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Dominant and Shared T Cell Receptor β Chain Variable Regions of T Cells Inducing Synovial Hyperplasia in Rheumatoid Arthritis

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Previously, we demonstrated the presence of at least two distinct subpopulations of patients with rheumatoid arthritis (RA) employing a cell-transfer experiment using severe combined immunodeficient (SCID) mice. One group of patients, whose T cells derived from the rheumatoid joints, induced synovial hyperplasia (SH) in the SCID mice (the positive group). The other group did not display the induction of SH (the negative group). TCR/Vβ gene usage analysis indicated that some dominant T cell subpopulations were oligoclonally expanding only in the rheumatoid joints, and not in the periphery of the patients of the positive group. Moreover, these T cell subpopulations were not seen in the joints of patients in the negative group or in non-RA patients. In addition, the preferential uses of certain TCR/Vβs (Vβ8, Vβ12, Vβ13, and Vβ14) genes were demonstrated in these T cells. In this study, to investigate whether these T cells are driven by a certain antigen(s), the third complementarity determining regions (CDR3s) of TCR/Vβ, especially Vβ8 and Vβ14 PCR products, were cloned and sequenced. As a result, a dominant CDR3 sequence, CASS-PRERAT-YEQ, was found in Vβ14+ T cells from the rheumatoid joint of a patient (Patient 1) of the positive group with a Vβ14 skew. The identical CDR3 sequence also predominated in Vβ14+ T cells from the rheumatoid joint of another patient (Patient 7) of the positive group with a Vβ14 skew. In addition, in the patients (Patients 4, 7, 8) of the positive group with a Vβ8 skew, other dominant CDR3 sequences, CASS-ENS-YEQ and CASS-LTEP-DTQ, were found as in the case of Vβ14. However, no identical CDR3 sequences were detected dominantly in the joints of the patients in the negative group or in non-RA patients. A Vβ14+ T cell clone (TCL), named G3, with the identical CDR3 sequence, CASS-PRERAT-YEQ, was isolated successfully from Patient 1, and cell transfer of G3 with autologous irradiated peripheral mononuclear cells induced SH in the SCID mice. Taken together, these results suggest that T cells inducing SH, thought to be pathogenic for RA, might be driven by a certain shared antigen(s).

Key Words: **TCR/Vβ; CDR3; sequence analysis; T cell clone; antigen.**

RA is one of the most common chronic inflammatory diseases characterized by destructive polyarthritis (1). Although its etiology remains unknown, several lines of evidence suggest the critical role of CD4+ T cells in the pathogenesis (2). However, the causative antigen(s), which stimulates the pathogenic T cells has not been identified yet. So that, one would think one of the ultimate goals of studies in RA is to identify the causative antigen(s). Analysis of TCR has been thought by many investigators to be a promising approach to identify the pathogenic T cells on the basis of the antigen-driven nature of the response. In fact, this approach has been extensively attempted in RA as well as in other T cell mediated autoimmune diseases (3–11).

Abbreviations used: TCR/Vβ, T cell receptor b chain variable regions; SH, synovial hyperplasia; RA, rheumatoid arthritis; CDR, complementarity determining region; SCID, severe combined immunodeficiency; TCL, T cell clone; SF, synovial fluid; ST, synovial tissue; RT-PCR, reverse transcription-polymerase chain reaction; MNC, mononuclear cell; AGPC, acid guanidinium thiocyanate phenol chloroform; SEB, staphylococcus Enterotoxin B.

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Although most investigators have described the presence of oligoclonal T cell subpopulations in the rheumatoid joints, these results have not been conclusive yet and sometimes conflicting. On the other hand, recent growing evidences have suggested the presence of non-T cell mediated mechanism in the pathogenesis of RA (12–14). Therefore, the approach to identify the pathogenic T cells by analysis of TCR could be applied for only the patients with T cell mediated RA but not the patients with non-T cell mediated RA, since it stems from the observation that the TCR repertoire of T cells responsive to a given antigen is typically limited.

Previously, we reported a new means to distinguish T cell mediated RA from non-T cell mediated RA by a cell-transfer experiment using SCID mice (15). The analysis of TCR repertoire in the separated groups, T cell mediated and non-T cell mediated RA revealed some distinct skews in TCR/Vβ gene usage of the synovial T cells from the patients with T cell mediated RA but not those from the patients with non-T cell mediated RA. Moreover, these T cells with a skewed TCR/Vβ were expanding oligoclonally in the rheumatoid joints but not in the periphery. In addition, the cell-transfer experiment demonstrated these T cells could induce SH.

In this study, to investigate whether or not these T cells with a certain skewed TCR/Vβ are driven by a certain antigen(s), the CDR3 segments of the skewed TCR/Vβs were sequenced.

METHODS

Patients and synovial samples. Human experimentation (use of patients' synovial fluids and synovial tissues) in this study has been approved since May 27, 1992 by the Ethical Committee of Osaka University Medical School. We obtained samples from 41 patients with informed consent according to the guidelines of the Ethical Committee. All patients with RA fulfilled the 1987 standard diagnostic criteria proposed by the American Rheumatism Associations, being followed at Osaka-Minami National Hospital or the Department of Medicine III, Osaka University Medical School. Synovial fluids (SFs) were obtained by intra-articular puncture. Synovial tissues (STs) were obtained from the affected joints at the surgical treatment. At the time of sampling all patients had active inflammatory diseases. The patients with T cell-mediated RA were determined by the cell-transfer experiment using SCID mice as described previously (15). Eleven patients were selected as T cell mediated RA (the positive group) out of 41 patients. Nine out of 11 positive patients were subjected to further TCR/Vβ genes usage analysis by reverse transcription-PCR (RT-PCR).

