

# Restricted Heterogeneity of T Cell Receptor Transcripts in Rheumatoid Synovium

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## Abstract

RA is characterized by massive proliferation of synovial tissue, accompanying infiltration of the tissue with CD4<sup>+</sup> T lymphocytes, and a genetic linkage to the MHC antigen HLA-DR4. Since T cells are restricted by class II MHC molecules such as DR4, this suggests a direct role for these CD4<sup>+</sup> cells in pathogenesis. To investigate T cell receptor (TCR) usage in RA, we used oligonucleotide primers specific for each of the major alpha and beta TCR subfamilies to amplify cDNA derived from whole synovium or synovial tissue T cell lines in a family-specific manner. Detection of amplified DNA was facilitated by utilizing oligonucleotide probes derived from the constant regions of the TCRs. The TCR repertoire present in the synovial T cell lines was quite heterogeneous, with an average of 15 alpha chains and 15.8 beta chains detected. When synovial tissue was analyzed, the predominant TCR subfamilies detected tended to be more restricted, with an average of 4.6 alpha chains and 8.6 beta chains detected. This compared with an average of six alpha chains and 12 beta chains in nonrheumatoid synovial samples. The average percentage of synovia positive per TCR  $\beta$  family was significantly lower for RA versus non-RA specimens (46.1 vs 65.6%,  $P = 0.034$ ). These findings indicate that while a polyclonal population of T cells is present in RA synovium, the predominant patterns of TCR transcript expression may be somewhat more restricted, suggesting that TCR-based therapy of RA is possible. (*J. Clin. Invest.* 1992; 90:326–333.) Key words: rheumatoid arthritis • T cell receptors • polymerase chain reaction • T lymphocytes • autoimmunity

## Introduction

Rheumatoid arthritis is characterized pathologically by the proliferation of synovial fibroblast-like and macrophage-like cells, and infiltration of the synovium with lymphocytes, predominantly T cells of the helper (CD4<sup>+</sup>) phenotype (1, 2). Such CD4<sup>+</sup> T cells are typically activated by an antigenic peptide complexed with class II MHC molecules (HLA-DR/DP/

DQ). Immunogenetic analysis reveals that RA is associated with HLA-DR4, and more specifically with glutamine/lysine residues at amino acids 70/71 of the HLA-DR $\beta$  chain (3–8). This association is reminiscent of similar associations seen in experimental models of autoimmunity, such as experimental autoimmune encephalomyelitis, a model for multiple sclerosis triggered by autoreactive T cells reactive to myelin basic protein and specific MHC class II antigens (9–12). The observation of a restriction to certain MHCs in such experimental systems often correlates with a restricted repertoire of T cell antigen receptors which respond to that MHC + antigen (13). This has also been documented in multiple sclerosis T cell lines derived from humans (14, 15). In experimental systems, antibodies directed to the relevant T cell receptors, or immunization with peptides derived from these T cell receptors, is capable of ameliorating the disease (10, 11). These observations suggest that similar strategies may be applied to human T cell mediated autoimmune diseases.

Molecular characterization of human T cell receptors has been greatly aided recently through the application of the polymerase chain reaction (PCR)<sup>1</sup>. By utilizing oligonucleotide primers specific for the different T cell receptor variable region families, family specific amplification is possible (14–16). This technique can be applied to the identification of T cell receptors of interest, as well as aid in the cloning and sequencing of these receptors. The purpose of these studies is to apply PCR with family specific primers to characterize the predominant T cell receptor families present in rheumatoid synovium, with the eventual goal of cloning and sequencing these receptors.

## Methods

**Synovial tissue and cell lines.** Tissue was obtained at the time of joint surgery and was handled aseptically at all times. The tissue was rinsed in sterile PBS, and placed in a petri dish. The superficial layer snipped off with scissors and minced with a sterile scalpel. The minced tissue was placed in 20 ml PBS with 5% Hepes buffer, 0.4 g hyaluronidase (type I-S), 0.04 g DNA-ase I (type II from bovine pancreas) and 1.2 g collagenase (type Z) (all from Sigma Chemical Co., St. Louis, MO) with 1% FCS, and stirred continuously for 90 min at 37°C. The large chunks of tissue were decanted, and the cells centrifuged and washed twice in culture media [RPMI 1640 with pen/strep, L-glutamine, sodium pyruvate, nonessential amino acids, Hepes buffer,  $5 \times 10^{-5}$  M  $\beta$ -mercaptoethanol (all from Gibco Laboratories, Grand Island, NY), and 10% FCS (HyClone Laboratories, Logan, UT)]. T cells were purified by standard nylon wool chromatography (17), cultured overnight at  $1 \times 10^6$ /ml in culture media, and the nonadherent cells were sepa-

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1. Abbreviations used in this paper: PCR, polymerase chain reaction; TCR, T cell receptor.

rated, centrifuged, and maintained in culture. Stimulation of the cells was with either phytohemagglutinin (1% solution, from Sigma), IL-2 (Amgen Biologicals, Thousand Oaks, CA), or media alone. Cells were stimulated for 3–5 d, and then maintained for 1–3 wks in 10 U/ml IL-2 before analysis.

