Restricted Usage of T Cell Receptor V_α/J_α Gene Segments with Different Nucleotide but Identical Amino Acid Sequences in HLA-DR3$^+$ Sarcoidosis Patients

Johan Grunewald,* Thomas Hultman,† Anders Bucht,‡ Anders Eklund,§ and Hans Wigzell*

*Microbiology and Tumorbiology Center (MTC), Karolinska Institute, Stockholm, Sweden

†Department of Biochemistry and Biotechnology, The Royal Institute of Technology, Stockholm, Sweden

‡Department of Pharmacology, Pharmacia AB, Uppsala, and Department of Clinical Immunology, Uppsala University Hospital, Uppsala, Sweden

§Institution of Medicine, Pulmonary Section, Karolinska Hospital, Stockholm, Sweden

ABSTRACT

Background: Sarcoidosis is a granulomatous disease characterized by the accumulation of activated T cells in the lungs. We previously showed that sarcoidosis patients expressing the HLA haplotype DR3(17),DQ2 had increased numbers of lung CD4$^+$ T cells using the T cell receptor (TCR) variable region (V) α 2.3 gene segment product. In the present study, the composition of both the TCR α- and β-chains of the expanded CD4$^+$ lung T cells from four DR3(17),DQ2$^+$ sarcoidosis patients was examined.

Materials and Methods: TCR α-chains were analyzed by cDNA cloning and nucleotide sequencing. TCR β-chains were analyzed for Vβ usage by flow cytometry using TCR V-specific monoclonal antibodies or by the polymerase chain reaction (PCR) using Vβ- and Cβ-specific primers. Jβ usage was analyzed by Southern blotting of PCR products and subsequent hybridization with radiolabeled Jβ-specific probes.

Results: Evidence of biased J_α gene segment usage by the α-chains of Vα 2.3$^+$ CD4$^+$ lung T cells was found in four out of four patients. Both different α-chain nucleotide sequences coding for identical amino acid sequences and a number of identically repeated α-chain sequences were identified. In contrast, the TCR β-chains of FACS-sorted V_α 2.3$^+$ CD4$^+$ lung T cells were found, with one exception, to have a nonrestricted TCR V_β usage.

Conclusions: The finding of V_α 2.3$^+$ CD4$^+$ lung T cells with identical TCR α-chain amino acid sequences but with different nucleotide sequences strongly suggests that different T cell clones have been selected to interact with a specific sarcoidosis associated antigen(s). The identification of T cells with restricted TCR usage, which may play an important role in the development of sarcoidosis, and the possibility of selectively manipulating these cells should have important implications for the treatment of the disease.

INTRODUCTION

Sarcoidosis is characterized by the formation of non-caseating granulomas in affected organs, depressed cell-mediated immunity and elevated B lymphocyte activity. Ninety percent of patients with sarcoidosis have involvement of the intrathoracic organs (1). The CD4$^+$ T cells, which accumulate in lungs and can be analyzed through bronchoalveolar lavage (BAL), show signs of being activated and are implicated in the

Address correspondence and reprint requests to: Dr. Johan Grunewald, Microbiology and Tumorbiology Center (MTC), Karolinska Institute, S-171 77 Stockholm, Sweden.

pathogenesis of sarcoidosis (2,3). In order to specifically identify an antigen, T cells need to have the antigen presented as peptides in the context of the major histocompatibility complex (MHC) (in humans called HLA) molecules (4). Most T lymphocytes use $\alpha\beta$ T cell receptor (TCR), consisting of α and β polypeptide chains for antigen recognition. Each chain is composed of a constant and a variable part, the latter made up of random combinations of V (variable), D (diversity; only the β-chain) and J (joining) gene segments (5). T cells with preferential use of particular TCR V genes have been described in several human diseases, such as multiple sclerosis (6), rheumatoid arthritis (7,8) and inflammatory vascular diseases (9,10). If T cells with restricted TCR V gene usage prove to be important for the development of the disease, a highly selective type of immune intervention may be undertaken (6). In animal models, it has been possible to selectively manipulate T cells with a restricted TCR V gene usage to succesfully prevent and modulate disease (11,12).

