Diversity in Junctional Sequences Associated with the Common Human Vγ9 and Vδ2 Gene Segments in Normal Blood and Lung Compared with the Limited Diversity in a Granulomatous Disease

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Summary

The T cell receptor (TCR) junctional regions (N regions) of the common human Vγ9 and Vδ2 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 $V\gamma9^+$ γ/δ^+ T cells are often observed. In normal individuals, the vast majority (86%) of blood Vγ9 transcripts used the JγP gene segment. In contrast to this restriction of J region usage, there was a large diversity of the junctional region, with <20% of blood Vγ9 junctional regions showing identical sequences for any one normal individual. For the blood Vδ2 transcripts in normal individuals, there was restriction of J region usage, with 93% using Jδ1. The junctional regions were even more diverse than for Vγ9, 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 Vγ9 transcripts examined were identical, suggesting a response of some γ/δ 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 Vγ9 transcripts, as well as Vδ2 transcripts, sharing identical sequences. For Vγ9 blood transcripts, two individuals showed 84 and 56% of junctional region sequences to be identical, respectively. Similarly, blood Vδ2 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 Vγ9 junctional sequences, lung Vγ9 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 γ/δ T cells may play a specific role in granuloma formation in sarcoidosis, as has been suggested in other granulomatous diseases.

T 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 α and β 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 γ and δ chains. Although the TCR-γ/δ uses similar recombination processes, unlike the broad repertoire of genetic elements that can potentially define the TCR-α/β, the human γ/δ repertoires are severely limited, with only eight functional Vγ and five Jγ segments, and an even smaller number of V, D, and J segments defining the δ locus (4, 5). Further, in normal individuals, the usage of TCR-γ/δ appears to be even more restricted, in that >60% of blood γ/δ^+ T cells use Vγ9 elements, as detected by the mAb TiγA (6). In addition, analysis of $V\gamma9^+$ T cell clones demonstrates that most TCRs using Vγ9 are Vγ9-JγP-Cγ1 paired with a δ chain using the Vδ2 elements (7, 8). In this context, the diversity of the TCR-γ/δ is primarily based on the variable deletion or addition of nucleotides to the junctional regions (N regions) during rearrangement of the γ and δ 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 γ/δ^+ 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 γ and δ 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 TiγA$^+$ (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 TiγA$^+$ 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. . , N2. . , 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 (21). The patients with sarcoidosis included five individuals diagnosed as previously described (22). All had pulmonary sarcoidosis as diagnosed by an intrathoracic biopsy showing noncaseating granulomata. 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 66 ± 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 × 10^6 for the normals and 63 ± 14 × 10^6 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-δ1 (all γ/δ^+ T cells, FITC-conjugated; T Cell Sciences, Cambridge, MA) (26); TCS-δ1 (a Vδ1/Jδ1 determinant, FITC-conjugated, T Cell Sciences) (27); TiγA (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% (vol/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 $MgCl_2$ 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 Vγ9 primer with a PstI cutting site (PVG9; 5'ATCTGCCAGGCACTGTCAGAAAGGAATC-3') and a Cγ primer with a SstI cutting site (PCG7; 5'-TAGAGCTCTATGTTCCAGCCTTCTGGAG-3'); and for Vδ2, a Vδ2 primer with a PstI site (PVD2: 5'-GACTGCAGGAAGACCCAAGGTAACAC-3') and a Cδ primer (PCD1; 5'-GTTATCTTGGATGACACGAG-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 Vδ2 cDNA with PstI and EcoRI (the amplified Cδ 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 (Geneclean; 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 dideoxy chain 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-Jγ 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 sequence of the Vγ9 gene (PVNJ9; 5′TACGTGGCCTTGTGGGAACGGGAA-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′-CTGTCTTTATTGGAGGAAAGATAAT-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 ^{32}P-labeled 274-bp Cγ region cDNA probe. This probe was synthesized by PCR using PCG2 (5′-AACAACTTGATGCAGATGTTTCC-3′) and PCG3 (5′-TCATGTCTGACGATACATCTGTG-3′) and purified by electroelution (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 sarcoid 1, blood lymphocytes were stained with Leu-4/PE and TCR-δ1/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). RNA 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 sarcoids 1–5, respectively). For all sarcoid individuals, the proportions 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 TiγA$^+$ T cells (TiγA$^+$/CD3$^+$: 38, 17, and 58%, for sarcoid 1, 2, and 4, respectively). These same individuals had no relative increase in TCS-δ1$^+$ blood T cells (TCS-δ1$^+$/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 Vδ2 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 Cγ 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 Vδ2, the N region, Dδ region, N region, entire Jδ region, and ~70 bp of the 5′ end of the Cδ region were sequenced, but only the junctional region is presented in the tables, along with the specific Jδ region. Also, for both Vγ9 and Vδ2 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 Jδ sequence did not correspond to a known genomic Jγ or Jδ sequence, respectively) (34–40).

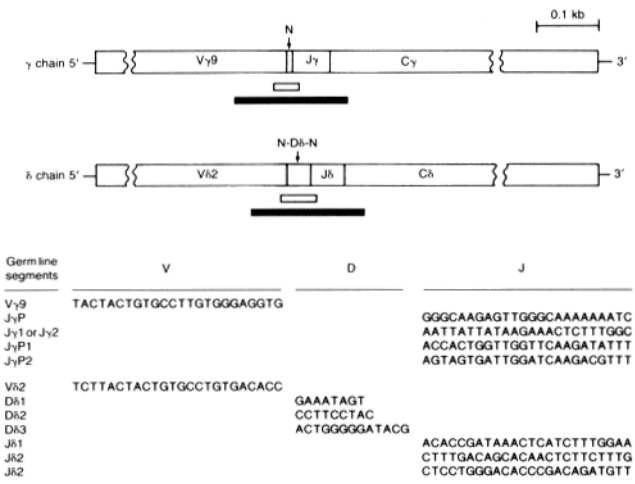

Figure 1. Germline sequences of the Vγ9, Jγ, Vδ2, Dδ, and Jδ segments. At the top is a configuration of a Vγ9 cDNA (Vγ9-N-Jγ-Cγ), and below that is a configuration of a Vδ2 cDNA (Vδ2-N-Dδ-N-Jδ-Cδ). The closed bar below each cDNA indicates the region sequenced. The primers used to amplify the cDNA for sequencing were located at the 5′ and 3′ ends of the closed bars (primers PVG9 and PCG7 for Vγ9; PVD2 and PCD1 for Vδ2; see Materials and Methods). Open bars indicate the sequences of Vγ9 or Vδ2 cDNAs, as presented in Tables 1–6. Below the diagrams of the cDNAs are the germline sequences of the 3′ portion of Vγ9 and the 5′ portion of 5 Jγ gene segments (for the region shown, Jγ1 and Jγ2 are identical). Also shown are the 3′ portion of Vδ2, the 3 Dδ segments, and the 5′ portion of 3 Jδ gene segments.

