Diversity in Functional Sequences Associated with the Common Human \( \gamma \delta \) and \( \delta \) Gene Segments in Normal Blood and Lung Compared with the Limited Diversity in a Granulomatous Disease

By Naoaki Tamura, Kenneth J. Holroyd, Tyrone Banks, Martha Kirby, Hiroshi Okayama, and Ronald G. Crystal

From the Pulmonary Branch, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, Maryland 20892

Summary

The T cell receptor (TCR) junctional regions (N regions) of the common human \( \gamma \) and \( \delta \) gene segments were sequenced from the blood and lung of normal individuals (195 transcripts) and a group of individuals with sarcoidosis (220 transcripts), a granulomatous disease in which increased numbers of \( \gamma \delta \)-positive T cells are often observed. In normal individuals, the vast majority (86%) of blood \( \gamma \) transcripts used the \( \gamma P \) gene segment. In contrast to this restriction of \( J \) region usage, there was a large diversity of the junctional region, with <20% of blood \( \gamma \) junctional regions showing identical sequences for any one normal individual. For the blood \( \delta \) transcripts in normal individuals, there was restriction of \( J \) region usage, with 93% using \( J61 \). The junctional regions were even more diverse than for \( \gamma \), with a unique sequence observed in each transcript examined. Compared with blood, sequences from the normal lung showed a small increase in identical junctional regions, particularly in one individual where 46% of \( \gamma \) transcripts examined were identical, suggesting a response of some \( \gamma \delta \) T cells to antigens found in the lung in the normal state. In marked contrast to normals, some individuals with sarcoidosis had large numbers of \( \gamma \) transcripts, as well as \( \delta \) transcripts, sharing identical sequences. For \( \gamma \) blood transcripts, two individuals showed 84 and 56% of junctional region sequences to be identical, respectively. Similarly, blood \( \delta \) transcripts showed 43, 33, and 25% identical junctional region sequences in three individuals. In the sarcoid patient with the most striking over-representation of blood \( \gamma \) junctional sequences, lung \( \gamma \) transcripts showed increased (67%) use of the same junctional region sequence as in blood. This limited diversity of TCR junctional regions among some individuals with sarcoidosis suggests a response from specific stimuli, possibly antigenic, and that \( \gamma \delta \) T cells may play a specific role in granuloma formation in sarcoidosis, as has been suggested in other granulomatous diseases.

The lymphocytes recognize antigens through the TCR, a CD3-associated heterodimeric surface complex that defines the specificity of the T cell (1–3). In normal individuals, most (>90%) of blood T cells express a TCR composed of \( \alpha \) and \( \beta \) chains, proteins defined by a large repertoire of genetic elements that recombine during T cell ontogeny, permitting a broad diversity of antigen recognition (1–3). The remaining T cells have TCRs composed of \( \gamma \) and \( \delta \) chains. Although the TCR-\( \gamma \delta \) uses similar recombination processes, unlike the broad repertoire of genetic elements that can potentially define the TCR-\( \alpha \beta \), the human \( \gamma \delta \) repertoires are severely limited, with only eight functional \( \gamma \) and five \( J \) segments, and an even smaller number of \( V \), \( D \), and \( J \) segments defining the \( \delta \) locus (4, 5). Further, in normal individuals, the usage of TCR-\( \gamma \delta \) appears to be even more restricted, in that >60% of blood \( \gamma \delta \) T cells use \( \gamma 9 \) elements, as detected by the mAb Tr\( \gamma \)A (6). In addition, analysis of \( \gamma 9 \) T cell clones demonstrates that most TCRs using \( \gamma 9 \) are \( \gamma 9 \)-\( J \gamma P-Cy1 \) paired with a \( \delta \) chain using the \( \delta 2 \) elements (7, 8). In this context, the diversity of the TCR-\( \gamma \delta \) is primarily based on the variable deletion or addition of nucleotides to the junctional regions (N regions) during rearrangement of the \( \gamma \) and \( \delta \) loci (9–11).

The reason for the increased representation and association of these specific gene segments is not clear. In the context that <5% of human postnatal thymic \( \gamma \delta \) clones have this pattern of gene usage, it does not appear to result from a restriction of recombinational possibilities at the gene level nor a restriction of the protein pairing possibilities of \( \gamma \) and \( \delta \) chains (12). Rather, it is consistent with the concept that
these cells are exported from the thymus during fetal development and/or are subsequently expanded by restricted antigen recognition or other pressures. Further, put in the setting of data suggesting that γ/δ T cells play a role in the response to mycobacteria and parasites (13–18), and can proliferate in response to mycobacterial heat shock proteins (16–18), it is conceivable that the extensive usage of Vγ9 paired with Vδ2 elements among normal blood γ/δ T cells may reflect early post-thymic exposure to classes of antigens such as heat shock proteins, or other developmental pressures to expand T cells with such specificities.

The present study was designed to help understand these concepts by evaluating the sequences of the junctional segments of Vγ9 and Vδ2 mRNA transcripts in normal individuals and from individuals with sarcoidosis. The relevance of the sarcoidosis group relates to the recent observation that: (a) 35% of individuals with sarcoidosis have increased numbers of γ/δ T cells, and >70% of these cells are TrγA+ (19); and (b) sarcoidosis is a systemic granulomatous disorder for which the etiology is unknown, but has long been considered to relate in some fashion to mycobacteria and/or similar microorganisms (20). By analyzing a total of 281 Vγ9 transcripts and 134 Vδ2 transcripts of normals and individuals with sarcoidosis, we observed a broad diversity of Vγ9 junctional sequences in the normals, but a striking overrepresentation of specific junctional region sequences in a subgroup of the individuals with sarcoidosis. Further, the junctional regions of Vδ2 transcripts in these same sarcoid individuals also showed an overrepresentation of certain sequences, although less than with Vγ9, suggesting the TrγA+ T cells observed in these individuals are expanded post-thymically in response to specific pressures.

**Materials and Methods**

**Source of T Lymphocytes.** Two populations of individuals were evaluated: normals and individuals with sarcoidosis. The normals included nine individuals (referred to as "normal 1," "normal 2," etc.; with the individual cDNA clones defined as N1, ... , N9, respectively). Five individuals were used as a source of blood T cells and four for lung T cells. None had a history of lung disease, and all had normal chest x-rays and lung function. Bronchoalveolar lavage of four normals showed a normal number of cells recovered and normal cell differential. The patients with sarcoidosis included five individuals diagnosed as previously described (22). All had pulmonary sarcoidosis as diagnosed by an intrathoracic biopsy showing noncaseating granuloma. The average age was 38 ± 5 yr (all data are presented as mean ± SEM, and all statistical comparisons were made using the two-tailed student’s t test). There were four males and one female; three were nonsmokers, two were ex-smokers. None was receiving therapy at the time of evaluation or within the previous two mo. All had chest x-rays with diffuse reticulonodular infiltrates and hilar adenopathy, and all had positive gallium-67 scans. Lung function tests (23) revealed vital capacity 64 ± 18% predicted, total lung capacity 79 ± 6% predicted, ratio of forced expiratory volume in 1 s to forced vital capacity 106 ± 10% predicted, and diffusing capacity 65 ± 7% predicted. As is typical for such individuals, bronchoalveolar lavage analysis revealed an elevation of the average proportion of lymphocytes (37 ± 11%) and the ratio of CD4+ (helper/inducer T cells) to CD8+ (suppressor/cytotoxic T cells) T cells (10.3 ± 6.5). The CD4+/CD8+ ratio for the blood T cells in the same individuals was 1.5 ± 0.8 (24).

Blood mononuclear cells were obtained from heparinized blood by Ficoll-Hypaque (LSM; Organon Teknika Corp., Durham, NC) gradient centrifugation. Lung mononuclear cells were obtained from bronchoalveolar lavage fluid as previously described (21). The recovery of lung mononuclear cells was 15 ± 6 x 10⁶ for the normals and 63 ± 14 x 10⁶ for the individuals with sarcoidosis.

**mAbs and Flow Cytometry.** The phenotype of blood and lung T cells was determined by two-color immunofluorescence and flow cytometry (FACS 440; Becton Dickinson & Co., Mountain View, CA) with the mAbs Leu-4 (CD3, pan T cell, PE conjugated); Leu-3 (CD4, FITC conjugated); Leu-2 (CD8, FITC conjugated); TCR-1 (WT31, all α/β T cells, FITC conjugated) (25); (all these mAbs were from Becton Dickinson & Co.); TCR-αβ (all γ/δ T cells, FITC-conjugated; T Cell Sciences, Cambridge, MA) (26); TCS-81 (a Vβ1/Jβ1 determinant, FITC-conjugated, T Cell Sciences) (27); TrγA (recognizing a Vγ9-encoded epitope of γ/δ T cells; kindly provided by T. Hercend, Institut Gustave-Roussy, Villejuif, France) (28). Indirect immunofluorescence was performed by using FITC-conjugated goat anti-mouse Ig (Becton Dickinson & Co.) as a second antibody. Control antibodies included isotype-matched PE-conjugated, FITC-conjugated, and unconjugated nonrelevant mouse myeloma antibodies (control FITC, control PE, control Ig; Becton Dickinson & Co.).

**Analysis of Vγ9 and Vδ2 mRNA Transcripts.** Total cellular RNA from blood or lung mononuclear cells was extracted using the guanidine/cesium chloride method (29). Briefly, the cells were lysed as a pellet in 5.5 M guanidine isothiocyanate with 0.5% (w/vol) 2-ME. The lysates were layered on a cushion of 5.7 M cesium chloride, 100 mM EDTA, and the RNA was collected after centrifugation (180,000 g, 12 h, 20°C). The pellet was dissolved in 10 mM Tris-HCl, pH 7.4, 5 mM EDTA, and 1% SDS, precipitated with ethanol, then redissolved in water with an RNase inhibitor (RNasin; Promega Biotec, Madison, WI), and stored in liquid nitrogen vapor until use. When small numbers of lymphocytes were evaluated, as in the case of the normal lung, cytoplasmic RNA was extracted as follows. Cells were suspended in 200 μl of 10 mM Tris-HCl, pH 7.5, 10 mM NaCl, 3 mM MgCl₂ with RNasin. Then, 20 μl of 10% NP-40 was added. The mixture was vortexed, and after centrifugation (12,000 g, 5 min), the supernatant was recovered and mixed with 200 μl of 1% SDS, 20 mM EDTA, pH 7.5, 0.6 M NaCl, 20 mM Tris-HCl, pH 7.5. After phenol/chloroform extraction, RNA was ethanol precipitated.

