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Research Article

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T Cell Receptor Vβ Gene Bias in Rheumatoid Arthritis

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Abstract

Polymerase chain reaction (PCR) technology was employed to examine peripheral blood and synovial T cells in patients with rheumatoid arthritis (RA) for biased utilization of T cell receptor (TCR) variable region (V) genes. Oligonucleotide primers specific for individual TCR Vβ gene families were used to amplify TCR gene products in a semiquantitative assay of their relative utilization in unselected T cell populations. Mean Vβ expression in 24 RA peripheral blood samples was very similar to that in a panel of 15 normal subjects, except for a slight decrease in Vβ13.2 expression. Vβ utilization in 8 RA synovial tissue samples and 13 synovial fluid samples was compared to simultaneously obtained blood samples. Although heterogeneous patterns of skewed Vβ utilization were observed, several significant trends emerged. By a number of approaches to data analysis, a statistically significant increase in expression of Vβ6 and Vβ15 in synovial T cells was documented. In addition, increased synovial expression of Vβ14 was found, but only in the synovial fluid samples. Reduced expression of Vβ1, Vβ4, Vβ5.1, Vβ10, Vβ16, and Vβ19 was also observed in synovial T cells. These results indicate that biased Vβ gene utilization in different peripheral compartments of RA patients can be observed in unselected T cell populations, and are consistent with the conclusion that populations of T cells expressing these Vβ gene products may be involved in the pathogenesis of the disease. (*J. Clin. Invest.* 1993. 92:2688–2701.) Key words: polymerase chain reaction • rheumatoid arthritis • synovial T cells • T cell receptor • T cell repertoire

Introduction

Rheumatoid arthritis (RA) is an idiopathic disease characterized by chronic inflammation of synovium, local destruction of cartilage and bone, and systemic illness. Several lines of evidence implicate T cells which respond to antigenic stimuli via their α/β T cell receptor (TCR)[1] in the disease process (1). The majority of RA patients express products of a set of alleles at the B1 locus of the HLA-DR region, HLA-DRB1*0401 (HLA-DR4, Dw4), *0404/*0408 (DR4, Dw14), *0405 (DR4, Dw15), *0101 (HLA-DR1), and *1402 (HLA-DRw14, Dw16), that are associated with increased risk for acquiring the disease (2–7). These class II MHC molecules share a sequence of amino acids in the third hypervariable region (residues 70–74) of the HLA-DRβ1 chain, indicating that this epitope may confer disease susceptibility (8). Because the major roles of class II molecules are to bind and present antigenic peptides to CD4+ T cells or to exert an influence on thymic selection of the CD4+ T cell repertoire, these findings are compatible with the conclusion that the introduction of an unidentified pathogenic antigen into the synovial membrane of an immunogenetically susceptible host triggers a CD4+ T cell–mediated autoimmune response resulting in the manifestations of RA.

A number of studies have established the ability of antigen or autoantigen plus MHC combinations to select for T cells expressing particular TCR genes (9). There is also evidence that the variable region (V) gene expression profile of peripheral T cells can be influenced by the MHC molecules encountered during thymic education (10–13). In addition, it has been postulated that superantigens, characterized in part by their ability to stimulate T cells utilizing particular Vβ genes, may play a role in the development of autoimmunity including RA (14, 15). Thus, there are several factors that could lead to the development of biased TCR expression in RA.

Initial studies sought evidence of biased TCR expression in RA by looking for the presence of oligoclonal T cells. Evidence of oligoclonality manifested by the presence of rearranged bands detected by Southern blotting was rarely found in T cells directly isolated from synovial fluid samples (16, 17). Moreover, T cell clones established in culture conditions designed to allow outgrowth of all cells rarely exhibited identical rearrangements (18, 19). However, some studies were able to detect oligoclonality of synovial T cells selected for the ability to grow in IL-2 alone (20, 21). Even when oligoclonality was claimed, however, there was no consensus on the specific dominant rearrangement in different patient samples (22, 23). Taken together, these studies indicate that there is minimal oligoclonality in the rheumatoid synovium, although selection for IL-2–responsive T cells may induce outgrowth of a limited number of clones. A major limitation of these analyses, however, is that examination of cells for identical rearrangement patterns might not detect the predominance of an antigen- or superantigen-reactive T cell population utilizing particular Vα or Vβ gene families.

Several studies have examined RA synovial fluid or synovial tissue for restricted expression of TCR Vα or Vβ. Some have interpreted their results to indicate that the number of Vβ, but not Vα, gene families expressed in synovial T cells is limited (24), whereas others have reported that, at least in patients with early disease, a limited number of Vα, but not Vβ, gene families are expressed (25). Although variable numbers of Vα and Vβ products were also detected in other studies of synovial

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1. *Abbreviations used in this paper:* C or V, constant or variable region (gene); DMARD, disease-modifying antirheumatic drug; NSAID, nonsteroidal antiinflammatory drug; RF, rheumatoid factor; RT, reverse transcriptase.

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fluid or synovial tissue samples (14, 26), two recent analyses of synovial fluid T cells detected all of the Vβ families in every patient (27, 28). Thus, no consensus concerning the potential biased expression of Vβ or Vα in RA synovium has emerged from these studies, most of which examined small numbers of patients. Some studies included comparisons of V gene expression in rheumatoid peripheral blood and synovial T cells. Analyses of increased synovial T cell expression of individual V gene families have generally demonstrated heterogeneous patterns (27–30). However, Paliard et al. (14) reported that, in nine of nine RA patients, expression of Vβ14 was greater in synovial fluid T cells than in the peripheral blood. An increase in Vβ14 expression in synovial fluid T cells in comparison to peripheral blood was found in one other study (29), but not in two others (27, 28). Therefore, the question of whether there is biased representation or activation of T cells expressing particular Vα or Vβ products in rheumatoid synovium remains unresolved.

The present report of 24 RA patients used semiquantitative PCR amplification of cDNA with Vβ-specific primers to test the possibility that T cells in the peripheral blood, synovial fluid, or synovial tissue exhibit biased Vβ gene utilization. Several approaches to data analysis indicate biased Vβ gene utilization by T cells in patients with RA. Although the differences are small, several statistically significant trends were noted, with either enrichment or depletion of particular Vβ genes noted in the synovium or the peripheral blood. These data indicate that biased Vβ gene family utilization in different peripheral compartments of rheumatoid patient can be observed in unselected T cell populations and are consistent with the conclusion that T cells expressing these particular TCR gene products may be involved in the pathogenesis of RA.

Methods

Subjects. 24 patients who fulfilled the 1987 American College of Rheumatology criteria (31) for RA were studied. Synovial tissue was obtained from 8 patients undergoing synovectomy or joint replacement surgery, and synovial fluid was obtained from 11 patients undergoing therapeutic arthrocentesis. Synovial fluid was obtained from two of these patients on two occasions for a total of 13 synovial fluid samples. Peripheral blood was obtained from each of these patients, and from 5 similar patients from whom analysis of synovial T cells was not successful. Peripheral blood and synovial tissue or synovial fluid samples were obtained from 4 control patients with other arthritides. The disease characteristics, age, sex, and medications used by these subjects at the time samples were obtained are presented in Table I. The presence or absence of circulating rheumatoid factors was determined by nephelometry, except for a few cases in which a latex fixation test was used. Peripheral blood samples from 15 subjects who did not have arthritis served as normal controls.

HLA typing. Peripheral blood lymphocytes were used for serologic HLA class II typing by the lymphocytotoxicity method (32). Oligonucleotide typing of HLA class II genes was then performed according to previously published methods (33, 34). DNA was extracted from lymphocytes as described (35), and amplified by PCR using previously described primers for DRβ (33), DQβ (36), DPβ (36), and DR4 subtyping. Previously described oligonucleotides were used for probing dot blots of the DRβ (33), DPβ (36), DQβ (34), and DR4 (36) PCR products.

