Clin Exp Immunol 2002; **129**:140–149

Conserved CDR 3 region of T cell receptor BV gene in lymphocytes from bronchoalveolar lavage fluid of patients with idiopathic pulmonary fibrosis

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(Accepted for publication 25 February 2002)

SUMMARY

Idiopathic pulmonary fibrosis (IPF) is an inflammatory lung disease characterized by the accumulation of inflammatory cells and deposition of collagen, resulting in lung remodelling. High numbers of T cells are present in bronchoalveolar lavage fluid (BALF) of IPF patients, although the characteristics of these cells are yet to be determined. To elucidate the pathogenic mechanisms of IPF, we analysed the T cell receptor (TCR) of BALF lymphocytes in three patients with IPF and three healthy subjects as control. TCR repertoire of BALF lymphocytes and T cell clonality were examined by family PCR and Southern blot analysis, and single-strand conformation polymorphism (SSCP), respectively. We observed that the TCR repertoire in the lung was heterogeneous, both in the control subjects and three patients with IPF. SSCP analysis demonstrated an increase in the number of accumulated T cell clones in BALF of two of the three patients, but not in the healthy subject. Furthermore, junctional sequence analysis showed the presence of conserved amino acid motifs (ETGRSG, LAxG, QGQ, GxQP, GRxG, VAR, PGT, GTI, GGT, TGR, LxLxQ, SGQ) in the TCR-CDR 3 region of BAL lymphocytes in patients with IPF, whereas only two amino acid motifs (VTTG, GGE) were found in the control. Our findings suggest that T cells in BALF of patients with IPF expand oligoclonally in the lung, suggesting antigen stimulation of these cells.

Keywords clonality IPF pathogenesis T cell receptor T lymphocytes

INTRODUCTION

Idiopathic pulmonary fibrosis (IPF) is a chronic and progressive pulmonary disorder, associated with both inflammation and fibrosis of the lung parenchyma [1,2]. Recent advances in biotechnology in the field of bronchoalveolar lavage (BAL) have provided considerable information on the cellular components of alveoli in this disease [3–5]. Pulmonary damage and fibrosis represent the consequences of immune response and inflammatory process. Previous studies have shown that lymphocytes (especially T cells) and alveolar macrophages play a central role in the pathogenesis of IPF, although the mechanism that triggers these cells has not been elucidated [6–8].

T cells recognize antigens in the context of MHC on antigen-presenting cells (APC) through an antigen receptor, the T cell receptor (TCR). Several groups investigating TCR genes in autoimmune diseases such as rheumatoid arthritis [9], Sjögren's syndrome [10,11] and multiple sclerosis [12], among other diseases, have demonstrated that T cells accumulate oligoclonally in the inflamed lesion. Furthermore, conserved amino acid motifs have been observed in the CDR3 region of TCR gene, whereas there was no skewed usage of TCR genes [10–13].

Several studies have examined TCR genes of BAL fluid (BALF) T cells in patients with various lung diseases such as sarcoidosis and bronchial asthma. Moller and colleagues [14] demonstrated an increased number of TCR BV8 T cells in BALF of patients with sarcoidosis. Zissel *et al*. [15] showed the predominant usage of TCR BV5, BV8, BV12, BV13S3 and BV19 genes in BALF. Bellocq and coworkers [16] found a number of TCR BV19-positive T cells in BALF of patients with sarcoidosis. In asthmatic patients, Hodges *et al*. [17] reported expansion of TCR BV5S2/3-positive T cells in BALF. In contrast, there was no dominant usage of TCR BV genes in BALF T cells in patients with non-atopic asthma [18]. These findings support the hypothesis that BALF T cells of patients with sarcoidosis and atopic bronchial asthma may be induced by antigen on antigen-presenting cells. To date, there are no reports on the TCR

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gene of BALF T cells, or possible triggering factors in patients with IPF.

The present study was conducted in order to investigate the pathogenesis of IPF. Our experiments included an analysis of the TCR BV repertoire and clonality of T cells infiltrating the lung. Our results showed that T cells in BALF of patients with IPF expand oligoclonally, suggesting antigen-driven stimulation. Furthermore, highly conserved amino acid sequence motifs were identified in the TCR BV CDR3 region of accumulated BALF T cells. These findings indicate that BALF T cells in patients with IPF recognize a limited epitope on antigens. Based on these findings, we discuss possible pathogenic mechanisms of IPF.