TCR/Vβ gene usage analysis. Total RNA was extracted from the infiltrating mononuclear cells (MNC) (SFMNC; synovial fluid MNC, STMNC; synovial tissue MNC) by acid guanidinium thiocyanate phenol chloroform (AGPC) method as described elsewhere (16). Vβ gene segment usage was determined by RT-PCR with each Vβ specific primer and a Cβ specific primer as previously described (15). Amplified products were separated on 5–15% polyacrylamide gels. The quantitative analysis of the amplified products with the ^{32}P end-labeled 3′ primers was made by Image analyzer (Fujix BAS 2000, Fuji Film I&I Co. Tokyo, Japan). The selective expression of TCR/Vβ genes was determined by normalization of the density of the Vβ bands to that of the Cβ band as an internal control.

TABLE 1

HLA-DR Haplotypes and Skewed TCR/Vβs in RA Patients of the Positive Group

Patient	HLA-DR	Skewed TCR/Vβ
1	DR2/DR4	Vβ 14
2	DR4/DRw15	Vβ 8
3	DR1/DRw8.1	Vβ 12, Vβ 13
4	DR4/-	Vβ 8, Vβ 13
5	DR9/-	Vβ 12, Vβ 13, Vβ 17
6	DR4/-	Vβ 8, Vβ 12, Vβ 13
7	DR4/-	Vβ 8, Vβ 14
8	DRw8.2/DRw15	Vβ 7, Vβ 8
9	DR9/-	Vβ 6, Vβ 12

Note. 11 of 41 RA patients were selected as patients in the positive group for the cell-transfer experiment using SCID mice as reported previously (15). 9 out of 11 positive patients were subjected to TCR/Vβ gene usage analysis in synovial MNCs by RT-PCR as described under Methods. Skewed TCR/Vβs were defined when relative expression of a certain Vβ to Cβ exceeded 20%. HLA-DR haplotypes were determined serologically.

TCR/Vβ CDR3 segment sequence analysis. cDNA from the MNC or T cell clones (G3) was amplified with a Cβ primer and the appropriate Vβ primer as previously described (15). The amplified PCR products were ligated into PCR II Vector using TA cloning Kit (Invitrogen, San Diego, CA) according to the manufacturer's instructions. The ligation mixture was transformed into *E. coli* cell. Ampicillin-resistant colonies were selected and miniprep DNA was prepared by standard methods. The plasmid DNA was then sequenced directly by the dideoxy chain termination method (17–18). The sequence data were confirmed by the repeated experiments (more than twice) using the samples prepared at different time points.

T cell cloning. STMNCs from Patient 1, a patient of the positive group with a Vβ14 skew were activated *in vitro* with 100 μg/ml of Staphylococcus Enterotoxin B (SEB), a superantigen which can stimulate Vβ14+ T cells for 72 h at a concentration of 1×10^6 cells/ml and then, cloned at a concentration of one cell/well in IL-2 (50 U/ml of rIL-2; Takeda Pharmaceutical Co., Osaka, Japan) containing medium by the limiting dilution method in the presence of 3×10^4 cells/well of irradiated autologous PBMNC. After 10 days, growing wells were selected under microscopic observation and expanded with Con A stimulation and subsequent culture in IL-2 containing medium. Established T cell clones (or lines) were maintained by periodical stimulation with Con A and subsequent culture with IL-2.

Cell-transfer experiment using SCID mice. 2×10^5 T cell clones (G3, D2 and G2) from Patient 1 with 2×10^6 irradiated autologous PBMNC, or 1×10^6 STMNCs and PBMNCs (with or without PHA stimulation) from the same patient suspended in 50 μl of HBSS, were transferred directly into bilateral knee joints of the posterior legs of the SCID mice by intra-articular injection as described previously (15). Four weeks after the cell-transfer, the SCID mice were killed with anesthesia. Bilateral knee joints of the posterior legs were removed and subjected to histopathological examinations as previously described (15).

TABLE 2

TCR/Vβ CDR3 Segment Sequences of Skewed Vβs (Vβ14 and Vβ8) in the RA Patients of the Positive Group