Cell preparation for TCR analysis. PBMC and synovial fluid mononuclear cells were prepared from heparinized samples by density gradient centrifugation over ficoll diatrizoate gradients. These cells were either used directly for RNA preparation or further enriched for T cells by rosetting with neuraminidase-treated sheep erythrocytes and/or passage over a nylon wool column. Lymphocytes were prepared as described (37) from those synovial tissue samples not used directly for RNA preparation. Briefly, tissue was minced with scissors and digested with 1 mg/ml collagenase for 1–2 h at 37°C, and then passed rapidly over a nylon wool column.

RNA and cDNA preparation. RNA was directly prepared from synovial tissue samples by homogenization in guanidine thiocyanate with a tissue homogenizer followed by ultracentrifugation through a CsCl gradient as described (38). Cell preparations were homogenized in guanidine thiocyanate by repeated passage through a small gauge needle. Total RNA was extracted from cell homogenates as previously described (39). 1–6 μg of RNA was converted to cDNA by incubation with 8 U avian myeloblastosis virus reverse transcriptase (RT) and 20 μg/ml oligo-dT primers in the presence of actinomycin D (50 μg/ml) for 1 h at 42°C. The cDNA was heated to 95°C for 5 min and then diluted to a final volume equal to the greater of 25 μl per 1 μg of RNA, or 80 μl.

TCR β-chain PCR. In preliminary experiments, each cDNA was examined, alongside a positive control, in constant region (C) amplifications to determine the relative amount of TCR-derived cDNA. Cβ amplifications were done with primers 5′Cβ: GTGTTTGAGCCA-TCAGAA-GCA and 3′Cβ6: AAGCCACAGTCTGCTCTACC. For the RNA samples from patients RA5 through RA24 (Table I) and from all of the control subjects a negative control cDNA, prepared from RNA without added RT, was tested with Cβ region primers to ensure there was no contaminating TCR DNA. Products were sampled after 20, 25, 30, and 35 cycles to determine the number of cycles through which the specific product accumulated exponentially (see Fig. 1 *A*). The subsequent semiquantitative assay utilized the 22 Vβ family–specific 5′ primers designed by Choi et al. (40) and the reverse primer 3′Cβ4: ACCCACCAGCTCAGCTCCA in separate simultaneous PCR reactions. The number of cycles used (usually 22–30) was designed to stop the reactions while all of the products were still accumulating exponentially, as determined in the preliminary experiment. These steps were taken to ensure that each PCR product was proportional to the amount of corresponding mRNA. In addition, when possible, all of the reagents were aliquoted from a master mix so that pipetting variability did not produce artifacts in the PCR. In each experiment a negative control, with no cDNA added, was run in parallel for all primer pairs. PCR amplification of cDNA samples from subjects RA-1, RA-2, CP-4, and one of the sets of synovial fluid and peripheral blood samples from RA-3 was performed in individual tubes in a reaction volume of 50 μl. Amplification of samples from RA-7, NC-11, and one of two sets of synovial fluid mononuclear cell and PBMC samples from RA-11 was carried out in triplicate in tubes. PCR amplification of the remaining 60 cDNA samples was carried out in triplicate 20-μl reactions using 96-well microtiter plates in a model PTC-100-96 thermal controller (MJ Research, Watertown, MA). Triplicate amplifications were performed to further the reproducibility of the assay. The standard error of the three replicate determinations was usually < 10% of the corresponding mean. The reaction mixture contained 0.2 mM dNTPs (20 nM each of dATP, dCTP, dGTP, and dTTP), 1.5 mM $MgCl_2$, 0.3 μM oligonucleotide primers, and 20–40 U/ml Taq DNA polymerase (Promega Corp., Madison, WI). The temperatures used in the PCR were 94°C for denaturation, 50°C for annealing, and 72°C for polymerization. The duration of each step was 1 min.

Determination of Vβ gene family utilization. A sample of each PCR amplification was transferred to a nylon membrane (Zeta-Probe GT, Bio-Rad Laboratories, Richmond, CA) by alkaline transfer using a slot blot apparatus. Blots were incubated in hybridization fluid (38) containing 6× sodium chloride, sodium citrate (SSC), 10% Denhardt's reagent, 100 μg/ml denatured, fragmented salmon sperm DNA, and 0.5% SDS for at least 1 h at 37°C. The specific β-chain products were then detected by incubation of the blots for 3–24 h at 37°C in hybridization fluid containing [γ-^{32}P]-labeled β probe: ATTCTCCCACAC-CCAAAAGG, derived from the Cβ region. The β probe oligonucleotide was end-labeled with T4 polynucleotide kinase using standard

Table I. Subjects Studied

Donor	Diagnosis	Age/ duration	Race/Sex	RF	Disease characteristics	Medications	DR	DQ	Samples
RA patients									
RA-1	RA	52/1	B/F	+	nodular, erosive, class 3	NSAID	2, 6, w52	w1	blood
RA-2	RA	73/7	W/F	+	erosive, class 3	NSAID, Gold	4, 11, w52, w53	w7(3)	blood
RA-3	RA	56/2	B/F	+	erosive, class 3	NSAID	3, 11, w52	w4, w7(3)	SF, blood*
RA-4	RA	62/25	B/F	+	erosive, class 3	NSAID, D-Pen	4, 8, w52, w53	w7(3)	blood
RA-5	RA	62/17	W/F	+	nodular, erosive, class 3	NSAID, MTX	1, 4, w53	w1, w7	tissue, blood
RA-6	RA	37/1	B/F	+	class 2	NSAID, HCQ	4, 11, w52, w53	w1, w3	SF, blood
RA-7	RA	58/12	B/M	+	nodular, erosive, class 3	NSAID, Gold	4, 13, w52, w53	w1, w3	SF, blood
RA-8	RA	26/4	W/F	+	erosive, class 3	NSAID, MTX	3, 7, w52, w53	w2	tissue, blood
RA-9	RA	57/5	As/F	−	erosive, class 3	NSAID	2, 12, w52	w7, w1	blood
RA-10	RA	61/19	W/F	+	erosive, class 3	MTX, Pred	7, 8, w52, w53	w2, w4	tissue, blood
RA-11	RA	63/2	B/F	+	erosive, class 3	NSAID, PRED, MTX	3, 13, w52	w1, w2?	SF, blood*
RA-12	RA	26/1	L/F	+	class 2	NSAID	4, 7, w53	w2, w3	SF, blood
RA-13	RA	59/2	B/F	+	erosive, class 2	NSAID, MTX	2, 13, w52	w1, w7(3)	SF, blood
RA-14	RA	47/4	W/F	+	nodular, erosive, class 3	NSAID, MTX, HCQ, Pred	3, 4, w52, w53	w2, w3	blood
RA-15	RA	21/3	LA/F	+	erosive, class 3	NSAID	2, 4, w53	w7(3), w1	SF, blood
RA-16	RA	24/1	LA/F	−	erosive, class 3	NSAID	7, 10, w53	w1, w2	SF, blood
RA-17	RA	40/2	LA/F	−	class 2	NSAID	1, 8, w52	w1	tissue, blood
RA-18	RA	57/10	B/F	+	nodular, erosive, class 3	NSAID, Gold	4, 6, w52, w53	w1, w3	SF, blood
RA-19	RA	66/10	B/F	+	erosive, class 3	NSAID, Gold	3, 11, w52, w53	w4, w7(3)	SF, blood
RA-20	RA	70/3	W/F	+	nodular, erosive, class 2	NSAID, PRED, MTX	3, 4, w52, w53	w2, w3	tissue, blood
RA-21	RA	57/3	B/F	+	erosive, class 3	NSAID	ND‡		tissue, blood
RA-22	overlap	50/12	W/F	+	erosive, class 3	NSAID	6, 7, w52, w53	w1, w2	tissue, blood
RA-23	RA	42/1	W/F	−	nodular, class 2	NSAID, HCQ	3, 7, w52, w53	w2	tissue, blood
RA-24	RA	56/12	W/F	+	nodular, erosive, class 2	NSAID, CyA	1, 4, w53	w1, w3	SF, blood
Arthritic controls									
CP-1	OA	44/6	B/M	−		NSAID	ND		SF, blood
CP-2	OA	76/20	W/M			NSAID	ND		blood
CP-3	JCA	24/20	W/M	−	erosive, class 2	NSAID, MTX	ND		tissue, blood
CP-4	JCA	27/23	W/F	−	erosive, class 2	Pred, NSAID	11, w53	w7(3)	SF, blood
Normal controls									
NC-1		30	B/F				ND		blood
NC-2		31	W/F				12, 13, w52	w1, w7(3)	blood
NC-3		41	B/F				8, 10, w52	w1, w7	blood
NC-4		53	W/F				11, 10, w52	w1, w7	blood
NC-5		36	B/F				6?, 11, w52, w53	w1, w3	blood
NC-6		29	W/M				7, −	w1, w2	blood
NC-7		36	W/M				7, 11, w52, w53	w7, w9	blood
NC-8		37	W/M				1, 4, w53	w1, w3(w7)	blood
NC-9		28	W/M				4, w6, w52, w53	w1, w3NT	blood
NC-10		31	W/F				7, 9, w52, w53	w1, w2	blood
NC-11		30	A/F				2, 4, w53	w1, w3	blood
NC-12		33	W/M				3, 4, w52, w53	w2, w3	blood
NC-13		25	W/F				8, −, w52	w7(3)	blood
NC-14		32	B/M				3, 11, w52	w2, w7(3)	blood
NC-15		32	W/M				3, 4, w52, w53	w2, w3	Blood

