Common T Cell Receptor Clonotype in Lacrimal Glands and Labial Salivary Glands from Patients with Sjögren's Syndrome

Isao Matsumoto,* Kazuo Tsubota,‡ Yoshiyuki Satake,‡ Yasuhiko Kita,* Ryutaro Matsumura,§ Hideyuki Murata,* Takashi Namekawa,* Kusuki Nishioka,‖ Itsuo Iwamoto,* Yasushi Saitoh,* and Takayuki Sumida‖

**Second Department of Internal Medicine, School of Medicine, Chiba University, ‡Department of Ophthalmology, Tokyo Dental College, Chiba, §Department of Internal Medicine, Toho University Sakura Hospital, Chiba, and ‖Division of Rheumatology and Molecular Immunology, Institute of Medical Science, St. Marianna University School of Medicine, Kawasaki 216, Japan*

Abstract

Sjögren's syndrome (SS) is an autoimmune disease characterized by lymphocytic infiltration into lacrimal and salivary glands leading to symptomatic dry eyes and mouth. Immunohistological studies have clarified that the majority of infiltrating lymphocytes around the lacrimal glands and labial salivary glands are CD4 positive αβ T cells. To analyze the pathogenesis of T cells infiltrating into lacrimal and labial salivary glands, we examined T cell clonotype of these cells in both glands from four SS patients using PCR–single-strand conformation polymorphism (SSCP) and a sequencing method. SSCP analysis showed that some infiltrating T cells in both glands expand clonally, suggesting that the cells proliferate by antigen-driven stimulation. Intriguingly, six to sixteen identical T cell receptor (TCR) Vβ genes were commonly found in lacrimal glands and labial salivary glands from individual patients. This indicates that some T cells infiltrating into both glands recognize the shared epitopes on autoantigens. Moreover, highly conserved amino acid sequence motifs were found in the TCR CDR3 region bearing the same TCR Vβ family gene from four SS patients, supporting the notion that the shared epitopes on antigens are limited. In conclusion, these findings suggest that some autoreactive T cells infiltrating into the lips and eyes recognize restricted epitopes of a common autoantigen in patients with SS. (*J. Clin. Invest.* 1996. 97: 1969–1977.) Key words: labial salivary glands • lacrimal glands • Sjögren's syndrome • SSCP • T cell receptor Vβ gene

Introduction

Sjögren's syndrome (SS)[1] is an autoimmune disease characterized by lymphocytic infiltration into lacrimal and salivary glands leading to symptomatic dry eyes and mouth (1). Immunohistochemical studies have demonstrated that the majority of infiltrating lymphocytes around the labial salivary and lacrimal glands are CD4 positive αβ T cells (2–4). We analyzed the repertoire of the T cell receptor (TCR) Vβ gene on T cells infiltrating the lips of SS patients using the quantitative polymerase chain reaction (PCR) and reported that the TCR Vβ repertoire is not restricted, although the Vβ2 and Vβ13 genes are predominantly expressed in the lips of these patients (5). Further analysis of the junctional sequence of the TCR Vβ2 and Vβ13 genes in expanded T cells showed relatively limited junctional usage and conserved amino acids at position 106 in the complementary determining region 3 (CDR3) region (6, 7). Our studies on the repertoire of TCR Vα using double-step inverse PCR also demonstrated that the TCR Vα repertoire of infiltrating T cells is restricted in individual subjects with SS (8). These findings suggest that T cells infiltrating into the lips of SS patients expand by recognizing a restricted epitope on the major histocompatibility complex (MHC) through TCR, rather than by stimulation of superantigens.

In contrast to labial salivary glands, T cells infiltrating into the lacrimal glands of SS patients are heterogeneous and there are no shared T cells in lacrimal and labial salivary glands as determined by the RT-PCR and sequencing methods (9). In the present study, to address the question of whether infiltrating T cells in lacrimal glands and labial salivary glands of patients with SS recognize shared epitopes on autoantigens, the T cell clonotype in both glands was examined by a PCR–single-strand conformation polymorphism (SSCP) method (10). We observed a number of clonally expanded T cells in both glands as compared with peripheral blood lymphocytes (PBL). These results support the notion that T cells infiltrating the lips and eyes expand by antigen-driven stimulation. Surprisingly, several identical TCR Vβ genes were detected in lacrimal and labial salivary glands from individual patients. This suggests that some infiltrating T cells in lacrimal glands and labial salivary glands recognize shared epitopes on autoantigens. Moreover, highly conserved amino acid sequence motifs were found in the CDR3 region of TCR clones bearing the same Vβ family gene. This indicates limited common epitopes on autoantigens in both glands. A possible mechanism involved in the progression of SS is discussed.

Address correspondence to Dr. Takayuki Sumida, Division of Rheumatology and Molecular Immunology, Institute of Medical Science, St. Marianna University School of Medicine, 2-16-1 Sugao, Miyamae-ku, Kawasaki 216, Japan. Phone: 81-44-977-8111 ext. 4109; FAX: 81-473-33-2154.

Received for publication 5 October 1995 and in revised form 14 February 1996.

J. Clin. Invest.

0021-9738/96/04/1969/09 $2.00
Volume 97, Number 8, April 1996, 1969–1977

1. *Abbreviations used in this paper:* CDR3, complementarity determining region 3; SS, Sjögren's syndrome; SSCP, single-strand conformation polymorphism; TCR, T cell receptor.