			Joint (SFMNC or STMNC)				Periphery (PBM NC)			
			CDR3				CDR3			
Patient	HLA-DR	Skewed Vβ	Vβ	NDN	Jβ	Freq.	Vβ	NDN	Jβ	Freq.
1	DR2/DR4	Vβ14	CASS	PRERAT	YEQ (Jβ 2.7)	15/15	CAS	SRGVPE	GYT (Jβ 1.2)	1/16
							CASS	LGSGA	QPQ (Jβ 2.1)	2/16
							CASS	RTGTIP	YNE (Jβ 2.1)	1/16
							CASS	STRQGSR	SYN (Jβ 2.1)	1/16
							CAS	TLLAGGRS	SYN (Jβ 2.2)	1/16
							CASS	PGNYPTGE	LFF (Jβ 2.2)	1/16
							CASS	PGTYPTGE	LFF (Jβ 2.2)	2/16
							CASS	PGTILPGK	LFF (Jβ 2.2)	1/16
							CASS	PGTILPGE	LFF (Jβ 2.2)	2/16
							CASS	PGTIYRE	LFF (Jβ 2.2)	2/16
							CASS	PTSGGTI	DTE (Jβ 2.3)	1/16
							CASS	PGTHP	TDT (Jβ 2.3)	1/16
7	DR4/-	Vβ14	CASS	PRERAT	YEQ (Jβ 2.7)	9/10				
			CASS	PRERATC	EQY (Jβ 2.7)	1/10	N.D.			
8	DRw8.2/DRw15	Vβ8	CASS	ENS	YEQ (Jβ 2.1)	12/13	CASS	GRD	YGY (Jβ 1.2)	1/13
			CASS	LTGGIS	TY (Jβ 2.1)	1/13	CASS	RGQV	YGY (Jβ 1.2)	1/13
							CAS	KTGGSPD	QPQ (Jβ 1.5)	1/13
							CAS	SPQT	NQP (Jβ 1.5)	1/13
							CASS	PQQTGQVVY	BQF (Jβ 2.1)	1/13
							CASS	LTPGVGA	DTQ (Jβ 2.3)	1/13
							CAS	SPRV	QET (Jβ 2.5)	1/13
							CASS	SRITSP	YEQ (Jβ 2.7)	1/13
							CASS	LSASS	YEQ (Jβ 2.7)	1/13
							CASS	LAGT	YEQ (Jβ 2.7)	1/13
							CASS	PSGL	YEQ (Jβ 2.7)	1/13
							CASS	LTTSA	YEQ (Jβ 2.7)	1/13
							CAS	RPTGQGD	YEQ (Jβ 2.7)	1/13
7	DR4/-	Vβ8	CASS	ENS	YNE (Jβ 2.1)	4/5				
			CASS	LTGTGI	SYE (Jβ 2.1)	1/5	N.D.			
4	DR4/-	Vβ8	CASS	SLGQG	DTE (Jβ 1.1)	2/10				
			CASS	LTEP	DTQ (Jβ 2.3)	4/10	N.D.			
			CASS	LYLR	TDT (Jβ 2.3)	2/10				
			CASS	ENS	YEQ (Jβ 2.1)	2/10				
2	DR4/DRw15	Vβ8	CASS	PSRD	SPL (Jβ 1.6)	1/13	CASS	EKGQDK	TEA (Jβ 1.1)	1/10
			CASS	LTEP	DTQ (Jβ 2.3)	11/13	CASS	SGGYG	EAF (Jβ 1.1)	2/10
			CASS	LYLR	DTQ (Jβ 2.3)	1/13	CAS	SPSRD	SPL (Jβ 1.6)	2/10
							CASS	AGHY	SPL (Jβ 1.6)	1/10
							CASS	LGGGPRQF	EQF (Jβ 2.1)	2/10
							CAS	SPDSSH	EQY (Jβ 2.7)	2/10

Note. CDR3s of Vβs (Vβ14 and Vβ8) PCR products of synovial and peripheral T cells derived from two patients with Vβ14 skew (Patient 1 and Patient 7) and four patients with Vβ8 skew (Patient 8, Patient 7, Patient 4, and Patient 2) were cloned and sequenced as described under Methods. Predicted amino acid sequences are shown. Underlined sequences were common among the multiple patients. SFMNC, synovial fluid mononiclear cell; STMNC, synovial tissue mononuclear cell; N.D., not done.

RESULTS

HLA-DR haplotypes and skewed TCR/Vβs in RA patients of the positive group. In previous study, we demonstrated the presence of at least two distinct subpopulations of RA patients by the cell-transfer experiments using SCID mice. One is a group of the patients, whose T cells derived from the joints induced SH in the SCID mice (the positive group). The other is a group did not display the induction of SH (the negative group). Eleven out of 41 patients were selected as a patient of the positive group. Nine out of 11 positive patients were subjected to TCR/Vβ genes usage analysis by RT-PCR. TCR/Vβ gene usage analysis suggested that some T cell subpopulations, inducing SH were oligoclonally expanding in the rheumatoid joints of the patients of the positive group but not of the negative group.

HLA-DR haplotypes and skewed TCR/Vβs in 9 pa-

TABLE 3

TCR/Vβ CDR3 Segment Sequences of Vβ14 and Vβ8 in Synovial T Cells of the RA Patients of the Negative Group