Abbreviations used in this table: CyA, cyclosporine A; D-pen, D-penicillamine; HCQ, hydroxychloroquine; JCA, juvenile chronic arthritis; MTX, methotrexate; OA, osteoarthritis; Pred, prednisone.

* Synovial fluid and peripheral blood were obtained on two separate occasions from this patient.

‡ ND, not determined.

methodology (38). Blots were washed in a solution of 6× SSC and 0.5% SDS for 20 min at 50°C. Hybridized probe was quantified with the Radioanalytic Imaging System (AMBIS Systems Inc., San Diego). Background counts from areas of slot blots containing no detectable bands (water controls) were subtracted with the AMBIS software. Accordingly, the amount of hybridized probe is expected to be linearly related to the amount of PCR product. The specificity of these amplifications was confirmed by separating samples of products by electrophoresis on agarose gels, transferring to nylon membranes, and probing as described above. In preliminary experiments it was determined that all $V\beta$ products detectable on slot blots produced a single band of the correct size on the agarose gel blots.

The data are expressed as the mean of the triplicate samples (where applicable) of each $V\beta$ amplification divided by the sum of the 22 $V\beta$ amplifications. The proportion of each $V\beta$ PCR product to the sum of all the $V\beta$ products will, by design, reflect the proportion of total TCR mRNA that encodes a particular $V\beta$ gene family. Control $C\beta$ amplifications were performed in each experiment, but were not used to normalize the individual $V\beta$ data because the sum of the individual $V\beta/C\beta$ ratios was not consistent from one cDNA to another. Conse-

quently, differences in an individual Vβ/Cβ ratio from one cDNA to another might reflect this inconsistency rather than true skewing. In contrast, when the data were normalized with the sum of the 22 Vβ amplifications repeat experiments performed on the same cDNA produced nearly identical Vβ expression profiles (see Results). This consistency allowed meaningful comparisons of different cDNAs.

The ability of this semiquantitative assay to detect selective expansion or activation of T cells bearing a particular Vβ gene was demonstrated in preliminary experiments and in experiments published elsewhere. Peripheral T cells stimulated with monoclonal antibodies directed against specific Vβ proteins in the presence of IL-2 produced at least a twofold increase in the amount of the appropriate Vβ PCR product even though there was no detectable increase in the number of cells in culture (data not shown), demonstrating that increased amounts of mRNA resulting from activation of Vβ-specific T cells could be detected. In addition, proliferation driven by Vβ-specific superantigens resulted in a marked increase of the appropriate Vβ PCR product (41). Finally, addition of small numbers of monoclonal T cell populations (5%) to T cells derived from the peripheral blood of a normal donor was easily detected (data not shown). These preliminary results confirmed that the semiquantitative PCR could detect changes in both the activation state and number of T cells expressing specific Vβ gene products in a heterogeneous population.

Statistical analysis. Vβ expression in the peripheral blood and synovium of RA patients and in the peripheral blood of control subjects was not normally distributed for every Vβ gene family. Therefore, Wilcoxon's rank sum test was applied to comparisons of Vβ expression in RA patients and control groups, and Wilcoxon's signed rank test was applied to comparisons of Vβ expression in simultaneously obtained peripheral blood and synovial samples. Student's t test was applied to comparisons of Vβ expression in peripheral blood and synovium in each experiment performed with triplicate PCR amplifications. Regression analysis was used to examine the overall similarity of Vβ expression profiles in different experiments. The coefficient of determination (r^2) is reported, with $r^2 = 1.0$ indicating perfect correlation, and $r^2 = 0$ indicating no correlation.

Results

Patient population. Samples were obtained from 24 RA patients, 4 patients with other arthritides, and 15 normal donors. The clinical characteristics of these subjects are presented in Table I. A large majority of the RA patients had erosive synovitis and all of them were functional class II or III (42). Rheumatoid factor (RF) was detected in the serum of 20 RA patients. A majority (15/24) of the RA patients was taking a disease-modifying antirheumatic drug (DMARD) at the time the samples were obtained. Of the 23 patients on whom serologic HLA-typing was available, 12 expressed either HLA-DR1 or HLA-DR4 or both. 5 of the 15 normal subjects tested positive for either or both of these markers. Of the 8 HLA-DR4+ patients who were DNA typed, 7 expressed at least one of the susceptibility conferring alleles DRB1 *0401, *0404, or *0408. For clarity of presentation, serologic typing of HLA-A, B, and C, and DNA analysis of DP, DQ, and DR4 subtypes, are not shown.

RA peripheral blood. Fig. 1 illustrates the protocol used in these experiments for the analysis of Vβ gene family expression in unselected T cell populations. The preliminary analysis of the peripheral blood cDNA of one subject indicated that TCR Cβ product accumulated exponentially for 30 PCR cycles (Fig. 1 *A*). Because the Cβ primer pair amplifies all TCR β chain–derived cDNA, the individual Vβ PCR products amplified from this cDNA would also accumulate exponentially for at least 30 cycles. The subsequent experiment utilized 28 PCR cycles for the triplicate amplification of this cDNA with the 22