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

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*	V	N	J	Jγ region	In-frame‡
Normal 1	17	3	N1B.G1-3	GCCTTGTGGGAGGTG		CAAGAGTTGGGC	JP	+
		2	N1B.G4-5	GCCTTGTGGGAG	CAACTGG	AAGAGTTGGGC	JP	+
		1	N1B.G6	GCCTTGTGGGA	ACGGG	AAGAGTTGGGC	JP	+
		1	N1B.G7	GCCTTGTGGGAGGT	AGGC	GAGTTGGGC	JP	+
		1	N1B.G8	GCCTTGTGGGAGG	CAGGGGC	AGAGTTGGGC	JP	+
		1	N1B.G9	GCCTTGTGGGAGGT	A	CAAGAGTTGGGC	JP	+
		1	N1B.G10	GCCTTGTGGGAG	CTTGTCC	AAGAGTTGGGC	JP	+
		1	N1B.G11	GCCTTGTGGGAG	CAG	GAGTTGGGC	JP	+
		1	N1B.G12	GCCTTGTGGGAGGT	A	AAGAAA	J1	+
		1	N1B.G13	GCCTTGTGGGAGGT	AAGGGAA	AAGAAA	J2	+
		1	N1B.G14	GCCTTGTGGGAGGTG	CATTCTG	ATTATAAGAAA	J2	+
		1	N1B.G15	GCCTTGTGGGAGGT	A	AAGAAA	J2	+
		1	N1B.G16	GCCTTGTGGGAGG	CCCGCTCCGG	GAGTTGGGC	JP	-
		1	N1B.G17	GCCTTGTGGGAGGT	TCAGG	TATAAGAAA	J1	-
Normal 2	16	2	N2B.G1-2	GCCTTGTGGGAGGTG		CAAGAGTTGGGC	JP	+
		1	N2B.G3	GCCTTGTGGGAGG	C	AGAGTTGGGC	JP	+
		1	N2B.G4	GCCTTGTGGGAGGT	TCCCCGGGTTC	TGGGC	JP	+
		1	N2B.G5	GCCTTGTGGGAGGTG	GGG	GAGTTGGGC	JP	+
		1	N2B.G6	GCCTTGTGGGAGGTG	CAG	GAGTTGGGC	JP	+
		1	N2B.G7	GCCTTGTGGGAG	CAATATCT	GCAAGAGTTGGGC	JP	+
		1	N2B.G8	GCCTTGTGGGA	T	CAAGAGTTGGGC	JP	+
		1	N2B.G9	GCCTTGTGGGAGGTG	AAT	GAGTTGGGC	JP	+
		1	N2B.G10	GCCTTGTGGGAGG	CCGGAGACGC	GCAAGAGTTGGGC	JP	+
		1	N2B.G11	GCCTTGTGGGAGGTG	CCCGG	AGAGTTGGGC	JP	+
		1	N2B.G12	GCCTTGTGGGAGG	AC	AATTATTATAAGAAA	J2	+
		1	N2B.G13	GCCTTGTGGGA	CCTGGGGGGAG	ATTATTATAAGAAA	J2	+
		1	N2B.G14	GCCTT	AC	ATTATTATAAGAAA	J2	+
		1	N2B.G15	GCCTTGTGGGAGGTG	TAATATC	GCAAGAGTTGGGC	JP	-
		1	N2B.G16	GCCTTGTGGGAGGTG	ACGG	TATAAGAAA	J2	-
Normal 3	13	2	N3B.G1-2	GCCTTGTGGGAGGTG		CAAGAGTTGGGC	JP	+
		1	N3B.G3	GCCTTGTGGGAGGTG	AAC	GAGTTGGGC	JP	+
		1	N3B.G4	GCCTTGTGGGAG	CGC	GAGTTGGGC	JP	+
		1	N3B.G5	GCCTTGTGGGA	ATC	AGAGTTGGGC	JP	+
		1	N3B.G6	GCCTTGTGGGAGGTG	CACTTG	GAGTTGGGC	JP	+
		1	N3B.G7	GCCTTGTGGGAGGT	TGG	AGAGTTGGGC	JP	+
		1	N3B.G8	GCCTTGTGGGAG	TTCC	AGTTGGGC	JP	+
		1	N3B.G9	GCCTTGTGGGAGG	AACTG	GAGTTGGGC	JP	+
		1	N3B.G10	GCCTTGTGGGAGGT	CCAC	GAGTTGGGC	JP	+
		1	N3B.G11	GCCTTGTGG	TGC	CAAGAGTTGGGC	JP	+
		1	N3B.G12	GCCTTGTGGGAGGTG	CGTGG	GTTGGGC	JP	+
		1	N3B.G13	GCCTTGTGGGAG	AC	GCAAGAGTTGGGC	JP	+
Normal 4	20	2	N4B.G1-2	GCCTTGTGGGAGGTG		CAAGAGTTGGGC	JP	+
		1	N4B.G3	GCCTTGTGG		GAGTTGGGC	JP	+
		1	N4B.G4	GCCTTGTGG	TGC	CAAGAGTTGGGC	JP	+
		1	N4B.G5	GCCTTGTGGGAGGTG	TT	AGAGTTGGGC	JP	+
		1	N4B.G6	GCCTTGTGGGAGG	A	GCAAGAGTTGGGC	JP	+
		1	N4B.G7	GCCTTGTGGGAG	ACT	CAAGAGTTGGGC	JP	+
		1	N4B.G8	GCCTTGTGGG	GGGCAGC	GTTGGGC	JP	+
		1	N4B.G9	GCCTTGTGGGAGGTG	CACTTG	GAGTTGGGC	JP	+
		1	N4B.G10	GCCTTGTGGGAGGTG	CACACCACCGCAACG	GAGTTGGGC	JP	+
		1	N4B.G11	GCCTTGTGGG	TT	CAAGAGTTGGGC	JP	+
		1	N4B.G12	GCCTTGTGGGAGGTG	TTGTCTA	AGTTGGGC	JP	+
		1	N4B.G13	GCCTTGTGGGAG	CGTCTAGGGGG	AGAGTTGGGC	JP	+
		1	N4B.G14	GCCTTGTGGGA	TAC	AGAGTTGGGC	JP	+
		1	N4B.G15	GCCTTGTGGGA	ATC	AGAGTTGGGC	JP	+
		1	N4B.G16	GCCTTGTGGGAG	TCCGCGGGG	GAGTTGGGC	JP	+
		1	N4B.G17	GCCTTGTGGGAG	TTA	CAAGAGTTGGGC	JP	+
		1	N4B.G18	GCCTTGTGGGAGGTG	CG	AGAGTTGGGC	JP	+
		1	N4B.G19	GCCTTGTGGGAGG	GCCCGGCGA	ATTATAAGAAA	J2	+
		1	N4B.G20	GCCTTGTGGGAGGT	TGCGAAGG	GAGTTGGGC	JP	-
Normal 5	15	2	N5B.G1-2	GCCTTGTGGGA	ACGGG	AAGAGTTGGGC	JP	+
		1	N5B.G3	GCCTTGTGGGAGGTG	CGGCG	AGAGTTGGGC	JP	+
		1	N5B.G4	GCCTTGTGGGAGGTG	CATGAG	GAGTTGGGC	JP	+
		1	N5B.G5	GCCTTGTGGG	TTGTCGACCAAC	AGTTGGGC	JP	+
		1	N5B.G6	GCCTTGTGGGAGGT	AGGC	GAGTTGGGC	JP	+
		1	N5B.G7	GCCTTGTGGGAGG	GG	GAGTTGGGC	JP	+
		1	N5B.G8	GCCTTGTGGGAGGTG	CGG	GAGTTGGGC	JP	+
		1	N5B.G9	GCCTTGTGGGAGGT	TTC	AGAGTTGGGC	JP	+
		1	N5B.G10	GCCTTGTGGGAGGTG	CAGTGG	GAGTTGGGC	JP	+
		1	N5B.G11	GCCTTG		TATTATAAGAAA	J2	+
		1	N5B.G12	GCCTTGTGGGAG	CG	TTATAAGAAA	J2	+
		1	N5B.G13	GCCTTGTGGGAGGTG	CGGCGAG	GAGTTGGGC	JP	-
		1	N5B.G14	GCCTTGTGGGAGG	GTAGGC	GAGTTGGGC	JP	-
		1	N5B.G15	GCCTTGTGGGAGGTG	ACGG	TATAAGAAA	J2	-