**Fluorescence-activated cell sorter (FACS®) analysis.** After culture, cells were centrifuged, washed, and resuspended in FACS® media (1% bovine serum albumin in PBS with 0.1% sodium azide), at  $1 \times 10^6$  cells per 100  $\mu$ l. Primary antibody was added for 20–40 min on ice. After an additional two washings, the cells were subjected to second antibody (fluorescein isothiocyanate-conjugated goat anti-mouse Ig [Sigma]; at a 1:100 dilution), then washed twice again. The cells were analyzed at the University of Pennsylvania Cancer Center fluorescence activated cell sorter facility. Percent positive was determined by comparing the samples to a no primary antibody control. Antibodies used were OKT3 anti-CD3 (Ortho Diagnostic Systems, Inc., Raritan, NJ), Leu3a anti-CD4 (Becton Dickinson, San Jose, CA), and OKT8 (Ortho), at the dilutions suggested by the suppliers.

**RNA extraction and cDNA synthesis.** Tissue was homogenized in guanidinium isothiocyanate (GITC) solution, or cells resuspended in GITC solution, and vortexed for 30 s. 0.1 ml 2 M sodium acetate pH 4 was added, the solution vortexed, followed by 1 ml diethylpyrocarbonate (DEP)-water-saturated phenol, the sample mixed, then 0.2 ml SE-VAG, thorough vortexing, and the solution transferred to sterile eppendorf tubes. Each sample was then incubated on ice for 20 min, microfuged for 10 min, and the top layer recovered, RNA precipitated with 2.5 vol of 100% ethanol and 1/10 vol 1 M sodium acetate pH 5.5 in dry ice/ethanol for 30 min. The solutions were microfuged for 15 min, the supernatant was decanted, the pellets were washed in 70% ethanol, and rotary evaporated. The dried pellets were resuspended in 50  $\mu$ l DEP-water and RNA quantified spectrophotometrically.

For reverse transcription, 1–20  $\mu$ g of RNA in 10  $\mu$ l was used to synthesize cDNA primed with random hexamers in the following reaction mixture: 3  $\mu$ l Maloney Murine Leukemia Virus reverse transcriptase with 6  $\mu$ l 5 $\times$  reverse transcriptase buffer, 1.5  $\mu$ l RNase inhibitor, and 3  $\mu$ l 0.1 M dithiothreitol (all from Gibco/Bethesda Research Laboratories, Gaithersburg, MD), 3  $\mu$ l random hexamers (from Pharmacia LKB Biotechnology, Piscataway, NJ), and either 1 or 3  $\mu$ l 100 mM dNTPs (25 mM in each dNTP, from Boehringer Mannheim GmbH, Mannheim, Germany). After a 10-min preincubation at 25°C, the reaction was carried out for 1 h at 42°C, then 95°C for 5 min followed by storage at –20°C until use.

**PCR amplification of T cell receptor variable regions.** cDNA was amplified using the primers listed in Table I, with  $V\alpha/\beta_n$  and  $C\alpha/\beta_{mid}$  at 0.2 nM concentrations. cDNA was amplified using *Taq* polymerase and standard reaction conditions suggested by the manufacturer (Perkin-Elmer Cetus Corp., Norwalk, CT). The reaction mixture contained 10  $\mu$ l of 10 $\times$  reaction buffer, 16  $\mu$ l 1.25 mM dNTPs (final concentration 200  $\mu$ M in each dNTP), 5  $\mu$ l of each oligonucleotide primer at 20  $\mu$ M (final 1  $\mu$ M in each primer), 5  $\mu$ l of DNA, 0.5  $\mu$ l *Taq* polymerase, and 58.5 ml distilled/deionized water. Primers were synthesized by the Wistar Institute's oligonucleotide synthesis facility. The program used was DEGEN2, which uses five initial low temperature cycles for low stringency (95°C for 1 min, 37°C for 2 min, 52°C for 2 min), followed by higher stringency for 40 cycles (95°C for 1 min, 52°C for 2 min, 72°C for 2 min), and a final 5-min 72°C elongation phase. For some experiments, the initial 20 cycles of DEGEN2 was used followed by additional increments of five higher stringency cycles (95°C for 1 min, 52°C for 2 min, 72°C for 2 min), with PCR product removed after each increment of five cycles for analysis. Products were analyzed by electrophoresis on 2–3% agarose gels stained with ethidium bromide.