MATERIALS AND METHODS

Patients and histological examination

Three patients with IPF were referred to Tokyo Medical University Hospital. Each individual met the criteria for IPF diagnosis, including clinical features, laboratory findings on chest X-ray and chest CT and endoscopic biopsies. We also recruited three healthy subjects who had no respiratory-related complaints and a negative chest X-ray. The clinical characteristics of the three patients with IPF and the healthy subject*s* are summarized in Table 1. Written informed consent was obtained from all patients. A transbronchial lung biopsy was performed, and the tissue was stained with haematoxylin and eosin. No open lung biopsy was performed. Typing of HLA-DR alleles was performed using PCR combined with dot-blot hybridization with sequence-specific oligonucleotide probes (PCR-SSOP) following the protocol of the Eleventh Histocompatibility Workshop [10].

Bronchoalveolar lavage and peripheral blood lymphocytes

BAL was performed on the involved lung segment (right lower lobe and posterior segment) of the three patients with IPF and three healthy control subjects. After topical anaesthesia, the fiberoptic bronchoscope (Olympus type BF20, Olympus Co., Tokyo, Japan) was advanced into the described segment and wedged, and 50 ml sterile 0·9% saline at 37°C was injected through the bronchoscope. The latter process was performed three times. The volume of BALF recovered from the involved segment was approximately 100 ml. Cells in BALF were passed through sterile gauze to remove debris. BALF from patients was centrifuged at 20 ***g*** and 4°C for 10 min, and washed twice with PBS. Following cell count, part of the cell mass was subjected to flow cytometric analysis. A number of cells (2×10^5) were stained with MoAb against Leu 4 (anti-CD3), Leu 3a (anti-CD4) and Leu 2a (anti-CD8) (Becton Dickinson, Mountain View, CA, USA). After flow cytometry, PBLs from patients with IPF were obtained by Ficoll-Hypaque density gradient centrifugation, and analysed immediately.

PCR, Southern blot analysis and SSCP

Total RNA from BALF cells was prepared with Isogen (Nippon Gene Co., Tokyo, Japan). PCR and cDNA synthesis were performed as described previously by Sumida *et al*. [11]. Briefly, first-strand cDNA was synthesized from 1 μg total RNA in a 20-μl reaction mixture containing an oligo(dT) primer by avian myeloblastosis virus reverse transcriptase. Amplification was performed with *Taq* polymerase in 50 μl standard buffer, using 0·2 μl cDNA (corresponding to 10 ng total RNA), with primers specific for 20 different TCR BV genes and BC gene. Sequences of the primers were obtained from previously published data [10]. Denaturing was performed at 95°C for 1·5 min, annealing at 60°C for 1·0 min and extension at 72°C for 1·0 min for 30 cycles in a DNA Thermal Cycler (Perkin-Elmer Corporation, Norwork, CT, USA). One-tenth of each amplified PCR product was subjected to 2% agarose gel electrophoresis and transferred to a nylon membrane. Membranes were hybridized further with

Table 1. Characteristics of study population

	IPF- I	IPF-2	IPF-3	HS-1	HS-2	HS-3
Age (years)	70	65	80	52	32	28
Sex	M	F	M	M	M	M
Smoking	(+)	(−)	(+)	(+)	(+)	(−)
%VC (%predicted value)	97·7	80·5	106·9	ND	118·0	133·4
FEV1%	79·5	88	77·3	ND	80·5	97·4
%DLco	48·2	57·9	74·6	ND	91·7	90·9
PaO_2 (mmHg)	89·7	85·9	81·3	ND	ND	ND
Chest radiograph						
diffuse reticular infiltrate pattern	(+)	(+)	(+)	(−)	(−)	(−)
BAL analyses						
Total cell count ($\times 10^5$/ml)	5	1·2	7	1	2·6	0·8
Differential count (%)						
Macrophages	87·2	81·7	92·1	90·5	93·0	90·0
Lymphocytes	3·7	15·2	4·5	3·2	3·5	8·0
Neutrophils	6·8	2·9	1·8	4·9	3·0	2·0
Eosinophils	2·3	0·2	1·6	1·4	0·5	0
CD4/CD8 ratio	1·9	1·4	2·3	2·2	0·6	4·7
Absolute number of lymphocytes*	1·9	1·8	3·2	0·3	0·9	0·6

Diagnosis of IPF was based on clinical criteria for IPF defined by The Ministry of Health and Welfare research group in Japan. *$\times 10^4$/ml.

digoxygenin-labelled TCR BC probe, and visualized using the DIG luminescent detection kit (Boehringer Mannheim, Mannheim, Germany). The digoxygenin-labelled TCR BC probe was synthesized employing the PCR DIG probe synthesis kit (Boehringer Mannheim), with 5′-TCR BC (5′-GAGGATCTGA GAAATGTGACT-3′) and 3′-TCR BC (5′-CAAGCACACAC GAGGGTAGCCT-3′) primers. For individual single-strand conformation polymorphism (SSCP) assays, amplified DNA was diluted (1 : 20) in a denaturing solution (95% formamide, 10 mM EDTA, 0·1% bromophenol blue, 0·1% xylene cyanol) at 90°C for 2 min. Diluted samples (2 : 1) were subjected to electrophoresis in non-denaturing 5% polyacrylamide gels containing 10% glycerol [19]. Gels were run at 35 W constant power for 2 h. Following electrophoresis, DNA was transferred to Immobilon-S (Millipore Intertech, Bedford, MA, USA) and hybridized with biotinylated TCR BC probe (5′-A (AC) AA (GC) GTGTTCCCACCCGAG GTCGCTGTGTT-3′), streptavidin, biotinylated alkaline phosphatase and a chemiluminescent substrate system (Plex™ Luminescence kit, Millipore).