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 (Jγ).

* 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.

‡ 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 sequences.

nucleotides. One normal individual (normal 1) showed three clones with the same Vγ9-junctional sequence (5'-GCCTTG-TGGGAGGTA-3'; N1B.G9, N1B.G12, N1B.G15) but three different Jγ gene segments.

Vγ9 mRNA Transcripts in Lung T Cells of Normals. A total of 64 Vγ9 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 Vγ9 transcripts with in-frame sequences, 37 clones (62%) used the JγP gene segment, 18 used Jγ2 (30%), three used JγP2 (5%), two used JγP1 (3%), and none used Jγ1. 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 Vγ9 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).

Vγ9 mRNA Transcripts in Blood and Lung T Cells of Individuals with Sarcoidosis. For the individuals with sarcoidosis,

Table 2. *Junctional Sequences of Vγ9-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	Jγ region	In-frame‡
Normal 6	16	3	N6L.G1-3	GCCTTGTGGGA	CGCGAG	AAGAGTTGGGC	JP	+
		2	N6L.G4-5	GCCTTGTGGGAGGT	A	CAAGAGTTGGGC	JP	+
		2	N6L.G6-7	GCCTTGTGGGAG	AC	GCAAGAGTTGGGC	JP	+
		1	N6L.G8	GCCTTGTGGGAG	CGGGAGCGGGCG	GAGTTGGGC	JP	+
		1	N6L.G9	GCCTTGTGGG	GGGTGCACGGGC	TGGGC	JP	+
		1	N6L.G10	GCCTTGTGGGAGGT	AGGC	GAGTTGGGC	JP	+
		1	N6L.G11	GCCTTGTGGG	TAGATGGG	GAGTTGGGC	JP	+
		1	N6L.G12	GCCTTGTGGG	GAAGGGCAG	AGAAA	J2	+
		1	N6L.G13	GCCTTGTGGGAGGTG	AGGG	ATTATAAGAAA	J2	+
		1	N6L.G14	GCCTTGTGGGAGGTG	CGCGAG	ACTGGTTGGTTC	JP1	+
		1	N6L.G15	GCCTT	TAAA	GATTGGATC	JP2	+
		1	N6L.G16	GCCTTGT	A	AAA	J2	-
Normal 7	19	3	N7L.G1-3	GCCTTGTGGGAGGTG	GC	GCAAGAGTTGGGC	JP	+
		2	N7L.G4-5	GCCTTGTGGGAGGT	CCCCTCCCC	A	J2	+
		2	N7L.G6-7	GCCTTGTGGGAGGT	CCA	GCAAGAGTTGGGC	JP	+
		2	N7L.G8-9	GCCTTGTGGGAGGTG		CAAGAGTTGGGC	JP	+
		1	N7L.G10	GCCTTGTGGGAGGTG	CA	AGTTGGGC	JP	+
		1	N7L.G11	GCCTTGTGGG	TTGTCGAC	CAAGAGTTGGGC	JP	+
		1	N7L.G12	GCCTTGTGGGAGG	CA	CAAGAGTTGGGC	JP	+
		1	N7L.G13	GCCTTGTGGGAGGTG	GGG	GAGTTGGGC	JP	+
		1	N7L.G14	GCCTTGTGGGAG	ACCTCCGGG	GAGTTGGGC	JP	+
		1	N7L.G15	GCCTTGTGGGAGGTG	GCGGC	AGAGTTGGGC	JP	+
		1	N7L.G16	GCCTTGTGGGAGG	CGGAAGAGC	TGGGC	JP	+
		1	N7L.G17	GCCTTGTGGGAGGT	AGG	GAAA	J2	+
		1	N7L.G18	GC	TGCC	GGTTGGTTC	JP1	+
		1	N7L.G19	GCCTTG	GAG	GAAA	J2	-
Normal 8	14	2	N8L.G1-2	GCCTTGTGGGAGG	CGCAGGGG	GAGTTGGGC	JP	+
		2	N8L.G3-4	GCCTTGTGGGAGGTG		CAAGAGTTGGGC	JP	+
		2	N8L.G5-6	GCCTTGTGGGAGG	CGG	AAGAGTTGGGC	JP	+
		1	N8L.G7	GCCTTGTGGG	TTGTCGAC	CAAGAGTTGGGC	JP	+
		1	N8L.G8	GCCTTGTGGGAGGT	TCTT	GAGTTGGGC	JP	+
		1	N8L.G9	GCCTTGTGGGAGGT	A	CAAGAGTTGGGC	JP	+
		1	N8L.G10	GCCTTGTGGGAG	CCCCGGTTGGG	GTTGGGC	JP	+
		1	N8L.G11	GCCTTGTGGGAGG	CCAGG	GAGTTGGGC	JP	+
		1	N8L.G12	GCCTTGTGGGAGGTG	TCAAATTATAACAC	A	J2	+
		1	N8L.G13	GCCTTGTGGGAG	CCCGGG	AATTATTATAAGAAA	J2	+
		1	N8L.G14	GCCTTGTGGGAGG	GGAGG	AAGAAA	J2	+
Normal 9	15	6	N9L.G1-6	GCCTTGTGGGAGGTG	CG	TTATAAGAAA	J2	+
		3	N9L.G7-9	GCCTTGTG	CCGGGGGG	AGAAA	J2	+
		2	N9L.G10-11	GCCTTGTGGGAGGT	TG	TAGTGATTGGATC	JP2	+
		1	N9L.G12	GCCTTGTGGGAGGTG	CCTC	ATTATAAGAAA	J2	+
		1	N9L.G13	GCCTTGTGGGAGGTG		CAAGAGTTGGGC	JP	+
		1	N9L.G14	GCCTTGTGGG	GTACA	(TCTTTGG)‡	J2	-
		1	N9L.G15	GCCTTGTGGGAGGTG	CGGGAG	GAAA	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, γ chain, clones 1, 2, and 3, etc.