**TABLE 1 T CELL RECEPTOR PCR PRIMERS**

Name	Sequence	Name	Sequence
V $\alpha$ 1	CTGAGGTGCAACTACTCA	V $\beta$ 1	AAGAGAGAGCAAAAGGAAACATTCTTGAAC
V $\alpha$ 2	GTGTTCCAGAGGGAGCCATTGCC	V $\beta$ 2	GCTGCAAGGCCACATACGAGCAAGGCGTCG
V $\alpha$ 3	GGTGAACAGTCAACAGGGAGA	V $\beta$ 3	AAAATGAAAGAAAAAGGAGATATTCTTGAG
V $\alpha$ 4	ACAAGCATTACTGTACTCCTA	V $\beta$ 4	CTGAGGCCACATATGAGAGTGGATTGTGCA
V $\alpha$ 5	GGCCCTGAACATTTCAGGA	V $\beta$ 5	CAGAGAAACAAAGGAAACTTCCCTGGTCGA
V $\alpha$ 6	GTCACCTTCTAGCCTGCTGA	V $\beta$ 6	GGGTGCGGCAGATGACTCAGGGCTGCCAA
V $\alpha$ 7	AGGAGCCATTGTCCAGATAAA	V $\beta$ 7	ATAAATGAAAGTGTGCCAAGTCGCTTCTCA
V $\alpha$ 8	GGAGAGAATGTGGAGCAGCATC	V $\beta$ 8	AACGTTCCGATAGATGATTACAGGGATGCCC
V $\alpha$ 9	ATCTCAGTGCTTGTGATAATA	V $\beta$ 9	CATTATAAATGAAACAGTTCCAAATCGCTT
V $\alpha$ 10	ACCCAGCTGGTGGAGCAGAGCCCT	V $\beta$ 10	CTTATTTCAGAAAGCAGAAATAATCAATGAG
V $\alpha$ 11	AGAAAGCAAGGACCAAGTGTT	V $\beta$ 11	TCCACAGAGAAGGGAGATCTTTCCTCTGAG
V $\alpha$ 12	CAGAAGGTAACCTCAAGCGCAGACT	V $\beta$ 12	GATACTGACAAAGGAGAAGTCTCAGATGGC
V $\alpha$ 13	GCTTATGAGAACACTGCGT	V $\beta$ 14	GTGACTGATAAGGGAGATGTTTCCTGAAGGG
V $\alpha$ 14	GCAGCTTCCCTTCCAGCAAT	V $\beta$ 15	GATATAAACAAAGGAGAGATCTCTGATGGA
V $\alpha$ 15	AGAACCTGACTGCCAGGAA	V $\beta$ 16	CATGATAATCTTTATCGACGTGTTATGGGA
V $\alpha$ 16	CATCTCCATGGACTCATATGA	V $\beta$ 17	TTTCAGAAAGGAGATATAGCTGAAGGGTAC
V $\alpha$ 17	GACTATACTAACAGCATGT	V $\beta$ 18	GATGAGTCAGGAATGCCAAAGGAACGATTT
V $\alpha$ 18	TGTCAGGCAATGACAAGG	V $\beta$ 19	CAAGAAACGGAGATGCACAAGAAGCGATTC
		V $\beta$ 20	ACCGACAGGCTGCAGGCAGGGGCTCCAGC
*C $\alpha$ 3'	AATAGGTCGAGACACTTGTCACTGGA	*C $\beta$ 1 3'	CCCTAGCAGGATCTCATAGAGGATGGTGGC
		*C $\beta$ 2 3'	CCCTAGCAAGATCTCATAGAGGATGGTGGC
*C $\alpha$ mid	CTTGCTCACTGGATTTAGATCTCTCAGCTG	*C $\beta$ mid	CTCTGCTTCTGATGGCTCAAACACAGCGAC
*C $\alpha$ 5'	GTACACGGCAGGGTCAGGGTTCTGGATATT	*C $\beta$ 1 5'	CTCGGGTGGGAACACCTTGTTCAGGTCTC
		*C $\beta$ 2 5'	CTCGGGTGGGAACACGTTTTTCAGGTCTC

\*Denotes antisense primer. C $\beta$ 1 & C $\beta$ 2 primers were used mixed together in equimolar concentrations.

**Transfer and probing.** Agarose gels were transferred to nylon filters (Genescreen Plus) by capillary transfer overnight (18, 19). Hybridization was with either C $\alpha$ 5' or C $\beta$ 5' primers noted in Table I. Oligonucleotide labeling employed 100 ng DNA, 75  $\mu$ Ci [ $^{32}$ P]ATP, 2.5  $\mu$ l 10 $\times$  kinase buffer (500 mM Tris HCL pH 7.6, 100 mM MgCl<sub>2</sub>, 50 mM dithiothreitol, 1 mM spermadine, 1 mM EDTA), 10 U T4 DNA kinase adjusted to a final volume of 25  $\mu$ l with distilled water. Labeling was carried out by incubation at 37°C for 30 min before use. Blots were prehybridized in 5 $\times$  standard saline citrate (SSC), 5 $\times$  Denhardt's solution, 0.1% SDS for 1–1.5 h at 55°C in seal-a-meal bags, most of the solution poured off,  $^{32}$ P-labeled oligonucleotide added (75  $\mu$ Ci) and hybridized for 2–3 h at 42°C or overnight at 4°C, the blots washed once in 2 $\times$  SSC, 0.1% SDS for 20 min at 45°C, then three times in 5 $\times$  SSC, 0.1% SDS for 20 min at 45°C, and exposed to Kodak XRP film at –70°C for 2–72 h.