In sarcoidosis, several groups have shown a preferential TCR V gene usage by lung T cells and have suggested that there is a specific sarcoidosis-associated antigen in the lungs of these patients (13–21). We showed previously that $CD4^+$ cells bearing the V_α 2.3 gene segment on the α-chain of the TCR specifically accumulate in the lungs of sarcoidosis patients of HLA type DR3(17),DQ2, suggesting that, in this subgroup of patients, T cells expressing this particular V gene element may have a role in the development of the disease (19,20). In the present study, a more extensive analysis of the TCR expressed by these $CD4^+$ T cell was undertaken. In particular, the TCR α-chains and the V_α 2.3-associated Vβ- and Jβ-gene segments of the β chains have been analyzed using different approaches: cloning and nucleotide sequencing of the rest of the TCR α-chains; PCR amplifications using V_β- and C_β-specific primers to identify the V_α 2.3-associated V_β gene segments; a recently developed PCR based technique using radiolabelled J_β-specific probes to determine the usage of V_β-associated J_β gene segments (22); and flow cytometric triple staining techniques.

MATERIALS AND METHODS

Subjects

Paired samples of PBL and lung T cells recruited by BAL were obtained from 5 HLA-DR3(17),$DQ2^+$ Caucasian patients (three males and two females; ages 24–42 years, median 37 years) with biopsy-proven sarcoidosis. Samples from Patient 3, with persistent clinical disease activity over a period of 1 year, were obtained by BAL on two separate occasions, when the patient received no medication and 30 mg prednisolone/every other day, respectively. BAL samples showed signs of lung restricted V_α 2.3^+ $CD4^+$ T cell expansions at both times. The other four patients received no medication. Three patients were never smokers and two exsmokers (quit >5 years ago). These sarcoidosis patients were included in a previous report (20), where more detailed clinical information, including bronchoalveolar lavage procedures, is given.

Monoclonal Antibodies

Anti-TCR V_α 2.3-, V_β 3-, V_β 5.1-, V_β 5.2/5.3-, V_β 5.3, V_β 6.7, V_β 8.1-, and V_β 12-specific MAb were provided by T Cell Sciences, Inc. (Cambridge, MA, U.S.A.). MAb 6D6 (V_α 12.1) was a kind gift of Dr. H. DerSimonian and Dr. M. Brenner (23). The V_β 2-, V_β 13-, and V_β 17-specific MAb were purchased from Immunotech (Luminy, France).

PE or PerCp-conjugated leu-3a (CD4) Mab were obtained from Becton-Dickinson (Mountain View, CA, U.S.A.). $F(ab')_2$ fragments of rabbit anti-mouse Ig, used as secondary antibodies, were conjugated with either FITC or PE (Dakopatts A/S, Glostrup, Denmark). Normal mouse serum (NMS) from BALB/c mice was used for negative control (in all cases $\leq$0.5%) at a dilution of 1:500 in PBS.

Immunofluorescence and Flow Cytometry (Fluorescence-Activated Cell Sorting)

Cells were first stained with the V_α 2.3-specific Mab (F1), detected with FITC-conjugated $F(ab')_2$ fragments of rabbit anti-mouse Ig, and, after a blocking step using NMS, PE-conjugated anti-CD4 Mab was added (described in detail in Ref. 19). To analyze TCR V_α 2.3-associated V_β segments, cells from Patients 3 and 5 were incubated with the respective anti-TCR V_β segment-specific MAb for 30 min, washed twice, and PE-conjugated $F(ab')_2$ fragments of rabbit anti-mouse Ig were added to detect bound antibodies. After three washes, NMS (diluted 1:500) was used to block remaining rabbit anti-mouse Ig before adding the FITC-conjugated F1 (anti-V_α 2.3), as well as PerCp-conjugated (anti-CD4) MAb. For positive separation of V_α 2.3^+ $CD4^+$ BAL T cells, a FACStar PLUS (Becton Dickinson),

with standard optical equipment and a 90-μm nozzle diameter (24) was used, giving a sorting purity of >98%.

Sequencing of TCR α-Chains

Total RNA was extracted from 0.5×10^6 FACS sorted V_α 2.3^+ T cells, using a RNA isolation kit (Stratagene, La Jolla, CA, U.S.A.), and cDNA synthesized using random hexamer primers, by the reverse transcriptase (RT) reaction according to the manufacturer's recommendation (BRL, Gaithersburg, MD, U.S.A.). Amplification of cDNA was performed as described previously (25), using a V_α 2.3 sense primer (5′-AAGCAT GCTCCTGGACCCTTCAATGTTCC-3′), including a Sph I cleavage site at the 5′ end (underlined), in combination with a C_α-antisense primer (5′-GGGTCGACTAGGCAGACAGACTTGTCACT-3′) with a Sal I cleavage site (underlined). The PCR profile used was the following: 94°C (60 sec), 55°C (60 sec) and 72°C (60 sec) for 38 cycles, with a final extension of 5 min. After PCR amplification, PCR products were cleaved with Sal I/Sph I and loaded on a low melting agarose gel. DNA bands corresponding to 500 bp were subsequently cut out from the gel, purified using Geneclean kit (Bio 101, La Jolla, CA, U.S.A.) and ligated into a Sal I/Sph I-cleaved pUC18 plasmid. Alternatively, PCR fragments amplified using primers without restriction enzyme cleavage sites were inserted blunt ended into a linearized pUC18 plasmid. The vector was inserted into competent *Escherichia coli* (JM 105) and grown on ampicillin/streptomycin plates. Positive colonies were picked using blue/white selection, and further PCR amplified and biotin labeled using pUC18 primers as described (26).