			Vβ14				Vβ8			
			CDR3				CDR3			
Patient	HLA-DR	Cell source	Vβ	NDN	Jβ	Fre	Vβ	NDN	Jβ	Fre
19	DR4/-	SFMNC	CASS	PRERAT	YEQ (Jβ 2.7)	1/12	CASS	LRPT	YEQ (Jβ 2.7)	1/8
			CASSC	SDRGVPE	GYT (Jβ 1.2)	3/12	CASS	LSSGS	SYE (Jβ 2.7)	2/8
			ASS	LGSGA	QPQ (Jβ 1.5)	2/12	CASC	RRRT	STID (Jβ 2.3)	1/8
			CASS	FRGG	SYE (Jβ 2.7)	2/12	ASS	LISGAR	BQY (Jβ 2.7)	1/8
			CASS	LTSRV	YEQ (Jβ 2.7)	1/12	CASS	LEAGGPQ	TQY (Jβ 2.3)	1/8
			CASS	QLEAG	BQY (Jβ 2.7)	1/12	CAS	ATGG	NSP (Jβ 1.6)	1/8
			CASS	KGGA	YEQ (Jβ 2.7)	1/12	CASS	LGDS	QBQ (Jβ 2.7)	2/8
			CA	NSTGEG	YEQ (Jβ 2.7)	1/12				
			CA	IGGAGLS	SYE (Jβ 2.7)	1/12				
21	DR4/DR1	SFMNC	CASS	KQGT	TGY (Jβ 1.2)	1/14	CASS	SA	NYG (Jβ 1.2)	5/11
			CASS	SRAT	YGY (Jβ 1.2)	1/14	CASS	DWPSGRHWG	GEL (Jβ 2.2)	1/11
			CAS	HSTGD	YGY (Jβ 1.2)	1/14		LA		
			CASS	LGGG	QPQ (Jβ 1.5)	1/14	CASS	FRR	YDT (Jβ 2.3)	1/11
			CASS	LYE	YNE (Jβ 2.1)	1/14	CAS	TPGRD	SYE (Jβ 2.7)	1/11
			CASS	LCRD	TGE (Jβ 2.2)	1/14	CASS	SNREG	YNE (Jβ 2.1)	1/11
			CASS	LSLARN	YEQ (Jβ 2.7)	1/14	CASS	SYEGQA	YNS (Jβ 1.6)	1/11
			CASS	SGGLAGK	YBQ (Jβ 2.7)	1/14	CAS	HRAF	NQP (Jβ 1.5)	1/11
			CASS	LDPAFV	GPG (Jβ 2.7)	1/14				
			CASS	FGGS	BQY (Jβ 2.7)	1/14				
			CASS	RSRAAH	BQY (Jβ 2.7)	1/14				
			CASS	YL	YBQ (Jβ 2.7)	1/14				
			CASS	FEGQGRN	BQY (Jβ 2.7)	1/14				
			CASS	NPTIKYIR	YBQ (Jβ 2.7)	1/14				
32	DR4/-	SFMNC	CASS	FTD	YGY (Jβ 1.2)	1/15	CASS	SLGQGDT	EAF (Jβ 1.1)	1/14
			CASS	LSTGY	YGY (Jβ 1.2)	1/15	CASS	PTGV	TDT (Jβ 2.3)	1/14
			CASS	TTG	YGY (Jβ 1.2)	1/15	CASS	TGTTSGG	TDT (Jβ 2.3)	2/14
			CASS	LDL	NQP (Jβ 1.5)	1/15	CASS	YNTGLIM	ETQ (Jβ 2.5)	2/14
			CASS	GQAGS	PQH (Jβ 1.5)	1/15	CASS	LIASGRD	EQF (Jβ 2.1)	1/14
			CASS	VTGTF	NSP (Jβ 1.6)	1/15	CASS	TSGS	IQY (Jβ 2.4)	1/14
			CASS	TAS	YNE (Jβ 2.1)	1/15	CASS	TGLAGM	SYE (Jβ 2.7)	1/14
			CASS	LDS	YNE (Jβ 2.1)	1/15	CASS	PRDRG	TEA (Jβ 2.1)	1/14
			CASS	KLAGY	SNY (Jβ 2.1)	1/15	CASS	RPQGH	EQY (Jβ 2.7)	1/14
			CASS	SGEGF	EQY (Jβ 2.7)	1/15	CASS	SLET	EQY (Jβ 2.7)	1/14
			CASS	VGFRA	YEQ (Jβ 2.7)	1/15	CASS	ARQE	NSP (Jβ 1.6)	1/14
			CASS	PQVN	YEQ (Jβ 2.7)	1/15	CAS	RLHPGTE	QYF (Jβ 2.3)	1/14
			CASS	LWPG	EQY (Jβ 2.7)	1/15				
			CASS	TIPGQG	YEQ (Jβ 2.7)	1/15				
			CASS	TASPTGVG	EQY (Jβ 2.7)	1/15				
39	DR4/-	SFMNC	CASS	YEQETQY	FGP (Jβ 2.5)	1/4	CASS	LPPLGYGY	TGE (Jβ 2.2)	1/6
			CASS	KLAG	EQY (Jβ 2.7)	1/4	CAS	TG	KDI (Jβ 2.4)	1/6
			CASS	PGLAG	YEQ (Jβ 2.7)	1/4	CASS	YEQETQY	FGP (Jβ 2.5)	1/6
			CASS	TGLNI	QYF (Jβ 2.7)	1/4	CASS	KLAG	EQY (Jβ 2.7)	1/6
							CASS	PGLAG	YEQ (Jβ 2.7)	1/6
							CASS	TGKDI	QYF (Jβ 2.7)	1/6

Note. CDR3s of Vβ14 and Vβ8 PCR products of synovial T cells derived from three patients of the negative group with HLA-DR4 haplotypes were cloned and sequenced as described under Methods. SFMNC, synovial fluid mononuclear cell.

tients of the positive group are summarized in Table 1. Five out of 9 patients were positive for DR4. The skewed TCR/Vβs were not common but preferential to certain Vβs (Vβ8, Vβ12, Vβ13 and Vβ14). In addition, although there were no obvious associations between HLA-DR haplotypes and skewed Vβs, two patients (Patient 1, 7) with a Vβ14 skew were positive for DR4.