To sequence Vγ9 and Vδ2 mRNA transcripts in the blood and lung T cell RNA preparations, the following strategy was used. First-strand cDNA was synthesized from the extracted RNA by using cloned M-MLV reverse transcriptase (Bethesda Research Laboratory, Gaithersburg, MD) and oligo(dT) (1 h, 37°C). Aliquots of the resulting mixture were used as template for amplification by PCR with Taq DNA polymerase (Perkin-Elmer Cetus, Norwalk, CT) using the following primers: for Vγ9, a Vy9 primer with a PstI site (PVG9; 5′-ATCTGGCAAGGCATGTCAAGAAGATTAC-3′) and a Cy primer (PCG7; 5′-CTCTGCTATGTCCAGCTTCTCGAG-3′); for Vδ2, a Vy9 primer with a PstI site (PVD2: 5′-GACTGCAGGAAGACC-AAAGGTTACAC-3′) and a Cy primer (PCD1; 5′-GTTATCTTGAGTACGACAG-3′). The reaction was carried out in a 100-μl volume under recommended conditions for 45 cycles, consisting of denaturation (94°C, 30 s); annealing (54°C, 30 s); and extension (72°C, 1 min) using a thermal cycler (Perkin-Elmer Cetus) (30). Amplified products were phenol/chloroform extracted and...
ethanol precipitated. After digestion of the Vγ9 cDNA with SstI and PstI, and the Vβ2 cDNA with PstI and EcoRI (the amplified C6 region includes an EcoRI cutting sequence), respectively, the cDNA was purified by 1.8% agarose gel electrophoresis, and the appropriate sized band was cut out and extracted (Gene clean; Bio101, La Jolla, CA). The digested, purified cDNA was ligated to M13mp19 plasmids, and plaques containing appropriate inserts were isolated randomly. Sequencing was done by the dyeoxy termination method with T7 DNA polymerase (Sequenase; United States Biochemical Corp., Cleveland, OH) and universal primer (31).

**Dot Blot Hybridization.** To confirm the presence of multiple copies of identical cDNAs in some individuals with sarcoidosis, the relative numbers of specific Vγ9-N-JγP junctional region transcripts compared with total Vγ9-N-JγP transcripts was evaluated using allele-specific amplification, followed by dot blot hybridization (32). After synthesis of first-strand cDNA from the RNA preparations of T cells of normals and individuals with sarcoidosis, PCR was performed with two sets of primers for 25 cycles under the same conditions as described above. An allele-specific amplification primer was constructed according to the specific junctional region of the Vγ9 gene (PVNJ9; 5'TACGTGGCCTTGTTGG-GAAAGGGA-3'), identified as being present in abundance in some individuals with sarcoidosis (see Results). Two combinations of primers were used for PCR amplification, PVG9 and PCG5 (5'-CTGCTTTATTGAGAAAGATAAT-3'), and PVNJ9 and PCG5. The combination of PVG9-PCG5 will amplify all Vγ9 mRNA transcripts, while the combination of PVNJ9-PCG5 will amplify only the cDNA that has the specific junctional region sequence of Vγ9 mRNA transcripts. Amplified cDNAs were then dot blotted on a nitrocellulose membrane, and hybridized with a 32P-labeled 274-bp Cy region cDNA probe. This probe was synthesized by PCR, using PCG2 (5'-AACAACTTGATGCAGATGCA-3') and PCG3 (5'-TCAATCTGACGACATCACCTGTT-3') and purified by electrophoresis (see Fig. 1). The relative number of Vγ9 mRNA transcripts can then be compared visually.

**Single Cell Analysis.** For single cell analysis of Vγ9 mRNA transcripts from sarcoïd blood lymphocytes were stained with Leu-4/PE and TCR-51/FITC, and double positive cells (γ/δ T cells) were sorted by FACS 440 so that each well of a 96 well U-shaped plastic plate contained one cell (33). DNA was then prepared as described above, and Vγ9 transcripts were amplified by PCR.

**Results**

FACS analysis of the blood and lung T cells of sarcoid individuals showed many with an increase in the proportion of γ/δ+ T cells (γ/δ+/CD3+) in blood (47, 27, 23, 59, and 7% for sarcoid 1-5, respectively). For all sarcoid individuals, the proportion of γ/δ+ T cells in the lung was <10%, although the increase in lung lymphocyte numbers in these individuals resulted in a total increase in the number of γ/δ+ T cells in the lung compared with normals. Of the sarcoid individuals with >10% γ/δ+ blood T cells, several showed an increase in TrγA+ T cells (TrγA+/CD3+: 38, 17, and 58%, for sarcoid 1, 2, and 4, respectively). These same individuals had no relative increase in TCS-81+ blood T cells (TCS-81+/CD3+: 1, 11, and 1%, for sarcoid 1, 2, and 4, respectively). Sarcoid 3 could not be tested due to insufficient biologic material.

A total of 415 mRNA transcripts were sequenced, including 281 Vγ9 transcripts and 134 Vβ2 transcripts. For Vγ9 transcripts, the region sequenced (see Fig. 1) included ~100 bp of the 3' end of Vγ9, the N region, the entire Jγ region, and ~70 bp of the 5' end of the Cy region. Because of space limitations, only the junctional regions are shown in the tables, with the specific Jγ segment identified. Likewise, ~100 bp of the 3' end of Vβ2, the N region, Dβ region, N region, entire Jβ region, and ~70 bp of the 5' end of the Cβ region were sequenced, but only the junctional region is presented in the table, along with the specific Jβ region. Also, for both Vγ9 and Vβ2 transcripts, the sequence is indicated as “in-frame” (e.g., a potentially productive transcript based on sequence analysis) or not “in-frame” (e.g., likely a nonproductive transcript based on a frame shift such that the Jγ or Jβ sequence did not correspond to a known genomic Jγ or Jβ sequence, respectively) (34-40).

Vγ9 mRNA Transcripts in Blood T Cells of Normals. A total of 81 Vγ9 transcripts were evaluated in blood T cells of five individuals (Table 1). Of these, 73 (90%) of all Vγ9 transcripts were in-frame (i.e., likely productive) sequences. Of the 73 clones with productive sequences, 63 clones (86%) used the JγP gene segment, nine clones (12%) used Jγ2, and one clone (2%) used Jγ1; no clones used JγP1 or Jγ2. Each normal showed a few identical junctional sequences used in more than one transcript, but the vast majority were different, showing a large diversity in the Vγ-N-Jγ junctional region sequences. Most of the identical junctional regions showed only a nucleotide deletion of the 3' portion of the Vγ9 gene segment or the 5' portion of the Jγ gene segment, with no addition of

![Figure 1. Germline sequences of the Vγ9, Jγ, Vβ2, Dβ, and Jβ segments.](image-url)
Table 1. Junctional Sequences of Vγ9-containing mRNA Transcripts in Blood T Cells of Normal Individuals

| Individual | Number of cloned sequences | Number of clones with this sequence | Clone<sup>a</sup> | V | N | Jy | Jy region | In-frame<sup>1</sup> |
|------------|---------------------------|-----------------------------------|----------------|---|---|-----|----------|-----------------|
| Normal 1   | 17                        | 3                                 | K1B.G1-3       | GCCTTGTGGAGGGTC | CAAGAGTTGGGC | JP | +       |
|            |                           |                                   |                 |               |               |     |         |
| Normal 2   | 16                        | 2                                 | K1B.G1-2       | GCCTTGTGGAGGGTC | CAAGAGTTGGGC | JP | +       |
|            |                           |                                   |                 |               |               |     |         |
| Normal 3   | 13                        | 2                                 | K1B.G1-2       | GCCTTGTGGAGGGTC | CAAGAGTTGGGC | JP | +       |
|            |                           |                                   |                 |               |               |     |         |
| Normal 4   | 20                        | 2                                 | K1B.G1-2       | GCCTTGTGGAGGGTC | CAAGAGTTGGGC | JP | +       |
|            |                           |                                   |                 |               |               |     |         |
| Normal 5   | 15                        | 2                                 | K1B.G1-2       | GCCTTGTGGAGGGTC | CAAGAGTTGGGC | JP | +       |

Sequences shown include the 3' region of the Vγ9 element (V), the N region, the 5' region of the Jγ element (J), and the specific Jγ element (Jy).

<sup>a</sup> The clones were sequenced randomly, but for convenience, they are numbered so that identical sequences have consecutive numbers; N1B.G1-3, normal individual 1, blood T cells, γ chain, clones 1, 2, and 3; N1B.G4, same but clone 4, etc.

<sup>1</sup> In-frame +, true mRNA transcript; -, nonproductive transcript based on sequence being frame shifted such that the Jγ region did not correspond to a known genomic Jγ sequence.
nucleotides. One normal individual (normal 1) showed three clones with the same \( V_y9 \)-junctio nal sequence (5'-GCCTTG-TGGAGGTA 3'; N1B9, N1B12, N1B15) but three different \( J_y \) gene segments.

\( V_y9 \) mRNA Transcripts in Lung T Cells of Normals. A total of 64 \( V_y9 \) transcripts were analyzed in lung T lymphocytes of four normals (Table 2). Almost all (60 clones, 94%) showed in-frame sequences. In general, the pattern of sequences was similar to that in blood, with a large diversity among the lung sequences. Of the lung \( V_y9 \) transcripts with in-frame sequences, 37 clones (62%) used the \( J_yP \) gene segment, 18 used \( J_y2 \) (30%), three used \( J_yP2 \) (5%), two used \( J_yP1 \) (3%), and none used \( J_y1 \). Among each individual, most sequences were unique, but more sequences shared the same junctional regions than that observed in blood of normals. The most striking example was normal 9, who showed six transcripts out of 13 (46%); clones N9L.G1-6 with the same sequence. However, for the other three individuals, at most, 19% (3 of 16, clones N6L.G1-3, normal 6), 16% (3 of 16, clones N7L.G1-3, normal 7), or 14% (2 of 14 for three sets of clones, normal 8) were identical. Taken together, of the 60 in-frame \( V_y9 \) sequences evaluated in the four normals, the most that any one junctional sequence was observed was 10% (six clones [N9L.G1-6] for normal 9); this sequence was not observed in any other normal (lung or blood, Tables 1 and 2).