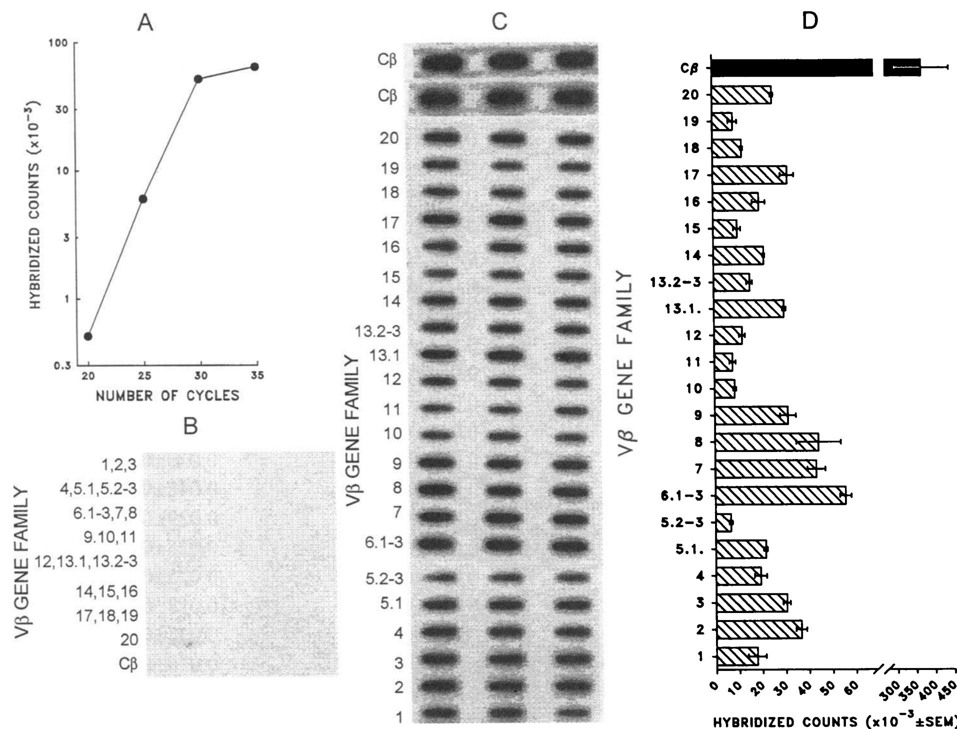

Figure 1. Determination of T cell Vβ gene family utilization in PBMC from patient RA-23. (*A*) PBMC cDNA was amplified with 5′Cβ and 3′Cβ6 for 35 PCR cycles. Samples were removed after 20, 25, 30, and 35 cycles, transferred to a slot blot, and hybridized with radiolabeled βprobe. Net hybridized counts are depicted on the *y*-axis. (*B* and *C*) PCR amplifications of H_2O controls containing no cDNA (*B*) and of PBMC cDNA (*C*) were performed for 28 cycles. Amplification of a control cDNA, prepared from RNA without the addition of RT, with Cβ primers produced no detectable product after 28 cycles (data not shown). (*B*) Area of the slot blot containing samples of the H_2O controls for the PCR experiment depicted in C. (*C*) PBMC cDNA was amplified in triplicate using the 5′ primers depicted on the left. Each slot represents a separate PCR reaction. The slot blot was hybridized with radiolabeled βprobe, washed, and analyzed with the AMBIS system. The image is presented in log mode for clarity. (*D*) Net hybridized counts (mean±SEM) were determined for each triplicate and are presented on the *x*-axis. The area of the slot blot depicted in *B* was used as the sample background.

Vβ gene family specific 5′ primers. The hybridized slot blots of this experiment are shown in Fig. 1, *B* and *C*. Quantifying of the net hybridized counts, depicted in Fig. 1 *D*, indicated a low degree of well-to-well variability in the triplicate PCR amplifications. The normalized data from this experiment are depicted in Fig. 2. Each cDNA in this report was subjected to a similar analysis.

Circulating T cells from the blood of 24 RA patients with active disease were examined for biased TCR expression in comparison with TCR expression in the peripheral blood of 15 normal donors. Although two blood samples were available from 2 RA subjects, only one sample from each was used in this analysis. Restricted Vβ gene family utilization was not observed in RA patients nor in normal donors. For example, all of the 22 Vβ products were detected at levels exceeding 1% of the total in the blood of patient RA-23 (Fig. 1 *C*, and Fig. 2). At least 16 of the 22 Vβ products represented 1% or more of the total in every blood sample; the mean for all of the blood samples was 20 Vβ products > 1% (data not shown). Every Vβ family comprised 1% or more of the total in the peripheral blood of 90% or more of the subjects tested, except for Vβ10 and Vβ11 which were expressed to this level in 56% and 51%, respectively. The patterns of TCR expression were heterogeneous in both RA patients and controls. The data summarized in Table II indicate that the mean expression of each Vβ gene family was not markedly different in RA patients and normal donors. However, a statistically significant decrease in Vβ13.2 expression in RA patients was observed. Comparison of the peripheral blood T cell Vβ expression in the 20 RF+ to those of the normal donors produced results very similar to those obtained by analyzing all of the RA patients (data not shown).

To address the possibility that the RA patients who express HLA-DR1 or HLA-DR4 could represent a distinct subset, peripheral blood Vβ expression by these patients was compared to that of normal donors expressing either of these markers and the results are summarized in Table II. As in the previous comparisons Vβ13.2 expression was lower in RA patients. Vβ8 expression was also significantly lower in the patients than in the controls. In addition, RA patients in this group expressed significantly higher levels of Vβ2 and Vβ5.1 than did this normal donor group.

The potential effect of disease duration was explored by comparing peripheral blood Vβ expression in subjects having RA for 2 yr or less, and those having RA for more than 2 yr, to each other and to the normal subjects. There were no statistically significant differences between the group of 16 RA patients with longer disease duration and normal donors, nor between this group of patients and the group of 8 with shorter disease duration. However, Vβ13.2 expression (3.0±0.5%) was lower in the patients with RA for 2 yr or less than in the normal donors (Table II) ($P < 0.004$), whereas Vβ20 expression (4.1±0.5%) was higher in this group of RA patients ($P < 0.039$).

Similarly, the possibility that peripheral blood Vβ expression differed in patients taking nonsteroidal antiinflammatory drugs (NSAIDs) alone and patients taking DMARDs was ex-

Table II. Vβ Gene Family Utilization in Peripheral Blood of RA patients and Normal Donors

	All donors		Donors expressing HLA-DR1 or HLA-DR4	
Vβ gene family	Normal donors (n = 15)	RA patients (n = 24)	Normal donors (n = 5)	RA patients (n = 12)
Vβ1	0.040±0.006	0.044±0.004	0.033±0.006	0.041±0.003
Vβ2	0.075±0.013	0.091±0.007	0.052±0.009	0.095±0.010†
Vβ3	0.053±0.010	0.060±0.009	0.085±0.018	0.080±0.015
Vβ4	0.075±0.010	0.066±0.005	0.066±0.007	0.071±0.009
Vβ5.1	0.043±0.006	0.047±0.004	0.029±0.004	0.053±0.006‡
Vβ5.2–3	0.034±0.006	0.035±0.004	0.027±0.008	0.038±0.004
Vβ6.1–3	0.102±0.007	0.106±0.008	0.093±0.006	0.089±0.005
Vβ7	0.096±0.007	0.093±0.007	0.099±0.010	0.088±0.012
Vβ8	0.086±0.007	0.078±0.006	0.101±0.010	0.070±0.007‡
Vβ9	0.044±0.005	0.049±0.004	0.054±0.006	0.049±0.006
Vβ10	0.014±0.003	0.013±0.001	0.013±0.004	0.011±0.002
Vβ11	0.012±0.002	0.013±0.002	0.015±0.004	0.013±0.004
Vβ12	0.029±0.005	0.028±0.003	0.035±0.007	0.030±0.005
Vβ13.1	0.057±0.006	0.051±0.004	0.053±0.004	0.048±0.007
Vβ13.2	0.053±0.004	0.038±0.004*	0.053±0.005	0.029±0.004*
Vβ14	0.034±0.003	0.027±0.003	0.033±0.004	0.025±0.003
Vβ15	0.023±0.003	0.027±0.003	0.021±0.004	0.025±0.004
Vβ16	0.020±0.002	0.018±0.002	0.020±0.002	0.018±0.002
Vβ17	0.043±0.006	0.032±0.004	0.042±0.009	0.037±0.005
Vβ18	0.020±0.003	0.023±0.002	0.026±0.005	0.026±0.004
Vβ19	0.021±0.003	0.027±0.006	0.020±0.005	0.032±0.011
Vβ20	0.028±0.004	0.035±0.005	0.030±0.004	0.033±0.005

Data represent mean±SE of the value Vβ/ΣVβ determined for each case.
Wilcoxon's rank sum test, ‡ $P \leq 0.023$, * $P \leq 0.01$, in comparison to corresponding group of normal donors.