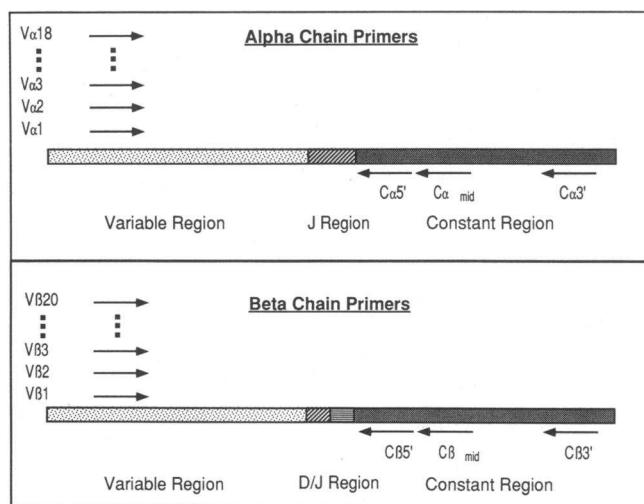
**Statistics.** The standard error of occurrence of each TCR V region family was calculated by the formula:

$$100 \sqrt{\frac{p[1-p]}{n}}$$

where  $n$  is the number of samples analyzed, and  $p$  is the proportion of positives. The frequency of occurrence of a particular TCR V region family was considered significantly increased if it was  $\geq 2$  standard errors higher than the mean for all V regions of that type ( $\alpha$  or  $\beta$ ). Comparisons of rheumatoid and nonrheumatoid synovial TCR expression employed the Student's  $t$  test, with correlations between TCR alpha and TCR beta expression employing Chi<sup>2</sup> analysis (20).

## Results

**PCR primers.** We used primers derived from the human TCR alpha and beta constant regions in conjunction with primers specific for individual variable region families (14–16). The primers used in these studies are listed in Table I, and their relative positions on the coding strand of cDNA indicated in Fig. 1. The constant region primers were designed as antisense primers to allow their use to prime PCR reactions as well as probes for blotting. Variable region primers were designed to act in a family specific manner as has been previously reported (14–16).



**Figure 1.** T cell receptor specific oligonucleotides and their relative location. → “sense” (coding strand) primer; ← “antisense” (noncoding strand) primer.

The PCR program used in these studies used a low stringency initial five cycles, followed by 40 cycles at higher stringency. The rationale for using this program was twofold. As these studies were designed to investigate the range of T cell receptors expressed in RA synovium, and all TCR V regions have not yet been sequenced, related TCR families which have sequences related to the primers used here may also be amplified in the initial low stringency cycles. 40 cycles of amplification were then used to amplify even low frequency transcripts. This should help overcome the potential problem of sampling error, which is possible from surgical specimens. Thus, if local accumulations of specific TCR bearing T cells are present, and such a local accumulation is missed in the surgical specimen, their presence still may be detected if they are also present at lower frequency in the surgical specimen examined. Preliminary experiments with these primers using the DEGEN2 program revealed that all of them (except V $\beta$ 16) are effective in amplifying TCR V regions from PHA stimulated peripheral blood mononuclear cells, but that only the appropriate V region primers amplified Jurkat cell cDNA TCR ([21] and data not shown).

**Synovial T cell lines.** RNA was extracted and cDNA synthesized from both whole synovium and PHA stimulated synovial T cell lines maintained in IL-2 for 1–2 wk before analysis. Synovial T cell lines derived in this manner have been previously described (17) and represent a phenotypically mixed population, including CD8+ and CD4+ cells. FACS® analysis was available for four of these cell lines at the time of analysis; data are shown in Table II. In three samples, CD4+ cells predominated, whereas CD8+ cells were more prevalent in a fourth line.

T cell receptor transcripts were amplified from cDNA derived from these T cell lines. All rheumatoid synovia were obtained at the time of joint surgery, and thus represented late disease. cDNA was split into equal portions and amplified with the middle constant region primers (C $\beta$ <sub>mid</sub> or C $\alpha$ <sub>mid</sub>) in combination with each of the respective individual variable region primers noted in Table I (e.g., C $\beta$ <sub>mid</sub> + C $\beta$ 1, C $\beta$ <sub>mid</sub> + C $\beta$ 2, . . . C $\beta$ <sub>mid</sub> + C $\beta$ 20; C $\alpha$ <sub>mid</sub> + C $\alpha$ 1, C $\alpha$ <sub>mid</sub> + C $\alpha$ 2, . . . C $\alpha$ <sub>mid</sub> + C $\alpha$ 18). After electrophoresis and transfer, these were probed with C $\beta$ 5' or C $\alpha$ 5', respectively. Results for the synovial T cell lines are shown in Fig. 2. An average of 15 alpha chain and 15.8 beta chain families were detected in these cell lines. This suggests that a quite heterogeneous population of T cells is present in synovium. However, as these cell lines were initially expanded with PHA, it is possible that the proportion of the various TCR subsets alter during culture. In addition, the ability of PHA to activate resting T cells raises concern about the relative proportion of activated T cells following stimulation compared with before stimulation. Therefore, similar analyses

**Table II.** Phenotype of Synovial Tissue T Cell Lines

Patient	Control	CD3+	CD4+	CD8+
		%		
EP <sub>1</sub>	1	90	25	54
NJ <sub>1</sub>	1	80	81	9
MW <sub>1</sub>	1	99	77	28
HR <sub>1</sub>	3	98	74	15