Sequencing of cDNA encoding TCR BV genes

Complementary DNA, encoding TCR BV genes from BALF and PBLs, was purified from polyacrylamide gels for subjection to SSCP and amplified by PCR using the primers described above. PCR products were ligated to plasmids using the TA cloning kit (Invitrogen, San Diego, CA, USA), transformed into competent INVαF′ *Escherichia coli* cells and grown under appropriate conditions. After selection of TCR BC-positive colonies, plasmid DNA was purified by alkaline lysis for DNA sequencing. Sequencing reactions were performed using an automated DNA sequencer (model 377 A, Applied Biosystems, Foster City, CA, USA).

RESULTS

Heterogeneous TCR BV repertoire of BALF T cells in patients with IPF

To analyse the pathogenesis of IPF, we examined the TCR repertoire of BALF T cells from three patients with IPF (IPF-1, -2, and -3) using the family PCR method. PBLs from identical patients were used as a control. Clinical profiles of IPF patients are summarized in Table 1. Chest CT revealed diffuse reticulonodular opacities, honeycombing and ground-glass attenuation. Histological examination of lung biopsies from patients with IPF showed a large number of mononuclear cells in the inflamed alveolar septa. The pathological changes in the interstitial septa, alveolar spaces, bronchial mucosa and pleura were similar to those in lungs with UIP. Infectious agents or parasites were not observed, and there was no evidence of vasculitis in biopsy specimens. TCR analysis showed expression of the majority of TCR BV family genes in both BALF T cells and PBL in all patients (Fig. 1). These results suggest that the TCR BV repertoire of T cells in the lung is heterogeneous and that there is no restricted predominant usage of TCR BV genes. HLA typing of two patients with IPF showed that one was the DR B1*0405, 0405 allele and the other was the DR B1*0901, 1502 allele.

Lung-specific T cell clones in patients with IPF

TCR BV genes in BALF and peripheral T cells were examined by PCR-SSCP in order to investigate the clonality of pulmonary T cells in patients with IPF. Lung-specific bands that were found in several TCR BV genes of the three IPF patients. These bands were detected mainly in TCR BV 3, 11 and 15 genes. The number of bands encoding TCR BV genes in the lung is summarized in Table 2. We observed a significant increase in the number of expanded clones in BALF from IPF-1 and -3 patients (26 and 24 clones), compared with the healthy subjects (six clones) ($P < 0{\cdot}05$). In contrast, there was no difference in the number of T cell clones in the lung between IPF-2 patients and healthy controls. These results suggest that some T cells accumulate in the lungs of patients with IPF, suggesting that these cells proliferate by antigen stimulation.

Conserved amino acid sequence motifs in the CDR3 region of TCR BV genes from BALF-specific T cells of IPF patients

To examine the amino acid sequences of the CDR3 region in the TCR BV gene, we focused on the lung-specific T cell clones on SSCP analysis. DNAs encoding the TCR BV genes from BALF-specific bands were eluted from gels, following which the corresponding CDR3 regions were sequenced. As shown in Table 3, the CDR3 region of the lung-specific accumulated T cell clones contained conserved amino acid motifs. A LAxG motif was found in BV3-2 and BV6-1 clones from IPF-1 and BV8-2 and BV16-1 clones from the IPF-2 patient. Furthermore, in the IPF-2 patient, QGQ, GxQP, GRxG and EIGRSG motifs were found in BV6-1 and BV9-1, BV11-1 and BV11-2, BV15-2, BV 20-1 and BV20-2, and BV20-1 and BV20-2, respectively. For the IPF-3 patient, VAR, PGT, TGR, SGQ, LxLxQ, GGT, PGT, GTI sequences were observed in BV1-1 and BV1-2, BV2-1 and BV15-1, BV2-2 and BV18-2, BV3-1 and BV3-2, BV3-1 and BV11-1, BV10-2 and