DNA Sequencing

Amplified biotin-labeled clones were subjected to solid-phase sequencing as described (27,28). Briefly, the PCR mixture was immobilized on streptavidin coupled magnetic beads (Dynabeads; Dynal, Oslo, Norway). After washing and denaturation, single-stranded DNA was eluted and subsequently neutralized. Sequencing reactions were performed using AutoRed T7 sequencing kit (Pharmacia, Uppsala, Sweden) in a robot workstation (Biomek-1000; Beckman, Palo Alto, CA, U.S.A.). Samples were loaded on a sequencer (Pharmacia) with detection of fluorescent bands during electrophoresis. A total of 118 clones were sequenced (31 from Patient 1, 51 from Patient 2, 18 from Patient 3, and 18 from Patient 4).

PCR Amplification of V_α 2.3-Associated TCR β-Chains

Total RNA and cDNA was prepared as described above, and amplification of cDNA was performed using TCR V_β- and C_β-specific primers (for detailed information, see Ref. 25), and a PCR profile as above. The amplified products were separated on an ethidium bromide stained 2.0% agarose gel to show the expected size (270 bp), and subsequently blotted over night (Southern blot) onto Hybond-N nylon filters (Amersham, United Kingdom). After prehybridization of filters, hybridizations were performed over night using a 5′ end-labeled (using 5′-[γ-^{32}P]-ATP; Amersham) C_β-specific oligonucleotide probe (25). After washing the filters, they were exposed to Hyperfilm MP (Amersham) overnight at −70°C. Gel scanning technique (2400 GelScan XL; Pharmacia-LKB) was used for quantification of exposed films.

Analysis of J_β Gene Segment Usage

V_β 7-, V_β 12-, V_β 13.1-, and V_β 18-amplified PCR products of Patient 2 were transferred to Hybond-N nylon filters, using a Bio-Dot SF microfiltration unit (Bio-Rad, Hercules, CA, U.S.A.). Filters were then treated as above, and hybridizations were performed overnight using 5′-(γ-^{32}P)-ATP end-labeled J_β-specific oligonucleotide probes. Washing, film-exposure, and gel scanning (for quantification) were performed as described above and in detail in Ref. 22.

RESULTS

Flow Cytometric Analyses

TCR V_α 2.3 was used by 31.3, 12.8, 21.8 (mean value), 15.4, and 27.6% of $CD4^+$ T cells obtained by bronchoalveolar lavage (BAL T cells) from Patients 1–5, respectively. The cells were analyzed at the time disease was discovered, except in Patient 3, in whom BAL T cells were obtained at different times for PCR analyses and for flourescence-activated cell sorting (FACS) analyses.

TCR J_α Usage

TCR J_α usage by V_α 2.3$^+$ TCR α-chains, obtained from V_α 2.3$^+$ CD4$^+$ BAL T cells of Patients 1–4, was found to be restricted. In Patient 1, J_α family 17 gene segments were used by about 50% of the TCR α-chains. In Patients 3 and 4, a restricted usage of J_α family 1 gene segments was noted in particular, with frequencies of about 35 and 50%, respectively.

In addition, a preference for certain individual J_α gene segments by V_α 2.3$^+$ TCR α-chains was noted (Table 1). In Patient 1, 26% of the TCR α-chains used the J_α 17.3 gene segment and, within this group, two distinct nucleotide sequences were found twice (Table 1). In comparison, the J_α 17.3 gene segment has a normal usage of 3% in PBLs (29). The reported normal usage in PBL for the J_α 9.11, 15.2, and 16.6 gene segments is 1.3, 1, and 4%, respectively (29). These J_α gene segments were preferentially used in Patient 2, in whom J_α 9.11 was used by 18%, J_α 15.2 by 14%, and J_α 16.6 by 14% of the α-sequences (Table 1). Interestingly, identical J_α 9.11 nucleotide sequences were found in Patients 1 and 2. Moreover, several different nucleotide sequences were found to code for identical amino acid sequences (Table 2). In two different cloning experiments, 6/7 J_α 15.2 as well as 6/7 J_α 16.6 nucleotide sequences were found to be identical.