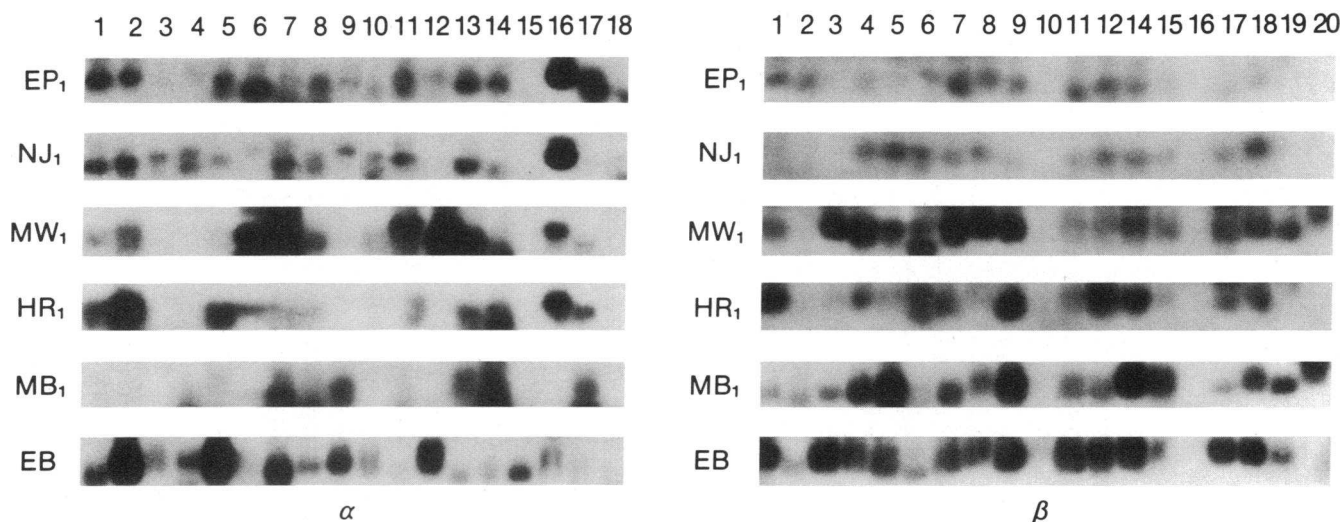


Figure 2. TCR transcripts in RA synovial T cell lines. Rheumatoid synovial T cell lines were developed by initial culture in PHA for 3–5 d, then maintained in IL-2 at 10 U/ml. After 1–3 wk of passage, the cells were frozen, and RNA was later extracted for analysis of TCR expression as outlined in Methods. The sample designations are shown on the left, with the corresponding TCR alpha and beta family-specific primers used indicated above each lane. Note: MB, cells were not PHA-stimulated.

were performed on whole, unstimulated rheumatoid synovium.

**Rheumatoid synovium.** The results for the whole synovia or freshly isolated, unstimulated synovial T cells analyzed similarly are shown in Tables III–V. The intensity of the bands detected was quite variable. To further evaluate the technique, cDNA was pooled from four synovia, and amplified with these primers for increasing numbers of cycles (Fig. 3). Note that the intensity of some bands which appeared in early cycles faded relative to the intensity of bands which arose at later cycles. Thus, the intensity of the bands can not be taken as an indicator of the relative abundance of the corresponding cDNA.

The frequency of occurrence of each TCR variable region is tabulated for rheumatoid synovial tissue in Tables III and IV. Similar analysis of nonrheumatoid synovial tissue and synovial

fluid is shown in Table V. As the nonrheumatoid samples also included synovial fluid analysis, any comparison of these results assumes that the fluid and tissue T cell receptor repertoires are similar. With this in mind, analysis of Tables III–V reveals that an average of  $4.6 \pm 1.64$  alpha chain and  $8.4 \pm 1.64$  beta chain families were detected in the rheumatoid synovia studied by this technique. In comparison, an average of  $6.0 \pm 2.49$  alpha chain and  $11.8 \pm 1.69$  beta chain families were present in the nonrheumatoid synovial tissue and fluid samples examined. When analyzed statistically, the average number of T cell receptor alpha or beta families expressed per rheumatoid synovium did not differ significantly from the average number of T cell receptor alpha or beta families expressed per nonrheumatoid synovial sample (Student's *t* test).

The average percentage of synovia positive per TCR family

Table III. T Cell Receptor Alpha Chain Expression in 10 Rheumatoid Synovia

Patient	V $\alpha$ 1	V $\alpha$ 2	V $\alpha$ 3	V $\alpha$ 4	V $\alpha$ 5	V $\alpha$ 6	V $\alpha$ 7	V $\alpha$ 8	V $\alpha$ 9	V $\alpha$ 10	V $\alpha$ 11	V $\alpha$ 12	V $\alpha$ 13	V $\alpha$ 14	V $\alpha$ 15	V $\alpha$ 16	V $\alpha$ 17	V $\alpha$ 18	Total detected
	<i>n</i>																		
MH	+			+	+	+	+	+	+	+		+	+	+			+	+	13
JL						?						±					+		1
AG																			0
RLP																			0
MG	+			+			+				+						+		5
HR	+						+										+		3
MW				+										+					2
MB					+	+		+				+				+	+	+	7
MJ	+																		1
EB	+	+	+		+	+	+	+	+	+	+	+		+	+		+		14
Number																			
+	5	1	1	3	3	3	4	3	2	2	2	3	1	3	1	1	6*	2	$4.6 \pm 1.64^\ddagger$
																			$25.6 \pm 3.4\%$

\* > 2 Standard errors from mean.