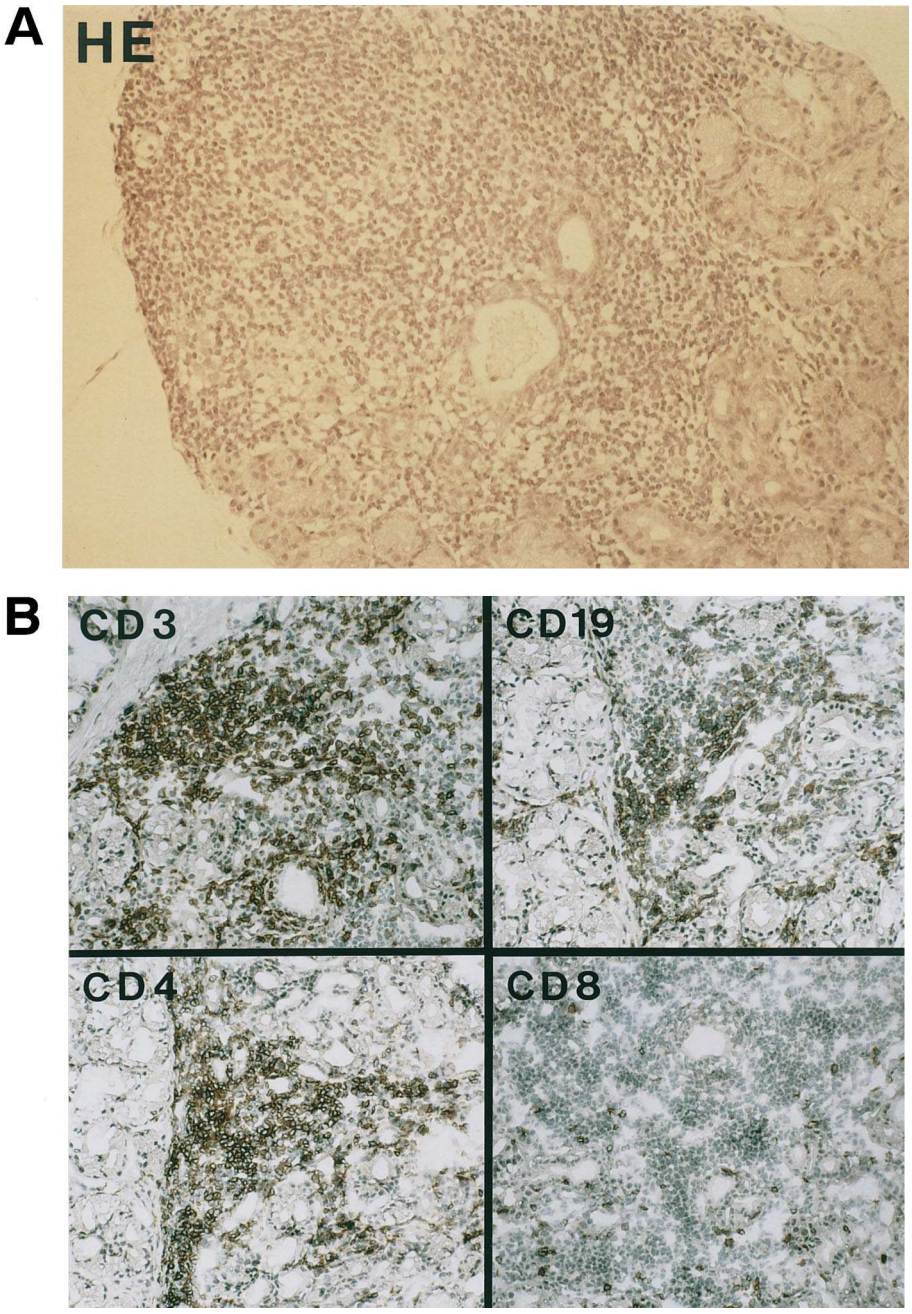

Figure 1. Infiltration of $CD4^+$ T cells into the lacrimal glands of patients with SS. Histological examination (H-E staining) of lacrimal glands from SS patients shows remarkable infiltration of mononuclear cells around the lacrimal glands (*A*, ×200). Immunochemical studies using mAbs against CD3, CD19, CD4, and CD8 demonstrated that the majority of infiltrating cells were $CD4^+$ T cells (*B*).

Methods

Patients, lacrimal gland, and labial salivary gland biopsies. Four SS patients were referred to Chiba University Hospital and met the criteria for a diagnosis of SS. The criteria for diagnosis included dry eye without reflex tearing (11, 12), xerostomia, and grade 4 mononuclear cell infiltration of the salivary and lacrimal glands identified on biopsy specimens (13, 14). Typing of the HLA-DR and -DQ alleles was performed using PCR combined with dot-blot hybridization with a sequence-specific oligonucleotide probe (PCR-SSOP) following the protocol of the Eleventh Histocompatibility Workshop (7).

Histological and immunohistochemical examination. Tissue samples from the labial and lacrimal glands of SS patients were fixed in buffered-formalin, embedded in paraffin, and stained with hematoxylin and eosin (H-E). For immunostaining, a portion of a sample was snap frozen, and cryostat sections were cut and stained with anti-CD3, anti-CD19, anti-CD4, or anti-CD8 mAbs (Becton Dickinson, Mountain View, CA). Cryostat sections were incubated with biotinylated rabbit anti-mouse immunoglobulins (DAKO, Denmark), then with streptABComplex/HRP (DAKO), and finally with a peroxidase substrate.