Table 2. Accumulated T cell clones in BALF of patients with IPF

TCR BV gene	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Total
IPF -1	0	0	2	0	0	3	0	0	3	1	7	0	0	0	2	0	2	2	4	0	26
IPF -2	1	1	1	0	0	0	0	1	0	0	1	0	0	0	1	1	0	0	0	1	8
IPF -3	1	1	3	0	1	1	1	0	0	2	1	0	3	3	3	1	0	2	0	1	24
Healthy subject 1	1	2	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	2	6
Healthy subject 2	0	0	0	0	0	1	0	1	0	0	1	0	0	1	1	0	0	1	0	0	6
Healthy subject 3	0	0	1	0	0	0	1	0	0	1	0	0	0	1	0	0	0	1	0	1	6

Numbers represent T cell clones accumulated in BAL from patients with IPF. The distinct bands encoding TCR BV genes on SSCP were described as the number of BAL-specific T cell clones.

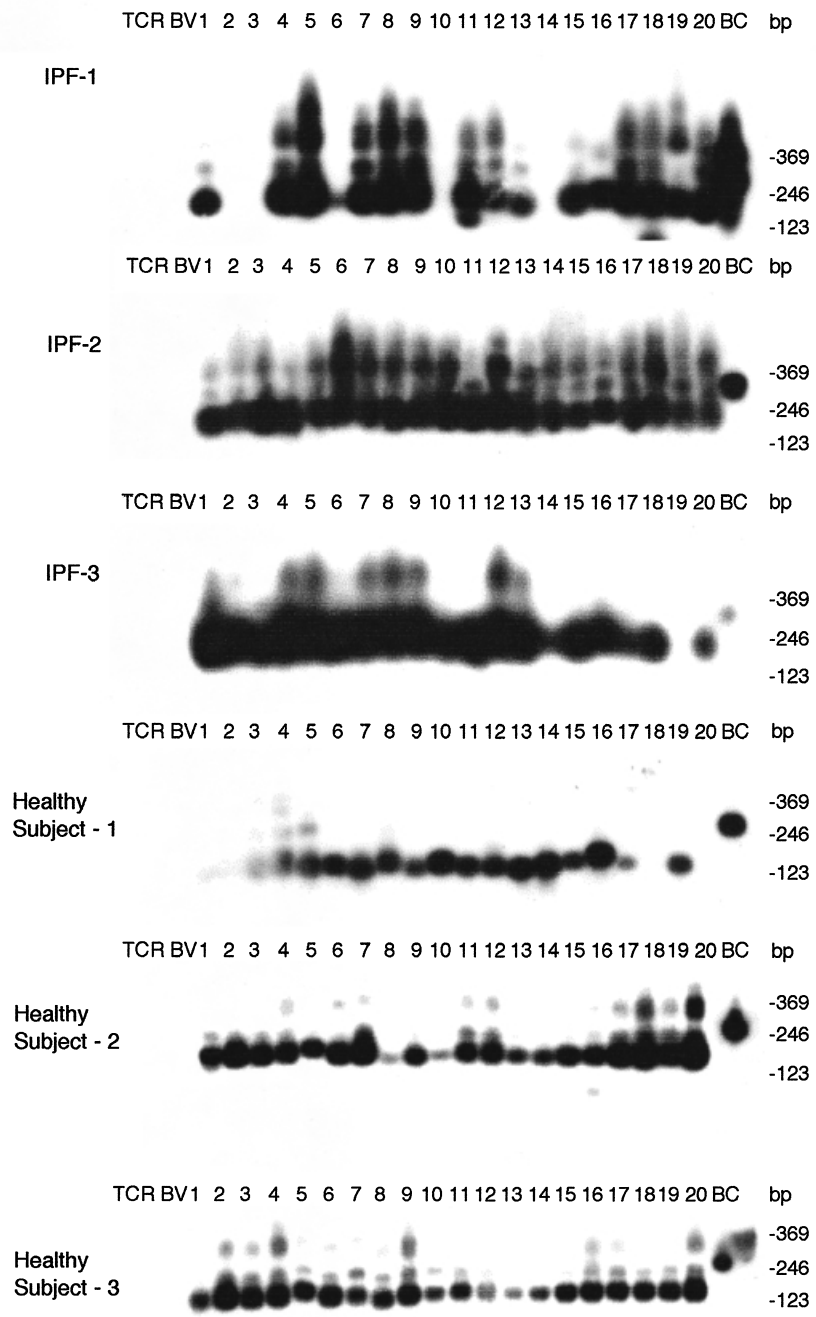

Fig. 1. TCR BV gene repertoire of BALF T cells from three patients with IPF and three healthy subjects. PCR, Southern blot analysis and SSCP methods are described in Patients and methods. The numbers indicate the family of TCR BV genes.