‡ 16 bp of the 5' portion of the Jγ2 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′-TGGGAACGGGAAGAGTT-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 S4B.G1-5 [5 of 21, 24%] to N1B.G7 and N5b.G6 [Table 1]; compare S4B.G6-9 [4 of 21, 19%] to N1B.G1-3, N2B.G1-2, N3B.G1-2, N4B.G1-2, N7L.G8-9, 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 Jγ1 and one clone each used Jγ2, 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′-GCCTTGTGGGAACGGG-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 allele-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 S1L.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. *Junctional 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	Jγ region	In-frame
Sarcoid 1	31	26	S1B.G1-26	GCCTTGTGGGA	ACGGG	AAGAGTTGGGC	JP	+
		2	S1B.G27-28	GCCTTGTGGGAGGTG		CAAGAGTTGGGC	JP	+
		1	S1B.G29	GCCTTGTGG		CAAGAGTTGGGC	JP	+
		1	S1B.G30	GCCTTGTGG	AATC	GAGTTGGGC	JP	-
		1	S1B.G31	GCCTTGTGG	AGGT	GCAAGAGTTGGGC	JP	-
Sarcoid 2	27	15	S2B.G1-15	GCCTTGTGGGA	ACGGG	AAGAGTTGGGC	JP	+
		1	S2B.G16	GCCTTGTGGGAGGTG	CGGCG	AGAGTTGGGC	JP	+
		1	S2B.G17	GCCTTGTGGGAG	TC	GCAAGAGTTGGGC	JP	+
		1	S2B.G18	GCCTT	ATGGGACCGATG	GTTGGGC	JP	+
		1	S2B.G19	GCCTTGTGGGAGGTG	CGG	GAGTTGGGC	JP	+
		1	S2B.G20	GCCTTGTGGGAGGTG	GC	GCAAGAGTTGGGC	JP	+
		1	S2B.G21	GCCTTGTGGGAG	GGG	GAGTTGGGC	JP	+
		1	S2B.G22	GCCTTGTGG		CAAGAGTTGGGC	JP	+
		1	S2B.G23	GCCTTGTGGGAGGTG		CAAGAGTTGGGC	JP	+
		1	S2B.G24	GCCTTGTGGGAGGTG	CAGGC	AGAGTTGGGC	JP	+
		1	S2B.G25	GCCTTGTGGGAGGTG	TGG	GGGCAAGAGTTGGGC	JP	+
		1	S2B.G26	GCCTTGTGGGAGG	CCCC	GCAAGAGTTGGGC	JP	+
		1	S2B.G27	GCCTTGTGGGAGG	GGAGG	AAGAAA	J2	+
Sarcoid 3	21	3	S3B.G1-3	GCCTTGTGGGA	ACGGG	AAGAGTTGGGC	JP	+
		1	S3B.G4	GCCTTGTGGGAG	TCT	CAAGAGTTGGGC	JP	+
		1	S3B.G5	GCCTTGTGGGAGG	CAGGTTTGGG	GTTGGGC	JP	+
		1	S3B.G6	GCCTTGTGGGAGG		AAGAGTTGGGC	JP	+
		1	S3B.G7	GCCTTGTGGG	TGGTGTTAGG	AGAGTTGGGC	JP	+
		1	S3B.G8	GCCTTGTGGGA	AATA	CAAGAGTTGGGC	JP	+
		1	S3B.G9	GCCTTGTGGGAGGTG	CT	GCAAGAGTTGGGC	JP	+
		1	S3B.G10	GCCTTGTGGGAGG	CAG	AAGAGTTGGGC	JP	+
		1	S3B.G11	GCCTTGTGGGAGGT	CTTAAAT	TTGGGC	JP	+
		1	S3B.G12	GCCTTGTGGG	TGGCGGTAAG	GTTGGGC	JP	+
		1	S3B.G13	GCCTTGTGGGAGGTG	CAG	GAGTTGGGC	JP	+
		1	S3B.G14	GCCTTGTGGGAG	ACCCATGG	GTTGGGC	JP	+
		1	S3B.G15	GCCTTGTGGGAGGTG	CTGAGT	GAGTTGGGC	JP	+
		1	S3B.G16	GCCTTGTGGGAGG	C	GCAAGAGTTGGGC	JP	+
		1	S3B.G17	GCCTTGTGGGAGGT	TAGCTCTCTTTGGGG	GTTGGGC	JP	+
		1	S3B.G18	GCCTTGTGGGAGG	CGTCGCA	GCAAGAGTTGGGC	JP	+
		1	S3B.G19	GCCTTGTGGG	GGGGGGT	TAAGAAA	J1	+
		1	S3B.G20	GC	TGCC	GGTTGGTTC	JP1	+
		1	S3B.G21	GCCTTGTGGGAGGTG	CATTTGG	GAAA	J1	-
Sarcoid 4	21	5	S4B.G1-5	GCCTTGTGGGAGGT	AGGC	GAGTTGGGC	JP	+
		4	S4B.G6-9	GCCTTGTGGGAGGTG		CAAGAGTTGGGC	JP	+
		1	S4B.G10	GCCTTGTGGGAGGT	AGGCGT	GTTGGGC	JP	+
		1	S4B.G11	GCCTTG	CGGGAGGCACCTTTGGG	GTTGGGC	JP	+
		1	S4B.G12	GCCTTGTGGGAG		CAAGAGTTGGGC	JP	+