PCR, Southern blot analysis, and SSCP analysis. Total RNA (5–10 μg) from labial salivary glands, lacrimal glands and peripheral blood lymphocytes (PBL) from each SS patient was prepared with Isogen (Nippon Gene, Co. Ltd., Tokyo, Japan). cDNA synthesis and PCR were carried out using methods described elsewhere (5). Briefly, first strand cDNAs were synthesized in a 20-μl reaction mixture containing oligo(dT) primer by avian myeloblastosis virus reverse transcriptase from 1 μg of total RNA. Amplification was performed with Taq polymerase in 50 μl of standard buffer using 0.2 μl of cDNA (corresponding to 10 ng of total RNA) with 20 different Vβ and Cβ primers. The sequences of the Vβ and Cβ primers were ob-

Table I. HLA Typing of Patients with Sjögren's Syndrome

	HLA					
	DR				DQ	
Patient	B1	B3	B4	B5	A1	B1
SS-54	0802, 1502			0102		0302, 0601
SS-81	0406, 0410		0101		03	0402, 0302
SS-82	0403, 0405		0101		03	0401, 0302
SS-86	0406		0101		03	0302

tained from previously published data (5). Oligonucleotides were synthesized with a DNA synthesizer (Applied Biosystems, Foster City, CA). Denaturing was done 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 on a DNA thermal cycler (Perkin-Elmer Corporation, Norwalk, CT). For Southern blot analysis, one-tenth of the PCR products was subjected to 2% agarose gel electrophoresis and hybridized with a ^{32}P-labeled PstI fragment of the JUR-β2 gene (Cβ) probe (5). For SSCP assay, amplified DNA was diluted (1:20) in a denaturing solution (95% formamide, 10 mM EDTA, 0.1% Bromophenol blue, 0.1% xylene cyanol) and held at 90°C for 2 min. The diluted sample (2 μl) was electrophoresed in nondenaturing 5% polyacrylamide gels containing 10% glycerol (10). The gels were run at 35W constant power for 2 h. After electrophoresis, the DNA was transferred to Immobilon-S (Millipore Intertech, Bedford, MA) and hybridized with biotinylated Cβ probe (5′-A(AC)AA(GC)GTGTTCCCACCCGAGGTCGCTGTGTT-3′), streptavidin, biotinylated alkaline phosphatase, and a chemiluminescent substrate system (Plex™ Luminescence kit, Millipore, Intertech).

Sequencing of cDNAs encoding TCR Vβ genes. Complementary DNAs encoding the common TCR Vβ genes from SS patients (SS-54, SS-81, SS-82, and SS-86) were purified from the polyacrylamide gels for SSCP and amplified by PCR, using primers with an EcoRI cutting site specific for each Vβ gene as described elsewhere (5, 6). Amplified DNAs were purified by phenol extraction, precipitated with ethanol, and digested with excess amounts of EcoRI. Fragments with the expected sizes of the cDNAs were enriched by preparative low-melting-point agarose gel electrophoresis. The recovered DNA fragments were ligated to M13mp19 plasmids obtained by EcoRI digestion. Phages were grown on TG-1 *Escherichia coli* cells. After hybridization with a Cβ probe (5), a single phage was allowed to grow, and recombinant phage DNA was purified for DNA sequence determination. Sequencing reactions were performed by automated sequencing (Applied Biosystems).

Statistical analysis. The statistical significance of the results was determined using the *t* test.

Results

Infiltration of CD4 positive T cells into lacrimal glands. Histological examination of the lacrimal glands of SS patients showed a remarkable infiltration of mononuclear cells into the glands (Fig. 1 *A*). Immunohistochemical studies using mAbs against CD3, CD19, CD4, and CD8 demonstrated that the infiltrating cells are mainly $CD3^+$ T cells, and of those the majority are $CD4^+$ T cells (Fig. 1 *B*). HLA typing of four patients with SS showed a decreased frequency of the DR B3 allele (0/4) and an increased frequency of the DR B4*0101 allele (3/4) (Table I).

Accumulation of multiple T cell clonotypes in lacrimal glands and labial salivary glands from patients with SS. PCR-Southern blot analysis showed that TCR Vβ repertoire on T cells infiltrating in lacrimal glands from SS patients was heterogenous and different among four patients, whereas TCR Vβ2 and Vβ13 genes were relatively over-expressed on T cells in labial salivary glands from all SS patients (Fig. 2). To analyze T cell clonotypes in lacrimal and labial salivary glands from patients with SS, the TCR Vβ clonotypes in eyes, lips, and PBLs from four SS patients were examined by the PCR-SSCP method. Table II shows that smeared bands were found in all Vβ genes from PBLs, while several distinct bands were detected in most Vβ genes from T cells infiltrating into the lacrimal and labial salivary glands. The numbers of bands in the TCR Vβ genes from lacrimal glands were significantly increased (mean±SE, 3.6±0.8; $P < 0.001$, 9.9±1.3; $P < 0.001$, 6.1±0.8; $P < 0.05$, and 7.2±0.7; $P < 0.001$) in all four SS patients compared with PBLs (1.7±0.3, 1.5±0.4, 2.0±0.4, and 1.6±0.4). Some of the T cells in labial salivary glands (4.8±0.9; $P < 0.05$, 6.7±0.8; $P <$