BV20-1, BV10-2 and BV15-1, and BV14-2 and BV16-1, respectively. In contrast, BALF-specific bands of healthy subjects revealed only two motifs, VTGG and GGE. A VTGG motif was found in BV1-2 clone from healthy subject 1 and BV11-2 clone from healthy subject 2 and a GGE motif was found in BV2-1 clone from healthy subject 1 and BV18-2 clone from healthy subject 2 (Table 3). These amino acid motifs were not detected in patients with IPF. The conserved amino acids motifs in TCR BV CDR3 region of BAL T cells in patients with IPF was summarized in Table 4. These findings suggest that accumulated T cells in the lungs of patients with IPF recognize a limited epitope on antigens.

DISCUSSION

IPF is an inflammatory lung disease characterized by the accumulation of lymphocytes and other inflammatory cells, resulting in lung remodeling. T cells comprise the major population of lymphocytes in pulmonary tissue and BALF [15,16,21]. In the present study of TCR genes, we provide evidence that some T

Table 3. TCR BV CDR3 region of BAL T cells in patients with IPF

(1) IPF-1

	V	N-D-N	J	
Clone	92	96	106	
BV3-1	C A S S	L G V S A N	T E A	BJ1S1
	TGTGCCAGCAGT	TTAGGAGTATCAGCGAAC	ACTGAAGCT	
BV3-2	C A S S	[L A] T [G] D	G Y	BJ1S2
	TGTGCCAGCAGT	TTAGCGACAGGGGAT	GGCTAC	
BV6-1	C A S	G [L A] [Q G]	[Q] P Q	BJ1S5
	TGTGCCAGC	GGTTTAGCCCAGGGT	CAGCCCCAG	
BV6-2	C A S S	V	G Y	BJ1S2
	TGTGCCAGCAGC	GTGGT	GGCTAC	
BV9-1	C A S S	[Q G Q] G T	N T E A	BJ1S1
	TGTGCCAGCAGC	CAAGACAGGGAACGACG	AACACTGAAGCT	
BV9-2	C A S S	P G	T E A	BJ1S1
	TGTGCCAGCAGC	CCAGGC	ACTGAAGCT	
BV11-1	C A S	S G R M	N T E A	BJ1S1
	TGTGCCAGC	TCGGGACGGATG	AACACTGAAGCT	
BV11-2	C A S	V R A G H	Q P Q	BJ1S5
	TGTGCCAGCA	GTGCGAGCGGGACATAT	CAGCCCCAG	
BV15-1	C A T S	D P G F	T E A	BJ2S7
	TGTGCCACCAGT	GATCCCGGATTT	TACGAGCAG	
BV15-2	C A T S	D S V	T D T Q	BJ2S3
	TGTGCCACCAGT	GACTCCGTC	ACAGATACGCAG	
BV17-1	C A S S	L R L S G K S D	Q P Q	BJ1S5
	TGTGCCAGTAGT	CTACGACTGTCGGGGAAATCAGAC	CAGCCCCAG	
BV17-2	C A S S	L S N T	E T V	BJ2S5
	TGTGCCAGCAGT	TTATCGAATACA	GAGACCCAG	
BV18-1	C A S S	P R V S	E Q	BJ2S7
	TGTGCCAGCTCA	CCACGGGTTTCC	GAGCAG	
BV18-2	C A S S	L R W A P A	Y E Q	BJ2S7
	TGTGCCAGCTCA	CTGCGGTGGGCCCCGGCT	TACGAGCAG	
BV19-1	C A S	P S G	E Q	BJ2S1
	TGTGCCAGCAG	CCAAGCGGCT	GAGCAG	

(2) IPF-2

	V	N-D-N	J	
Clone	92	96	106	
BV1-1	C A S S	V S D A G A	Q P Q	BJ1S5
	TGTGCCAGCAGC	GTTTCGGACGCGGGGGCT	CAGCCCCAG	
BV1-2	C A S S	V E G Q G R	D T Q	BJ2S3
	TGTGCCAGCAGC	GTAGAAGGACAGGGAAGA	GATACGCAG	
BV2-1	C S A S	V T S L I	I Q	BJ2S1
	TGCAGTGCTAGT	GTGACTAGTTTGATT	ATACAG	

Table 3. *Continued*

(2) IPF-2 *Continued*.