\( V_y9 \) mRNA Transcripts in Blood and Lung T Cells of Individuals with Sarcoidosis. For the individuals with sarcoidosis,

| Table 2. Junctional Sequences of \( V_y9 \)-containing mRNA Transcripts in Lung T Cells of Normal Individuals |
|---------------------------------------------------------------|
| Individual | Number of cloned sequences | Number of clones with this sequence | Clone* | \( V \) | \( N \) | \( J \) | \( J_y \) region | In-frame*
| Normal 6 | 16 | 3 | N6L.G1-3 | GCCTTGCGGAGA | CCGAG | AAGATTGGGCG | JP + |
| | | 2 | N6L.G4-5 | GCCTTGCGGAGGAT | A | CAAGATTGGGCG | JP + |
| | | 2 | N6L.G6-7 | GCCTTGCGGAGAC | GCAAGATTGGGCG | JP + |
| | | 1 | N6L.G8 | GCCTTGCGGAGGAG | GCGAGCGCGCGG | GAGATTGGGCG | JP + |
| | | 1 | N6L.G9 | GCCTTGCGGAGGAGG | GGGTGCACGGCG | TGGGC | JP + |
| | | 1 | N6L.G10 | GCCTTGCGGAGGAGGAT | AGGCC | GAATTTGGCG | JP + |
| | | 1 | N6L.G11 | GCCTTGCGGAGGAGGAT | TACGTTGAG | GAATTTGGCG | JP + |
| | | 1 | N6L.G12 | GCCTTGCGGAGGAGGAG | GAAAGAGC | AGAAA | J2 + |
| | | 1 | N6L.G13 | GCCTTGCGGAGGAGGAGG | TGGGC | JP + |
| | | 1 | N6L.G14 | GCCTTGCGGAGGAGGAGG | CGCGAG | GATTTGGAGTC | JP2 + |
| | | 1 | N6L.G15 | GCCTGCGGAGGAGGAGGAGG | TAAA | GATTTGGATC | JP2 + |
| | | 1 | N6L.G16 | GCCTGGCGGAGGAGGAGGAGG | A | AAA | J2 - |
| Normal 7 | 19 | 3 | N7L.G1-3 | GCCTTGCGGAGGAGGAGG | GC | GCAAGATTGGGCG | JP + |
| | | 2 | N7L.G4-5 | GCCTTGCGGAGGAGGAGG | CCCCTCCC | A | J2 + |
| | | 2 | N7L.G6-7 | GCCTTGCGGAGGAGGAGG | CCA | GAAGATTGGGCG | JP + |
| | | 1 | N7L.G8-9 | GCCTTGCGGAGGAGGAGG | CA | GATTTGGCG | JP + |
| | | 1 | N7L.G10 | GCCTTGCGGAGGAGGAGG | GCGAGCGCGCGG | GAGATTGGGCG | JP + |
| | | 1 | N7L.G11 | GCCTTGCGGAGGAGGAGG | TTCTGCA | CAAGATTGGGCG | JP + |
| | | 1 | N7L.G12 | GCCTTGCGGAGGAGGAGG | CA | GATTTGGCG | JP + |
| | | 1 | N7L.G13 | GCCTTGCGGAGGAGGAGG | GGG | GATTTGGCG | JP + |
| | | 1 | N7L.G14 | GCCTTGCGGAGGAGGAGG | ACCCGCAG | GATTTGGCG | JP + |
| | | 1 | N7L.G15 | GCCTTGCGGAGGAGGAGG | GCCG | GATTTGGCG | JP + |
| | | 1 | N7L.G16 | GCCTTGCGGAGGAGGAGG | CGAGAGGCG | TGGGC | JP + |
| | | 1 | N7L.G17 | GCCTTGCGGAGGAGGAGG | AGG | GAAA | J2 + |
| | | 1 | N7L.G18 | GCCTTGCGGAGGAGGAGG | TGCC | GATTTGGTC | JP1 + |
| | | 1 | N7L.G19 | GCCTTGCGGAGGAGGAGG | GAG | GAAA | J2 - |
| Normal 8 | 14 | 2 | N8L.G1-2 | GCCTTGCGGAGGAGGAGG | CCCCGG | GATTTGGCG | JP + |
| | | 2 | N8L.G5-4 | GCCTTGCGGAGGAGGAGG | CGAGCGGG | GAATTTGGCG | JP + |
| | | 2 | N8L.G5-6 | GCCTTGCGGAGGAGGAGG | CCGGGG | AAGATTGGGCG | JP + |
| | | 1 | N8L.G7 | GCCTTGCGGAGGAGGAGG | TCTCGAC | CAAGATTGGGCG | JP + |
| | | 1 | N8L.G8 | GCCTTGCGGAGGAGGAGG | TCTT | GAATTTGGCG | JP + |
| | | 1 | N8L.G9 | GCCTTGCGGAGGAGGAGG | A | CAAGATTGGGCG | JP + |
| | | 1 | N8L.G10 | GCCTTGCGGAGGAGGAGG | CCGGCGGCGGG | GATTTGGCG | JP + |
| | | 1 | N8L.G11 | GCCTTGCGGAGGAGGAGG | CGCGG | GATTTGGCG | JP + |
| | | 1 | N8L.G12 | GCCTTGCGGAGGAGGAGG | TCAAAATTAAACAC | A | J2 + |
| | | 1 | N8L.G13 | GCCTTGCGGAGGAGGAGG | CGCGG | GAATTTGGACAGAAGGAA | J2 + |
| | | 1 | N8L.G14 | GCCTTGCGGAGGAGGAGG | GGAGG | GAAAGAGC | J2 + |
| Normal 9 | 15 | 6 | N9L.G1-6 | GCCTTGCGGAGGAGGAGG | CG | TTAAAGGGAAG | J2 + |
| | | 3 | N9L.G7-9 | GCCTTGCGGAGGAGGAGG | CGG | AGAAA | J2 + |
| | | 2 | N9L.G10-11 | GCCTTGCGGAGGAGGAGG | TTGAC | TATTTGGACG | JP2 + |
| | | 1 | N9L.G12 | GCCTTGCGGAGGAGGAGG | CCTG | ATTTAAAGGGAAG | J2 + |
| | | 1 | N9L.G13 | GCCTTGCGGAGGAGGAGG | GCAGG | GATTTGGACG | JP2 + |
| | | 1 | N9L.G14 | GCCTTGCGGAGGAGGAGG | CCGGGG | AATTAAAGGGAAG | J2 + |
| | | 1 | N9L.G15 | GCCTTGCGGAGGAGGAGG | GGAGG | GAAAGAGC | J2 + |

Sequences shown include the same regions described in Table 1.

* The clones are numbered in the same fashion as described in Table 1; N6L.G1-3, normal individual 6, lung T cells, \( \gamma \) chain, clones 1, 2, and 3, etc.

† 16 bp of the 5' portion of the \( J_y2 \) gene segment was deleted. See Fig. 1 for the location.
A total of 115 Vγ9 transcripts from the blood T cells were sequenced (Table 3). Like the normals, most Vγ9 sequences in blood were in-frame (107 of 115, 93%). However, in contrast to normals, there was a dramatic similarity among Vγ9 junctional region sequences in a subgroup of these individuals. Two (sarcoids 1 and 2) showed a marked overrepresentation of Vγ9 transcripts with the same junctional region sequence (5'-TGAGACGGAAGATTTC-3'). In this regard, for sarcoid 1, 26 of 31 clones (84%) shared this sequence (clones S1B.G1-26), while for sarcoid 2, 15 of 27 clones (56%) had the identical sequence (clones S2B.G1-15). Further, other sarcoid individuals (sarcoids 3 and 5) also showed three clones (S3B.G1-3) or one clone (S5B.G1) with the same sequence. The one sarcoid individual (sarcoid 4) that did not show transcripts with the same junctional region sequence had an increase in number of transcripts with sequences observed in normals (compare S4.B.G1-5 [5 of 21, 24%] to N1B.G7 and N5B.G6 [Table 1]; compare S4.B.G6-9 [4 of 21, 19%] to N1B.G1-3, N2B.G1-2, N3B.G1-2, N4B.G1-2, N7L.G6-9, N8L.G3-4, and N9L.G13 [Tables 1 and 2]; as well as other sarcoidosis patients, S1B.G27-28, and S5B.G5 [Table 3]). Most of Vγ9 transcripts of blood T cells (100 of 107 clones, 93%) that were in-frame sequences showed use of JγP, while three clones (3%) used Jγ2 and one clone each used Jγ1, Jγ1, and Jγ2. In sarcoid 1, only two other in-frame sequences were found (other than the identical sequence previously mentioned), indicating overall limited diversity of the Vγ9 transcripts. In contrast to sarcoid 1, the other four sarcoidosis individuals showed large overall diversity in the junctional region sequences, i.e., except the identical sequences described above, the rest of the transcripts showed different sequences from each other. As seen in the normal blood, nonproductive Vγ9 sequences among the sarcoid blood T cells mainly used the JγP gene segment.

The limitation of the availability of the biological material limited the analysis of lung T cells among the sarcoid individuals. However, for the one individual that could be analyzed (sarcoid 1), the individual with the most dramatic overrepresentation of the sequence 5'-GCCTTGTGGGGAACGGG-3' (see Table 3), the lung T cells showed the same dramatic overrepresentation for this junctional region sequence (Table 4). In this regard, 14 of the 21 clones (67%, all in-frame) had the identical sequence. Of the 21 transcripts analyzed, all but one used the JγP gene segment.

To confirm the presence of a large number of identical Vγ9 transcripts in individuals with sarcoidosis compared with normals, the cDNA populations were evaluated by allelic-specific amplification using a primer specific for the junctional region observed in abundance in individuals sarcoid 1 and 2 (Fig. 2). The same Cγ primer, PCG5, was used in combination with either PVG9, a primer for all Vγ9 sequences, or PVNJ9, an allele-specific primer with the specific junctional region sequence of the Vγ9 gene found in patients with sarcoidosis clones S1B.G1-26, S2B.G1-15, S3B.G1-3, S5B.G1, and SIL.G1-14, and in normal clones N1B.G6 and N5B.G1-2. Consistent with the sequencing data, dot blot hybridization of amplified cDNA from sarcoid 1 showed almost the same density of the amplified cDNA by the PVNJ9-PCG5 combination as by the PVG9-PCG5 combination in both blood (Fig. 2, lanes 1 and 2) and lung (Fig. 2, lanes 3 and 4). Sarcoid 2 showed the same result in the blood (Fig. 2, lanes 5 and 6). Sarcoid 4, who did not have any transcripts with this specific sequence, showed a marked discrepancy in the density of the total Vγ9 transcripts compared with the junctional region–specific transcripts, consistent with the finding that the junctional region–specific sequence must be rare in this individual (Fig. 2, lanes 7 and 8). Normal individuals also showed no overrepresentation of this junctional region–specific sequence (Fig. 2, lanes 9–12).