‡ Average number of TCR  $\alpha$  families detected per synovium  $\pm$  standard error

§ Average percentage of synovia positive per TCR  $\alpha$  family  $\pm$  standard error

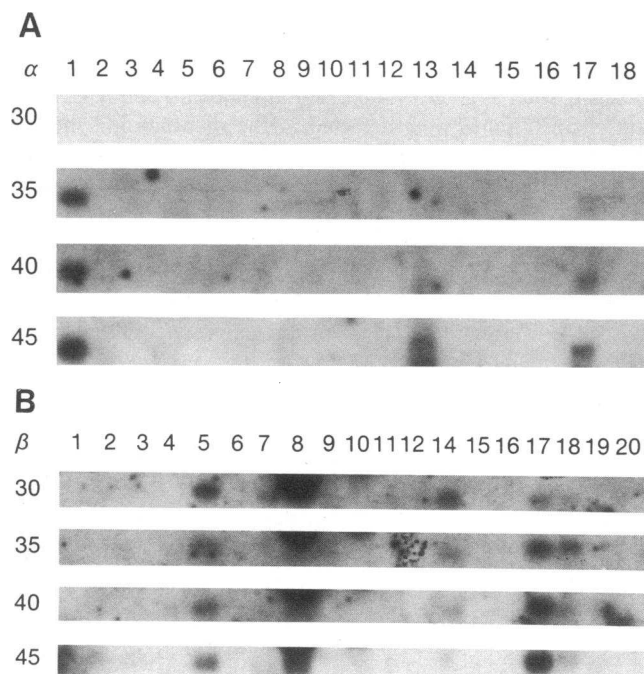
Table IV. T Cell Receptor Beta Chain Expression in 10 Rheumatoid Synovia

Patient	V $\beta$ 1	V $\beta$ 2	V $\beta$ 3	V $\beta$ 4	V $\beta$ 5	V $\beta$ 6	V $\beta$ 7	V $\beta$ 8	V $\beta$ 9	V $\beta$ 10	V $\beta$ 11	V $\beta$ 12	V $\beta$ 14	V $\beta$ 15	V $\beta$ 17	V $\beta$ 18	V $\beta$ 19	V $\beta$ 20	Total detected
	<i>n</i>																		
MH	+	+		+	+	+	+	+	+		+	+	+		+	+	+		14
JL							+	?			+	+	+	+	+		+		7
AG	+		+		+		+	?			+	+	+		+		+		9
RLP											+	+			+				3
MG			+		+		+	+	+	+		+	+		+	+			10
HR	+			+	+	+	+	?	+	?	+	+	+	+	+		+		12
MW		+	+	+	+			+	+	+	+	+	+	+	+		+		13
MB							+	?		?			+						2
MJ								?		?									0
EB	+	+		+	+	+	+	+	+			+	+	+	+	+	+		14
Number																			8.4 $\pm$ 1.64 <sup>‡</sup>
+	4	3	3	4	6	3	7	4	5	2	6	8*	8*	4	8*	3	6	0	46.7 $\pm$ 5.3%

\* > 2 standard errors from mean.

<sup>‡</sup> Average number of TCR  $\beta$  families detected per synovium $\pm$ standard error

Average percentage of synovia positive per TCR  $\beta$  family $\pm$ standard error



**Figure 3.** Effect of PCR cycle number on TCR transcripts identified. RNA was extracted and cDNA was synthesized from rheumatoid synovial tissues obtained at the time of joint surgery. (A) Alpha chain primers: cDNA from rheumatoid synovia MB, MJ, AG, and MG were pooled, and aliquots amplified for five low stringency cycles followed by 25, 30, 35, or 40 high stringency cycles (corresponding to 30, 35, 40, or 45 cycles noted.) Aliquots were removed following each time point, electrophoresed and probed as noted. The V $\alpha$  family specific primers are shown above each lane. (B) Beta chain primers: cDNA from rheumatoid synovia MH, MW, HR, and JL were pooled, and aliquots amplified for five low stringency cycles followed by 25, 30, 35, or 40 high stringency cycles (corresponding to 30, 35, 40, or 45 cycles noted). Aliquots were removed following each time point, electrophoresed and probed as noted. The V $\beta$  family specific primers are shown above each lane.

may also be compared for rheumatoid versus nonrheumatoid synovia. This should give an indication of the probability that an "average" TCR family would be detected in a collection of synovial samples. In this analysis for the alpha chains there are 25.6 $\pm$ 3.4% rheumatoid versus 33.3 $\pm$ 5.11% nonrheumatoid synovia positive per TCR family ( $P$  = not significant, Student's  $t$  test). For the beta chain, there are 46.7 $\pm$ 5.3% rheumatoid versus 65.6 $\pm$ 7.01% nonrheumatoid synovia positive per TCR family ( $P$  = 0.036, Student's  $t$  test). This indicates that while the number of TCRs detected per synovium was not significantly different for rheumatoid versus nonrheumatoid synovia, the frequency with which any given TCR  $\beta$  family was found in the collection of rheumatoid synovia was significantly lower compared with nonrheumatoid synovia.