In Patient 3, the J_α 1.8 (17%) and J_α 9.7 (17%) gene segments were found to be preferentially used. The J_α 9.7 gene segment (normally used at a frequency of 3% in PBLs [29]), included 3/3 identically repeated nucleotide sequences (Table 1). Similarily, in Patient 4 a high number of J_α 1.8 gene segments (39%) was identified. Here, 5/7 J_α 1.8$^+$ clones with different nucleotide sequences had identical amino acid sequences, while a sixth clone coded for an amino acid sequence with matching properties (Tables 1 and 3). Amino acid sequences with similar properties were also seen when comparing J_α 1.4$^+$ clones from Patients 1 and 4, and J_α 15.3$^+$ clones from Patient 2. In both cases, an arginine was found to be substituted with a lysine (both positively charged).

TCR V_β and J_β Usage

TCR V_β usage by V_α 2.3$^+$ CD4$^+$ BAL T cells was investigated using two different approaches. First, for Patients 3 and 5 triple staining techniques and flow cytometry were used. All TCR V_β genes investigated (which included V_β 2, V_β 3, V_β 5.1, V_β 5.2/5.3, V_β 5.3, V_β 6.7, V_β 8.1, V_β 12, V_β 13, and V_β 17) were found to be expressed by the V_α 2.3$^+$ CD4$^+$ BAL T cells. In these cells, TCR V_β usage did not differ significantly from the respective total BAL CD4$^+$ T cell populations, showing no preferential V_β use (data not shown). In another approach, V_β- and C_β-specific primers and the PCR technique were used to amplify cDNA synthesized from RNA of V_α 2.3$^+$ CD4$^+$ BAL T cells of patients. For Patients 1, 3, and 4, a polyclonal pattern was observed, with detectable signals from all 22 investigated V_β chains (Table 4). In contrast, Patient 2 showed a more restricted V_β usage, with strong signals for V_β 7, V_β 12, V_β 13.1, and V_β 18 in particular. In addition, weak signals were detected for V_β 3, V_β 5.2, V_β 6, V_β 10, V_β 14, V_β 17, and V_β 19. When comparing the V_β signals obtained for all four patients, an overall preference for V_β 7 and V_β 18 was noted (Table 4).

The TCR J_β segments associated with V_β 7, V_β 12, V_β 13.1, and V_β 18, from Patient 2 were analyzed using a recently developed technique (22). With this method, a semiquantification of the J_β gene segment usage in relation to the respective V_β expressing cell population was obtained. A highly restricted J_β segment usage was noted in all four samples (Fig. 1). V_β 7 had an unusual preference for J_β 2.1, while V_β 12, V_β 13.1, and V_β 18 all preferentially used J_β 2.3.

DISCUSSION

The previously described absolute positive correlation between lung restricted V_α 2.3$^+$ CD4$^+$ T cell expansions and expression of HLA-DR3(17),DQ2 in sarcoidosis patients with clinical disease, prompted us to suggest the presence of a specific sarcoidosis-associated antigen in the lungs of these (HLA-DR3$^+$) sarcoidosis patients (19,20). In order to understand more about these lung accumulated V_α 2.3$^+$ CD4$^+$ T cells, presumably reacting to a specific antigen, we have analyzed the composition of the rest of the TCR molecules expressed by these cells.

Use of J_α segments by the α-chains of V_α 2.3$^+$ CD4$^+$ lung T cells was strongly biased, with a preference for certain J_α segments. Notably, in Patients 3 and 4 there was even a preference for the same J_α (J_α 1.8) gene segment. At the clonal level, identical VJ α-sequences were repeatedly found in each (i.e., four out of four) patient, most pronounced in Patient 2 who also showed

TABLE 1. TCR J_α gene segment usage by V_α 2.3$^+$ lung accumulated CD4$^+$ BAL T cells recovered from four sarcoidosis patients (Patients 1–4) and the number of identical nucleotide α-sequences found repeatedly within individual J_α segments