Vδ2 mRNA Transcripts in Blood T Cells of Normals. A total of 50 Vδ2 transcripts were evaluated in the blood T cells of three normals (Table 5). Of these, 44 clones (88%) had in-frame sequences. In the 44 transcripts with in-frame sequences, 41 clones used Jδ1 (93%), three clones used Jδ3 (7%), while none used Jδ2. All 50 clones showed different junctional region sequences, suggesting marked diversity.

Vδ2 mRNA Transcripts in Blood T Cells of Individuals with Sarcoidosis. For the individuals with sarcoidosis, 84 Vδ2 clones from four patients were analyzed (Table 6). Of these, 81 clones (96%) had in-frame sequences. Among the 81 clones with in-frame sequences, 70 (86%) used Jδ1, 11 (14%) used Jδ3, and none used Jδ2, the same distribution seen in normals. In contrast to normal individuals, however, there were several sequences that were found in two or more clones. Of the 28 transcripts sequenced in sarcoid 1, more than half were overrepresented, with identical sequences observed in seven clones (25%), six clones (21%), and three clones (11%), respectively, although the sequences of the three sets of clones were all different. In sarcoid 2, 10 of 23 clones (43%) showed the same junctional sequence, although it was different from that observed in sarcoid 1. Further, six clones (33%) and three clones (17%) out of 18 transcripts in sarcoid 3 showed the same junctional sequences, respectively, although different from sarcoid 1 or sarcoid 2.

Although a striking overrepresentation of both Vγ9 and Vδ2 transcripts was observed in several individuals with sarcoidosis, it was not possible to determine if these overrepresented Vγ9 and Vδ2 transcripts were from the same single T cells. We attempted to answer this question by isolating single γ/δ T cells from the blood of sarcoid 1, the individual with the most marked overrepresentation of sequences (Tables 3, 4, and 6). Of the 24 cells evaluated at the single cell level, eight (33%) showed positive amplification of cDNA using the primers PVG9 and PCG5, the same combination as described above. However, attempts to sequence the amplified cDNAs was unsuccessful, and insufficient biological material was available to repeat the single-cell sorting and subsequent analysis.

Discussion

Although there is evidence that the TCR-γ/δ can initiate T cell activation and proliferation through recognition of specific antigens (15–18), the role of γ/δ T cells in health...
**Table 3. Functional Sequences of Vγ9-containing mRNA Transcripts in Blood T Cells of Individuals with Sarcoidosis**

| Individual | Number of cloned sequences | Number of clones with this sequence | Clone* | V  | N  | J  | Jy region | In-frame |
|------------|---------------------------|------------------------------------|--------|----|----|---|----------|----------|
| Sarcoid 1  | 31                        | 25                                 | SIB.G1-26 | GCCCTGGGAA  | ACGGG | AAGAGTTGGGC | JP       | +        |
|            |                            |                                    | SIB.G27-28 | GCCCTGGGAAAGG | IG | CAAGAGTTGGGC | JP       | +        |
|            |                            |                                    | SIB.G29   | GCCCTGGGAGG | AATC | GAGTTGGGC | JP       | -        |
|            |                            |                                    | SIB.G30   | GCCCTGGGAGG | AGGTGCTTGG | GAGTTGGGC | JP       | -        |
| Sarcoid 2  | 27                        | 15                                 | S2B.G1-15 | GCCCTGGGAAAGG | ACGGG | AAGAGTTGGGC | JP       | +        |
|            |                            |                                    | S2B.G16   | GCCCTGGGAAAGG | GCGGG | AAGAGTTGGGC | JP       | +        |
|            |                            |                                    | S2B.G17   | GCCCTGGGAAAGG | GC | GCAAGAGTTGGGC | JP       | +        |
|            |                            |                                    | S2B.G18   | GCCCTGGGAAAGG | ATGGAGCCTGG | GAGTTGGGC | JP       | -        |
|            |                            |                                    | S2B.G19   | GCCCTGGGAAAGG | CAG | GAGTTGGGC | JP       | +        |
|            |                            |                                    | S2B.G20   | GCCCTGGGAAAGG | GC | GCAAGAGTTGGGC | JP       | +        |
|            |                            |                                    | S2B.G21   | GCCCTGGGAAAGG | GGG | GAGTTGGGC | JP       | +        |
|            |                            |                                    | S2B.G22   | GCCCTGGGAAAGG | AGGTGCTTGG | GAGTTGGGC | JP       | +        |
|            |                            |                                    | S2B.G23   | GCCCTGGGAAAGG | C4C | GCAAGAGTTGGGC | JP       | +        |
|            |                            |                                    | S2B.G24   | GCCCTGGGAAAGG | CAG | GCAAGAGTTGGGC | JP       | +        |
|            |                            |                                    | S2B.G25   | GCCCTGGGAAAGG | CAG | GCAAGAGTTGGGC | JP       | +        |
|            |                            |                                    | S2B.G26   | GCCCTGGGAAAGG | C4C | GCAAGAGTTGGGC | JP       | +        |
|            |                            |                                    | S2B.G27   | GCCCTGGGAAAGG | GGGGG | AAGAA | J2       | -        |
| Sarcoid 3  | 21                        | 3                                  | S3B.G1-3  | GCCCTGGGAAAGG | ACGGG | AAGAGTTGGGC | JP       | +        |
|            |                            |                                    | S3B.G4    | GCCCTGGGAAAGG | CGG | AAGAGTTGGGC | JP       | +        |
|            |                            |                                    | S3B.G5    | GCCCTGGGAAAGG | CAG | GAGTTGGGC | JP       | +        |
|            |                            |                                    | S3B.G6    | GCCCTGGGAAAGG | GC | GCAAGAGTTGGGC | JP       | +        |
|            |                            |                                    | S3B.G7    | GCCCTGGGAAAGG | CTGGTTT | GAGTTGGGC | JP       | +        |
|            |                            |                                    | S3B.G8    | GCCCTGGGAAAGG | AATA | GCAAGAGTTGGGC | JP       | +        |
|            |                            |                                    | S3B.G9    | GCCCTGGGAAAGG | CTGGTTT | GAGTTGGGC | JP       | +        |
|            |                            |                                    | S3B.G10   | GCCCTGGGAAAGG | CAG | GAGTTGGGC | JP       | +        |
|            |                            |                                    | S3B.G11   | GCCCTGGGAAAGG | CTGAGCTT | GAGTTGGGC | JP       | +        |
|            |                            |                                    | S3B.G12   | GCCCTGGGAAAGG | TGGCTGGT | GAGTTGGGC | JP       | +        |
|            |                            |                                    | S3B.G13   | GCCCTGGGAAAGG | CAG | GAGTTGGGC | JP       | +        |
|            |                            |                                    | S3B.G14   | GCCCTGGGAAAGG | AATCC | GAGTTGGGC | JP       | +        |
|            |                            |                                    | S3B.G15   | GCCCTGGGAAAGG | CAG | GAGTTGGGC | JP       | +        |
|            |                            |                                    | S3B.G16   | GCCCTGGGAAAGG | CAG | GAGTTGGGC | JP       | +        |
|            |                            |                                    | S3B.G17   | GCCCTGGGAAAGG | CAG | GAGTTGGGC | JP       | +        |
|            |                            |                                    | S3B.G18   | GCCCTGGGAAAGG | C4C | GAGTTGGGC | JP       | +        |
|            |                            |                                    | S3B.G19   | GCCCTGGGAAAGG | CAG | GAGTTGGGC | JP       | +        |
|            |                            |                                    | S3B.G20   | GCCCTGGGAAAGG | CAG | GAGTTGGGC | JP       | +        |
|            |                            |                                    | S3B.G21   | GCCCTGGGAAAGG | CAG | GAGTTGGGC | JP       | +        |
| Sarcoid 4  | 21                        | 5                                  | S4B.G1-5  | GCCCTGGGAAAGG | AGGCC | GAGTTGGGC | JP       | +        |
|            |                            |                                    | S4B.G6-9  | GCCCTGGGAAAGG | AGGCC | GAGTTGGGC | JP       | +        |
|            |                            |                                    | S4B.G10   | GCCCTGGGAAAGG | AGGCC | GAGTTGGGC | JP       | +        |
|            |                            |                                    | S4B.G11   | GCCCTGGGAAAGG | AGGCC | GAGTTGGGC | JP       | +        |
|            |                            |                                    | S4B.G12   | GCCCTGGGAAAGG | AGGCC | GAGTTGGGC | JP       | +        |
|            |                            |                                    | S4B.G13   | GCCCTGGGAAAGG | AGGCC | GAGTTGGGC | JP       | +        |
|            |                            |                                    | S4B.G14   | GCCCTGGGAAAGG | AGGCC | GAGTTGGGC | JP       | +        |
|            |                            |                                    | S4B.G15   | GCCCTGGGAAAGG | AGGCC | GAGTTGGGC | JP       | +        |
|            |                            |                                    | S4B.G16   | GCCCTGGGAAAGG | AGGCC | GAGTTGGGC | JP       | +        |
|            |                            |                                    | S4B.G17   | GCCCTGGGAAAGG | AGGCC | GAGTTGGGC | JP       | +        |
|            |                            |                                    | S4B.G18   | GCCCTGGGAAAGG | AGGCC | GAGTTGGGC | JP       | +        |
|            |                            |                                    | S4B.G19   | GCCCTGGGAAAGG | AGGCC | GAGTTGGGC | JP       | +        |
|            |                            |                                    | S4B.G20   | GCCCTGGGAAAGG | AGGCC | GAGTTGGGC | JP       | +        |
|            |                            |                                    | S4B.G21   | GCCCTGGGAAAGG | AGGCC | GAGTTGGGC | JP       | +        |
| Sarcoid 5  | 15                        | 1                                  | S5B.G1    | GCCCTGGGAAAGG | AGGCC | GAGTTGGGC | JP       | +        |
|            |                            |                                    | S5B.G2    | GCCCTGGGAAAGG | AGGCC | GAGTTGGGC | JP       | +        |
|            |                            |                                    | S5B.G3    | GCCCTGGGAAAGG | AGGCC | GAGTTGGGC | JP       | +        |
|            |                            |                                    | S5B.G4    | GCCCTGGGAAAGG | AGGCC | GAGTTGGGC | JP       | +        |
|            |                            |                                    | S5B.G5    | GCCCTGGGAAAGG | AGGCC | GAGTTGGGC | JP       | +        |
|            |                            |                                    | S5B.G6    | GCCCTGGGAAAGG | AGGCC | GAGTTGGGC | JP       | +        |
|            |                            |                                    | S5B.G7    | GCCCTGGGAAAGG | AGGCC | GAGTTGGGC | JP       | +        |
|            |                            |                                    | S5B.G8    | GCCCTGGGAAAGG | AGGCC | GAGTTGGGC | JP       | +        |
|            |                            |                                    | S5B.G9    | GCCCTGGGAAAGG | AGGCC | GAGTTGGGC | JP       | +        |
|            |                            |                                    | S5B.G10   | GCCCTGGGAAAGG | AGGCC | GAGTTGGGC | JP       | +        |
|            |                            |                                    | S5B.G11   | GCCCTGGGAAAGG | AGGCC | GAGTTGGGC | JP       | +        |
|            |                            |                                    | S5B.G12   | GCCCTGGGAAAGG | AGGCC | GAGTTGGGC | JP       | +        |
|            |                            |                                    | S5B.G13   | GCCCTGGGAAAGG | AGGCC | GAGTTGGGC | JP       | +        |
|            |                            |                                    | S5B.G14   | GCCCTGGGAAAGG | AGGCC | GAGTTGGGC | JP       | +        |
|            |                            |                                    | S5B.G15   | GCCCTGGGAAAGG | AGGCC | GAGTTGGGC | JP       | +        |