	V	N-D-N	J	
Clone	92	96	106	
BV3-1	C A S S	F G Q	N T E A	BJ1S5
	TGTGCCAGCAGT	TTTGGACAG	AACACTGAAGCT	
BV3-2	C A S S	R D I Y	T E A	BJ1S1
	TGTGCCAGCAGC	CGCGACATATAC	ACTGAAGCT	
BV8-1	C A S	T L R	Y E Q	BJ2S7
	TGTGCCAGCGG	ACCCTTAGGC	TACGAGCAG	
BV8-2	C A S S	L A L G A	Y G Y	BJ1S2
	TGTGCCAGCAGT	CTGGCTTTGGGGGCT	TATGGCTAC	
BV11-1	C A S	G G Q	Q P Q	BJ1S5
	TGTGCCAGCA	GAAGGGGGTAT	CAGCCCCAG	
BV11-2	C A S R	M G F	Q P Q	BJ1S5
	TGTGCCAGCAGA	ATGGGGTTT	CAGCCCCAG	
BV15-1	C A T S	D R L I G P	N E K L	BJ1S4
	TGTGCCACCTCT	GACCGATTGATGGGCCCT	AATGAAAAACTG	
BV15-2	C A T S	E T G R Q G T	T G E L	BJ2S2
	TGTGCCACCAGT	GAAACCGGGAGACAGGGTACC	CCAGGGGAGCTG	
BV16-1	C A S S	Q A A P G L A R	G E L	BJ2S2
	TGTGCCAGCAGC	CAAGCGGCTCCGGGACTGGCACGA	GGGGAGCTG	
BV20-1	C A	S R E I G R S G	N E Q	BJ2S1
	TGTGCC	AGCAGAGAAATAGGTAGGAGTGGG	AATGAGCAG	
BV20-2	C A W S	E I G R S G S K	N E Q	BJ2S1
	TGTGCCAGCAGT	GAAATAGGTAGGAGCGGGAGTAAG	AATGAGCAG	

(3) IPF-3

	V	N-D-N	J	
Clone	92	96	106	
BV1-1	C A S S	V A R T S I N	E Q	BJ2S5
	TGTGCCAGCAGC	GTAGCGAGGACTAGCATTAAT	GAGCAG	
BV1-2	C A S S	V A R G	S E A	BJ1S1
	TGTGCCAGCAGC	GTAGCGCGGGGC	TCTGAAGCT	
BV2-1	C A A R	P G T	D Y G Y	BJ2S1
	TGTAGTGCTAGA	CCAGGGACG	GACTATGGCTAC	
BV2-2	C A A	R E T G R	D T Q	BJ2S3
	TGCAGTGCC	AGGGAAACAGGCCGA	GATACCCAT	
BV3-1	C A S S	S G Q L V	L T Q	BJ2S5
	TGTGCCAGCAGT	AGCGGACAGCTACAA	GAGACCCAG	
BV3-2	C A S S	S G Q E T	Q	BJ2S3
	TCAGGACAAGAG	TCAGGACAAGAGACC	CAG	
BV3-3	C A S S	L C Q A P F N	Q	BJ2S7
	TGTGCCAGCAGT	TTATGGCAGGCCCCCTTCAAT	CAG	
BV5-1	C A S S	S K Y R E P G	E Q	BJ2S7
	TGTGCCAGCAGC	TCCAAATACAGGGAACCGGGA	GAGCAG	

Table 3. *Continued*

(3) IPF-3 *Continued*.

	V	N-D-N	J	
Clone	92	96	106	
BV6-1	C A S S	S L T G V F N	N Q	BJ2S1
	TGTGCCAGCAGC	TCGCTAACGGGAGTCTTCAAT	AATCAA	
BV7-1	C A S	S I Q G S T	G Y	BJ1S2
	GCCAGCAGC	CTGATACAGGGGTCTACT	GGCTAC	
BV10-1	C A S S	K A P G Q G P R C	A	BJ1S1
	TGTGCCAGCAGC	AAAGCACCGGGACAGGGTCCTCGCTGC	GCT	
BV10-2	C A S S	M [G G T] G D [P G	T] Q	BJ2S3
	TGTGCCAGCAGC	ATGGGTGGGACAGGGGACCCGGGG	ACCCAG	
BV11-2	C A S	S E [L] T [L] I [Q]	E T Q	BJ2S3
	TGTGCCAGC	AGTGAACTAACCGATATCCAA	GATACCCAG	
BV13-1	C A S S	L L G	I S P L	BJ1S1
	TGTGCCAGCTCT	TTGCTGGGG	GATTCACCCCTC	
BV13-2	C A G S	L G V D	G N T I	BJ1S3
	TGTGCCGGCTCG	CTCGGGGTGGAT	GGAAACACCATA	
BV13-3	S A S S	C N T G A G N	S P L	BJ1S6
	AGTGCCAGCAGT	TCAAACACAGGGGCCGGGAAT	TCACCCCTC	
BV13-3	C A S A	S L A Y T	E Q	BJ2S7
	TGTGCCTCCGCA	TCCCTTGCGTATACG	GAGCAT	
BV14-1	C A S S	P P A A G	L N T E A	BJ1S1
	TGTGCCAGCAGT	CCCCCTGCGGCAGGG	TTGAACACTGAAGCT	
BV14-2	C A S S	Y R R S E [G T I] T	Y	BJ1S2
	TGGGCCAGCAGT	TATCGCCGATCCGAGGGGACTATAACC	TAC	
BV14-3	C A S S	F G G	P Y N Q	BJ1S2
	TGTGCCAGCAGT	TTTGGCGGG	CCCTACAATGAG	
BV15-1	C A S S	[P G T] S G R	S D T Q	BJ2S3
	TGTGCCACCAGT	CCCGGGACTAGCGGGAGG	TCAGATACGCAG	
BV15-2	C A S S	D	S G E L	BJ2S2
	TGTGCCACCAGT	GAT	TCCGGGGAGCTG	
BV15-3	C A S S	A A S	A F D Q	BJ2S7
	TGTGCCACCAGT	GCCGCCAGC	GCCTACGATCAG	
BV16-1	C A S S	Q V G [G T I]	R	BJ1S1
	TGTGCCAGCAGC	CAAGTCGGGGGGACGATA	CGA	
BV18-1	C A S	S R G V	N E Q	BJ2S1
	TGTGCCAGCTC	TCGCGGGGGGTCC	AATGAGCAG	
BV18-2	C A S S	P T Q [T G R]	N E K L	BJ1S4
	TGTGCCAGCTCA	CCGACACAGACAGGACGG	AATGAAAAACTG	
BV20-1	C A S D	[G G T] C L	T E A	BJ1S1
	TGTGCCTGGGAT	GGTGGGACATGCCTC	ACTGAAGCT	