	Patient			
Jα Segment	**1**	**2**	**3**	**4**
1.3		2		
1.4	1	1	2	2
				2 identical
1.5	2		1	
1.8		2	3 (17%)	7 (39%)[b]
				2 identical
3.1				1
3.2	1			1
4.1	2			
	2 identical			
6.1		2		
		2 identical		
9.5		1		
9.7			3 (17%)	
			3 identical	
9.11	1	9 (18%)[a]	1	1
		2 identical		
9.14	1			2
				2 identical
9.15	1	2		
		2 identical		
14.1	2	1		1
14.4		1		
15.1		1		
15.2		7 (14%)	1	
		6 identical		
15.3	2	2	1	2
16.3		3 (6%)	1	
16.6	3 (10%)	7 (14%)	1	
		6 identical		
17.2		1		
17.3	8 (26%)	3 (6%)		
	2 + 2 identical	2 identical		
17.5	1			
17.6			2	
17.7	1			
17.9	2	4 (8%)	1	
	2 identical	3 identical		
17.10	3 (10%)	2	1	1

Individual J_α family members used ≥3 times are marked by shadowing.

[a] 2 + 4 of the amino acid sequences were identical (see Table 2).

[b] 5 of the amino acid sequences were identical (see Table 3).

TABLE 2. Identical or similar TCR VJ α sequences found in V_α 2.3$^+$ J_α 9.11$^+$ lung accumulated CD4$^+$ BAL T cells recovered from two sarcoidosis Patients (Patients 1 and 2)

Sequence	V_α 2.3				J_α 9.11							
	C	V	V		D	Y	G	G	S	Q	G	N
2.3 + 2.14 + 1.10	5′-TGT	GTG	GTG	—	GAT	TAT	GGA	GGA	AGC	CAA	GGA	AAT
2.30	5′-TGT	GTG	GTG	—	GAT	TAC	GGA	GGA	AGC	CAA	GGA	AAT
2.43	5′-TGT	GTG	GTG	—	GAT	TAC	GGA	GGA	AGC	CAG	GGA	AAT
	C	V	V		P	S	G	G	S	Q	G	N
2.8	5′-TGT	GTG	GTC	—	CCA	TCA	GGA	GGA	AGC	CAA	GGA	AAT
2.37	5′-TGT	GTG	GTC	—	CCA	TCA	GGA	GGG	AGC	CAA	GGA	AAT

Nucleotides that alter between sequences are underlined. The identical sequences 2.3/2.14 and 1.10 are from two different patients (Patients 2 and 1, respectively). Note that the tyrosine (Y) at the second 5′ position of Jα 9.11, in the upper group of sequences, is substituted with a serine (S), with similar properties, in sequences 2.8 and 2.37.

evidence of restricted TCR V_β and J_β usage (see below). In several cases identical VJ α-nucleotide sequences were identified in different cDNA cloning experiments, rendering it unlikely that the results could be due to technical errors. Moreover, the same VJ α-nucleotide sequence (J_α 9.11), repeated twice in Patient 2, was also identified in Patient 1. These findings suggest that some degree of oligoclonal expansion of V_α 2.3$^+$ CD4$^+$ T cells has occurred in the lungs of this group of sarcoidosis patients.

Another highly relevant discovery was the identification of different nucleotide sequences that code for the same amino acid sequence, such as the V_α 2.3–J_α 1.8 sequences in Patient 4. Also, different amino acid sequences retaining very similar properties were found in several cases, both intra- and inter-individually. We consider these findings to provide strong evidence that the V_α 2.3$^+$ CD4$^+$ lung T cells, originally derived from different T cell clones, have been selected for their ability to interact with a specific antigen.

TCR V_β usage by V_α 2.3$^+$ CD4$^+$ BAL T cells of five patients was also analyzed. In four of these patients, nonrestricted V_β usage was noted, although Patient 5 was investigated only using a restricted panel of anti-TCR V_β-specific Mab. Evidence for a polyclonal V_β usage by the V_α 2.3$^+$ CD4$^+$ BAL T cells is also provided by our previous results, in which the panel of anti-TCR V_β Mab showed more or less normal reactivities in

TABLE 3. Indentical or similar TCR VJ α sequences found in V_α 2.3$^+$ J_α 1.8$^+$ lung accumulated CD4$^+$ BAL T cells recovered from a sarcoidosis patient (Patient 4)

Sequence	V_α2.3				J_α 1.8				
	C	V	V		R	H	M	D	S
4.3	5′-TGT	GTG	GTG	—	AGA	CAC	ATG	GAT	AGC
4.12	5′-TGT	GTG	GTG	—	AGG	CAC	ATG	GAT	AGC
4.20	5′-TGT	GTG	GTT	—	CGC	CAC	ATG	GAT	AGC
4.14 + 4.25	5′-TGT	GTG	GTC	—	CGC	CAC	ATG	GAT	AGC
	C	V	V		R	Y	M	D	S
4.23	5′-TGC	GTG	GTA	—	CGG	TAC	ATG	GAT	AGC

Nucleotides that alter between sequences are underlined. Note that the substitution of histidine (H), in the group of sequences 4.3–4.25, to a tyrosine (Y), in sequence 4.23, constitutes only a minor change in the properties of the amino acid sequence.