When the frequency of specific TCR families is analyzed, several rheumatoid TCR families appeared more frequently than others. Specifically, V $\alpha$ 17 was present in 6/10 rheumatoid synovia, and V $\alpha$ 1 was present in 5/10. V $\beta$ 14 was seen in 8/10 rheumatoid synovia, as were V $\beta$ 17 and V $\beta$ 12, while V $\beta$ 7 was seen in 7/10. The frequency of V $\beta$ 12, 14, and 17 were  $\geq$  2 standard errors above the mean values for all TCR V $\beta$ s detected in rheumatoid synovia, and V $\alpha$ 17 was  $\geq$  2 standard errors above the mean values for all TCR V $\alpha$ s detected in rheumatoid synovia. This suggests the presence of these variable regions in rheumatoid synovia from many different patients. However, when nonrheumatoid synovial samples were analyzed similarly, V $\beta$ 5, 14, 17, and 18 were  $\geq$  2 standard errors above the mean values for all TCR V $\beta$ s detected, with none of the alpha chain families significantly higher by this analysis. (Note that these calculations employed the standard error of each TCR family expressed according to the formula in the Methods, not the standard error of the average number of synovial samples expressing given TCR families shown in Tables III–V). Comparison of the rheumatoid and nonrheumatoid synovia by this method indicates that the frequency of expression of V $\beta$ 1, 2, 3, 4, 5, & 18 and V $\alpha$ 2 and 5 in rheumatoid synovia was  $\geq$  2 standard errors below that seen in nonrheu-

Table V. T Cell Receptor Alpha and Beta Chain Expression in Five Nonrheumatoid Synovia

Patient*																			Total detected
Dx	V $\alpha$ 1	V $\alpha$ 2	V $\alpha$ 3	V $\alpha$ 4	V $\alpha$ 5	V $\alpha$ 6	V $\alpha$ 7	V $\alpha$ 8	V $\alpha$ 9	V $\alpha$ 10	V $\alpha$ 11	V $\alpha$ 12	V $\alpha$ 13	V $\alpha$ 14	V $\alpha$ 15	V $\alpha$ 16	V $\alpha$ 17	V $\alpha$ 18	n
RN DJD	+	+		+	+	+		+		+							+	+	9
AJ DJD	+	+			+			+									+		5
WJ AS <sup>§</sup>	+	+	+	+	+		+	+	+		+	+	+	+		+		+	14
JP1 <sup>‡</sup> AS <sup>§</sup>																	+		1
JP2 <sup>§</sup> AS								+											1
Number																			
+	3	3	1	2	3	1	1	4	1	1	1	1	1	1	0	1	3	2	6.0 $\pm$ 2.49 <sup>†</sup> 33.3 $\pm$ 5.11%
	V $\beta$ 1	V $\beta$ 2	V $\beta$ 3	V $\beta$ 4	V $\beta$ 5	V $\beta$ 6	V $\beta$ 7	V $\beta$ 8	V $\beta$ 9	V $\beta$ 10	V $\beta$ 11	V $\beta$ 12	V $\beta$ 14	V $\beta$ 15	V $\beta$ 17	V $\beta$ 18	V $\beta$ 19	V $\beta$ 20	Total detected
																			n
RN DJD	+	+		+	+							+	+		+	+			8
AJ DJD	+		+	+	+		+	?	+	?	+	+	+		+	+	+		12
WJ AS <sup>§</sup>	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+		16
JP1 <sup>§</sup> AS	+	+	+	+	+		+	+	+	+	+	+	+		+	+	+		15
JP2 <sup>§</sup> AS					+			+	+	+	+		+		+	+			8
Number																			
+	4	3	3	4	5 <sup>‡</sup>	1	3	3	4	2	4	4	5 <sup>‡</sup>	1	5 <sup>‡</sup>	5 <sup>‡</sup>	3	0	11.8 $\pm$ 1.69 <sup>†</sup> 65.6 $\pm$ 7.01%

\* Patient indicated with diagnosis. DJD = degenerative joint disease; AS = ankylosing spondylitis. <sup>‡</sup> JP1 and JP2 represent synovial fluid obtained 10 days apart from the same individual. <sup>§</sup> Synovial fluid used for RNA isolation (all others from synovial tissue). <sup>||</sup> > 2 standard errors above mean.

<sup>†</sup> Average number of TCR  $\alpha$  or  $\beta$  families detected per synovium  $\pm$  standard error

Average percentage of synovia positive per  $\alpha$  or  $\beta$  TCR family  $\pm$  standard error

matoid samples. None of the TCR families were expressed in rheumatoid synovia  $\geq 2$  standard errors more frequently than in nonrheumatoid synovia.