Table 3. *Continued*

(4) healthy subject-1

	V				N-D-N						J					
Clone	92				96						106					
BV1-1	C	A	S	S	A	G	T				N	Q	E	T	Q	BJ2S5
BV1-2	C	A	S	S	V	T	G	G	S	L	N	E	Q			BJ2S1
BV2-1	C	S	A	K	G	E	R	G	G		E	Q				BJ2S1
BV2-2	C	S	A	R	I	G	T				Q	E	T	Q		BJ2S5
BV2-3	C	S	A		D	R	N				Q	E	T	Q		BJ2S5
BV2-4	C	S	A	S	K	T	G	T			G	E	L			BJ2S2
BV8-1	C	A	S	S	L	G					Y	E	Q			BJ2S7
BV20-1	C	A	W		K	R	E	S			E	Q				BJ2S1
BV20-2	C	A			F	T	G	Q	G	A	S	N	Q	P	Q	BJ1S5
BV20-3	C	A			S	R	D	R	G	L	N	Q	P	Q		BJ1S5

(5) healthy subject-2

	V				N-D-N						J					
Clone	92				96						106					
BV6-1	C	A	S	S	H	S	G	R	E	R	Y	N	E	Q		BJ2S1
BV8-1	C	A	S		Q	G					M	N	T	E	A	BJ1S1
BV8-2	C	A	S	S	F	G	A				E	Q				BJ2S7
BV8-3	C	A	S	S	F	S	G	T	S	G	N	E	Q			BJ2S1
BV11-1	C	A	S		V	M	M	T	G		T	E	A			BJ1S1
BV11-2	C	A	S		V	T	G	G			T	G	E	L		BJ2S2
BV14-1	C	A	S	S	L	V	G	G	R		T	D	D	Q		BJ2S3
BV14-2	C	A	S	S	W	R	G				T	G	E	L		BJ2S2
BV15-1	C	A	T	S	G	P	A	D	E	N	G	E	Q			BJ2S1
BV15-2	C	A	T	S	D	G					Y	E	Q			BJ2S7
BV18-1	C	A	S	S	P	G	A	G			S	S	Y	E	Q	BJ2S7
BV18-2	C	A	S	S	P	Q	G	G			E	Q				BJ2S1
BV18-3	C	A	S	S	P	P	G	P	L		S	Y	N	E	Q	BJ2S1

(6) healthy subject-3

	V				N-D-N						J					
Clone	92				96						106					
BV3-1	C	A	S	S	P	E	P	Q	G	V	R	T	Q			BJ2S5
BV7-1	A	S	S		Y	S	S	G				Y	E	Q		BJ2S7
BV7-2	A	S	S	Q	D	G	G	G	G			N	T	G	E	L	BJ2S2
BV10-1	C	A	S	S	S	P	L	G	A			P	Q			BJ1S5
BV10-2	C	A	S	S	K	T	E	R	E			Y	E	Q		BJ2S7

Table 3. *Continued*

(6) healthy subject-3 *Continued*.