TCR/Vβ CDR3 segment sequence analysis in the skewed Vβs (Vβ14 and Vβ8) of the RA patients of the positive group. To examine whether or not T cell subpopulations with a certain skewed Vβ from the rheumatoid joints of the patients of the positive group were driven by certain antigen(s), CDR3 segments of the skewed Vβs (Vβ14 and Vβ8) PCR products of synovial

TABLE 4

TCR/Vβ CDR3 Segment Sequences of Vβ14+ and Vβ8+ Synovial T Cells in the Non-RA Patients

			Vβ14				Vβ8			
			CDR3				CDR3			
Patient	HLA-DR	Cell source	Vβ	NDN	Jβ	Freq.	Vβ	NDN	Jβ	Freq.
Polyarthritis	DR9/DRw12	SFMNC	CA	IGDSTV	NTE (Jβ 1.1)	1/16				
			CA	ISGGRDAD	TEA (Jβ 1.1)	1/16				
			CA	IGSREHL	SYN (Jβ 2.1)	1/16				
			CA	IREEVGES	SYN (Jβ 2.1)	2/16				
			CA	TSHQA	NEQ (Jβ 2.1)	2/16	N.D.			
			CASS	YGV	EQF (Jβ 2.1)	1/16				
			CA	ISDVLQREGR	DTE (Jβ 2.3)	1/16				
			CASS	EILED	IQY (Jβ 2.4)	1/16				
			CA	IRPGTGD	EQY (Jβ 2.7)	1/16				
			CA	ISENSGSGE	QYF (Jβ 2.7)	2/16				
			CA	ITTTGARIP	YEQ (Jβ 2.7)	1/16				
			CASS	LRALQG	SYE (Jβ 2.7)	2/16				
OA#1	DRw13/-	SFMNC	CASS	FGSG	YEQ (Jβ 2.7)	1/11	CASS	LSG	NEQ (Jβ 2.1)	2/10
			CASS	FGSP	YEQ (Jβ 2.7)	2/11	CASS	FG	TDT (Jβ 2.3)	2/10
			CAS	FSSG	YEQ (Jβ 2.7)	1/11	CASS	LGGNQ	EQY (Jβ 2.5)	2/10
			CASS	FSSP	YEQ (Jβ 2.7)	2/11	CAS	SRITSP	YEQ (Jβ 2.7)	4/10
			CASS	LVGGT	YEQ (Jβ 2.7)	2/11				
			CASS	SYRGGG	SPL (Jβ 1.5)	1/11				
			CASS	SGQGFG	QYF (Jβ 2.7)	1/11				
			CASS	VGSG	YEQ (Jβ 2.7)	1/11				
OA#2	DR4/DR9	SFMNC	CASS	PGQGAPLS	TEA (Jβ 1.1)	1/15	CASS	SSVGGD	TEA (Jβ 1.1)	2/15
			CASS	SRGY	TEA (Jβ 1.1)	1/15	CASS	SSTGGD	TEA (Jβ 1.1)	1/15
			CASS	S	NYG (Jβ 1.2)	4/15	CASS	PFGQGTKD	TEA (Jβ 1.1)	1/15
			CAS	RTTSRS	SYE (Jβ 2.7)	4/15	CASS	LTGVY	TEA (Jβ 1.1)	1/15
			CASS	SWTS	YEQ (Jβ 2.7)	2/15	CASS	AFGP	SGN (Jβ 1.3)	1/15
			CASS	LSSG	YEQ (Jβ 2.7)	1/15	CAS	TMT	STDST (Jβ 2.3)	1/15
			CAS	GSSSAS	SYE (Jβ 2.7)	1/15	CAS	RMT	D (Jβ 2.3)	1/15
			CASS	FQTPS	YEQ (Jβ 2.7)	1/15	CASS	PQE	TDS (Jβ 2.3)	1/15
							CASS	NDYGSLS	GAN (Jβ 2.6)	2/15
							CASS	NVRG	YEQ (Jβ 2.7)	1/15
							CASS	LERG	YEQY (Jβ 2.7)	1/15
							CASS	LVRG	BQ (Jβ 2.7)	1/15
							CAS	TAVQET	QYF (Jβ 2.7)	1/15
OA#3	DRw15/-	SFMNC	CASS	FTGFFG	QPQ (Jβ 1.5)	7/18	CASS	LS	SYE (Jβ 2.7)	13/14
			CASS	VRGG	SYE (Jβ 2.7)	1/18	CASS	LSS	YEQ (Jβ 2.7)	1/14
			CASS	LKGQGVYLG	NYG (Jβ 1.5)	4/18				
			CASS	ARGG	SYE (Jβ 2.7)	2/18				
			CASS	FTGFGQPR	HFG (Jβ 1.5)	1/18				
			CASS	FRGFG	QPQ (Jβ 1.5)	1/18				
			CASS	QRGGS	YEQ (Jβ 2.7)	1/18				
			CASS	SRDNLYK	QYE (Jβ 2.7)	1/18				
OA#4	N.T.	SFMNC	CASS	FGGVY	TEA (Jβ 1.1)	2/15	CASS	RWWKGP	TEA (Jβ 1.1)	1/13
			CASS	SPGRGN	EAF (Jβ 1.1)	1/15	CASS	LFPG	GYT (Jβ 1.2)	2/13
			CASS	LTA	YGY (Jβ 1.2)	1/15	CASS	EDTGDNF	GYT (Jβ 1.2)	1/13
			CASS	LEV	YGY (Jβ 1.2)	1/15	CASS	NGPRGVA	QPQ (Jβ 1.5)	1/13
			CASS	AQGK	YGY (Jβ 1.2)	1/15	CASS	PQV	YNE (Jβ 2.1)	1/13
			CASS	LLPPQA	GYT (Jβ 1.2)	1/15	CA	GSNQVAGS	TET (Jβ 2.3)	1/13
			CASS	LGGRD	QPQ (Jβ 1.5)	1/15	CASS	DQH	SYE (Jβ 2.7)	1/13
			CASS	GDIL	NSP (Jβ 1.6)	1/15	CASS	NGGTSLQ	QYF (Jβ 2.7)	1/13
			CASS	RRG	NSP (Jβ 1.6)	1/15	CASS	QVTGY	EQY (Jβ 2.7)	1/13
			CASS	LSLAGDP	KNI (Jβ 2.4)	1/15	CASS	LTENQET	QYF (Jβ 2.7)	1/13
			CA	IGTE	YEQ (Jβ 2.7)	1/15	CASS	LAGLN	EQY (Jβ 2.7)	1/13
			CASS	LGVAGG	YEQ (Jβ 2.7)	1/15	CASS	PGRT	YEQ (Jβ 2.7)	1/13
			CASS	GPGQRGD	EQY (Jβ 2.7)	1/15				
			CASS	TGLAV	SYE (Jβ 2.7)	1/15				