TABLE 4. TCR V_β genes associated with TCR V_α 2.3 in BAL $CD4^+$ expansions obtained from four sarcoidosis patients (Patients 1–4)

	TCR Vβ Gene Segments																				
Patient	**1**	**2**	**3**	**4**	**5.1**	**5.2**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13.1**	**13.2**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
1	++	+++	++	++	++	+++	+++	+++	+++	++	++	+	+	+	+	+++	++	++	++	+++	+++	++
2	−	−	+	−	−	+	+	+++	−	−	+	−	++	++	−	+	−	−	+	++	+	−
3	++	++	−	+	++	++	+++	+++	++	++	+	++	+	++	++	++	++	−	−	+++	++	+
4	++	++	++	++	++	++	++	+++	++	+++	+	+	++	+	++	++	++	++	+	++	+	+++

−, very weak/absent; +, weak; ++, normal; +++, strong.

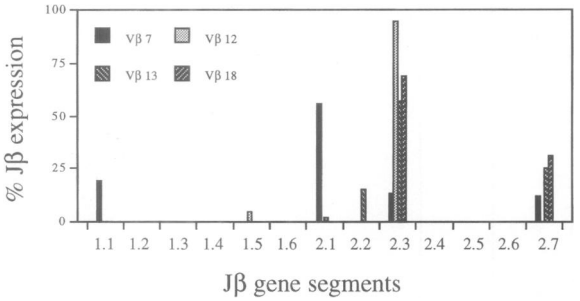

FIG. 1. TCR J_β gene usage for V_β 7, V_β 12, V_β 13, and V_β 18

All TCR V_β genes were associated with the V_α 2.3 gene segment and expressed by the $CD4^+$ BAL cells of Patient 2.

all cases with V_α 2.3^+ expansions (20). Similarly, two recently published reports showed a diverse TCR V_β repertoire in lung accumulated T cells of sarcoidosis patients (17,30).

An exception was found in Patient 2, in whom a preference for V_β 7, V_β 12, V_β 13.1, and V_β 18 was noted. These β-chains are most likely derived from oligoclonal T cell populations, since the related J_β usage showed a highly restricted pattern, with a strong bias for J_β 2.1 in association with V_β 7, and for J_β 2.3 in association with V_β 12, 13.1, and 18. Bellocq et al. also found V_β 7^+ lung T cells to be oligoclonal to a high degree (17). Interestingly, Patient 2 also had the most conspicuous TCR α-chain oligoclonality, with repeated nucleotide sequences of the J_α 9.11, J_α 15.7, and J_α 16.6 segments in particular. Thus, certain combinations of V_β–J_β segments, associated with certain V_α 2.3–J_α joinings, seemed to be the TCRs favourably used by the expanded $CD4^+$ BAL T cells in Patient 2. Clinically, we did not find any difference between this patient and the others.

The duration of disease might influence the TCR usage in antigen-responding T cells. A very restricted TCR V gene usage in the initial immune response may exist, but, with time, more heterogenous T cell populations may become attracted to the inflammatory focus. In experimental autoimmune encephalomyelitis (EAE), cryptic myelin basic protein (MBP) determinants became immunogenic (i.e., determinant spreading) with time, allowing for a more heterogenous T cell response (31,32). In sarcoidosis, the onset of disease in the majority of patients is insidious, making it difficult to evaluate any possible influence of disease duration. The genetic background of the individuals may also influence TCR V gene usage in health (33,34), as well as in disease (21). In addition to HLA-$DR3^+$, other HLA alleles, as well as other genes such as the Tap-genes (35), may be important here. Finally, although expanded lung T cells used a particular V_α gene and were strictly $CD4^+$, oligoclonal to some degree, and seemingly restricted by HLA-haplotype, the possibility of a superantigen-driven T cell response in sarcoidosis cannot be completely excluded by the present results. It has recently been suggested that superantigens with a low affinity to its specific TCR V gene segment product would preferentially stimulate $CD4^+$ cells, since these cells could, unlike $CD8^+$ cells, strengthen the avidity to the MHC class II bearing superantigen presenting cell. Moreover, a bias in the usage of other TCR gene products, which would improve the binding to such antigen presenting cell, could also be expected (36).