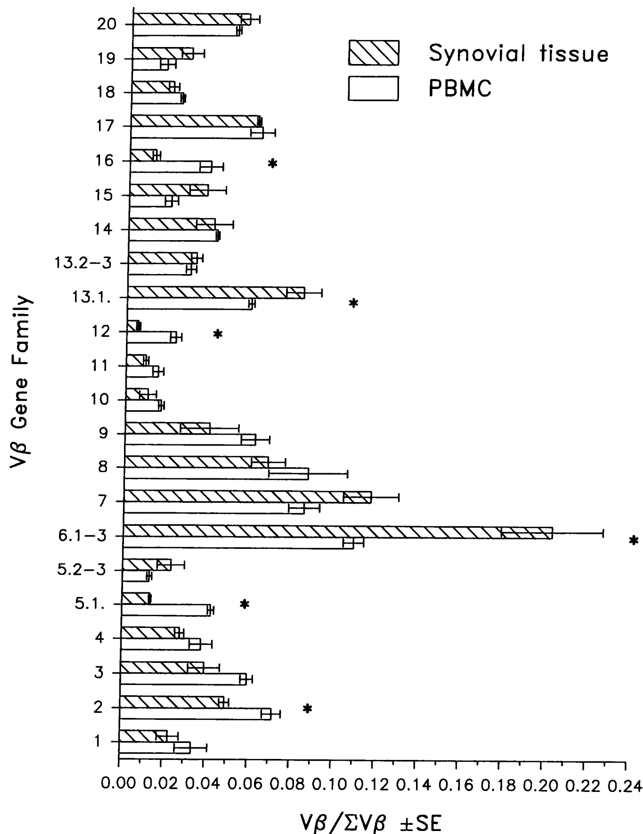

Figure 2. Comparison of T cell Vβ gene family utilization in synovial tissue and PBMC from patient RA-23. RNA prepared directly from synovial tissue and was compared to that isolated from blood drawn at the time of surgery. Significantly different ($P < 0.05$) levels of Vβ gene family expression are noted (*).

amined by comparing these groups to each other and to the normal donors. There were no statistically significant differences in the peripheral blood expression of any Vβ families between the group of 15 patients taking DMARDs and the normal donors. In comparison to normal donors (Table II), the group of 9 patients taking NSAIDs alone exhibited reduced expression of Vβ13.2 (3.4±0.5%, $P < 0.009$) and Vβ14 (2.2±0.4, $P < 0.026$). Vβ1 expression was slightly higher in the patients taking NSAIDs alone than in the patients also taking DMARDs (5.4±0.7% vs. 3.8±0.3%, $P < 0.026$).

Taken together, these results suggest that the entire group of RA patients exhibited differences of a minor degree in Vβ gene expression in their peripheral blood in comparison to the entire control group of normal donors, but greater differences were observed if the comparison was limited to groups which expressed similar MHC class II alleles. Moreover, these analyses suggested that peripheral blood Vβ expression in the group of patients who had RA for 2 yr or less, and in those taking NSAIDs alone, differed from that in normal donors to a greater degree than did the group with longer duration disease or those taking DMARDs as well. Peripheral blood was also obtained from 4 arthritis patients with diagnoses other than RA and the Vβ expression in these samples was compared to that of the normal donors. There were no statistically significant differences in the mean expression of any Vβ gene family between these two control groups (data not shown).

Rheumatoid synovium. The possibility of skewing of Vβ expression in rheumatoid synovium compared to peripheral blood was examined in 21 samples from 19 patients. A number of statistical analyses were carried out to validate the biases observed (see below). Initially, we sought evidence of biased Vβ expression by comparison of the mean utilization of the group of synovial samples to that of the group of corresponding blood samples. In addition, each case was examined for a 50% difference between the synovial and blood T cells in the level of expression of each Vβ family. Finally, those cases in which triplicate amplifications were carried out were examined for statistically significant differences in Vβ expression regardless of the magnitude of the disparity. Comparison of group means has the potential advantage of revealing significant trends apparent in the entire group that could have been of modest magnitude in individual cases. On the other hand, if there was marked heterogeneity within the group, biologically meaningful differences in individual cases might be obscured. Tabulation of the number of individual comparisons in which a bias was either statistically significant or exceeded 50% was performed to address this possibility. In order to examine the possibility that biased Vβ utilization may be limited to certain subsets of RA patients, these analyses were performed on: (*a*) the entire set of samples; (*b*) those in which either synovial tissue or synovial fluid was analyzed; (*c*) samples from RF+ patients; (*d*) samples from subjects known to express HLA-DR1 or DR4; (*e*) samples from patients with RA for 2 yr or less, or for more than 2 yr; (*f*) samples from patients taking DMARDs; and (g) samples from patients taking NSAIDs alone.

The Vβ expression of T cells in synovial tissue at the time of joint replacement or synovectomy was determined in eight RA patients, six of whom were seropositive for RF. Histologic examination was performed on four of these synovial specimens and confirmed the presence of an active inflammatory infiltrate in each case. Vβ expression in simultaneously obtained peripheral blood T cells was used for comparison. An example of this type of experiment is shown in Fig. 2. Results of the comparison of the expression of each Vβ family in the eight synovial tissue samples to that of the corresponding peripheral blood samples are presented in Table III. There were statistically significant increases in Vβ6.1-3 and Vβ15 expression in synovial tissue as compared to peripheral blood, and a significant decrease in synovial tissue expression of Vβ1 and Vβ5.1. Moreover, mean expression of Vβ6 and Vβ15 in RA synovial tissue was significantly greater, and Vβ5.1 expression significantly lower, than in normal donor peripheral blood ($P < 0.034$).

Synovial fluid samples obtained at the time of therapeutic arthrocentesis were also examined and compared to simultaneously obtained peripheral blood T cells in 13 RA cases (synovial fluid and blood were obtained from two patients on two occasions). 12 of the samples were obtained from RF+ patients. This analysis (Table III) revealed that Vβ6.1-3 and Vβ14 expression in the 13 synovial fluid T cell samples was significantly higher than that in the peripheral blood. On the other hand, synovial fluid expression of Vβ4, Vβ16, and Vβ19 was significantly lower than in the peripheral blood samples. In addition, mean expression of Vβ4, Vβ16, and Vβ19 in RA synovial fluid was significantly lower than in normal donor peripheral blood ($P < 0.014$).