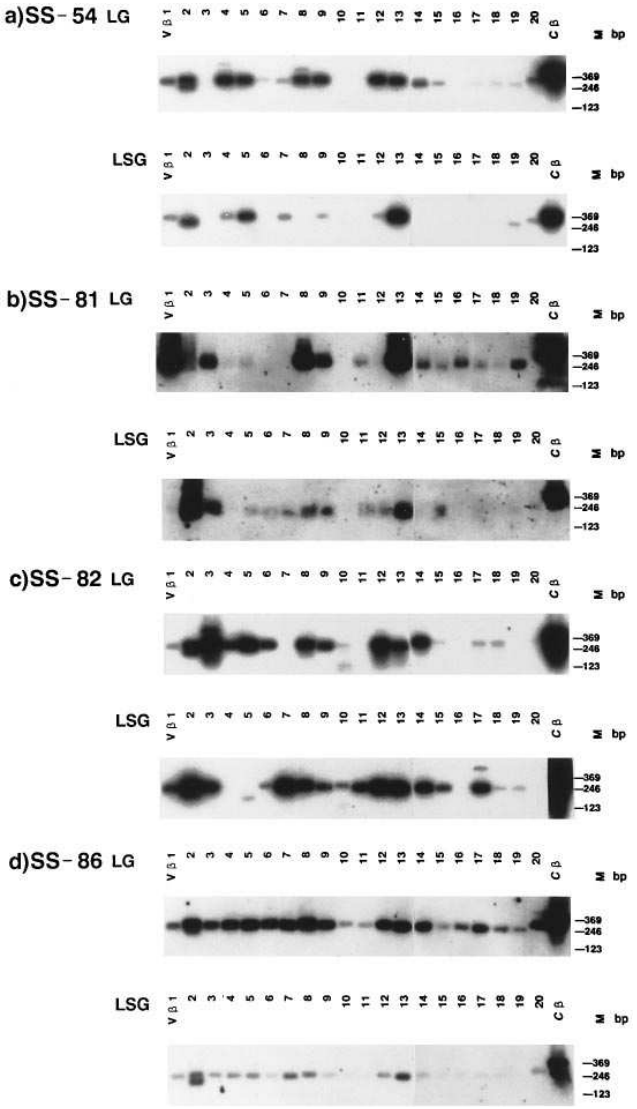

Figure 2. Heterogenous usage of the TCR Vβ gene on T cells infiltrating in lacrimal glands of SS patients. Four lacrimal glands (*LG*) and four labial salivary glands (*LSG*) biopsy specimens from SS patients (*a*, SS-54; *b*, SS-81; *c*, SS-82; and *d*, SS-86) were used for PCR. The TCR Vβ repertoire was examined by family-PCR Southern blot analysis as described in Methods.

Table II. Clonotype Analysis of TCR Vβ Expression in Lacrimal Glands, Labial Salivary Glands, and PBLs from SS Patients

	TCRVβ																				
SS	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	**mean**±SE
54																					
P	3	3	2	–	4	0	2	1	1	1	1	1	3	2	0	–	2	–	0	3	**1.7**±0.3
S	2	3	–	7	7	–	10	2	3	–	–	–	8	2	7	–	–	2	–	–	**4.8**±0.9*
L	1	6	–	3	7	–	5	–	1	–	–	4	5	–	–	–	0	–	–	–	**3.6**±0.8‡
81																					
P	4	4	1	2	0	2	5	2	1	0	–	3	0	2	–	1	0	0	0	0	**1.5**±0.4
S	7	1	5	6	12	5	2	4	4	10	9	7	13	8	12	–	9	4	3	–	**6.7**±0.8‡
L	16	11	5	–	8	11	12	7	4	8	4	5	19	8	10	–	22	8	–	–	**9.9**±1.3‡
82																					
P	0	3	3	1	2	4	–	4	3	1	–	3	3	3	0	0	–	0	3	1	**2.0**±0.4
S	10	6	4	–	3	–	3	3	10	17	3	8	3	4	1	–	8	0	–	–	**5.5**±1.1*
L	8	3	4	7	10	7	–	4	10	4	–	5	8	9	0	–	10	2	–	–	**6.1**±0.8‡
86																					
P	2	3	2	0	2	2	0	0	4	2	–	3	4	–	1	–	0	0	–	0	**1.6**±0.4
S	18	7	–	3	16	6	7	7	5	6	3	15	13	15	15	–	17	4	11	0	**9.3**±1.3‡
L	10	4	6	5	5	3	9	5	10	13	6	7	10	9	6	–	–	9	–	5	**7.2**±0.7‡

Numbers indicate the number of TCR clonotypes for each Vβ gene. (−) indicated no detectable TCR bands found even with longer exposure. *P*, PBL; *S*, labial salivary glands; *L*, lacrimal glands. *$P < 0.05$. ‡$P < 0.001$.

0.001, 5.5±1.1; $P < 0.05$, and 9.3±1.3; $P < 0.001$) also showed an accumulation of T cells compared with PBLs. These results suggest that T cells expand clonally in both eyes and lips, indicating that they are stimulated by antigens.

Common T cell clonotype in lacrimal and labial salivary glands from SS patient. As shown in Fig. 3, 6–16 identical bands bearing the TCR Vβ gene were commonly found in the lacrimal and labial salivary glands of four individual SS patients. Four paired common bands in labial salivary glands and in lacrimal glands of two SS patients were eluted from SSCP gels, and their junctional sequences of the cDNAs encoding TCR Vβ genes were analyzed. As shown in Table III, paired clones in both glands share an identical TCR CDR3 region, respectively. These findings indicate that some of the T cells in-