## Discussion

Many lines of evidence indicate that T cells are involved in the development of rheumatoid joint disease. These include the presence of lymphocytic infiltrates composed primarily of CD4<sup>+</sup> T cells in the synovium (1, 2, 22–24), the linkage of RA to HLA-DR4 which comprises a ligand for CD4<sup>+</sup> T cell receptors (3–8), and experimental models of arthritis and related autoimmune diseases that can be transferred by T cell lines (10, 12, 13, 25–34). Studies in both animal models and human rheumatoid arthritis indicate that anti-T cell reagents can be of

therapeutic efficacy (10, 11, 26, 35–41). However, if these reagents are nonspecific and delete too large a portion of the T cell repertoire, immunodeficiency may result.

A better therapeutic alternative is to delete only those T cells involved in the autoimmune response. Studies of rheumatoid T cell receptors suggest a polyclonal or oligoclonal population (3, 42–48). These studies generally employed Southern analysis searching for restriction fragment length polymorphisms to determine the oligoclonality of the populations analyzed. Two reports used PCR with family specific primers in analysis of rheumatoid synovial fluid lymphocyte TCRs (46, 49). In analysis of synovial fluid and peripheral blood lymphocytes from three patients with RA, Sottini et al. found evidence for restriction in TCR V $\beta$  transcripts but not V $\alpha$  transcripts (46). They suggested a role for V $\beta$ 7 in pathogenesis based on

its occurrence in 2/3 synovial fluids. In a similar analysis which also employed relative quantification of TCR transcripts, Paliard et al. (49) found a relative abundance of V $\beta$ 14 transcripts in synovial fluid with a corresponding decrease in V $\beta$ 14 in peripheral blood from RA patients. These studies indicate that while the T cell population present in rheumatoid synovial fluid is heterogeneous, based on T cell receptor analysis, that some T cell receptors are more common than others among the different patients.

The current study confirms some of these observations in RA synovial tissue, while also suggesting additional interpretations based on analysis of nonrheumatoid specimens. Since the synovial tissues described here were all obtained from patients undergoing joint surgery, they all represent long-standing RA. The data presented herein may not be directly applicable to synovial tissue from patients earlier in the disease course. However, in the setting of long standing RA, a polyclonal population might be expected in the synovium. In fact, while the number of TCR  $\alpha$  or  $\beta$  chain families detected per synovial sample was not significantly less for rheumatoid compared with nonrheumatoid synovial samples, the average number of synovial positive per TCR  $\beta$  chain family was significantly less compared with the nonrheumatoid specimens. As the nonrheumatoid specimens also included synovial fluid, these results should be interpreted with caution. However, this does indicate a greater restriction of TCR  $\beta$  expression among different rheumatoid synovia compared with the nonrheumatoids. A similar restriction may also exist during earlier stages of rheumatoid pathogenesis.

In addition, predominance of some TCR transcripts was found. Specifically, V $\alpha$  17, V $\alpha$ 1, V $\beta$ 12, V $\beta$ 14, V $\beta$ 17, and V $\beta$ 7 appeared in more than half of the rheumatoid synovial tissue samples studied (Tables III and IV). None of the samples lacked at least one of these T cell receptor chain families. However, some of these same TCR families were also found quite frequently in nonrheumatoid synovial tissue and fluid (Table V). Comparison of the rheumatoid and nonrheumatoid specimens indicates that there is no evidence for an increased frequency of expression of any given TCR alpha or beta family in the rheumatoid synovia. Instead, the frequency of some of the TCR families encountered was > 2 standard errors decreased for the rheumatoid compared with the nonrheumatoid specimens. This included V $\alpha$ 2, 5, & 8 and V $\beta$ 1, 2, 3, 4, 5, and 18. This suggests that these TCR families are not associated with the rheumatoid synovial lesion.

The high frequency with which V $\alpha$ 17, V $\alpha$ 1, V $\beta$ 12, V $\beta$ 14, V $\beta$ 17, and V $\beta$ 7 occurred compared with other TCR families in the rheumatoid synovia should be interpreted in light of the nonrheumatoid findings. The lower average expression of TCR families in the rheumatoid tissues suggests a greater potential role for those TCRs expressed more frequently. It is interesting to note that V $\beta$ 14 and 17 were also found in 100% of the nonrheumatoid specimens. The higher average frequency of TCR V region family expression in the nonrheumatoid specimens makes interpretation difficult, but this may indicate a relative predilection for T cells bearing these receptors to target synovial tissue as a general phenomenon. Such tissue-specific TCR expression has been reported for gamma-delta T cell receptors (50), but the significance remains unclear.

The potential existence of rheumatoid-specific T cells, possessing a functional alpha-beta TCR pair, can not be discounted. Such a T cell is likely to include at least one TCR

chain which is frequently encountered in multiple different rheumatoid synovial specimens. It is interesting to note that in rheumatoid synovia analyzed herein, there was a significant correlation between expression of V $\alpha$ 17 and V $\beta$ 14 ( $\chi^2 = 3.75$ ,  $P = 0.05$ ). This correlation was not apparent in the nonrheumatoid synovia, or for the other frequently expressed TCR families in the rheumatoid synovia. As these and other TCRs are further characterized the molecular basis for auto-reactivity and association with HLA-DR4 may be appreciated. Reagents and therapies designed to specifically target the T cells which bear these T cell receptors should be of use in further delineating the function of these cells, and may be efficacious in ameliorating some of the disease manifestations of rheumatoid arthritis.

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