TABLE 4—*Continued*

			Vβ14				Vβ8			
			CDR3				CDR3			
Patient	HLA-DR	Cell source	Vβ	NDN	Jβ	Freq.	Vβ	NDN	Jβ	Freq.
OA#5	N.T.	SFMNC	CASS	YWGAT	YEQ (Jβ 2.7)	5/7	CASS	SQGGS	EQF (Jβ 2.1)	1/15
			CASS	LTVDSS	YEQ (Jβ 2.7)	2/7	CAS	RP	GAN (Jβ 2.6)	1/15
							CAS	QGDTE	NSP (Jβ 1.6)	1/15
							CAS	RPASFQ	TFG (Jβ 2.6)	1/15
							CAS	RGTGSYRE	SRE (Jβ 2.7)	1/15
							CASS	LVRG	YEQ (Jβ 2.1)	3/15
							CASS	FGLD	NEQ (Jβ 2.7)	1/15
							CASS	LPIAW	SYE (Jβ 2.7)	1/15
							CASS	IVRG	YEQ (Jβ 2.7)	1/15
							CASS	LRQERLAGS	SYN (Jβ 2.1)	2/15
							CASS	GRFVQN	FGP (Jβ 2.7)	1/15
							CASS	LVTG	YEQ (Jβ 2.7)	1/15

Note. CDR3s of either Vβ14 or Vβ8 PCR products of synovial T cells derived from six non-RA patients (including five osteoarthritis patients) were cloned and sequenced as described under Methods. Predicted amino acid sequences are shown. N.T., not tested; N.D., not done; Freq., frequency.

T cells from two patients (Patient 1, 7) with a Vβ14 skew and three patients (Patient 4, 7, and 8) with a Vβ8 skew were cloned and sequenced as described in Methods. As a result shown in Table 2, a dominant CDR3 sequence, CASS-PRERAT-YEQ was found in Vβ14+ T cells from the rheumatoid joints of Patient 1 (15 positive out of 15 clones). On the other hand, the CDR3 sequences were heterogenous and no identical sequence with CASS-PRERAT-YEQ was not seen in Vβ14+ T cells from the periphery of the same patient. The identical sequence also predominated in the joint of the other patient (Patient 7) of the positive group with a Vβ14 skew. In three patients (Patients 4, 7, 8) of the positive group with a Vβ8 skew, other dominant sequences (CASS-ENS-YEQ and CASS-LTEP-DTQ) were found only in the joints as a case of Vβ14. Thus, three dominant CDR3 sequences were detected in synovial T cells derived from RA patients of the positive group with a Vβ14 or a Vβ8 skew and they were shared by multiple RA patients.

TABLE 5

T Cell Clones (or lines) from Patient 1 and Their TCR/Vβ

T cell clones (or lines)	Vβ
A1	12,15
A2	14,17
A5	14,17
B2	17
B3	14,17
B5	17
D2	17
E4	12,14,17
E5	12
E6	N.T.
F2	12
F4	12
F5	12,17
F6	15
G1	12,14
G2	17
G3	14
G4	20

Note. To isolate a T cell clone with the shared and dominant CDR3 sequence, STMNCs from Patient 1 were *in vitro* stimulated with SEB and subsequently cloned by the limiting dilution method as described under Methods. 18 clones (or lines) were established and their TCR/Vβs were determined by RT-PCR method. G3 (underlined) was a Vβ14 T cell clone. All T cell clones (or lines) were positive for CD4.

Dominant and shared CDR3 sequences are unique for synovial T cells with a skewed Vβ of RA patients of the positive group. To examine whether or not the dominant and shared CDR3 sequences are unique for synovial T cells with a skewed Vβ (Vβ14 or Vβ8) of RA patients of the positive group, CDR3 segment sequence analysis was performed in Vβ14+ or Vβ8+ synovial T cells from either three RA patients of the negative group with a matched HLA-DR haplotype (HLA-DR4) or seven non-RA patients, including six patients with osteoarthritis.