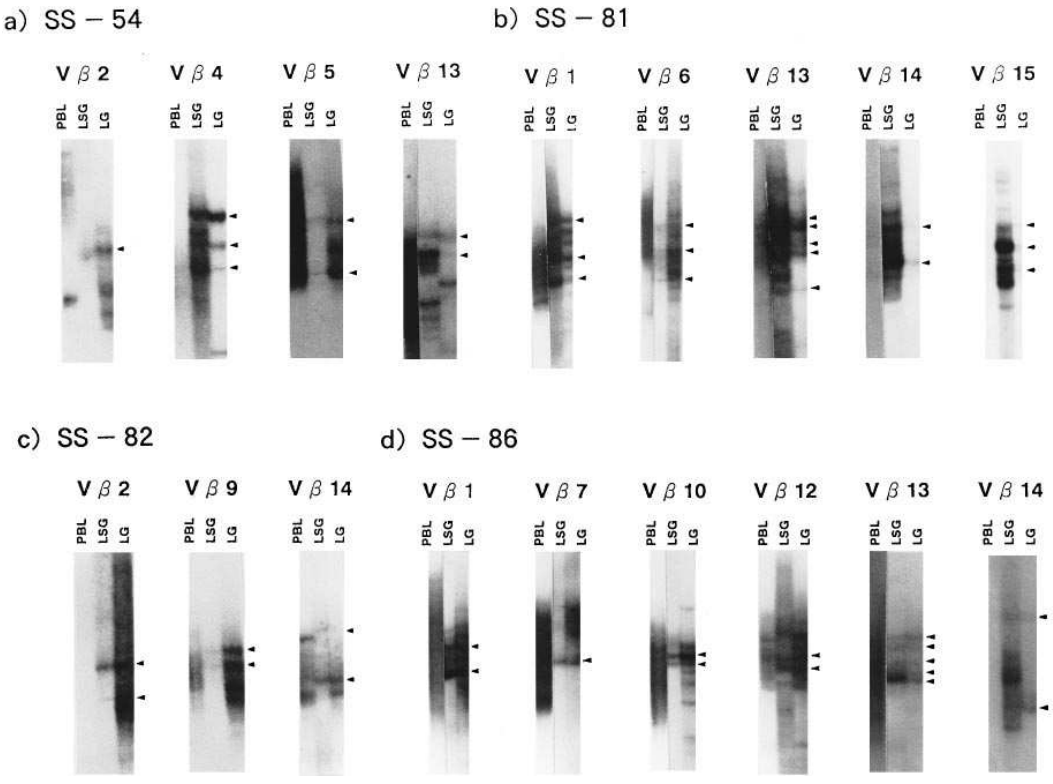

Figure 3. Common T cell receptor in lacrimal and labial salivary glands from SS patients. PCR products encoding the TCR Vβ genes from lacrimal glands (LG), labial salivary glands (LSG), and PBLs of two SS patients (*a*, SS-54; *b*, SS-81; *c*, SS-82; and *d*, SS-86) were analyzed by the SSCP method as described in Methods. 8 (SS-54), 16 (SS-81), 6 (SS-82), and 14 (SS-86) identical bands bearing the TCR Vβ gene were commonly found in both glands.

Table III. Junctional Sequences of Common TCR Vβ Genes from T Cells in Lacrimal Glands and Labial Salivary Glands of SS Patients

TCR clone		V 92	N-D-N	J 106	
SS-54					
LSG	Vβ4-1	TGCAGCGTTGAA	GATGGGACT	AGCACAGATACGCAG	Jβ2.3
LG	Vβ4-1	TGCAGCGTTGAA	GATGGGACT	AGCACAGATACGCAG	Jβ2.3
LSG	Vβ13-2	TGTGCCAGCAGT	TACTCCGCCGACATAAA	CGGGGAGCTG	Jβ2.2
LG	Vβ13-2	TGTGCCAGCAGT	TACTCCGCCGACATAAA	CGGGGAGCTG	Jβ2.2
SS-82					
LSG	Vβ14-1	TGTGCCAGCAGG	TCCCCTTCGGCAGGGT	TGAACACTGAAGCT	Jβ1.1
LG	Vβ14-1	TGTGCCAGCAGG	TCCCCTTCGGCAGGGT	TGAACACTGAAGCT	Jβ1.1
LSG	Vβ14-2	TGTGCCAGCAG	TTTATCGTTCAATGGCGGAACTGGG	GAGCAG	Jβ2.7
LG	Vβ14-2	TGTGCCAGCAG	TTTATCGTTCAATGGCGGAACTGGG	GAGCAG	Jβ2.7

Four paired bands from two SS patients (SS-54 and SS-82), which were commonly found in labial salivary glands (LSG) and in lacrimal glands (LG), were eluted from SSCP gels and their TCR Vβ junctional sequences were analyzed. Results showed that TCR Vβ CDR3 sequences of T cells in LSG were the same as those of paired clones in LG, respectively.

filtrating into lacrimal glands are the same clones as T cells in labial salivary glands in the individual patient, suggesting that the common T cells recognize the same autoantigens.

Highly conserved amino acid sequences of the common TCR CDR3 region in lacrimal and labial salivary glands of SS patients. Highly conserved amino acids were found in the CDR3 regions of common T cell clones expressing the same TCR Vβ family genes such as TCR Vβ4 (EDGTxTDTQ), Vβ5 (SLGGGxTDTQ), and Vβ13 (SYSAxINGEL) from patient SS-54 (Table IV). A conserved amino acid motif (GQVSNTGEL), (SLAGGAxEQ), and (SSYxPS) were found in the CDR3 region of two Vβ1 clones (Vβ1-2 and Vβ1-3), two Vβ6 clones (Vβ6-1 and Vβ6-2), and two Vβ13 clones (Vβ13-1 and Vβ13-2) from patient SS-81, respectively. The other two TCR clones (Vβ9-1 and Vβ9-2) shared amino acid sequences (SQxxR) in the CDR3 region of patient SS-82. The conserved amino acids motif (SKVG) was observed in two Vβ10 clones (Vβ10-1 and Vβ10-2) from SS-86 patient. These findings suggest that common T cells bearing the same TCR Vβ family gene recognize the same or similar epitopes on common antigens. In contrast, uncommon TCR Vβ genes from T cells in lacrimal glands of four SS patients (SS-54, SS-81, SS-82, and SS-86) showed no conserved amino acids motif in CDR3 region (Table V).

Discussion

The TCR Vβ repertoire of infiltrating T cells in patients with SS has been analyzed in several organs including labial salivary glands, lacrimal glands, and kidneys (5–9, 15–17). In labial salivary glands, the TCR repertoire of infiltrating T cells is heterogeneous, however, the Vβ2 and Vβ13 genes are predominantly expressed (5) and a restricted junctional usage of these TCR Vβ genes is used (6). The limited clonality of infiltrating T cells is in agreement with the results of Legras et al. (15), who studied cultured T cells from labial salivary glands of patients in the early stages of SS. In contrast, Mizushima et al. (9) reported using RT-PCR method that the TCR Vβ usage of infiltrating T cells in lacrimal glands is heterogeneous and there are no identical T cell clones between labial salivary glands and lacrimal glands in an individual SS patient. In this study, we used the PCR-SSCP method to show that at least 6–16 common TCRs are found in T cells infiltrating the labial salivary glands and lacrimal glands of four SS patients. Indeed, junctional sequence analysis showed that the common TCRs in both glands shared completely identical TCR CDR3 genes. The discrepancy between a previous report (9) and the present study might be due to the different method, since PCR-SSCP is better technology than RT-PCR to find out the small amount of common clonality.