As was the case with peripheral blood, restricted Vβ gene family expression was not observed in RA synovium. Levels of

Table III. Vβ Gene Family Utilization in RA Synovium and Simultaneously Obtained Peripheral Blood Samples

Vβ gene family	Synovial tissue samples (n = 8)		Synovial fluid samples (n = 13)		All synovial samples (n = 21)		HLA-DR-1/4 samples (n = 9)	
	Blood	Tissue	Blood	Fluid	Blood	Synovium	Blood	Synovium
Vβ1	0.045±0.008	0.028±0.004*	0.045±0.004	0.038±0.006	0.045±.004	0.034±.004*	0.044±.003	0.029±.004‡
Vβ2	0.084±0.012	0.114±0.022	0.097±0.009	0.107±0.010	0.092±.007	0.109±.010	0.096±.012	0.100±.016
Vβ3	0.044±0.014	0.023±0.003	0.058±0.012	0.065±0.015	0.052±.009	0.049±.010	0.064±.018	0.057±.020
Vβ4	0.065±0.005	0.041±0.009	0.075±0.008	0.040±0.005§	0.071±.006	0.040±.005§	0.080±.010	0.042±.005‡
Vβ5.1	0.040±0.003	0.022±0.006*	0.048±0.006	0.034±0.005	0.045±.004	0.030±.004‡	0.051±.006	0.024±.003‡
Vβ5.2–3	0.031±0.004	0.032±0.007	0.034±0.007	0.030±0.004	0.033±.005	0.031±.004	0.034±.004	0.028±.004
Vβ6.1–3	0.100±0.008	0.154±0.016*	0.096±0.008	0.132±0.016‡	0.098±.006	0.140±.012§	0.089±.007	0.138±.022‡
Vβ7	0.108±0.012	0.118±0.013	0.089±0.01	0.087±0.008	0.096±.008	0.099±.008	0.098±.015	0.098±.015
Vβ8	0.094±0.011	0.074±0.011	0.082±0.008	0.094±0.015	0.086±.007	0.086±.010	0.076±.009	0.091±.020
Vβ9	0.056±0.008	0.060±0.012	0.046±0.005	0.050±0.006	0.050±.004	0.054±.006	0.049±.008	0.049±.006
Vβ10	0.012±0.002	0.007±0.002	0.015±0.002	0.012±0.002	0.014±.001	0.010±.001*	0.011±.002	0.007±.002*
Vβ11	0.014±0.003	0.009±0.001	0.014±0.003	0.013±0.003	0.014±.002	0.011±.002	0.014±.005	0.013±.004
Vβ12	0.026±0.003	0.020±0.004	0.029±0.004	0.029±0.007	0.028±.003	0.025±.004	0.026±.006	0.024±.005
Vβ13.1	0.047±0.005	0.062±0.008	0.054±0.007	0.054±0.007	0.051±.004	0.057±.006	0.046±.008	0.056±.009
Vβ13.2	0.047±0.007	0.037±0.006	0.036±0.005	0.031±0.004	0.040±.004	0.033±.004	0.036±.007	0.034±.007
Vβ14	0.033±0.009	0.029±0.005	0.024±0.003	0.031±0.004‡	0.028±.004	0.030±.003*	0.022±.004	0.028±.006‡
Vβ15	0.018±0.002	0.037±0.005*	0.033±0.005	0.037±0.004	0.027±.004	0.037±.003*	0.024±.005	0.040±.003*
Vβ16	0.019±0.004	0.011±0.002	0.016±0.002	0.012±0.001*	0.017±.002	0.012±.001‡	0.017±.003	0.012±.002
Vβ17	0.037±0.007	0.030±0.005	0.030±0.004	0.033±0.007	0.033±.004	0.032±.005	0.038±.007	0.039±.009
Vβ18	0.022±0.002	0.026±0.005	0.020±0.002	0.021±0.002	0.021±.001	0.023±.002	0.022±.002	0.025±.005
Vβ19	0.023±0.004	0.026±0.007	0.024±0.004	0.011±0.002*	0.024±.003	0.017±.003*	0.026±.006	0.018±.007
Vβ20	0.038±0.007	0.040±0.007	0.035±0.005	0.041±0.009	0.036±.004	0.041±.006	0.037±.006	0.050±.012

Data represent mean±SE of the value Vβ/ΣVβ determined for each case.
Wilcoxon's signed rank test, * $P < 0.05$, ‡ $P \leq 0.01$, or § $P \leq 0.001$ in comparison to corresponding blood samples.

expression of at least 17 of the 22 Vβ products were > 1% in every synovial sample; the mean for the group was 20 Vβ products representing 1% or more of the total. Every Vβ family represented at least 1% of the total in the synovium of 90% or more of the RA patients, except Vβ10, Vβ11, Vβ12, Vβ16, and Vβ19 which were expressed at this level in 38%, 43%, 81%, 67%, and 53%, respectively. A comparison of the Vβ gene expression in all of the 21 synovial samples and the corresponding blood samples is presented in Table III. Vβ6.1-3 and Vβ15 were significantly overexpressed in rheumatoid synovium when all 21 samples were considered together ($P < 0.0005$ and $P < 0.05$, respectively). Although synovial T cell expression of Vβ14 expression was statistically significantly increased ($P < 0.02$), the difference in the means of the synovial and peripheral blood samples was extremely small (3.0% vs. 2.8%). The synovial T cell expression of Vβ1, Vβ4, Vβ5.1, Vβ10, Vβ16, and Vβ19 was significantly lower than expression in the corresponding RA peripheral blood samples ($P < 0.05$). In comparison to the peripheral blood of normal donors, the mean expression of Vβ6.1-3 and Vβ15 in the synovial samples was significantly greater ($P < 0.015$ and $P < 0.003$, respectively), and the mean expression of Vβ4 and Vβ16 was significantly lower ($P \leq 0.001$).

Similar results were obtained when the comparison was limited to the 18 RF+ cases (data not shown). If the analysis was limited to the nine cases where the samples were obtained from donors known to be HLA-DR1 or DR4+, increased synovial T cell Vβ6.1-3, Vβ14, and Vβ15 expression, and decreased synovial T cell Vβ1, Vβ4, Vβ5.1, and Vβ10 expression were again noted (Table III). Moreover, in the synovium of this group of patients the mean Vβ15 expression was significantly higher ($P < 0.01$), and the mean Vβ4 and Vβ10 expression was significantly lower ($P < 0.014$), than that in the peripheral blood of normal donors known to express these HLA antigens.

Individual analysis of rheumatoid synovium and peripheral blood in the (*a*) 9 samples from patients with RA for 2 yr or less; (*b*) 12 samples from patients with disease for more than 2 yr; (*c*) 8 samples from patients taking NSAIDs alone; and (*d*) 13 samples from patients also taking DMARDs, each revealed biased expression of Vβ gene families very similar to that seen in the entire group of RA patients. Restricted expression of Vβ gene families was not observed in any of these groups of synovial T cell samples.

Bias in individual cases. It was evident that all individual comparisons of synovial and peripheral blood T cells did not exhibit the same pattern of biased V gene expression. To explore the possibility that biased utilization of some Vβ families by synovial T cells occurs more frequently than anticipated from the data in Table III, we examined all individual comparisons of synovium and blood for differences in expression of 50% or more, reasoning that such a difference is of sufficient magnitude to be of potential biologic importance. In the example shown in Fig. 2, synovial tissue expression of Vβ5.2-3, Vβ6.1-3, Vβ15, and Vβ19 was at least 50% greater than that of peripheral blood in this patient (RA-23). On the other hand, Vβ2, Vβ5.1, Vβ9, Vβ10, Vβ11, Vβ12, and Vβ16 expression was at least 50% greater in peripheral blood.

The number of cases in which expression of a particular Vβ

gene family differed by 50% or more in synovial tissue and peripheral blood is shown in Table IV. There are at least two examples of biased expression of every Vβ gene family. Increased synovial tissue expression of Vβ15 and Vβ6.1-3 was frequently seen, whereas Vβ4, Vβ5.1, Vβ10, Vβ12, Vβ13.2, and Vβ16 expression was frequently greater in the peripheral blood. Biased expression of Vβ15 and Vβ5.1 in the synovial tissue was found in all three of the donors known to express DR1 or DR4 or both.