Sequences shown include the same regions described in Table 1.

* The clones are numbered in the same fashion as described in Table 1; SIB.G1-26, individual with sarcoidosis 1, blood T cells, γ chain, clones 1 to 26, etc.
Sequences shown include the same regions described in Table 1. *The clones are numbered in the same fashion as described in Table 1; S1L.G1-14, individual with sarcoidosis 1, lung T cells, 6 chain, clones 1-14, etc.

The analysis of 145 Vy9 mRNA transcripts and 50 V62 transcripts from blood and lung of normal individuals in the present study strongly supports this concept. While there was clearly limited diversity in the V and J gene segments used (for Vy9, 86% paired with JyP and 12% with Jy2; for V62, 93% paired with Jø), broad diversity was observed in the junctional regions. In this regard, for the 81 normal blood Vy9 transcripts, no more than three were identical in any one individual, and two identical transcripts were observed only six times. For the normal lung, the diversity was not quite so broad, but still impressive: of 64 Vy9 normal lung transcripts, 42 different sequences were observed, and except for six transcripts of one individual that were identical, lung T cells of other normals had at most only two or three identical sequences. For the blood V62 transcripts, an even broader diversity was observed. Of the 50 transcripts evaluated, all were different. Further, comparison of the sequences of the 145 Vy9 and 50 V62 transcripts with the Vy9 and the V62 transcripts of T cell clones in the literature revealed no identical junctional regions (34-45). Taken together, these data support the concept, despite limited genomic diversity, and even further limited actual use of genomic elements observed in normals, that there is large potential for possible diverse antigen recognition through the use of the deletion and addition of junctional sequences.

Although the normal lung showed a broad diversity in junctional regions for Vy9 transcripts, the diversity was some what less than that observed in blood. Several possibilities could explain this observation. First, the lung Vy9 transcripts were from T cells recovered from the pulmonary epithelial surface, a location that is in contact with the external environment, and thus, many antigens i.e., the small increase of identical transcripts observed among lung T cells, might result from antigen-driven expansion of some Vy9 T cells within

| Individual | Number of cloned sequences | Number of transcripts with this sequence | Clone* | V | N | J | Jy region | In-frame |
|------------|---------------------------|-----------------------------------------|--------|---|---|---|-----------|---------|
| Sarcoid 1  | 21                        | 14                                      | S1L.G1-14 | GCCTTGTTGGGAA CGG AAGAGTTGGG JP + |
| 2          |                           |                                         | S1L.G15-16 | GCCTTGTTGGGAGTG CAG GAGTTGGG JP + |
| 1          |                           |                                         | S1L.G17    | GCCTTGTTGGGAGTG CAG GAGTTGGG JP + |
| 1          |                           |                                         | S1L.G18    | GCCT GCTT GCAAGAGTTGGG JP + |
| 1          |                           |                                         | S1L.G19    | GCCTTGTTGGGAGTG CG AAGAGTTGGG JP + |
| 1          |                           |                                         | S1L.G20    | GCCTTGTTGGGAGG CAACTGG AAGAGTTGGG JP + |
| 1          |                           |                                         | S1L.G21    | GCCTTGTTGGGAGGT A AAGAAA J2 + |

Sequences shown include the same regions described in Table 1.

Table 4. Functional Sequences ofVy9-containing mRNA Transcripts in Lung T Cells of an Individual with Sarcoidosis

![Diagram](https://via.placeholder.com/150)

**Figure 2.** Relative number of total Vy9 transcripts compared with Vy9 transcripts containing a specific N region (junctional region) sequence observed in high abundance among Vy9 transcripts of some individuals with sarcoidosis. Comparisons were made by allele-specific amplification of cDNA using Vy9 specific primers and N region-specific primers. The primer combination of PVN9 and PCG5 was used for total Vy9 amplification (indicated by V above lanes), while the combination of PVN9 and PCG5 was used for N region-specific amplification (indicated by N above lanes). Amplified cDNAs were evaluated by dot blot hybridization with a 32P-labeled Cy probe (location indicated by closed bar ["probe"]) located in Cy exon 1 (Cy 1) where there is a shared sequence between Cy1 and Cy2. Sarcoids 1, 2, and 4 and normals 1 and 2; two correspond to the individuals thus numbered in the text and tables. Lane 1, Vy9-specific transcripts in blood of sarcoid 1; lane 2, N region-specific transcripts in blood of sarcoid 1; lane 3, Vy9-specific transcripts in the lung of sarcoid 1; lane 4, N region-specific transcripts in the lung of sarcoid 1; lane 5, Vy9-specific transcripts in the blood of sarcoid 2; lane 6, N region-specific transcripts in blood of sarcoid 2; lane 7, Vy9-specific transcripts in blood of sarcoid 4; lane 8, N region-specific transcripts in blood of sarcoid 4; lane 9, Vy9-specific transcripts in blood of normal 1; lane 10, N region-specific transcripts in lung of normal 1; lane 11, Vy9-specific transcripts in blood of normal 2; lane 12, N region-specific transcripts in blood of normal 2.
Table 5. Functional Sequences of V62-containing mRNA Transcripts in Blood T cells of Normal Individuals