		1	S4B.G13	GCCTTGTGGGAGG	CACCTTTGGG	GTTGGGC	JP	+
		1	S4B.G14	GCCTTGTGGGAGGTG	CGG	GAGTTGGGC	JP	+
		1	S4B.G15	GCCTTGTGGGAGGTG	GG	GAGTTGGGC	JP	+
		1	S4B.G16	GCCTTGTGGGAGGTG	CAG	GAGTTGGGC	JP	+
		1	S4B.G17	GCCTTGTGGGA	AAGTAGTCGTCCTGT	AGAGTTGGGC	JP	+
		1	S4B.G18	GCCTTGTGGGAGGTG	CAGGTGGG	AGAGTTGGGC	JP	+
		1	S4B.G19	GCCTTGTGGGAGGTG	CATGAG	GAGTTGGGC	JP	+
		1	S4B.G20	GCCTTGTGGGAGGT	CGCCCT	TTATTATAAG	J1	+
		1	S4B.G21	GCCTTGTGGGA	ATTTAGCAAGCG	CAAGAGTTGGGC	JP	-
Sarcoid 5	15	1	S5B.G1	GCCTTGTGGGA	ACGGG	AAGAGTTGGGC	JP	+
		1	S5B.G2	GCCTTGTGGGAG	AC	GCAAGAGTTGGGC	JP	+
		1	S5B.G3	GCCTTGTGGGAGGTG	CAG	GAGTTGGGC	JP	+
		1	S5B.G4	GCCTTGTGGGAGGTG	AAATCG	GAGTTGGGC	JP	+
		1	S5B.G5	GCCTTGTGGGAGGTG		CAAGAGTTGGGC	JP	+
		1	S5B.G6	GCCTTGTGGGAGGTG	CT	GCAAGAGTTGGGC	JP	+
		1	S5B.G7	GCCTTGTGGGAG	AGCCAAGAGTA	GGGC	JP	+
		1	S5B.G8	GCCTTGTGGGAGGTG	CGCCGAGAGC	TGGGC	JP	+
		1	S5B.G9	GCCTTGTGGGAGG	C	GCAAGAGTTGGGC	JP	+
		1	S5B.G10	GCCTTGTGGGAGG		ATAAGAAA	J1	+
		1	S5B.G11	GCCTTGTGGGAGGTG	GGC	AGTGATTGGATC	JP2	+
		1	S5B.G12	GCCTTGTGGGAGG	C	CAAGAGTTGGGC	JP	-
		1	S5B.G13	GCCTTGTGGGAGGTG	GG	AAGAGTTGGGC	JP	-
		1	S5B.G14	GCCTTGTGGGAG	ATG	CAAGAGTTGGGC	JP	-
		1	S5B,G15	GCCTTG	GAG	GAAA	J2	-

Sequences shown include the same regions described in Table 1.
* The clones are numbered in the same fashion as described in Table 1; S1B.G1-26, individual with sarcoidosis 1, blood T cells, γ chain, clones 1 to 26, etc.

Table 4. *Junctional Sequences of Vγ9-containing mRNA Transcripts in Lung T Cells of an Individual with Sarcoidosis*

Individual	Number of cloned sequences	Number of clones with this sequence	Clone*	V	N	J	Jγ region	In-frame
Sarcoid 1	21	14	S1L.G1-14	GCCTTGTGGGA	ACGGG	AAGAGTTGGGC	JP	+
		2	S1L.G15-16	GCCTTGTGGGAGGTG		CAAGAGTTGGGC	JP	+
		1	S1L.G17	GCCTTGTGGGAGGTG	CAG	GAGTTGGGC	JP	+
		1	S1L.G18	GCCT	GCTT	GCAAGAGTTGGGC	JP	+
		1	S1L.G19	GCCTTGTGGGAGGT	CG	AAGAGTTGGGC	JP	+
		1	S1L.G20	GCCTTGTGGGAG	CAACTGG	AAGAGTTGGGC	JP	+
		1	S1L.G21	GCCTTGTGGGAGGT	A	AAGAAA	J2	+

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, γ chain, clones 1–14, etc.

and disease is not well understood. One conceptual problem in regard to γ/δ function in antigen recognition relates to the apparent limited diversity of the number of genetic elements that can potentially recombine to direct the synthesis of γ and δ mRNA transcripts (4, 5, 9). Further, analysis of blood γ/δ T cells has shown that the choice of γ and δ gene segments that are actually used is limited, with the majority of γ/δ T cells using the Vγ9 segment (6–8). In addition, most rearranged Vγ9 gene segments utilize JγP and Cγ1 elements and pair with Vδ2 chains (7, 8). Thus, the specificity of TCR-γ/δ must lie elsewhere, likely in the junctional regions (N regions) between the V and J (for the γ chain) and V and D, D and D, and D and J (for the δ chain).