As a result, regarding Vβ14, although the identical sequence was detected in only one patient (Patient 19) of the negative group, it was not dominant (one positive out of 12 clones) and the CDR3 sequences were quite heterogeneous (Table 3). No identical sequence was detected in either the other RA patients (Pateint 21, 32) of the negative group (Table 3) or non-RA patients (Table 4). In the case of Vβ8, the CDR3 sequences were

TABLE 6

DNA Sequence and Predicted Amino Acid Sequence of TCR/Vβ CDR3 Segment of T Cell Clone, G3

Vβ	NDN	Jβ (2.7)
TGT-GCC-AGC-AGT-	-CCA-CGA-GAG-CGG-GCA-ACC-	-TAC-GAG-CAG-TAC-TTC
C A S S	P R E R A T	Y E Q Y F

Note. CDR3 of Vβ14 PCR products of G3 was cloned and sequenced as described under Methods. DNA sequence and predicted amino acid sequence are shown.

hetrogeneous and no identical sequence was detected (Table 3, 4). Thus, the shared and dominant CDR3 sequences were unique for Vβ14+ or Vβ8 synovial T cells from RA patients of the positive group.

Isolation of T cell clones (TCLs) from synovial T cells of Patient 1 with a Vβ14 skew of the positive group. To isolate a TCL with the shared and dominant CDR3 sequence, STMNCs from Patient 1 were *in vitro* stimulated with SEB and then, cloned by the limiting dilution method as described in Methods. Although some of which had not been cloned yet because of only one passage of limiting dilution culture, eighteen TCLs were established and their TCR/Vβs were determined by the RT-PCR method (Table 5). Among 18 TCLs, one TCL, named G3 was positive for Vβ14. All TCLs were positive for CD4 (data not shown).

Next, to examine whether or not the CDR3 sequence of G3 was identical for the shared and dominant sequence, the CDR3 PCR product of G3 was sequenced. As a result, the CDR3 sequence of G3 was identical for the shared and dominant sequence, CASS-PRERAT-YEQ as shown in Table 6.

G3 induces SH in the SCID mice. To examine whether or not G3 can induce SH, G3 was transferred into SCID mice with irradiated autologous PBMNC by intra-articular injection and the induction of SH were determined histopathologically as described in Methods. Two Vβ17 + TCLs from the same patients, G2 and D2 listed in Table 5, were used for control TCLs. As shown in Fig. 1 and Table 7, definite SH was observed in two out of three mice transferred with G3, while SH was not observed in any mice transferred with the

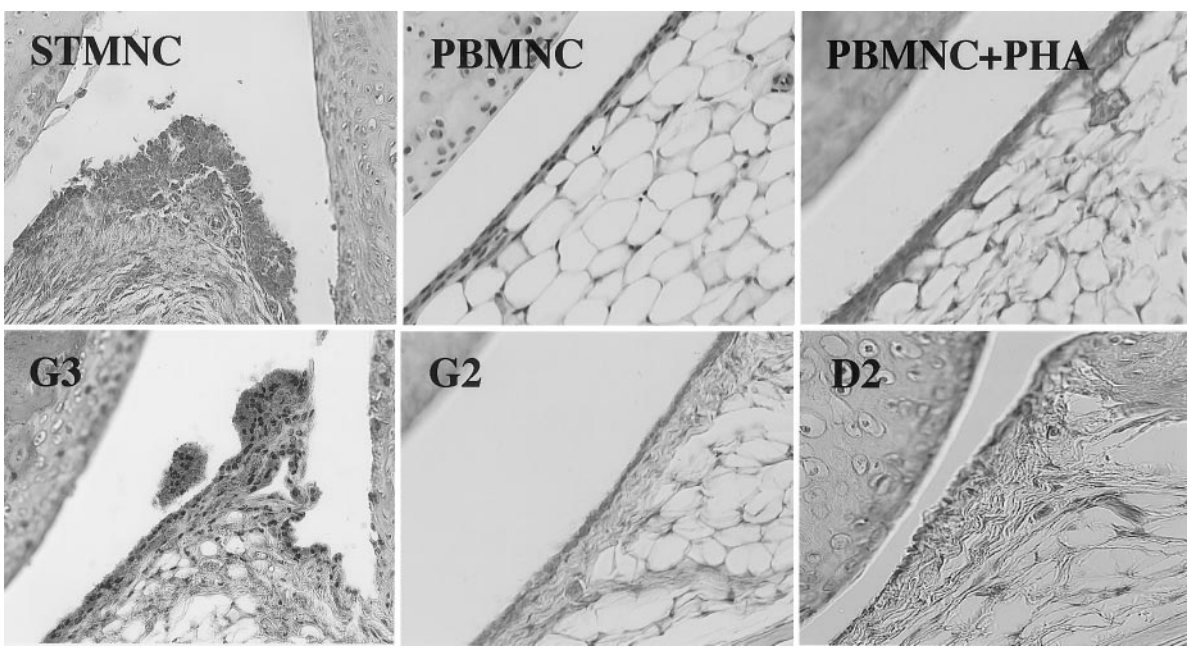

FIG. 1. TCL G3 Induces Synovial Hyperplasia. 2×10^5 of TCL G3 with 2×10^6 of irradiated autologous PBMNC suspended in 50 μl of saline were transferred directly into bilateral knee joints of the posterior legs of each SCID mouse by intra-articular injection as described previously (15). Four weeks after the cell transfer, the SCID mice were killed with anesthesia. Bilateral knee joints were removed and subjected to histopathological examinations as described previously (15). Two TCLs D2 and G2 (Vβ17+) derived from the same patient were used as a control. In addition, 1×10^6 of STMNCs and PBMNCs (with or without PHA stimulation) from the same patient were also transferred into the SCID mice following the same method. Representative histopathological changes of synovia of the injected knee joints in each group are shown.