| Individual | Number of cloned sequences | Number of clones with this sequence | Clone* | V | N-D-N-D-N | J | J6 region | In-frame |
|------------|----------------------------|------------------------------------|--------|---|------------|----|-----------|----------|
| Normal 1   | 19                         | 1                                  | N1B.D1 | TGTGACACC | CTGGGGAATCTGGGCACTGGGAGAGA | CACGGGATAA | J1       | +        |
|            |                             | 1                                  | N1B.D2 | TGTGACAGGGGGAGATGACCTGGGACCTGGGCACTGGGAGAGA | CACGGGATAA | J1       | +        |
|            |                             | 1                                  | N1B.D3 | TGTGACACC | CTGGGGAATCTGGGCACTGGGAGAGA | CACGGGATAA | J1       | +        |
|            |                             | 1                                  | N1B.D4 | TGTGACACC | CTGGGGAATCTGGGCACTGGGAGAGA | CACGGGATAA | J1       | +        |
|            |                             | 1                                  | N1B.D5 | TGTGACACC | CTGGGGAATCTGGGCACTGGGAGAGA | CACGGGATAA | J1       | +        |
|            |                             | 1                                  | N1B.D6 | TGTGACACC | CTGGGGAATCTGGGCACTGGGAGAGA | CACGGGATAA | J1       | +        |
|            |                             | 1                                  | N1B.D7 | TGTGACACC | CTGGGGAATCTGGGCACTGGGAGAGA | CACGGGATAA | J1       | +        |
|            |                             | 1                                  | N1B.D8 | TGTGACACC | CTGGGGAATCTGGGCACTGGGAGAGA | CACGGGATAA | J1       | +        |
|            |                             | 1                                  | N1B.D9 | TGTGACACC | CTGGGGAATCTGGGCACTGGGAGAGA | CACGGGATAA | J1       | +        |
|            |                             | 1                                  | N1B.D10| TGTGACACC | CTGGGGAATCTGGGCACTGGGAGAGA | CACGGGATAA | J1       | +        |
|            |                             | 1                                  | N1B.D11| TGTGACACC | CTGGGGAATCTGGGCACTGGGAGAGA | CACGGGATAA | J1       | +        |
|            |                             | 1                                  | N1B.D12| TGTGACACC | CTGGGGAATCTGGGCACTGGGAGAGA | CACGGGATAA | J1       | +        |
|            |                             | 1                                  | N1B.D13| TGTGACACC | CTGGGGAATCTGGGCACTGGGAGAGA | CACGGGATAA | J1       | +        |
|            |                             | 1                                  | N1B.D14| TGTGACACC | CTGGGGAATCTGGGCACTGGGAGAGA | CACGGGATAA | J1       | +        |
|            |                             | 1                                  | N1B.D15| TGTGACACC | CTGGGGAATCTGGGCACTGGGAGAGA | CACGGGATAA | J1       | +        |
|            |                             | 1                                  | N1B.D16| TGTGACACC | CTGGGGAATCTGGGCACTGGGAGAGA | CACGGGATAA | J1       | +        |
|            |                             | 1                                  | N1B.D17| TGTGACACC | CTGGGGAATCTGGGCACTGGGAGAGA | CACGGGATAA | J1       | +        |
|            |                             | 1                                  | N1B.D18| TGTGACACC | CTGGGGAATCTGGGCACTGGGAGAGA | CACGGGATAA | J1       | +        |
| Normal 2   | 16                         | 1                                  | N2B.D1 | TGTGACACC | CTGGGGAATCTGGGCACTGGGAGAGA | CACGGGATAA | J1       | +        |
|            |                             | 1                                  | N2B.D2 | TGTGACACC | CTGGGGAATCTGGGCACTGGGAGAGA | CACGGGATAA | J1       | +        |
|            |                             | 1                                  | N2B.D3 | TGTGACACC | CTGGGGAATCTGGGCACTGGGAGAGA | CACGGGATAA | J1       | +        |
|            |                             | 1                                  | N2B.D4 | TGTGACACC | CTGGGGAATCTGGGCACTGGGAGAGA | CACGGGATAA | J1       | +        |
|            |                             | 1                                  | N2B.D5 | TGTGACACC | CTGGGGAATCTGGGCACTGGGAGAGA | CACGGGATAA | J1       | +        |
|            |                             | 1                                  | N2B.D6 | TGTGACACC | CTGGGGAATCTGGGCACTGGGAGAGA | CACGGGATAA | J1       | +        |
|            |                             | 1                                  | N2B.D7 | TGTGACACC | CTGGGGAATCTGGGCACTGGGAGAGA | CACGGGATAA | J1       | +        |
|            |                             | 1                                  | N2B.D8 | TGTGACACC | CTGGGGAATCTGGGCACTGGGAGAGA | CACGGGATAA | J1       | +        |
|            |                             | 1                                  | N2B.D9 | TGTGACACC | CTGGGGAATCTGGGCACTGGGAGAGA | CACGGGATAA | J1       | +        |
|            |                             | 1                                  | N2B.D10| TGTGACACC | CTGGGGAATCTGGGCACTGGGAGAGA | CACGGGATAA | J1       | +        |
|            |                             | 1                                  | N2B.D11| TGTGACACC | CTGGGGAATCTGGGCACTGGGAGAGA | CACGGGATAA | J1       | +        |
|            |                             | 1                                  | N2B.D12| TGTGACACC | CTGGGGAATCTGGGCACTGGGAGAGA | CACGGGATAA | J1       | +        |
|            |                             | 1                                  | N2B.D13| TGTGACACC | CTGGGGAATCTGGGCACTGGGAGAGA | CACGGGATAA | J1       | +        |
|            |                             | 1                                  | N2B.D14| TGTGACACC | CTGGGGAATCTGGGCACTGGGAGAGA | CACGGGATAA | J1       | +        |
|            |                             | 1                                  | N2B.D15| TGTGACACC | CTGGGGAATCTGGGCACTGGGAGAGA | CACGGGATAA | J1       | +        |
| Normal 3   | 15                         | 1                                  | N3B.D1 | TGTGACACC | CTGGGGAATCTGGGCACTGGGAGAGA | CACGGGATAA | J1       | +        |
|            |                             | 1                                  | N3B.D2 | TGTGACACC | CTGGGGAATCTGGGCACTGGGAGAGA | CACGGGATAA | J1       | +        |
|            |                             | 1                                  | N3B.D3 | TGTGACACC | CTGGGGAATCTGGGCACTGGGAGAGA | CACGGGATAA | J1       | +        |
|            |                             | 1                                  | N3B.D4 | TGTGACACC | CTGGGGAATCTGGGCACTGGGAGAGA | CACGGGATAA | J1       | +        |
|            |                             | 1                                  | N3B.D5 | TGTGACACC | CTGGGGAATCTGGGCACTGGGAGAGA | CACGGGATAA | J1       | +        |
|            |                             | 1                                  | N3B.D6 | TGTGACACC | CTGGGGAATCTGGGCACTGGGAGAGA | CACGGGATAA | J1       | +        |
|            |                             | 1                                  | N3B.D7 | TGTGACACC | CTGGGGAATCTGGGCACTGGGAGAGA | CACGGGATAA | J1       | +        |
|            |                             | 1                                  | N3B.D8 | TGTGACACC | CTGGGGAATCTGGGCACTGGGAGAGA | CACGGGATAA | J1       | +        |
|            |                             | 1                                  | N3B.D9 | TGTGACACC | CTGGGGAATCTGGGCACTGGGAGAGA | CACGGGATAA | J1       | +        |
|            |                             | 1                                  | N3B.D10| TGTGACACC | CTGGGGAATCTGGGCACTGGGAGAGA | CACGGGATAA | J1       | +        |
|            |                             | 1                                  | N3B.D11| TGTGACACC | CTGGGGAATCTGGGCACTGGGAGAGA | CACGGGATAA | J1       | +        |
|            |                             | 1                                  | N3B.D12| TGTGACACC | CTGGGGAATCTGGGCACTGGGAGAGA | CACGGGATAA | J1       | +        |
|            |                             | 1                                  | N3B.D13| TGTGACACC | CTGGGGAATCTGGGCACTGGGAGAGA | CACGGGATAA | J1       | +        |
|            |                             | 1                                  | N3B.D14| TGTGACACC | CTGGGGAATCTGGGCACTGGGAGAGA | CACGGGATAA | J1       | +        |
|            |                             | 1                                  | N3B.D15| TGTGACACC | CTGGGGAATCTGGGCACTGGGAGAGA | CACGGGATAA | J1       | +        |

Sequences shown include the 3' region of the V62 element (V), the N region (N-D-N-D-N), the 5' region of the J6 element (J), and the specific J6 element (J6).

* The clones are numbered in the same fashion as described in Table 1; N1B.D1, normal individual 1, blood T cells, @ chain, clone 1, etc.

The broad diversity of junctional sequences among Vγ9 and Vδ2 transcripts in normals is in marked contrast to the limited diversity observed in some individuals with sarcoidosis. For Vγ9, this was particularly striking for two of five
Table 6. Functional Sequences of V62-containing mRNA Transcripts in Blood T Cells of Individuals with Sarcoidosis

| Individual | Number of cloned sequences | Number of clones with this sequence | Clone* | V | N-D-N-D-N | J | J0 region In-frame |
|------------|---------------------------|----------------------------------|--------|---|-----------|---|------------------|
| **Sarcoid 1** | 28                        | 7                                | S1B.D1-7 | TGGGAC | CCGAGTGGATAGGGATCCTGAGGACA | ACACCGATAAA | J1 + |
|            |                           | 6                                | S1B.D6-13 | TGGGAC | GCTGGAGGATACGGCTG | ACACCGATAAA | J1 + |
|            |                           | 3                                | S1B.D14-18 | TGGGAC | GCTGGAGGATACGGCTG | ACACCGATAAA | J1 + |
|            |                           | 2                                | S1B.D17-18 | TGGGAC | GCTGGAGGATACGGCTG | ACACCGATAAA | J1 + |
|            |                           | 1                                | S1B.D19 | TGGGAC | TCAGCTAGGGGATGCTGAGGACA | ACACCGATAAA | J1 + |
|            |                           | 1                                | S1B.D20 | TGGGAC | TCCTGCTGACCTGAGGATAAGG | ACACCGATAAA | J1 + |
|            |                           | 1                                | S1B.D21 | TGGGAC | ACCTAGCTGAGGATAAGG | ACACCGATAAA | J1 + |
|            |                           | 1                                | S1B.D22 | TGGGAC | TCAGCTAGGGGATGCTGAGGACA | ACACCGATAAA | J1 + |
|            |                           | 1                                | S1B.D23 | TGGGAC | GCTGGAGGATACGGCTG | ACACCGATAAA | J1 + |
|            |                           | 1                                | S1B.D24 | TGGGAC | CCACCTGGAGGATACGGCTG | ACACCGATAAA | J1 + |
|            |                           | 1                                | S1B.D25 | TGGGAC | TTCTGCTGACCTGAGGATAAGG | ACACCGATAAA | J1 + |
|            |                           | 1                                | S1B.D26 | TGGGAC | TCAGCTAGGGGATGCTGAGGACA | ACACCGATAAA | J1 + |
|            |                           | 1                                | S1B.D27 | TGGGAC | GCTGGAGGATACGGCTG | ACACCGATAAA | J1 + |
|            |                           | 1                                | S1B.D28 | TGGGAC | GCTGGAGGATACGGCTG | ACACCGATAAA | J1 + |
| **Sarcoid 2** | 23                        | 10                               | S2B.D1-10 | TGGGAC | GCTGGAGGATACGGCTG | ACACCGATAAA | J1 + |
|            |                           | 2                                | S2B.D11-12 | TGGGAC | GCTGGAGGATACGGCTG | ACACCGATAAA | J1 + |
|            |                           | 1                                | S2B.D13 | TGGGAC | CCGAGTGGATAGGGATCCTGAGGACA | ACACCGATAAA | J1 + |
|            |                           | 1                                | S2B.D14 | TGGGAC | GCTGGAGGATACGGCTG | ACACCGATAAA | J1 + |
|            |                           | 1                                | S2B.D15 | TGGGAC | GCTGGAGGATACGGCTG | ACACCGATAAA | J1 + |
|            |                           | 1                                | S2B.D16 | TGGGAC | GCTGGAGGATACGGCTG | ACACCGATAAA | J1 + |
|            |                           | 1                                | S2B.D17 | TGGGAC | GCTGGAGGATACGGCTG | ACACCGATAAA | J1 + |
|            |                           | 1                                | S2B.D18 | TGGGAC | GCTGGAGGATACGGCTG | ACACCGATAAA | J1 + |
|            |                           | 1                                | S2B.D19 | TGGGAC | GCTGGAGGATACGGCTG | ACACCGATAAA | J1 + |
|            |                           | 1                                | S2B.D20 | TGGGAC | GCTGGAGGATACGGCTG | ACACCGATAAA | J1 + |
|            |                           | 1                                | S2B.D21 | TGGGAC | GCTGGAGGATACGGCTG | ACACCGATAAA | J1 + |
|            |                           | 1                                | S2B.D22 | TGGGAC | GCTGGAGGATACGGCTG | ACACCGATAAA | J1 + |
|            |                           | 1                                | S2B.D23 | TGGGAC | GCTGGAGGATACGGCTG | ACACCGATAAA | J1 + |
| **Sarcoid 3** | 18                        | 6                                | S3B.D1-6 | TGGGAC | GCTGGAGGATACGGCTG | ACACCGATAAA | J1 + |
|            |                           | 3                                | S3B.D7-9 | TGGGAC | GCTGGAGGATACGGCTG | ACACCGATAAA | J1 + |
|            |                           | 1                                | S3B.D10 | TGGGAC | GCTGGAGGATACGGCTG | ACACCGATAAA | J1 + |
|            |                           | 1                                | S3B.D11 | TGGGAC | GCTGGAGGATACGGCTG | ACACCGATAAA | J1 + |
|            |                           | 1                                | S3B.D12 | TGGGAC | GCTGGAGGATACGGCTG | ACACCGATAAA | J1 + |
|            |                           | 1                                | S3B.D13 | TGGGAC | GCTGGAGGATACGGCTG | ACACCGATAAA | J1 + |
|            |                           | 1                                | S3B.D14 | TGGGAC | GCTGGAGGATACGGCTG | ACACCGATAAA | J1 + |
|            |                           | 1                                | S3B.D15 | TGGGAC | GCTGGAGGATACGGCTG | ACACCGATAAA | J1 + |
|            |                           | 1                                | S3B.D16 | TGGGAC | GCTGGAGGATACGGCTG | ACACCGATAAA | J1 + |
|            |                           | 1                                | S3B.D17 | TGGGAC | GCTGGAGGATACGGCTG | ACACCGATAAA | J1 + |
|            |                           | 1                                | S3B.D18 | TGGGAC | GCTGGAGGATACGGCTG | ACACCGATAAA | J1 + |
| **Sarcoid 4** | 15                        | 2                                | S4B.D1-2 | TGGGAC | GCTGGAGGATACGGCTG | ACACCGATAAA | J1 + |
|            |                           | 2                                | S4B.D3-4 | TGGGAC | GCTGGAGGATACGGCTG | ACACCGATAAA | J1 + |
|            |                           | 1                                | S4B.D5 | TGGGAC | GCTGGAGGATACGGCTG | ACACCGATAAA | J1 + |
|            |                           | 1                                | S4B.D6 | TGGGAC | GCTGGAGGATACGGCTG | ACACCGATAAA | J1 + |
|            |                           | 1                                | S4B.D7 | TGGGAC | GCTGGAGGATACGGCTG | ACACCGATAAA | J1 + |
|            |                           | 1                                | S4B.D8 | TGGGAC | GCTGGAGGATACGGCTG | ACACCGATAAA | J1 + |
|            |                           | 1                                | S4B.D9 | TGGGAC | GCTGGAGGATACGGCTG | ACACCGATAAA | J1 + |
|            |                           | 1                                | S4B.D10 | TGGGAC | GCTGGAGGATACGGCTG | ACACCGATAAA | J1 + |
|            |                           | 1                                | S4B.D11 | TGGGAC | GCTGGAGGATACGGCTG | ACACCGATAAA | J1 + |
|            |                           | 1                                | S4B.D12 | TGGGAC | GCTGGAGGATACGGCTG | ACACCGATAAA | J1 + |
|            |                           | 1                                | S4B.D13 | TGGGAC | GCTGGAGGATACGGCTG | ACACCGATAAA | J1 + |
|            |                           | 1                                | S4B.D14 | TGGGAC | GCTGGAGGATACGGCTG | ACACCGATAAA | J1 + |

