Strikingly, in these individuals, the increased proportions of sarcoid blood CD3+ $\alpha\beta-$ T cells persisted (Fig. 4), i.e., the exaggerated bias for CD3+ $\alpha\beta-$ T cells in this subgroup of sarcoid individuals reflected a chronic change in the normal immune homeostasis.

*Table I. Numbers of CD3+ $\alpha\beta+$ and CD3+ $\alpha\beta-$ Lymphocytes in Blood and Lung of Normal and Sarcoid Individuals**

	Normal		Sarcoid	
Category	Blood	Lung	Blood	Lung
		cells/μl		
Lymphocytes	1,440±140	4,200±1,000	1,130±120	11,800±1,800
CD3+ $\alpha\beta+$	1,380±140	4,100±1,000	950±130	11,500±1,800
CD3+ $\alpha\beta-$	58±12	78±14	192±45	240±60

* Data generated from the total and differential white blood cell counts, the total number and differential of cells of lung epithelial lining fluid recovered by bronchoalveolar lavage, and the proportions of CD3+ and $\alpha\beta+$ T cells determined by flow cytometry (see Methods).

Expression of the $\gamma\delta$ TCR in sarcoidosis. To confirm that the CD3+ $\alpha\beta-$ T cells in sarcoidosis are actually CD3+ $\gamma\delta+$ T cells, normal and sarcoid blood and lung T cells were evaluated using two-color direct immunofluorescence with the antibodies anti-CD3 (pan-T cell) and anti-TCRδ1 (all T cells having a $\gamma\delta+$ TCR). Importantly, the sarcoid individuals with elevated levels of CD3+ $\alpha\beta-$ T cells exhibited also increased levels of CD3+ TCRδ1+ T cells (Fig. 5). Furthermore, the numbers of blood and lung CD3+ TCRδ1+ T cells were similar to the numbers of blood and lung CD3+ $\alpha\beta-$ T cells, confirming that the CD3+ $\alpha\beta-$ sarcoid T cells do indeed express the $\gamma\delta+$ TCR.

To evaluate if the increased numbers of sarcoid $\gamma\delta+$ T lymphocytes were associated with preferential expansion of a particular $\gamma\delta+$ T cell subpopulation, lung and blood T lymphocytes from the subset of sarcoid individuals with elevated numbers of CD3+ $\gamma\delta+$ T cells were evaluated with two-color immunofluorescence using the antibodies that react respectively with a γ (anti-TiγA) and a δ (δTCS1) variable region

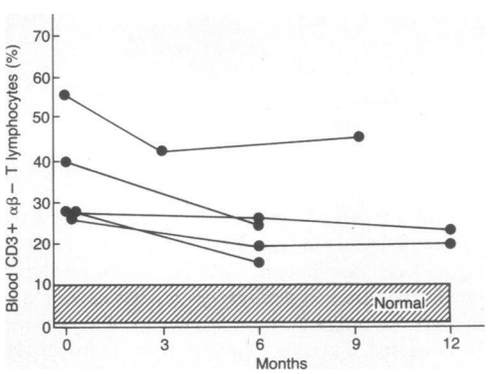

Figure 4. Chronicity of the increased proportions of blood CD3+ $\alpha\beta-$ T lymphocytes in individuals with sarcoidosis. The data was generated using dual color direct immunofluorescence and flow cytometric analysis as described in Fig. 1. Each set of lines represents one individual evaluated repetitively over a period of 6–12 mo. The shaded area corresponds to the range for normal individuals.