TABLE 7

Incidence of Synovial Hyperplasia (SH) in the Cell-Transfer Experiment

	Cells transferred					
	STMNC	PBMNC (−)	PBMNC (PHA)	G3	D2	G2
Incidence of SH Positive/total mice	3/6	0/3	0/3	2/3	0/3	0/3

Note. The cell-transfer experiments were performed using SCID mice as described under Methods. SH was assessed by histopathological examinations and "positive" was defined when SH was observed in at least one of the eight serial slices from the two injected knee joints of each mouse. STMNC, synovial tissue mononuclear cell; PBMNC, peripheral blood mononuclear cell; PHA, phytohemagglutinin. G3, D2, and G2 were CD4+ T cell clones derived from the same patients (Patient 1) as listed in Table 5. G3 was a Vβ14+ clone with the dominant and shared CDR3 sequence, CASS-Prerat-YEQ. D2 and G2 were Vβ17+ clones and used as a control.

control TCLs. Thus, G3 induced SH in the SCID mice. In addition, STMNCs from the same patient in which the clones with the dominant and shared CDR3 sequence, CASS-PRERAT-YEQ were dominated, also induced SH whereas PBMNCs from the same patient, in which the clones with such dominant and shared CDR3 sequence were not detected, did not induce SH under the condition with or without mitogen (PHA) stimulation. Taken together, these results suggest that T cells with the dominant and shared CDR 3 sequence, such as CASS-PRERAT-YEQ is important for the induction of SH.

DISCUSSION

In this study, we reported three dominant and shared CDR3 sequences on Vβ8+ or Vβ14+ T cell subpopulations oligoclonally expanding in the joints of multiple RA patients, thought to be a T cell-mediated RA patient. However, the identical sequence was not detected in the periphery of the same individuals. This suggests that such synovial T cell subpopulations of T cell-mediated RA patients might be driven by a certain local antigen of the joints. Moreover, the identical sequence was never detected in non-RA patients, suggesting that a certain antigen which drives such T cell subpopulations might be specific for RA. In addition, the identical sequence was not detected in two out of three RA patients of the negative group, thought to be a non-T cell mediated RA patient with a HLA-DR4 haplotype and it was not dominant although detected in the rest of the patients. The means to distinguish T cell mediated RA from non-T cell mediated RA employed in this study was a cell-transfer experiment using SCID mice as previously reported (15). Because of the limitations in the technique such as histopathological examinations and injections, a false negative cannot be excluded completely in this means. So that, one patient of the negative group in whom the identical sequence was detected, might not be a true patient of the negative group. Therefore, these result suggest that the dominant and shared CDR3 sequences might be associated with T cell mediation but there were no obvious associations with HLA-DR4 haplotype.

TCR analysis has been extensively attempted by many investigators to seek a pathogenic T cell in RA, and most of them have described the presence of oligoclonal T cell subpopulations in rheumatoid joints (3–11). Some of them have also shown CDR3 sequence data in such oligoclonal T cell subpopulations. So that, to consider the implication of the dominant and shared CDR3 sequences found in this study, we compared with the previous sequence data. Among them, we found an interesting CDR3 sequence. C. M. Weyand and J. J. Goronzy *et al.* have recently described the presence and role of unique oligoclonal CD4+ T cell subpopulations lacked expression of CD28 molecules in RA (19–23). These T cell subpopulations showed autoreactivity and association with extra-articular manifestations. In their series of the publications, they also demonstrated the preferential use of certain Vβs such as Vβ3, Vβ17, Vβ8, and Vβ14 in the above T cell subpopulations (19). In addition, they showed a dominant CDR3 sequence, as described CASS-PRRRAP-SYEQ (Jβ2.7) in such Vβ14+ T cell subpopulation (23). This sequence shows a strong homology with the dominant and shared sequence, CASS-PRERAT-YEQ (Jβ2.7) which we found in Vβ14+ T cell subpopulations of the patients of the positive group in this study. Both N-D-N regions of each CDR3 segment are composed by six amino acid residues with a similar motif. The T cell subpopulations described by them were not joint-specific but ubiquitous and shared in the multiple patients. On the other hand, we could not find the same sequence in the periphery. However, previously we demonstrated the induction of SH after the enrichment of Vβ14+ T cell subpopulations in the periphery of the RA patients of the positive group by a superantigen stimulation (our unpublished data). This suggests that Vβ14+ T cell inducing SH might be also present in the periphery although the frequency might be relatively lower compared with that in the joints. This might be a reason why we could not detect the identical sequence in the periphery. Thus, our results seem to be not conflicting with theirs. In addition, a Vβ14+ CD4+ TCL, named G3 with the identical CDR3 sequence was isolated and this TCL lacked expression of CD28 molecules (our unpublished observation). Moreover, G3 induced SH. Considering SH is known as one of the most characteristic histopathological features of RA, this TCL might

be a candidate as a pathogenic T cell in RA and the antigen(s) drives this TCL might be causative.

In conclusion, the present study suggests the presence of a certain causative antigen, which is shared with multiple RA patients in rheumatoid joints. In addition, it is possible to identify the causative antigen using the dominant and shared CDR3 sequence found in this study as a probe.

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