Sequences shown include the same regions described in Table 5.

* The clones are numbered in the same fashion as described in Table 1; S1B.D1-7, individual with sarcoidosis 1, blood T cells, δ chain, clones 1-7, etc.

individuals evaluated, in which 84 and 56% of the Vγ9 transcripts in blood were identical, respectively. Further, for one of these individuals, 67% of lung Vγ9 transcripts were identical and identical to those in blood of the same individual. Further, for Vδ2 transcripts, for the same two individuals with sarcoidosis with the striking overrepresentation of specific Vγ9 sequences, there was a marked overrepresentation of specific Vδ2 sequences. It is likely, therefore, although it cannot be proven with certainty from the available data, that these overrepresented TCR γ and δ chains are present on the same cell, thereby further limiting TCR diversity. In support of this concept are the data that: (a) the number of overrepresented sequences is so striking for both Vγ9 and Vδ2; (b) the γ/δ+ cells in these patients are predominantly V81, and in normals most γ/δ T cells that are V81 are Vδ2; and (c) Vγ9 most commonly pairs with Vδ2 in normals (7, 8). Since the data in this study were developed from RNA pooled from many cells before PCR, theoretically, one problem...
in interpreting the data is the possibility of an activated sub-population of the cells having increased numbers of mRNA transcripts, thereby skewing the results. T cells of the Jurkat tumor cell line activated with PMA have a 5-10-fold increase in their number of α and β mRNA transcripts. We have evaluated this concept in sarcoidosis for the β chain (48), and found that in the lung, the numbers of β chain transcripts is increased approximately threefold compared with blood, i.e., in active pulmonary sarcoid it is the lung T cells that are activated, while the blood T cells are relatively quiescent. This is consistent with a number of studies comparing lung and blood T cells in sarcoidosis (49-52). To our knowledge, no data are available on the level of γ and δ mRNA transcripts in activated T cells compared with resting T cells, and there are no data on whether the γ/δ lymphocytes are more activated in sarcoidosis patients than in normals. Putting all of the available data together, it is reasonable to conclude that the data in our study from blood, showing the marked overrepresentation of specific Vγ9 and Vδ2 transcripts, are not associated with an overrepresentation of transcripts from activated T cells prejudicing the selection for sequencing. Further, even if the analysis of RNA is taken from sarcoid patients who have an increased number of activated γ/δ cells compared with the normals, and if γ and δ chain RNA is increased in this subset of cells, then, since the stock of RNA used for PCR for each patient is a random sample of RNA extracted from a larger sample of millions of lymphocytes, the data of overrepresented sequences observed could not be due to a few-fold increase in transcripts due to activation of γ/δ lymphocytes. The ideal way to prove what percentage of cells have use of the particular γ and δ transcripts would be to perform single-cell analysis of γ and δ gene expression. We attempted this, but could not obtain material after PCR that could be sequenced.

The implications from the data are interesting for the emerging concepts regarding the function of the TCR-γ/δ and for the pathogenesis of sarcoidosis. Most importantly, put in the setting of studies demonstrating that γ/δ T cells play a role in the response to mycobacteria and parasites (13-18), and can proliferate to mycobacterial proteins, including heat shock proteins (16-18), it is a reasonable hypothesis that the γ/δ T cells in sarcoidosis are being used in a specific, possibly antigen-driven immune response against such antigens. Further, the limitation of junctional region diversity observed in some individuals with sarcoidosis may imply a response to a small number of epitopes, with, as a result, oligoclonal populations of γ/δ T cells. While the cause of sarcoidosis is unknown, it is a granulomatous disorder with morphology broadly similar to mycobacterial-induced disease. Further, there is a long history of scattered evidence in the literature of various mycobacteria being cultured from sarcoid tissue, or remnants of mycobacteria observed by EM in biopsy or autopsy specimens (53-55). In addition, mycobacterial antigens are not easily degradable, an observation consistent with the specificity of sarcoid individuals for reacting to the Kveim-Siltzbach "antigen," a very stable crude preparation extracted from sarcoid spleen (56). Finally, for the one sarcoid individual evaluated in lung and blood, the identical Vγ9 sequences were observed in both locations, consistent with the systemic nature of the disorder.

The data presented also have implications for the potential mechanisms by which normal individuals develop and maintain an overrepresentation of Vγ9-γ/yP-Cγ1 and Vδ2 usage and pairing in the normal γ/δ T cell repertoire. In the context that this overrepresentation is not seen in human postnatal thymic clones (12), the mechanism is not a restriction of recombinational possibilities at the gene level nor a restriction of the protein pairing of γ and δ chains. However, it is conceivable that the overrepresentation results from the Vγ9/Vδ2 cells responding and/or proliferating in response to a ubiquitous antigen or ligand. If so, to be consistent with the present study, such Vγ9/Vδ2 T cells would have to recognize the same group of antigens despite the extensive junctional regions (N region) diversity of Vγ9/Vδ2 TCR that was observed. Such a circumstance of specific TCR V region expansion, regardless of junctional region sequence, has been observed by "superantigens" such as staphylococcal enterotoxin (57, 58). Another plausible hypothesis is that the peripheral γ/δ T cell repertoire is populated by preferential export of Vγ9/Vδ2 T cells from the thymus during fetal development, a concept consistent with the knowledge that in the mouse fetal thymus there is an early population of T cells exported with TCR using a specific Vγ/Vδ-paired combination, although in this instance junctional region diversity is limited (59, 60).

We thank Bruno Balbi for help with the FACS analysis, and Cesare Saltini for helpful discussions.

Address correspondence to Ronald G. Crystal, Pulmonary Branch, National Heart, and Blood Institute, Bldg. 10, Rm. 6D03, National Institutes of Health, Bethesda, MD 20892.

Received for publication 2 January 1990 and in revised form 9 March 1990.

References

1. Davis, M.M., and P.J. Bjorkman. 1988. T-cell antigen receptor genes and T-cell recognition. Nature (Lond.). 334:395.
2. Kronenberg, M., G. Siu, L.E. Hood, and N. Shastri. 1986. The molecular genetics of the T-cell antigen receptor and T-cell antigen recognition. Annu. Rev. Immunol. 4:529.
3. Toyonaga, B., and T.W. Mak. 1987. Genes of the T-cell anti-
gen receptor in normal and malignant T-cells. 

4. Brenner, M.B., J.L. Strominger, and M.S. Krangel. 1988. The γδ T cell receptor. Adv. Immunol. 43:133.

5. Raulet, D.H. 1989. The structure, function, and molecular genetics of the γδ T cell receptor. Adv. Immunol. 7:175.

6. Triebel, F., F. Faure, M. Graziani, S. Jitsukawa, M-P. Lefranc, and T. Hercend. 1989. A unique VJ-C-rearranged gene encodes a γ protein expressed on the majority of CD3+ T cell receptor αβ circulating lymphocytes. J. Exp. Med. 167:694.

7. Born, J., A. Wicherink, J.J.M. Van Dongen, E. De Vries, W.M. Comans-Bitter, F. Wassenaar, and P.P. Volsen. 1989. Non-random expression of T cell receptor γ and δ variable gene segments in functional T lymphocyte clones from human peripheral blood. Eur. J. Immunol. 19:1559.

8. Triebel, F., F. Faure, F. Mami-Chouaib, S. Jitsukawa, A. Griscelli, C. Genevée, S. Roman-Roman, and T. Hercend. 1988. A novel human Vδ gene expressed predominantly in the ThyA fraction of γ/δ peripheral lymphocytes. Eur. J. Immunol. 18:2021.

9. Hata, S., K. Satyanarayana, P. Devlin, H. Band, J. McLean, J.L. Strominger, M.B. Brenner, and M.S. Krangel. 1988. Extensive junctional diversity of rearranged human T cell receptor δ genes. Science (Wash. DC). 240:1541.