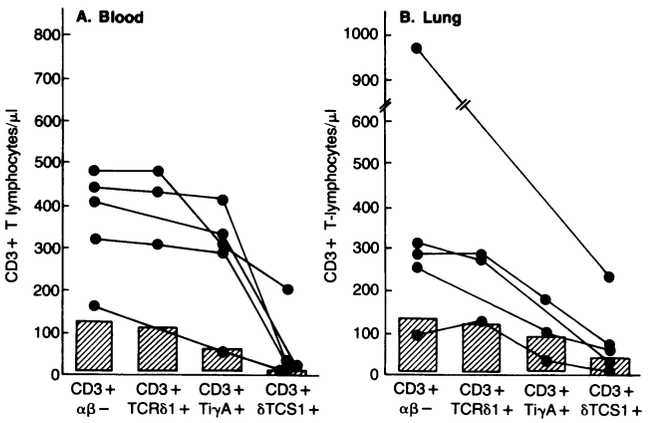

Figure 5. Numbers of CD3+ $\alpha\beta-$, CD3+ TCRδ1+, CD3+ TiγA+, and CD3+ δTCS1+ T lymphocytes in blood and lung epithelial lining fluid in a subset of sarcoid individuals with increased proportions of CD3+ $\alpha\beta-$ T lymphocytes. The data was generated as described in Fig. 3. The blood data is expressed per microliter of blood and the lung data per microliter epithelial lining fluid recovered by bronchoalveolar lavage. The shaded areas represent normal ranges for each parameter (normal data generated from the normal study population using identical methods as for the sarcoid patients).

and that define two subsets of $\gamma\delta+$ T cells. In this regard, evaluation of sarcoid blood and lung T cells using two-color indirect immunofluorescence with the antibodies anti-CD3 and anti-TiγA demonstrated that sarcoid individuals with increased numbers of blood CD3+ $\gamma\delta+$ T cells had also a marked increase in the numbers of blood CD3+ TiγA+ T cells (Fig. 5 *A*). The observation that all the cells of the $\gamma\delta+$ T cell subset defined by anti-TiγA use the Vγ9 region to form their TCR (64) suggests that in sarcoidosis the expansion in the numbers of circulating CD3+ $\gamma\delta+$ T lymphocytes is largely due to T cells expressing the Vγ9-variable segment of the γ gene. However, since TiγA+ cells are also the predominant blood CD3+ $\gamma\delta+$ T cell in normal individuals, the data obtained by antibody staining does not exclude a nonspecific expansion of all of the CD3+ $\gamma\delta+$ T cells. Interestingly, the majority of the elevated numbers of sarcoid lung CD3+ $\alpha\beta-$ T cells and CD3+ TCRδ1+ $\gamma\delta+$ T cells were not CD3+ TiγA+; i.e., although insufficient numbers of individuals were available to definitively evaluate this concept, it is possible that the elevated numbers of $\gamma\delta+$ T cells in sarcoid lung represent an overlapping, but different, subset of T-cells than found in blood.

In contrast to the data with anti-TiγA, evaluation of normal and sarcoid blood and lung T cells using two-color direct immunofluorescence with the antibodies anti-CD3 and anti-δTCS1, an antibody directed against a specific δ-chain determinant (56), showed that, as a group, sarcoid individuals did not have an increase of CD3+ δTCS1+ T cells in blood nor lung. In this regard, most sarcoid individuals with elevated proportions of blood CD3+ $\gamma\delta+$ T cells, had numbers of blood or lung CD3+ δTCS1+ T lymphocytes similar to normals (Fig. 5). There was one individual, however, who had a striking increase in the number of blood and lung CD3+ δTCS1+ T cells; i.e., there may be differences in the specific composition of the sarcoid CD3+ $\gamma\delta+$ T-cell populations on an individual basis.

Evaluation of sarcoid $\gamma\delta+$ T cells for specific rearrangements of the γ gene of the TCR. The presence of increased numbers of CD3+ $\gamma\delta+$ T cells in sarcoid individuals may be the result of an expansion of one or few $\gamma\delta+$ T cells (i.e., a monoclonal or oligoclonal expansion) or may be the result of an expansion of a large number of different $\gamma\delta+$ T cells. To evaluate the possibility that the expanded CD3+ $\gamma\delta+$ T cell populations observed show a biased use of the TCR, Southern blot analysis of sarcoid T cell DNA was carried out with Jγ and Vγ gene probes to determine the pattern of γ gene locus rearrangements. A predominant rearranged band, with a change in relative intensity of the band compared to normals, would be evidence for a biased use of a particular γ gene V-J pair. However, analysis of T cell DNA with BamHI, EcoRI, and KpnI, and hybridization with Jγ and Vγ9 probes failed to demonstrate any new bands (not shown). Thus, despite the increased numbers of $\gamma\delta+$ T cells, Southern analysis did not show any dominant γ gene rearrangement in sarcoid T cell DNA which would indicate a bias in the use of TCR by the $\gamma\delta+$ T cell population. However, because of the limitations of the Southern technique, this analysis does not exclude the possibility that overrepresentation of specific γ-chain usage does exist in some sarcoid individuals with increased numbers of $\gamma\delta+$ cells. Further, it does not exclude the possibility that there are biases in Vγ-Jγ junctional region (N region) sequences, where much of the γ/δ TCR heterogeneity is generated.