Synovial fluid and peripheral blood samples were obtained on two occasions 9 mo apart from subject RA-11. These two comparisons of synovial fluid and peripheral blood are depicted in Fig. 3. At both time points it is clear that T cell Vβ expression is not identical in the synovial fluid and peripheral blood. Regression analysis was used to determine the degree of overall similarity of Vβ expression. The coefficient of determination (r^2) for peripheral blood and synovial T cell Vβ expression was 0.78 at the first time point and 0.84 at the second time point. This degree of similarity was relatively high in comparison to that observed in similar experiments; the mean r^2 for the 21 comparisons was 0.57 with 95% confidence intervals of 0.47–0.67. It is also evident from Fig. 3 that peripheral blood (and synovial fluid) T cell Vβ expression was not identical at both time points. Between time points in RA-11 the degree of similarity (r^2) for peripheral blood Vβ expression was 0.66, and for synovial fluid Vβ expression $r^2 = 0.70$. The variability of Vβ expression in the peripheral blood of RA-11 with time was considerably less than that seen between this patient and the other RA patients where the mean $r^2 = 0.45$ (95% confidence interval of 0.38–0.52). In control experiments, the r^2 for repeat analysis of the same cDNA was 0.93 for the first RA-11 PBMC cDNA, and 0.91 for the second RA-11 PBMC cDNA. These results indicate that differences observed in comparisons of different cDNAs are unlikely to be artifactual.

Table IV. Skewed Vβ Gene Family Expression in RA Synovial Tissue and Fluid

Vβ gene family	Synovial tissue cases (n = 8)		Synovial fluid cases (n = 13)		All RF+ HLA-DR-1/4+ comparisons (n = 8)	
	Blood	Tissue	Blood	Fluid	Blood	Synovium
Vβ1	2*	0	6	2	5	0
Vβ2	1	3	0	2	1	2
Vβ3	4	1	2	2	3	2
Vβ4	5	1	9	0	5	0
Vβ5.1	6	1	7	1	6	0
Vβ5.2–3	2	2	4	2	1	1
Vβ6.1–3	0	4	0	2	0	4
Vβ7	1	2	2	2	1	2
Vβ8	3	1	1	2	0	2
Vβ9	2	1	1	3	0	1
Vβ10	5	1	7	2	4	0
Vβ11	3	0	5	3	3	0
Vβ12	4	1	4	4	2	2
Vβ13.1	0	3	2	2	1	2
Vβ13.2	4	0	3	1	1	1
Vβ14	1	0	0	2	0	2
Vβ15	0	6	3	6	0	3
Vβ16	4	0	5	0	2	0
Vβ17	2	1	4	3	3	1
Vβ18	1	3	3	1	0	0
Vβ19	3	2	8	1	3	1
Vβ20	2	1	1	2	0	1

* Number of cases in which expression was 50% greater in indicated compartment.

Expression of 10 Vβ families differed by 50% or more in the first comparison of peripheral blood and synovial fluid T cells in RA-11, whereas 7 Vβ families differed to this degree in the second comparison (Fig. 3). Decreased synovial T cell expression of Vβ4 and Vβ19 was seen on both occasions, consistent with the overall similarity of both peripheral blood and synovial fluid Vβ expression at the two timepoints. Samples were also obtained from subject RA-3 on two occasions 3 mo apart (data not shown). The coefficient of determination for synovial fluid and peripheral blood expression was 0.76 at the first time point and 0.63 at the second time point. The variability of peripheral blood Vβ expression in this patient with time ($r^2 = 0.77$) was less than that seen between individuals (see above). Synovial fluid T cell Vβ expression exhibited a similar degree of variability with time ($r^2 = 0.68$). A 50% increase in synovial fluid expression of Vβ7 and a 50% decrease in synovial fluid expression of Vβ12 were observed on both occasions. A 50% bias was observed in the expression of four other Vβ gene families in only the first comparison, and six others in only the second comparison.

A summary of the 13 synovial fluid analyses is presented in Table IV. Heterogeneous skewing continued to be noted as there are at least two examples of biased expression for each Vβ gene family. Similar to the synovial tissue comparisons, increased synovial fluid expression of Vβ15 and greater peripheral blood expression of Vβ4, Vβ5.1, Vβ10, and Vβ16 was frequently observed. In addition, there were several cases where synovial fluid expression of Vβ1 and Vβ19 was substantially less than that in the peripheral blood. There were only two cases in whom peripheral blood expression of Vβ14 was 50% less than in the synovial fluid. Only one of the six RF+, HLA-DR1 or DR4 patients exhibited a 50% or greater decrease in peripheral blood expression of Vβ14 in comparison to synovial fluid. Nevertheless, peripheral blood expression of Vβ14 was modestly but significantly lower than that of synovial fluid in this group of six patients (2.5% vs. 3.4%, $P = 0.031$), as it was for the entire group of synovial fluid samples (Table III).

Taking the synovial tissue and synovial fluid samples together synovial and peripheral blood TCR Vβ expression was compared in 21 cases, 18 of which were from seropositive patients. Increased expression of Vβ15, Vβ6.1-3, Vβ2, Vβ12, and Vβ13.1 was observed most frequently in synovial T cells, whereas increased expression of Vβ4, Vβ5.1, Vβ10, Vβ19, Vβ16 and Vβ1 was seen more frequently in peripheral blood (Table IV). When the samples were analyzed for larger differences between synovial and peripheral blood expression of individual Vβ gene families, biases continued to be noted. Thus, when twofold or greater differences were required, 9 of 18 RF+ patients exhibited increases in peripheral blood expression of Vβ4 (no cases of opposite bias), whereas 8 RF+ patients manifested greater expression of Vβ15 in synovial T cells (one case of opposite bias).

A comparison of synovial and peripheral blood T cell utilization of Vβ families was performed in eight seropositive RA patients who expressed HLA-DR1 or HLA-DR4 (Table IV).