10. Loh, E., S. Cwirla, A.T. Serfani, 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.

11. Casorati, G., G. De Libero, A. Lanzavecchia, and N. Migne. 1989. Molecular analysis of human γ/δ+ clones from thymus and peripheral blood. J. Exp. Med. 170:1521.

12. Janis, E.M., S.H.E. Kaufmann, R.H. Schwartz, and D.M. Pardoll. 1989. Activation of γδ T cells in the primary immune response to Mycobacterium tuberculosis. Science (Wash. DC). 244:713.

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 γδ by an antigen derived from Mycobacterium tuberculosis. Cell. 57:667.

14. Augustin, A., R.T. Kubo, and G.-K. Sim. 1989. Resident pulmonary lymphocytes expressing the γ/δ T cell receptor. Nature (Lond.). 339:544.

15. Holoshitz, J., F. Koning, J.E. Coligan, J. De Bruyn, and S. Strober. Isolation of CD4+CD8+ mycobacteria-reactive T lymphocyte clones from rheumatoid arthritis synovial fluid. Nature (Lond.). 339:226.

16. Haregewoin, A., G. Soman, R.C. Hom, and R.W. Finberg. 1989. Human γδ T cells respond to mycobacterial heat-shock protein. Nature (Lond.). 340:309.

17. Balbi, B., D.R. Moller, M. Kirby, K.J. Holroyd, and R.G. Crystal. 1990. Increased numbers of T lymphocytes with γδ-positive antigen receptors in a subgroup of patients with pulmonary sarcoidosis. J. Clin. Invest. 85:353.

18. Saltini, C., A.J. Hence, V.J. Ferrans, F. Basset, P.B. Bitterman, and R.G. Crystal. 1984. Accurate quantification of cells recovered by bronchoalveolar lavage. Am. Rev. Resp. Dis. 130:650.

19. Crystal, R.G., W.C. Roberts, G.W. Hunnighake, E.J. Gadek, J.D. Fulmer, and B.R. Line. 1981. Pulmonary sarcoidosis: a disease characterized and perpetuated by activated lung T-lymphocytes. Ann. Intern. Med. 94:737.

20. Fulmer, J.D., W.C. Roberts, E.R. Von Gal, and R.G. Crystal. 1979. Morphologic-physiologic correlates of the severity of fibrosis and degree of cellularity in idiopathic pulmonary fibrosis. J. Clin. Inve. 63:893.

21. 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.

22. Spits, H., J. Born, W. Tax, P.J.A. Capel, C. Terhorst, and J.E. De Vries. 1985. Characterization of a monoclonal antibody (WT-31) that recognizes a common epitope on the human T cell receptor for antigen. J. Immunol. 135:1922.

23. Band, H.F., 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). 236:682.

24. Faure, F., S. Jitsukawa, F. Triebel, and T. Hercend. 1988. Characterization of human peripheral lymphocytes expressing the CD3-γ-δ complex with anti receptor monoclonal antibodies. J. Immunol. 141:3357.

25. Jitsukawa, S., F. Faure, M. Lipincki, F. Triebel, and T. Hercend. 1987. A novel subset of human lymphocytes with a T cell receptor γ complex. J. Exp. Med. 166:1192.

26. Ghisla, V., R. Czvrunjakov, and C. Byus. 1974. Ribonucleic acid isolated by cesium chloride centrifugation. Biochemistry. 13:2633.

27. Okayama, H., D.T. Curiel, M.L. Brantley, M.D. Holmes, and R.G. Crystal. 1989. Rapid, nonradioactive detection of mutations in the human genome by allele-specific amplification. J. Lab. Clin. Med. 114:105.

28. Nolan, G.P., S. Fiering, J.F. Nicolas, and L.A. Herzenberg. 1988. Fluorescence-activated cell analysis and sorting of viable mammalian cells based on β-D-galactosidase activity after transduction of Escherichia coli lacZ. Proc. Natl. Acad. Sci. USA. 85:2603.

29. Huk, S., and M.-P. Lefranc. 1987. Rearrangements to the JP1, JP and JP2 segments in the human T-cell rearranging gamma gene (TRγ) locus. FEBS (Fed. Eur. Biochem. Soc.) Lett. 224:291.

30. Huk, S., P. Darivach, and M.-P. Lefranc. 1988. Variable region genes in the human T-cell rearranging gamma (TRG) locus: VγJ junction and homology with the mouse genes. EMBO (Eur. Mol. Biol. Organ.). J. 7:719.

31. 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.

32. Satyanarayana, K., S. Hata, P. Devlin, M.G. Roncarolo, J.E. De Vries, H. Spits, J.L. Strominger, and M.S. Krangel. 1988. Genomic organization of the human T-cell antigen-receptor γδ locus. Proc. Natl. Acad. Sci. USA. 85:8166.

33. Mami-Chouaib, F., S. Jitsukawa, F. Faure, B. Vasina, C.
Genevee, T. Hercend, and F. Triebel. 1989. cDNA cloning of functional T cell receptor $\gamma/\delta$ chains expressed in human peripheral blood lymphocytes. *Eur. J. Immunol.* 19:1545.

39. Takihara, Y., D. Tkachuk, E. Michalopoulos, E. Champagne, J. Reimmann, M. Minden, and T.W. Mak. 1988. Sequence and organization of the diversity, joining, and constant region genes of the human T-cell $\delta$-chain locus. *Proc. Natl. Acad. Sci. USA.* 85:6097.

40. Hata, S., K. Satyanarayana, P. Devlin, H. Band, J. McLean, J.L. Strominger, M.B. Brenner, and M.S. Krangel. 1988. Extensive junctional diversity of rearranged human T cell receptor $\delta$ genes. *Science (Wash. DC).* 240:1541.

41. LeFranc, M.-P., A. Forster, and T.H. Rabbitts. 1986. Genetic polymorphism and exon changes of the constant regionsof the human Tcell $\delta$-chainlocus. *Proc. Natl. Acad. Sci. USA.* 83:9596.

42. Takihara, Y., J. Reimmann, E. Michalopoulos, E. Ciccone, L. Moretta, and T.W. Mak. 1989. Diversity and structure of human T cell receptor $\delta$ chain genes in peripheral blood $\gamma/\delta$-bearing T lymphocytes. *J. Exp. Med.* 169:393.

43. Daraivach, P., and M.P. Lefranc. 1989. First genomic sequence of the human T-cell receptor $V\delta 2$ gene (TRDV2). *Nucleic Acid Res.* 17:4880.

44. Loh, E.L., J.F. Elliott, S. Cwirla, L.L. Lanier, and M.M. Davis. 1989. Polymerase chain reaction with single-sided specificity: analysis of T cell receptor $\delta$ chain. *Science (Wash. DC).* 243:217.

45. Hata, S., M. Clabby, P. Devlin, H. Spits, J.E. DeVries, and M.S. Krangel. 1989. Diversity and organization of human T cell receptor $\delta$ variable gene segments. *J. Exp. Med.* 169:41.

46. Asarnow, D.M., T. Goodman, L. LeFrancois, and J.P. Allison. 1989. Distinct antigen receptor repertoires of two classes of murine epithelium-associated T cells. *Nature (Lond.)* 341:60.

47. Saltini, C., M. Kirby, B.C. Trapnell, N. Tamura, and R.G. Crystal. 1990. Biased accumulation of T lymphocytes with "memory"-type CD45 leukocyte common antigen gene expression on the epithelial surface of the human lung. *J. Exp. Med.* 171:1123.

48. du Bois, R.M., B. Balbi, M. Kirby, and R.G. Crystal. 1989. T-lymphocyte accumulation in pulmonary sarcoidosis: evidence for persistent stimulation of sarcoid lung T-lymphocytes via the T-cell antigen receptor. *Am. Rev. Resp. Dis.* 139:A61.

49. Pinkston, P., P.B. Bitterman, and R.G. Crystal. 1983. Spontaneous release of interleukin-2 by lung T-lymphocytes in active pulmonary sarcoidosis. *N. Engl. J. Med.* 308:793.

50. Saltini, C., J.R. Spurzem, J.L. Lee, P. Pinkston, and R.G. Crystal. 1986. Spontaneous release of interleukin-2 by lung lymphocytes in active pulmonary sarcoidosis is primarily from the Leu$^+$ DR$^+$ T-cell subset. *77:1962.

51. Hunninghake, G.W., J.E. Gadek, R.C. Young Jr., 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.

52. Robinson, B.W.S., T. McIlmore, 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.

53. Cantwell, A.R. 1982. Histologic observations of variably acid-fast pleomorphic bacteria in systemic sarcoidosis: a report of 3 cases. *Growth.* 46:113.

54. Moscovic, E.A. 1982. Sarcoidosis and mycobacterial L-forms: histologic studies. In Cell Wall Deficient Bacteria. Gerald J. Domingue, editor. Addison-Wesley Publishing Company, Reading, MA. 299–319.

55. Vanek, J., and J. Schwarz. 1970. Demonstration of acid-fast rods in sarcoidosis. *Am. Rev Resp. Dis.* 101:395.

56. Teirstein, A.S., and L. K. Brown. 1988. The Kveim Siltzbach test in 1987. In Sarcoidosis and Other Granulomatous Diseases. C. Grassi, G. Rizzato, and E. Pozzi, editors. Excerpta Medica Inc., Lawrenceville, NJ. 7–19.

57. White, J., A. Herman, A.M. Pullen, R. Kubo, J.W. Kappler, and P. Marrack. 1989. The V$\beta$-specific superantigen staphylococcal enterotoxin B: stimulation of mature T cells and clonal deletion in neonatal mice. *Cell.* 56:27.

58. Kappler, J., B. Kotzin, L. Herron, E.W. Gelfand, R.D. Bigler, A. Boylston, S. Carrel, D.N. Ponett, Y. Choi, and P. Marrack. 1989. V$\beta$-specific stimulation of human T cells by staphylococcal toxins. *Science (Wash. DC).* 244:811.

59. Asarnow, D.M., W.A. Kuziel, M. Bonyhaid, 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.

60. Lafaille, J.J., A. Decloux, M. Bonneville, Y. Takagaki, and S. Tonegawa. 1989. Functional sequences of T cell receptor $\delta$ genes: implications for $\gamma\delta$ T cell lineages and for a novel intermediate of V-(D)-J Joining. *Cell.* 59:859.