The TCR CDR3 region interacts with a peptide presented by the MHC molecule in a computer modeling study (18). Analyses of TCR on antigen-specific T cells in several murine models with immunization have shown that amino acid residues in the TCR CDR3 region determine antigen specificity (19–21). Therefore, the presence of the common T cell clonotypes in both glands in our present study strongly suggests that common autoantigens are expressed on both tissues. These findings should be important evidence for analyzing the mechanism of immune response in the generation of SS. Highly conserved amino acid motifs in the CDR3 regions was detected in common T cell clones expressing the same TCR Vβ family gene. This supports the notion that common T cells bearing the same Vβ family gene recognize nearly the same epitopes on antigens. However, the same amino acid sequence motif in the TCR CDR3 region was not found in all four SS patients compared. This might be due to a different HLA-DR allele in each of these subjects, because the T cell epitope (peptide) consisting of the distinct amino acid motif seems to be presented on the different DR molecule.

The Jβ2 (Jβ2.1-Jβ2.7) genes were over-expressed in common TCR from four SS patients compared with the Jβ1 (Jβ1.1-Jβ1.6) genes. It is one possibility that these T cells are selected by particular common antigens and reveal the relatively conserved sequence motifs in CDR3 region, resulting in the predominant usage of the Jβ2 genes. Moreover, it is intriguing that TACTCC/G (YS) sequence has been detected in CDR3 region of two clones (Vβ13-1 and Vβ13-2) from SS-54 and one

Table IV. Amino Acid and Nucleotide Sequences of the Common TCR Vβ CDR3 Region of T Cells in Lacrimal Glands and Labial Salivary Glands of SS Patients

	V	N-D-N	J	
	92	96	106	
SS-54				
Vβ2	C S A R	D L T A G H	G E L	Jβ2.2
	TGCAGTGCTAGA	GATCTCACAGCCGGGCA	CGGGGAGCTG	
Vβ4-1	C S V E	D G T*	S T D T Q	Jβ2.3
	TGCAGCGTTGAA	GATGGGACT	AGCACAGATACGCAG	
Vβ4-2	C S V E	D G T R	T D T Q	Jβ2.3
	TGCAGCGTTGAA	GATGGGACTAGG	ACAGATACGCAG	
Vβ4-3	C S V E	N P T	S Y N E Q	Jβ2.1
	TGCAGCGTTGAA	AACCCGACT	TCCTACAATGAGCAG	
Vβ5-1	C A S S	L G G G R	T D T Q	Jβ2.3
	TGCGCCAGCAGC	TTGGGCGGCGGTAGG	ACAGATACGCAG	
Vβ5-2	C A S S	L G G G	S T D T Q	Jβ2.3
	TGCGCCAGCAGC	TTGGGCGGCGGT	AGCACAGATACGCAG	
Vβ13-1	C A S S	Y S A P I N	G E L	Jβ2.2
	TGTGCCAGCAGT	TACTCCGCCCCCATAAA	CGGGGAGCTG	
Vβ13-2	C A S S	Y S A D I N	G E L	Jβ2.2
	TGTGCCAGCAGT	TACTCCGCCGACATAAA	CGGGGAGCTG	
SS-81				
Vβ1-1	C A S S	S S	S Y N E Q	Jβ2.1
	TGTGCCAGCAGC	TCTAGT	TCCTACAATGAGCAG	
Vβ1-2	C A S S	T G Q V S	N T G E L	Jβ2.2
	TGTGCCAGCAGC	ACGGGACAGGTCT	CGAACACCGGGGAGCTG	
Vβ1-3	C A S E	G Q V S	N T G E L	Jβ2.2
	TGTGCCAGC	GAGGGACAGGTCT	CGAACACCGGGGAGCTG	
Vβ6-1	C A S S	L A G G A G	E Q	Jβ2.1
	TGTGCCAGCAGC	TTGGCGGGAGGGGCGGGC	GAGCAG	
Vβ6-2	C A S S	L A G G A D	E Q	Jβ2.7
	TGTGCCAGCAGC	TTGGCGGGAGGGGCGG	ACGAGCAG	
Vβ6-3	C A S S	L A P M G G	E Q	Jβ2.7
	TGTGCCAGCAGC	TTGGCACCTATGGGCGGC	GAGCAG	
Vβ13-1	C A S S	Y S P S G S G T D T Q		Jβ2.3
	TGTGCCAGCAGT	TACTCGCCTAGCGGGAGCG	GCACAGATACGCAG	
Vβ13-2	C A S S	Y G P S Y	G Y	Jβ1.2
	TGTGCCAGCAGT	TACGGACCTAGCTAC	GGCTAC	
Vβ13-3	C A S R	G S G G I P G G N E Q		Jβ2.1
	TGTGCCAGCAG	GGGTAGCGGGGGGATCCCCGGGGGC	AATGAGCAG	
Vβ13-4	C A S S	N S G P P	E T Q	Jβ2.5
	TGTGCCAGCAGT	AATAGCGGACCCCC	AGAGACCCAG	
Vβ13-5	C A S R	G Q G	N Y G Y	Jβ1.2
	TGTGCCAGCAG	GGGACAGGGG	AACTATGGCTAC	
Vβ14-1	C A S S	F P N A G	E Q	Jβ2.1
	TGTGCCAGCAG	TTTCCCGAATGCTGG	TGAGCAG	
Vβ14-2	C A S R	G G R S A	D T Q	Jβ2.3
	TGTGCCAGCAGG	GGCGGGAGAAGTG	CAGATACGCAG	
Vβ15-1	C A T S	E H R T S G N L	N E Q	Jβ2.1
	TGTGCCACCAGT	GAACACCGGACTAGCGGGAACCT	CAATGAGCAG	
Vβ15-2	C A T S	D P V P L A G	Y N E Q	Jβ2.1
	TGTGCCACCAGT	GATCCCGTCCCCTTAGCGGGA	TACAATGAGCAG	
Vβ15-3	C A T S	P G Q G S I A	G E L	Jβ2.2
	TGTGCCACCAGT	CCGGGACAGGGTTCGATTG	CCGGGGAGCTG	