The analysis of 145 Vγ9 mRNA transcripts and 50 Vδ2 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 Vγ9, 86% paired with JγP and 12% with Jγ2; for Vδ2, 93% paired with Jδ1), broad diversity was observed in the junctional regions. In this regard, for the 81 normal blood Vγ9 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 Vγ9 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 Vδ2 transcripts, an even broader diversity was observed. Of the 50 transcripts evaluated, all were different. Further, comparison of the sequences of the 145 Vγ9 and 50 Vδ2 transcripts with the Vγ9 and the Vδ2 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 Vγ9 transcripts, the diversity was some what less than that observed in blood. Several possibilities could explain this observation. First, the lung Vγ9 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 γ/δ T cells within

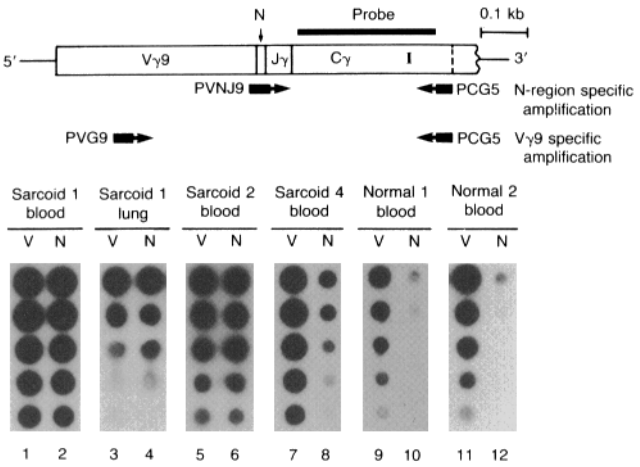

Figure 2. Relative number of total Vγ9 transcripts compared with Vγ9 transcripts containing a specific N region (junctional region) sequence observed in high abundance among Vγ9 transcripts of some individuals with sarcoidosis. Comparisons were made by allele-specific amplification of cDNA using Vγ9 specific primers and N region–specific primers. The primer combination of PVG9 and PCG5 was used for total Vγ9 amplification (indicated by *V* above lanes), while the combination of PVNJ9 and PCG5 was used for N region–specific amplification (indicated by *N* above lanes). Amplified cDNAs were evaluated by dot blot hybridization with a ^{32}P-labeled Cγ probe (location indicated by closed bar ["probe"]) located in Cγ exon I (Cγ I) where there is a shared sequence between Cγ1 and Cγ2. Sarcoids 1, 2, and 4 and normals 1 and 2; two correspond to the individuals thus numbered in the text and tables. Lane *1*, Vγ9-specific transcripts in blood of sarcoid 1; lane *2*, N region–specific transcripts in blood of sarcoid 1; lane *3*, Vγ9-specific transcripts in the lung of sarcoid 1; lane *4*, N region–specific transcripts in the lung of sarcoid 1; lane *5*, Vγ9-specific transcripts in the blood of sarcoid 2; lane *6*, N region–specific transcripts in blood of sarcoid 2; lane *7*, Vγ9-specific transcripts in blood of sarcoid 4; lane *8*, N region–specific transcripts in blood of sarcoid 4; lane *9*, Vγ9-specific transcripts in blood of normal 1; lane *10*, N region–specific transcripts in lung of normal 1; lane *11*, Vγ9-specific transcripts in blood of normal 2; lane *12*, N region–specific transcripts in blood of normal 2.