In conclusion, we have examined the detailed composition of the TCR α- and β-chains of expanded V_α 2.3^+ $CD4^+$ lung T cells of HLA-$DR3^+$ patients with pulmonary sarcoidosis. Evidence for a restricted α-chain usage was found, with convergence at the nucleotide level towards the same or similar amino acid sequences. On the other hand, the V_β usage by the same cells was, with one exception, nonrestricted. These results indicate that oligoclonal T cells expand as a result of antigen recognition in the lungs of this group of sarcoidosis patients, with the TCR α-chain being the most important (T cell) component in such a specific interaction.

ACKNOWLEDGMENTS

We thank Dr. Anders Johannisson for FACS sortings and Dr. Vida Hodara for help with V_β-J_β analyses. This work was supported by the Swedish Heart-Lung Foundation, the Swedish Cancer Society, and the Karolinska Institute.

REFERENCES

1. Daniele PR, Dauber HJ, Rossman DM. (1980) Immunologic abnormalities in sarcoidosis. *Ann. Intern. Med.* **92:** 406–416.
2. Hunninghake G, Crystal R. (1981) Pulmonary sarcoidosis: A disorder mediated by excess helper T lymphocyte activity at sites of disease activity. *N. Engl. J. Med.* **305:** 429–434.
3. Pinkston P, Bitterman P, Crystal R. (1983)

Spontaneous release of interleukin-2 by lung T lymphocytes in active pulmonary sarcoidosis. *N. Engl. J. Med.* **308:** 793–800.
4. Davis M, Bjorkman P. (1988) T cell antigen receptor genes and T cell recognition. *Nature* **334:** 395–402.
5. Moss P, Rosenberg W, Bell J. (1992) The human T cell receptor in health and disease. *Annu. Rev. Immunol.* **10:** 71–96.
6. Martin R, McFarland FH, McFarlin ED. (1992) Immunological aspects of demyelinating diseases. *Annu. Rev. Immunol.* **10:** 153–187.
7. Paliard X, West S, Lafferty J, et al. (1991) Evidence for the effects of a superantigen in rheumatoid arthritis. *Science* **253:** 325–329.
8. DerSimonian H, Sugita M, Glass ND, et al. (1993) Clonal Vα 12.1+ T cell expansions in the peripheral blood of rheumatoid arthritis patients. *J. Exp. Med.* **177:** 1623–1631.
9. Abe J, Kotzin B, Meissner C, et al. (1993) Characterization of T cell repertoire changes in acute Kawasaki disease. *J. Exp. Med.* **177:** 791–796.
10. Grunewald J, Andersson R, Hansson G, et al. (1994) CD4+ and CD8+ T cell expansions using selected TCR V and J gene segments at the onset of giant cell arteritis. *Arthritis Rheum.* **37:** 1221–1227.
11. Acha-Orbea H, Mitchell DJ, Timmermann L, et al. (1988) Limited heterogeneity of T cell receptors from lymphocytes mediating autoimmune encephalomyelitis allows specific immune intervention. *Cell* **54:** 263–273.
12. Acha-Orbea H. (1991) Limited heterogeneity of autoantigens and T cells in autoimmune diseases? *Res. Immunol.* **142:** 487–490.
13. Moller D, Konishi K, Kirby M, Balbi B, Crystal R. (1988) Bias toward use of a specific T cell receptor β chain variable region in a subgroup of individuals with sarcoidosis. *J. Clin. Invest.* **82:** 1183–1191.
14. Forman JD, Silver R, Anikis M, et al. (1992) Preferential expression of a limited number of T cell receptor variable β-genes in active pulmonary sarcoidosis. *Am. Rev. Respir. Dis.* **145:** A414.
15. Forrester J, Newman L, Wang Y, King T, Kotzin B. (1993) Clonal expansion of lung Vδ1+ T cells in pulmonary sarcoidosis. *J. Clin. Invest.* **91:** 292–300.
16. Forrester J, Wang Y, Ricalton N, et al. (1994) TCR expression of activated T cell clones in the lungs of patients with pulmonary sarcoidosis. *J. Immunol.* **153:** 4291–4302.
17. Bellocq A, Lecossier D, Pierre-Audiegier C, Tazi A, Valeyre D, Hance A. (1994) T cell repertoire of T lymphocytes recovered from the lung and blood of patients with sarcoidosis. *Am. J. Respir. Crit. Care Med.* **149:** 646–654.
18. Tamura N, Holroyd K, Banks T, Kirby M, Okayama H, Crystal R. (1990) Diversity in junctional sequences associated with the common human V γ 9 and V δ 2 gene segments in normal blood and lung compared with the limited diversity in a granulomatous disease. *J. Exp. Med.* **172:** 169.
19. Grunewald J, Janson CH, Eklund A, et al. (1992) Restricted Vα 2.3 gene usage by CD4+ T lymphocytes in bronchoalveolar lavage (BAL) fluid from sarcoidosis patients correlates with HLA-DR3. *Eur. J. Immunol.* **22:** 129–135.
20. Grunewald J, Olerup O, Persson U, Öhrn M, Wigzell H, Eklund A. (1994) T cell receptor V gene usage by CD4+ and CD8+ T cells in bronchoalveolar lavage fluid and peripheral blood of sarcoidosis patients. *Proc. Natl. Acad. Sci. U.S.A.* **91:** 4965–4969.
21. Grunewald J, Shigematsu M, Nagai S, et al. (in press) T cell receptor V gene use in HLA-typed Japanese patients with pulmonary sarcoidosis. *Am. J. Respir. Crit. Care Med.*
22. Grunewald J, Tehrani MJ, Pisa E, Janson C, Andersson R, Wigzell H. (1992) Analysis of Jβ gene segment usage by CD4+ and CD8+ human peripheral blood T lymphocytes. *Int. Immunol.* **4:** 643–650.
23. DerSimonian H, Band H, Brenner M. (1991) Increased frequency of T cell receptor Vα 12.1 expression on CD8+ T cells: Evidence that Vα participates in shaping the peripheral T cell repertoire. *J. Exp. Med.* **174:** 639–648.
24. Johannisson A, Eriksson B, Amnéus H, Zetterberg G. (1992) Attempts to use the HPRT-assay as an automated short-term monitor for an acute exposure to mutagens. *Cell Biol. Toxicol.* **8:** 233–253.
25. Hodara LV, Jeddi-Tehrani M, Grunewald J, et al. (1993) HIV infection leads to differential expression of T cell receptor Vβ genes in CD4+ compared to CD8+ T cells. *A.I.D.S.* **7:** 633–638.
26. Bucht A, Söderström K, Hultman T, et al. (1992) T cell receptor diversity and activation markers in the Vδ1 subset of rheumatoid synovial fluid and peripheral blood T lymphocytes. *Eur. J. Immunol.* **22:** 567–574.