continued

Table IV. (*continued*)

	V	N-D-N	J	
	92	96	106	
SS-82				
Vβ2-1	C S A S G H R TGCAGTGCTAGT GGACATAGA	T D T Q ACAGATACGCAG		Jβ2.3
Vβ2-2	C S A T G A G S TGCAGTGCTA CTGGAGCGGGGA	T D T Q GCACAGATACGCAG		Jβ2.3
Vβ9-1	C A S S Q M N R G R A G G E Q TGTGCCAGCAGC CAAATGAACCGCGGGAGGGCCGGCGG TGAGCAG			Jβ2.1
Vβ9-2	C A S S Q S G R K P E Q TGTGCCAGCAGC CAAAGCGGGAGGAAGCC TGAGCAG			Jβ2.1
Vβ14-1	C A S R S P S A G L TGTGCCAGCAGG TCCCCTTCGGCAGGGT	N T E A TGAACACTGAAGCT		Jβ1.1
Vβ14-2	C A S S L S F N G G T G TGTGCCAGCAG TTTATCGTTCAATGGCGGAACTGGG	E Q GAGCAG		Jβ2.7
SS-86				
Vβ1-1	C A S S A T G TGTGCCAGCAGC GCGACTGGA	E Q GAGCAG		Jβ2.1
Vβ1-2	C A S I R A F TGTGCCAGCA TCCGGGCTTT	T G E L CACCGGGGAGCTG		Jβ2.2
Vβ7	A T P P D P D R H D GCCA CACCACCCGACCCGGACAGGCACG	G Y ATGGCTAC		Jβ1.2
Vβ10-1	C A S S K V G Q G A M G Q P Q TGTGCCAGCAGC AAAGTCGGGCAGGGGGCGATGGG TCAGCCCCAG			Jβ1.5
Vβ10-2	C A S S K V G Y W Q E T Q TGTGCCAGCAGC AAAGTTGGCTATTGG CAAGAGACCCAG			Jβ2.5
Vβ12-1	C A T R K I E S P N Q P Q TGTGCCACTAGA AAAATTGAGTCGCCT AATCAGCCCCAG			Jβ1.5
Vβ12-2	C A T N L A G R N Y E Q TGTGCCACTA ACCTTGCAGGGAGGAAT TACGAGCAG			Jβ2.7
Vβ13-1	C A S T Q Q G R R Y E Q TGTGCCAGCA CCCAACAGGGGAGGCGG TACGAGCAG			Jβ2.7
Vβ13-2	C A S S P G R W N E Q TGTGCCAGCAGT CCGGGCCGTTGG AATGAGCAG			Jβ2.1
Vβ13-3	C A S G T V G L Q E T Q TGTGCCAGC GGGACCGTGGGACTA CAAGAGACCCAG			Jβ2.5
Vβ13-4	C A S R S G A G Q G S Y E Q TGTGCCAGCAG AAGCGGGGCCGGACAGGGT TCCTACGAGCAG			Jβ2.7
Vβ13-5	C A S N A G T D T Q TGTGCCAGCA ACGCGGGG ACAGATACGCAG			Jβ2.3
Vβ14-1	C A G F N S G G C T D T Q TGTGCC GGATTCAACAGCGGGGGAT GCACAGATACGCAG			Jβ2.3
Vβ14-2	C A S S S L P G R D T Q TGTGCCAGCAG TTCTCTGCCAGGTCG AGATACGCAG			Jβ2.3

Junctional sequences of TCR Vβ genes, which were commonly found in labial salivary glands and in lacrimal glands, from four SS patients were analyzed. The single letter amino acid codes of the 3′ position of TCR Vβ, CDR3, and the 5′ position of the J region are given. Underlining represents conserved amino acid sequences.

TCR clone (Vβ13-1) from SS-81 patient. Although the mechanism for this identical N sequence among several TCR clones is not known, it might be due to selection of T cells bearing the similar CDR3 region against T cell epitope on common antigen.

What are the shared autoantigens recognized by the common auto-reactive T cells in both tissues? There are no reports on the same TCR Vβ CDR3 motifs in this study, however, there may be several possible candidates. First, certain cellular proteins in a cell are expressed on the cell surface and function as autoantigens. Recently, Namekawa et al. (22) identified a high frequency of Ro-SS-A 52kD reactive T cells in labial salivary glands from SS patients, suggesting that Ro/SS-A 52kD might act as an autoantigen for auto-reactive T cells in both glands. Ro/SS-A 52kD is a ribonucleoprotein particle distributed mainly in the cytoplasm of all cells; however, it is also expressed on the cell surface (23). Thus, Ro/SS-A protein could

Table V. Amino Acid and Nucleotide Sequences of Uncommon TCR Vβ CDR3 Region of T Cells in Lacrimal Glands from SS Patients