Table 5. *Junctional Sequences of Vδ2-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	Jδ region	In-frame
Normal 1	19	1	N1B.D1	TGTGACACC	CTGGGGGT	CACCGATAAA	J1	+
		1	N1B.D2	TGTGAC	GGGGTGGGGGATAGCCGTGG	CGATAAA	J1	+
		1	N1B.D3	TGTGACAC	AGTATTAGCTGGGGCCTCAAGGGCGT	ACACCGATAAA	J1	+
		1	N1B.D4	TGTGACACC	ATGGCTGTCGGGGGGATAGG	CACCGATAAA	J1	+
		1	N1B.D5	TGTGAC	CCCGCAGGTACTGGGGGACCGCAGT	ACACCGATAAA	J1	+
		1	N1B.D6	TGTGACAC	TTTGACTACTGGGTTTT	ACACCGATAAA	J1	+
		1	N1B.D7	TGTGACACC	TTGGGGGCGTCGT	ACACCGATAAA	J1	+
		1	N1B.D8	TGTGAC	TTACTGGGGGATG	ACACCGATAAA	J1	+
		1	N1B.D9	TGTGACACC	GTTGGGTTTACTTCCTCGT	ACACCGATAAA	J1	+
		1	N1B.D10	TGTGACACC	CTGGGGTTAGTCG	ACACCGATAAA	J1	+
		1	N1B.D11	TGTGACA	ACTTGCGGGACTGGGGGGTACAGG	ACACCGATAAA	J1	+
		1	N1B.D12	TGTGAC	GCTGTACTGGGGGATCCTTCGT	ACACCGATAAA	J1	+
		1	N1B.D13	TGTGACACC	GTTGGGGGAATTCAAAGA	AAA	J1	+
		1	N1B.D14	TGTGACAC	AGTATTAGCTGGGGCCTCAAGGGCGT	ACACCGATAAA	J1	+
		1	N1B.D15	TGTGAC	GGCCTGGTACTGGGGGATACGCGTCTAG	ACACCGATAAA	J1	+
		1	N1B.D16	TGTGAC	GGTCTGAGGCTGGGGGATACTAGAGGT	CCGATAAA	J1	-
		1	N1B.D17	TGTGACACC	GTCGGTCCGGGATACGCGAGGG	AAA	J1	-
		1	N1B.D18	TGTGAC	CCCGTTGGGGGATTA	ACACCGATAAA	J1	-
		1	N1B.D19	TGTGACAC	GTTGCCGGTCATGGGGAACGT	ACACCGATAAA	J1	-
Normal 2	16	1	N2B.D1	TGTGACACC	CTCCTTACTGGAGGCCCTGTTC	CCGATAAA	J1	+
		1	N2B.D2	TGTGAC	CTACTGGGGGAT	ACCGATAAA	J1	+
		1	N2B.D3	TGTGACACC	GTCGGGGCCGACCTCC	CCGATAAA	J1	+
		1	N2B.D4	TGTGACAC	GCTCCTGGGGGACTCGT	ACACCGATAAA	J1	+
		1	N2B.D5	TGTGA	GCCGTTACTGGGGGATAGATCACTCCCG	ACACCGATAAA	J1	+
		1	N2B.D6	TGTGACACC	GTGGGACCCGG	CACCGATAAA	J1	+
		1	N2B.D7	TGTGACACC	GTCTCTTTGGGGGATAAATCAAGTCGAG	CGATAAA	J1	+
		1	N2B.D8	TGTGACACC	GTTGGACAACTGGGGGACG	ACACCGATAAA	J1	+
		1	N2B.D9	TGTGA	GGGGATGGGGGATACGG	CCGATAAA	J1	+
		1	N2B.D10	TGTGACACC	CTGACTGGGGGATACGTAGTA	ACCGATAAA	J1	+
		1	N2B.D11	TGTGACACC	GTTGGGGCCT	ACACCGATAAA	J1	+
		1	N2B.D12	TGTGACAC	TCTTACTGGGGGCCCGGGCGG	TAAA	J1	+
		1	N2B.D13	TGTGACACC	CTACTGGGGGAGAG	CTCCTGGGACA	J3	+
		1	N2B.D14	TGTGAC	CGACTGGGGGATAGGGCCCG	CTCCTGGGACA	J3	+
		1	N2B.D15	TGTGACA	GGGTTCCTACGAGACGGGAACTGGGGGAT ACGAGAACACCGGACATTGCCAAGT	ACACCGATAAA	J1	-
		1	N2B.D16	TGTGAC	CCCTGGGGGTTA	ACACCGATAAA	J1	-
Normal 3	15	1	N3B.D1	TGTGAC	CCTGCCTTCCTCCGAAGTACTTC	CACCGATAAA	J1	+
		1	N3B.D2	TGTGACACC	CTGGGGGTAGTCG	ACACCGATAAA	J1	+
		1	N3B.D3	TGTGA	GGCGTTACTGGGGG	ACACCGATAAA	J1	+
		1	N3B.D4	TGTGA	TCCCCTCGGGGCGAACGGAGGGATGAC ACCTC	CCGATAAA	J1	+
		1	N3B.D5	TGTGACACC	CTCCCCCGAATGGACTT	CACCGATAAA	J1	+
		1	N3B.D6	TGTGAC	CCAGTACTGGGGGATACGCCACCCGGAT	ACACCGATAAA	J1	+
		1	N3B.D7	TGTGAC	CACGTACTGGGGGATACGGGGT	ACACCGATAAA	J1	+
		1	N3B.D8	TGTGAC	CCTCTAGACAACCC	TAAA	J1	+
		1	N3B.D9	TGTGAC	TCTTTGGGGGATTTT	GATAAA	J1	+
		1	N3B.D10	TGTGAC	GCGGTGGGGGAGT	ACACCGATAAA	J1	+
		1	N3B.D11	TGTGAC	CCAGTACTGGGGGATACGCCACCCGGCT	ACACCGATAAA	J1	+
		1	N3B.D12	TGTG	TGTCAGTCCGCCTTCCTTCATTTACTGG GGGATTTGAT	ACCGATAAA	J1	+
		1	N3B.D13	TGTGACACC	TTAAGGGAA	ACCGATAAA	J1	+
		1	N3B.D14	TGTGACAC	GGGGAACTGGGGGATAGAG	TCCTGGGACA	J3	+
		1	N3B.D15	TGTGACACC	GTGCGTGTCCAAAG	CTCCTGGGACA	J3	+

Sequences shown include the 3′ region of the Vδ2 element (V), the N region (N-D-N-D-N), the 5′ region of the Jδ element (J), and the specific Jδ element (Jδ).

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

the lung. In this context, for other sites exposed to the environment, limited junctional region diversity has been observed among γ/δ T cells in BALB/c mouse skin, although not in mouse intestinal epithelium (46). It is also relevant, in light of recent reports of γ/δ T cells responding to mycobacterial antigens, that the lung is a common site for mycobacterial disease. Second, there is evidence that normal lung T cell populations differ from blood T cells in regards to their relative state of differentiation. For example, ~90% of normal lung α/β T cells are CD45RO$^+$ (detected by the mAb UCHL1) (47), a surface antigen observed in antigen-primed memory T cells, suggesting that many T cells on the pulmonary epithelial surface have been exposed to specific antigens. If this is also true for the normal lung γ/δ T cell, it would not be surprising to have less diversity among the lung γ/δ population compared with blood.