The sensitivity of Southern blot analysis for detecting monoclonal γ gene rearrangements was assessed by mixing experiments using γ gene rearranged DNA from the Jurkat T-cell line and unrearranged germline DNA. These experiments demonstrated that γ gene rearranged T cell DNA could be detected when it composed up to 5–10% of the total DNA mixture (not shown). This control evaluates only the limit of detection of a new γ gene rearrangement completely absent in the background DNA. It does not evaluate the sensitivity or reproducibility of detecting a change in intensity of a rearranged band against a background of γ gene rearrangements of normal T cells, i.e., of detecting a bias in the use of a particular Vγ-Jγ pair already present in the normal T-cell population sufficiently to produce a band.

Discussion

By evaluating the proportions of CD3+ $\gamma\delta+$ T lymphocytes in individuals with pulmonary sarcoidosis, the present study demonstrates in blood and lung of a subgroup of sarcoid individuals a bias for T cells that use the $\gamma\delta$ T cell antigen receptor. This expansion of $\gamma\delta+$ T cells was most clearly defined in the blood where the $\gamma\delta+$ T cells were increased in total numbers and as a percentage of total CD3+ T lymphocytes. In the lung, the expansion of CD3+ T lymphocytes was evident in absolute numbers, but not as a relative percentage of all CD3+ T lymphocytes, most likely reflecting an increased expansion of $\alpha\beta+$ T cells relative to the $\gamma\delta+$ T cells in the lung. This may be due to differences in the numbers of $\gamma\delta+$ T cells present in the lung (compared with $\alpha\beta+$ T cells) before the initiation of the sarcoid inflammation or may be due to differences in trafficking; i.e., $\gamma\delta+$ T cells may more easily move from the alveolar surfaces to the blood than $\alpha\beta+$ T cells. Interestingly, in the subgroup of individuals with increased numbers of $\gamma\delta+$ T cells, the bias was chronic, persisting for at least 6 mo to 1 yr, sug-

gesting that it represented a stable feature of the immune abnormalities associated with the disease. Moreover, the expansion of γδ+ T cells in sarcoidosis reflected predominantly an expansion in the numbers of CD3+ TiγA+ T cells, i.e., T-cells expressing the Vγ9 element of the γ-chain on the cell surface. TiγA+ cells are also the predominant blood CD3+ γδ+ T cell in normal individuals (57), so that the data obtained by antibody staining does not exclude a nonspecific expansion of all of the γδ+ T cells regardless of their specificity. Indeed, despite the presence of an expanded population of Vγ9 T cells, Southern blot analysis using a Vγ9 probe and a Jγ probe failed to reveal the emergence of a predominant population of γδ+ T cells, suggesting that the sarcoid γδ+ T cells likely represent a heterogeneous population of cells with at least several different γ gene rearrangements. While a nonspecific expansion of all γδ+ T cells remains a possibility, this finding may be analogous to previous studies on αβ+ T cell receptor repertoire bias in sarcoid patients which have demonstrated no evidence of clonal rearrangements of Vβ8+ T cell DNA using Southern blot analysis (42) in patients with increased proportions of lung and blood Vβ8+ T cells. However, when the same sarcoid subjects were studied using more sensitive techniques, the existence of identical T cell receptor mRNA transcripts indicating oligoclonal populations of Vβ8+ T cells was demonstrated in the lung T cell populations of at least some of these individuals (65). Thus, it is still possible that the expansion of γδ+ T cells in a subgroup of sarcoid individuals represents a selective stimulation and proliferation of a limited number of γδ+ T cell clones.