27. Hultman T, Ståhl S, Hornes E, Uhlén M. (1989) Direct solid phase sequencing of genomic and plasmid DNA using magnetic beads as solid support. *Nucleic Acids Res.* **17:** 4937–4946.
28. Hultman T, Bergh S, Moks T, Uhlèn M. (1991) Bidirectional solid-phase sequencing of in vitro-amplified plasmid DNA. *BioTechniques* **10:** 84–93.
29. Moss P, Rosenberg W, Zintzaras E, Bell J. (1993) Characterization of the human T cell receptor α-chain repertoire and demonstration of a genetic influence on Vα usage. *Eur. J. Immunol.* **23:** 1153–1159.
30. Dohi M, Yamamoto K, Masuko K, et al. (1994) Accumulation of multiple T cell clonotypes in lungs of healthy individuals and patients with pulmonary sarcoidosis. *J. Immunol.* **152:** 1983–1988.
31. Lehmann VP, Forsthuber T, Miller A, Sercarz EE. (1992) Spreading of T cell autoimmunity to cryptic determinants of an autoantigen. *Nature* **358:** 155–157.
32. Lehmann P, Sercarz E, Forsthuber T, Dayan C, Gammon G. (1993) Determinant spreading and the dynamics of the autoimmune T cell repertoire. *Immunol. Today* **14:** 203–208.
33. Gulwani-Akolkar B, Posnett D, Janson CH, et al. (1991) T cell receptor V segment usage in peripheral T cells correlates with HLA type. *J. Exp. Med.* **174:** 1139–1146.
34. Akolkar P, Gulwani-Akolkar B, Pergolizzi R, Bigler R, Silver J. (1993) Influence of HLA genes on T cell receptor V segment frequencies and expression levels in peripheral blood lymphocytes. *J. Immunol.* **150:** 2761–2773.
35. Powis JS, Deverson VE, Coadwell WJ, et al. (1992) Effect of polymorphism of an MHC-linked transporter on the peptides assembled in a class I molecule. *Nature* **357:** 211–215.
36. Woodland D, Blackman A. (1993) How do T-cell receptors, MHC molecules and superantigens get together? *Immunol. Today* **14:** 208–212.

Contributed by H. Wigzell on November 24, 1994.