	V						N-D-N							J	
Clone	92				96							106			
BV14-1	C	A	S	S	L	S	E	M	G			N	E	Q	BJ2S1
BV18-1	C	A	S	S	R	P	G	S	G			T	D	T	Q BJ2S3
BV18-2	C	A	S	S	Q	T	E					N	I	Q	BJ2S4
BV20-1	C	A	W	S	P	L	A	L				E	T	Q	BJ2S5
BV20-2	C	A	W		A	L	G	I	A	S		N	E	Q	BJ2S1

The single letter amino acid sequences at 3′ position of TCR BV, CDR3, and 5′ position of the J region are given. Identical sequences are boxed.

Table 4. Conserved amino acid motifs in TCR BV CDR3 region of BAL T cells in patients with IPF

(1)	IPF	
	LAxG	(IPF-1; BV3-2, BV6-1, IPF-2; BV8-2, BV16-1)
	QGQ	(IPF-1; BV6-1, BV9-1)
	GxQP	(IPF-2; BV11-1, BV11–2)
	GRxG	(IPF-2; BV15-2, BV 20–1, BV20-2)
	EIGRSG	(IPF-2; BV20-1, BV20-2)
	VAR	(IPF-3, BV1-1, BV1-2)
	PGT	(IPF-3, BV2-1, BV15-1)
	TGR	(IPF-3, BV2-2, BV18-2)
	SGQ	(IPF-3, BV3-1, BV3-2)
	LxLxQ	(IPF-3, BV3-1, BV11-1)
	GGT	(IPF-3, BV10-2, BV20-1)
	PGT	(IPF-3, BV10-2, BV15-1)
	GTI	(IPF-3, BV14-2, BV16-1)
(2)	Healthy subject	
	VTGG	(healthy subject 1; BV1-2, healthy subject 2; BV11-2)
	GGE	(healthy subject 1; BV2-1, healthy subject 2; BV18-2)

cells accumulate in BALF, suggesting that cells in pulmonary lesions might expand by antigen stimulation in the context of HLA, rather than stimulation by superantigen, as suggested previously [20,21]. This conclusion is in agreement with the results of Lympany *et al*. [22], where the TCR repertoire of T cells in lung biopsy samples and BALF was analysed using RT-PCR, and individual TCR AV and BV expression bias was observed in subjects with fibrosing alveolitis. The findings are also similar to those observed in T cells from the lungs of patients with sarcoidosis [14–16] and atopic bronchial asthma [17]. Considered together, these findings suggest that the pathogenesis of IPF occurs in two steps. The first step includes the presentation of antigens on the HLA molecule. This is followed by accumulation of reactive T cells in the pulmonary lesion, which in turn induces inflammation, resulting in pulmonary fibrosis.

Using flow cytometry, Gruber *et al*. [23] showed a high proportion of $CD8^+$ T cells, low proportion of $CD4^+$ T cells and no change in $\gamma\delta$ T cells in IPF, compared with sarcoidosis. However, we observed a greater increase in the number of $CD4^+$ T cells compared to $CD8^+$ T cells, while $\gamma\delta$ T cells (data not shown) were not detected in BALF of all three patients (data not shown). The discrepancy in these results may be due either to individual observations during different stages of IPF or the presence of distinct pathogenic antigens.

Several conserved amino acid motifs were found in the CDR3 region of the TCR BV gene in clonally expanded BALF T cells of patients with IPF, suggesting that these T cells recognize highly limited epitopes on the antigen. The phenomenon appears to be disease-specific, because there were no corresponding conserved amino acids in BALF T cells of the control subjects. The restricted T cell epitope on the antigen was not dependent on HLA, because HLA-DR of the two IPF patients varies widely among the two patients. This finding suggests the presence of dominant and common T cell epitopes on the antigen. Although this study is dependent on small numbers of IPF patients, a further large-scale study would be necessary to confirm our conclusion.

The identity of antigens recognized by accumulated T cells in BALF of patients with IPF remains to be established. While previous studies have recognized candidate antigens in IPF, no direct evidence has been presented to date [24,25]. Recently, viral proteins such as cytomegalovirus and Epstein–Barr virus (EBV) proteins were considered [26–30]. Antibodies against cytomegalovirus and EBV were detected in patients with pulmonary fibrosis [27,28] and their viral products were expressed in immunostained pulmonary tissues [26,30]. Moreover, DNA and mRNA encoding cytomegalovirus and EBV were detected in patients with pulmonary fibrosis by RT-PCR and ISH [29,30]. These observations suggest that particular viral products are antigens recognized by T cells in pulmonary lesions, and may therefore play a crucial role in the pathogenesis of pulmonary fibrosis.

Therefore, determination of the amino acid sequences of T cell epitopes of the antigen is essential before any vaccine using an analogue peptide of the antigen is used for antigen-specific regulation of IPF.

ACKNOWLEDGEMENTS

We thank Miss Miyuki Nishihara for technical assistance, and Dr F. G. Issa (www.word-medex.com.au) for critical reading of the manuscript.

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