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. *Junctional Sequences of Vδ2-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	Jδ region	In-frame
Sarcoid 1	28	7	S1B.D1-7	TGTGAC	CCCCTAGAAGGAAACTGGGGAGCCA	ACACCGATAAA	J1	+
		6	S1B.D8-13	TGTGACAC	GCTGGGGGATACGGCGT	ACACCGATAAA	J1	+
		3	S1B.D14-16	TGTGACACC	CGTACTGGGGGGTCCCTGT	ACACCGATAAA	J1	+
		2	S1B.D17-18	TGTGACAC	TGGGGGGTCCA	ACACCGATAAA	J1	+
		1	S1B.D19	TGTGACAC	TACGGTACTGGGGATCGGGTCACCGT	ACACCGATAAA	J1	+
		1	S1B.D20	TGTGACACC	CTCGGTATGGGCCTGGGGGATAAGAG	CACCGATAAA	J1	+
		1	S1B.D21	TGTGACAC	AACTGGGGGATCTATTT	ACACCGATAAA	J1	+
		1	S1B.D22	TGTGAC	TCACTACTGGGGGCGCAC	AAA	J1	+
		1	S1B.D23	TGTGACA	GGGGGTGGGACG	ACACCGATAAA	J1	+
		1	S1B.D24	TGTGAC	CCCTTACTGGGGGGGATCTCTCTGT	ACACCGATAAA	J1	+
		1	S1B.D25	TGTGAC	TTTCTCCGCACTCCGGGACTACGGG	ACACCGATAAA	J1	+
		1	S1B.D26	TGTGAC	TCGTTGTCTGGGGCCGCTGC	CACCGATAAA	J1	+
		1	S1B.D27	TGTGACACC	GTCGGATCAACGGCCT	ATAAA	J1	+
		1	S1B.D28	TGTGAC	CCCAGTACTGGGGGATACTC	CTCCTGGGACA	J3	+
Sarcoid 2	23	10	S2B.D1-10	TGTGACACC	GTGGGGTCCAGACTGGGGATGT	ACACCGATAAA	J1	+
		2	S2B.D11-12	TGTGACACC	GTGTCCCGGCTGGGGGATACGG	CCGATAAA	J1	+
		1	S2B.D13	TGTGAC	CCCGTACTGGGGGACCGGCTACGGGG	CACCGATAAA	J1	+
		1	S2B.D14	TGTGACACC	GTAGGGGGGGATACGCCGAGGT	ACACCGATAAA	J1	+
		1	S2B.D15	TGTGACACC	GTGGGGGAAAGCT	ACACCGATAAA	J1	+
		1	S2B.D16	TGTGACACC	GTGGGGGGCAAGG	CCGATAAA	J1	+
		1	S2B.D17	TGTGACAC	GCTGGGGGATAATG	CCGATAAA	J1	+
		1	S2B.D18	TGTG	GCCCTATGGTACTGGGGGATACAGGGG	CCGATAAA	J1	+
		1	S2B.D19	TGTGACAC	GGCCCGGAGATTGT	ACACCGATAAA	J1	+
		1	S2B.D20	TGTGAC	CCCTTCGTTACGCGTG	ACACCGATAAA	J1	+
		1	S2B.D21	TGTGACACC	GTGGCCCGGCTGGGGGATACGG	CCGATAAA	J1	+
		1	S2B.D22	TGTGACAC	ACCGGGGGATAG	CTCCTGGGACA	J3	+
		1	S2B.D23	TGTGACACC	CCGGGGGATAG	CTCCTGGGACA	J3	+
Sarcoid 3	18	6	S3B.D1-6	TGTG	TCGAGCTGGAAAGAGGGGTGGA	GGACA	J3	+
		3	S3B.D7-9	TGTGAC	CCAATGGGGGATACGCTCCGTGG	A	J1	+
		1	S3B.D10	TGTGACACC	GTGGGGGGAT	ACACCGATAAA	J1	+
		1	S3B.D11	TGTGACACC	GTTTCAGGGATAAGTCG	CACCGATAAA	J1	+
		1	S3B.D12	TGTGAC	CCTATTAGCTACAAACTAATGGGGGAT	ACCGATAAA	J1	+
		1	S3B.D13	TGTGAC	CGGGTAGGTACTGGGGGACCCATCCATT	ACACCGATAAA	J1	+
		1	S3B.D14	TGTGAC	CTGGTTTCCTCTCATAGTCATACTGGGG ATTCGT	ACACCGATAAA	J1	+
		1	S3B.D15	TGTGAC	CCAGTACTGGGGGATACGCCACCCGGCT	ACACCGATAAA	J1	+
		1	S3B.D16	TGTGACACC	GTACTGGGGGATACGGGGT	ACACCGATAAA	J1	+
		1	S3B.D17	TGTGACACC	GTCGGGGGACCTTTAGTT	ACCGATAAA	J1	+
		1	S3B.D18	TGTGAC	TGTCAGTCCGCCTTCCTTCATTTACTGG GGGATTTGAT	ACCGATAAA	J1	-
Sarcoid 4	15	2	S4B.D1-2	TGTGAC	CCTCTAGACAACCC	TAAA	J1	+
		2	S4B.D3-4	TGTGAC	TCGAACTGGGGGATAGGACGT	TCCTGGGAGA	J3	+
		1	S4B.D5	TGTGAC	CCAGTACTGGGGGATACGCCACCCGGCT	ACACCGATAAA	J1	+
		1	S4B.D6	TGTGAC	CCTCTGGCCTGGGGGATATC	CACCGATAAA	J1	+
		1	S4B.D7	TGTGA	GAAGATAGGTGTCCGTCGG	GATAAA	J1	+
		1	S4B.D8	TGTGAC	TACCTAGGGACAACTTCGT	ACACCGATAAA	J1	+
		1	S4B.D9	TGTGAC	CCTCTGGCCTGGGGGATATC	CACCGATAAA	J1	+
		1	S4B.D10	TGT	TTTCCTCTGGGGGCCC	ACACCGATAAA	J1	+
		1	S4B.D11	TGTGA	GAAGATAGGTGTCCGTCGG	GATAAA	J1	+
		1	S4B.D12	TGTGACAC	AATGGGGGATACGCTCCGTGG	A	J1	+
		1	S4B.D13	TGTGACAC	GGTACTGGGGGCCGACCACAG	CTCCTGGGACA	J3	+
		1	S4B.D14	TGTGACAC	GCTGGGGGATACGC	CACCGATAAA	J1	-
		1	S4B.D15	TGTGAC	CTTTGGGACTCAG	CTCCTGGGACA	J3	-

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 $V\delta1^-$, and in normals most γ/δ T cells that are $V\delta1^-$ are $V\delta2^+$; 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 subpopulation 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-JγP-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).

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