	V	N-D-N	J	
	92	96	106	
SS-54				
Vβ4-1	C S A T G G	S Y N E Q		Jβ2.1
	TGCAGCGC AACTGGGGGT	TCCTACAATGAGCAG		
Vβ4-2	C S V E F Q	Y N E Q		Jβ2.1
	TGCAGCGTTGAA TTTCAA	TACAATGAGCAG		
Vβ4-3	C S V E L T D T	A K N I Q		Jβ2.4
	TGCAGCGTTGAA CTAACAGATAC	AGCCAAAAACATTCAG		
Vβ5-1	C A T A W A A V D	T D T Q		Jβ2.3
	TGCGCCA CAGCTTGGGCGGCGGTAGA	CACAGATACGCAG		
Vβ5-2	C A S G Q	T G E L		Jβ2.2
	TGCGCCAGC GGCCA	GACCGGGGAGCTG		
Vβ5-3	C A S C S Q G E T G I	S Y E Q		Jβ2.7
	TGCGCCAGC TGCAGCCAAGGTGAGACAGGGAT	CTCCTACGAGCAG		
Vβ13-1	C A S S P A P G F T	Y E Q		Jβ2.7
	TGTGCCAGCAGT CCCGCACCGGGTTTCA	CCTACGAGCAG		
Vβ13-2	C A S S L R R L G	S Y E Q		Jβ2.7
	TGTGCCAGCAGT TTACGACGGCTAGGA	TCCTACGAGCAG		
SS-81				
Vβ1-1	C A S S V S G	S T D T Q		Jβ2.3
	ACTGCCAGCAGC GTATCCGGG	AGCACAGATACGCAG		
Vβ1-2	C A S S P R A A E D A	D T Q		Jβ2.3
	AGTGCCAGCAGC CCTCGGGCAGCGGAGGATG	CAGATACGCAG		
Vβ6-1	C A S S L I L R G	E Q		Jβ2.1
	TGTGCCAGCAGC TTAATCCTTCGGGG	TGAGCAG		
Vβ6-2	C A S S V F I Q P	E Q		Jβ2.1
	TGTGCCAGCAGC GTATTCATTCAGCC	TGAGCAG		
Vβ13-1	C A S S G L L G T	Y N E Q		Jβ2.1
	TGTGCCAGCAGT GGCCTACTCGGGA	CCTACAATGAGCAG		
Vβ13-2	C A S R G P E	E T Q		Jβ2.5
	TGTGCCAGCAG AGGGCCAG	AAGAGACCCAG		
Vβ13-3	C A S G Q T A	Y E Q		Jβ2.7
	TGTGCCAGC GGACAGACAG	CCTACGAGCAG		
SS-82				
Vβ9-1	C A S S A R Y D	E Q		Jβ2.1
	TGTGCCAGCAGC GCAAGGTATG	ATGAGCAG		
Vβ9-2	C A S S G P R D I	S Y E Q		Jβ2.7
	TGTGCCAGCAGC GGCCCCAGGGACAT	CTCCTACGAGCAG		
Vβ9-3	C A S S I D F	Y E Q		Jβ2.7
	TGTGCCAGCAGC ATTGACTT	CTACGAGCAG		
SS-86				
Vβ10-1	C A S S V P N R	Q P Q		Jβ1.5
	TGTGCCAGCAGC GTCCCGAACCGA	CAGCCCCAG		
Vβ10-2	C A R E T G G	E L		Jβ2.2
	TGTGCCAG GGAGACAGGGGGA	GAGCTG		
Vβ10-3	C A S S Y R A G L	T Q		Jβ2.5
	TGTGCCAGCAGC TACAGAGCGGGCCT	GACCCAG		

Junctional sequences of TCR Vβ genes, which are only found in lacrimal glands, from four SS patients were analyzed. The single letter amino acid codes of the 3′ position of TCR Vβ, CDR3, and the 5′ position of the J region are given.

be an autoantigen recognized by auto-reactive T cells such as glutamic acid decarboxylase (GAD) in patients with insulin-dependent diabetes (24) and snRNP protein in mixed connective tissue disease (MCTD) patients (25). A second possibility is that Epstein-Barr virus (EBV) might be an autoantigen. The EBV genome has been detected in both salivary and lacrimal gland biopsies at high frequency (26, 27). Especially, the EBVgp110 protein has the sequence QKRAAQRAA, which

is highly homologous to the HLA-DRB1*0401 allele, and it is possible that gp110 triggers autoimmunity through a molecular mimicry mechanism (28). However, this is not likely in our study, since none of the four SS patients presented the HLA-DRB1*0401 allele and there is no direct evidence that EBV reactive T cells are present in both organs and involved in the generation of SS. Third, retroviruses such as human T cell leukemia virus type I (HTLV-I) (29–31), human immunodeficiency virus (HIV) type 1 (HIV-1) (32), or A-type retrovirus (33) might be etiologically associated with SS. Although RNA expression and the genomes of infectious and endogenous retroviruses have been detected in salivary glands, there are no reports in lacrimal glands. However, it is interesting that the YSA sequence motif in CDR3 region on common TCR from an SS-54 patient was found in TCR Vβ13 clone in a salivary gland from one patient with HIV-1 infection (43). Further examination on the expression of retroviruses in eyes is necessary to clarify this possibility.

In conclusion, some T cells recognize shared epitopes on autoantigens from both labial salivary and lacrimal glands from patients with SS. The establishment of T cell transfectants or T cell lines from infiltrating T cells in several organs should shed light on the targets recognized by auto-reactive T cells. Identification of autoantigens at the amino acid level may give rise to a new specific therapeutic approach to SS via the selective inactivation of auto-reactive T cells by vaccination with analog peptides.

Acknowledgments

We thank Miss Tomoko Nakakita for her technical assistance, and Dr. Margaret Dooley Ohto for her critical reading of the manuscript.

This work was supported in part by grants from the Japanese Ministry of Health and Welfare, and the Japanese Ministry of Science and Culture.

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