Relevance of finding γδ+ T cells in sarcoidosis. The precise role of γδ+ T-cells has not yet been clearly defined, but their evolutionary conservation suggests they are important to host immunologic defense mechanisms (66–69). Similar to αβ+ T cells, γδ+ T lymphocytes can perform a variety of activities, including proliferation after stimulation with anti-CD3 or anti-TCR clonotypic antibodies (70–72), recognition of allogeneic MHC class I or class II molecules (73, 74), proliferation in allogeneic mixed lymphocyte cultures (75), and release of lymphokines after stimulation (31, 67–69, 72, 73, 76). The presence of a subgroup of sarcoid individuals with an expansion of TiγA+ γδ+ T-cells, i.e., of T cells using the same variable region of the γδ T cell receptor (64), suggests that the expansion of sarcoid γδ+ T cells may be related to their TCR specificity. However, given the limited number of known functional Vγ regions (58), it is possible the γδ T cell antigen receptor may have a limited range of specificities, as compared with the αβ+ antigen receptor (36, 67–69). In this context, it has been hypothesized that γδ+ T cells are able to recognize less polymorphic regions of MHC molecules expressed on the cell surface as a consequence of infectious agents, metaplasia or cell damage (67–69), suggesting a role for γδ+ T cells in the immunosurveillance against infected, transformed, or cancerous cells. Consistent with this concept, there is recent evidence that individuals infected with the HIV or the EBV virus can have elevated numbers of circulating CD3+ αβ− T lymphocytes (77). In this regard, the expression of Vγ9+ T cells in sarcoid could be triggered, for example, by the presence of cells infected by a viral agent causing this disease. Consistent with this general concept, sarcoidosis has many clinical similarities with T cell leukemia/lymphoma due to the human HTLV-1 retrovirus (18, 74). Although previous studies have demonstrated no evidence for HTLV-1 infection in sarcoidosis (78), it is possible that a yet unknown virus or retrovirus may cause sarcoidosis.

Based on the frequent observation of non-MHC-restricted cytotoxicity displayed by in vitro stimulated CD3+ αβ− T cell lines and clones (26, 27, 71, 75), it has been hypothesized that γδ+ T lymphocytes might be "natural killer-like" T cells (67–69, 75). In regards to sarcoidosis, this is consistent with the observation of cells with increased natural killer-like activity in lung and blood of sarcoid individuals (79–81). Thus, it is plausible that the natural killer-like function may be associated with at least part of the exaggerated γδ+ T cell numbers observed in this subgroup of individuals with sarcoid.

Since γδ+ T cells likely represent a distinct T cell lineage (82), it is conceivable that in some sarcoid patients an abnormal process of T cell development results in the expansion of specific γδ+ T cells which might predispose to the disease, if coupled with exposure to a specific antigenic stimulation. An interesting animal model of this theory is the *lpr* mouse, in which a thymic defect modulates the emergence of an autoimmune syndrome associated with an accumulation of T cells expressing high levels of γ gene transcripts (83). In humans, other examples of increased proportions of circulating γδ+ T lymphocytes are observed in individuals with bone marrow transplantation (84) and congenital immunodeficiency states (26, 27), suggesting that the increased numbers of γδ+ T cells may represent a regulatory host response to the redistribution or insufficient numbers of αβ+ T lymphocytes.

Finally, together with other studies on biases in the usage of variable and constant β gene regions, the observation of a bias for γδ+ Vγ9+ T cells in a subset of patients with sarcoidosis suggests that sarcoidosis is a heterogeneous disease. Evaluation of possible correlations between sarcoid subgroups with bias for Vβ8 or Cβ1 T cells have not revealed to date any striking correlation with individuals expressing a bias for γδ+ T cells. Together, these data support the hypothesis that the exaggerated cellular immune response of sarcoidosis is initiated by the selective expansion of various αβ+ and γδ+ T cell subsets, presumably triggered by a variety of factors in different individuals and/or in each individual at different stages of the disease.

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