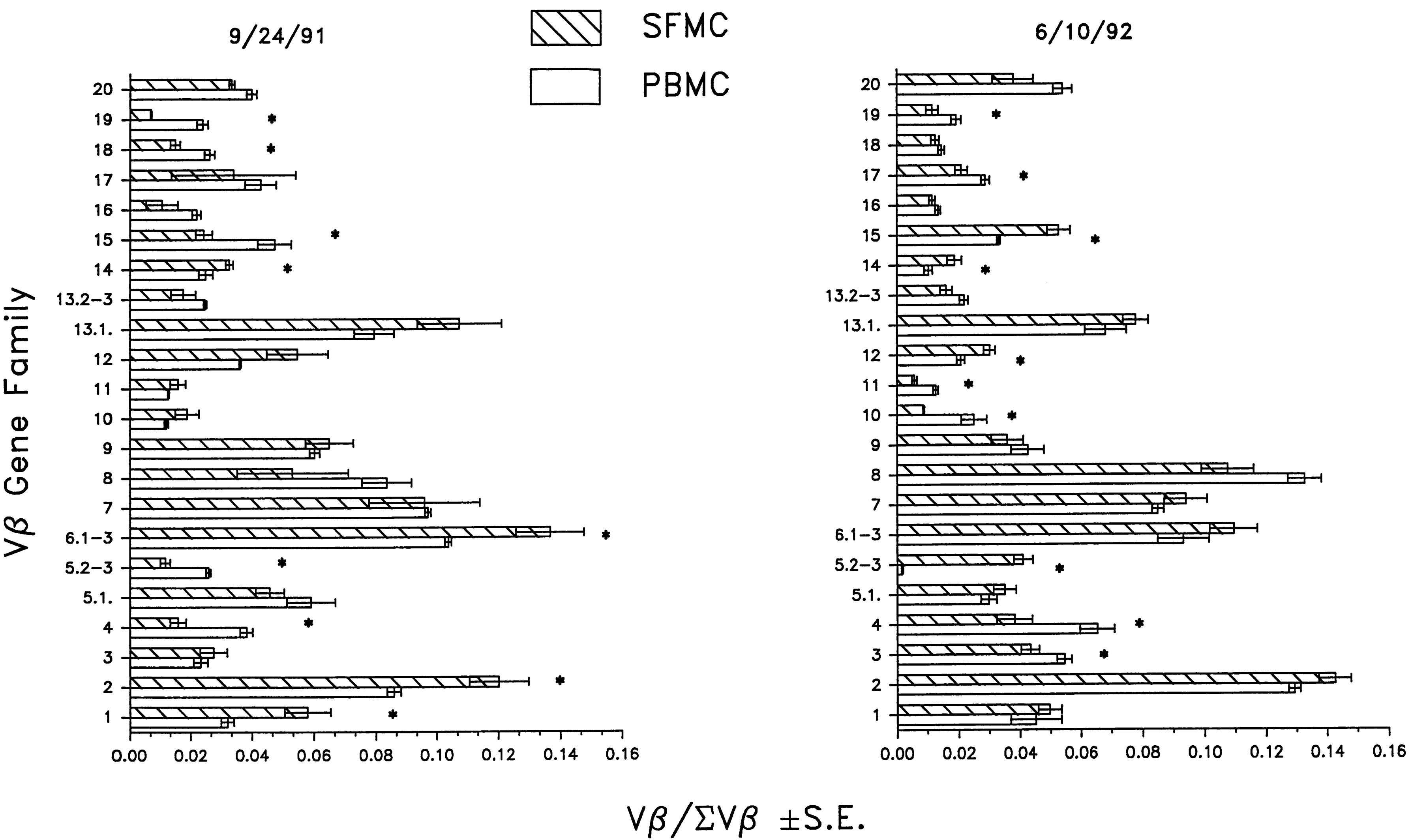

Figure 3. Comparison of T cell $V\beta$ gene family utilization in synovial fluid mononuclear cell and PBMC from patient RA-11 on two occasions. In both experiments significantly different ($P < 0.05$) levels of $V\beta$ gene family expression in SFMC and PBMC are noted (*).

Synovial T cell expression of Vβ15 was at least 50% greater than peripheral blood in five of these patients, whereas increased peripheral blood expression of Vβ1, Vβ4, Vβ5.1, Vβ10, and Vβ16 was seen in five, six, eight, six, and four cases, respectively.

In total, 19 comparisons of synovial and peripheral blood T cells were done using triplicate amplifications of cDNA with each primer pair. Statistical analysis could therefore be directly applied to these comparisons. Examination of the data in this way has the advantage of detecting biased Vβ gene expression that may be statistically significant but not of sufficient magnitude to meet the 50% cutoff used in the analyses presented above. For example, in the comparison shown in Fig. 2, synovial tissue T cell and peripheral blood T cell expression of Vβ2, Vβ5.1, Vβ6.1-3, Vβ12, Vβ13.1, and Vβ16 were significantly different ($P < 0.05$). The difference in Vβ2 expression, however, was < 50%. Similarly, in both of the experiments shown in Fig. 3 there were three examples of significant bias in which the magnitude of the difference was < 50%. Table V presents the number of cases where expression of individual Vβ families was significantly increased in one compartment or the other. Vβ2, Vβ6.1-3, and Vβ15 were the three families most frequently increased in synovial T cells, whereas there was a large number of cases of increased peripheral blood expression of Vβ1, Vβ4, Vβ5.1, Vβ10, and Vβ19. Similar trends were seen in the group of 17 RF+ cases (data not shown), and the eight comparisons from RF+, HLA-DR1/4+ cases (Table V).

Table V. Statistically Significantly Biased Vβ Gene Family Expression in synovium vs. Peripheral Blood of RA Patients

Vβ gene family	All comparisons (n = 19)		All RF+ HLA-DR-1/4+ comparisons (n = 8)	
	Blood	Synovium	Blood	Synovium
Vβ1	7*	2	5	0
Vβ2	3	7	1	2
Vβ3	5	3	3	2
Vβ4	12	1	5	0
Vβ5.1	11	1	6	0
Vβ5.2–3	4	3	1	1
Vβ6.1–3	0	10	0	4
Vβ7	2	3	1	2
Vβ8	1	3	0	2
Vβ9	1	2	0	1
Vβ10	7	1	4	0
Vβ11	5	2	3	0
Vβ12	5	4	2	2
Vβ13.1	3	3	1	2
Vβ13.2	4	3	1	1
Vβ14	1	4	0	2
Vβ15	1	7	0	3
Vβ16	5	0	2	0
Vβ17	6	1	3	1
Vβ18	2	1	0	0
Vβ19	9	1	3	1
Vβ20	3	1	0	1

* Number of comparisons done with triplicate PCR amplifications in which expression was significantly greater in the indicated compartment ($P < 0.05$).

Of note, Vβ6.1-3 was significantly overexpressed in 10 of 19 synovia (Table V), although this overexpression was > 50% in only 6 of 21 cases (Table IV). Similarly, there were only 2 of 13 cases where Vβ14 expression was 50% less in peripheral blood than synovial fluid (Table IV), but of the 12 synovial fluid comparisons done in triplicate, 4 exhibited a statistically significant decrease in peripheral blood Vβ14 expression (data not shown).

Discussion

The TCR repertoire of peripheral T cells is determined in part by the genetic elements which encode the TCR, by self-MHC antigen-directed positive and negative selection during thymic education, and presumably by the antigens or superantigens the individual has encountered. As RA is a chronic inflammatory disease with a genetic susceptibility linked to MHC class II genes, any or all of these determinants could produce a bias in the peripheral blood or synovial TCR repertoire. The present study provides the strongest evidence to date that biased Vβ gene utilization can be observed in unselected T cell populations from RA patients.

There was substantial subject-to-subject variability in peripheral blood Vβ gene expression both in normal donors and RA patients. Nevertheless, the mean expression of each Vβ gene family was not markedly different in RA patients, normal donors, or a small group of subjects with other arthritides. The mean expression of Vβ13.2 was slightly lower in RA patients, however. Small differences in the expression of other Vβ gene families were also observed in both the subset of patients with RA for 2 yr or less and in the subset of patients taking NSAIDs alone. However, a longitudinal study of a larger number of patients would be needed to determine whether TCR bias in peripheral blood T cells is blunted by passage of time or treatment with DMARDs.

It is possible that the subtle bias in the V gene expression of peripheral T cells in RA results from encounters with superantigens, chronic stimulation by antigen, or from other factors known to influence V gene expression, such as differences in the MHC antigens expressed by the normal donors and the RA patients (11). To address the possibility that expression of specific HLA molecules may influence Vβ gene utilization we compared peripheral blood Vβ expression by RA patients who expressed the disease susceptibility conferring MHC class II molecules HLA-DR1, or HLA-DR4, to that of normal donors expressing either of these markers. This analysis revealed several significant differences between these groups. Vβ13.2 and Vβ8 expression was lower in the patients than in the controls, whereas Vβ2 and Vβ5.1 expression was higher. The observation that a greater degree of bias was evident in a comparison in which the two groups shared some MHC alleles suggests that events related to the disease process itself, such as antigen or superantigen stimulation, alter the expression of TCR Vβ products in the peripheral blood of RA patients.

Paliard et al. (14) observed that the expression of Vβ14 in the peripheral blood of six of nine RA patients was undetectable or substantially diminished in comparison to peripheral blood of normal donors and arthritic controls. In the current study, the Vβ14 PCR product was detectable in every RA patient and normal donor examined, representing 0.9% or more of the total. The mean expression in RA patients was lower than that in the control group (2.7% vs 3.4%), but the differ-

ence did not achieve statistical significance ($P = 0.07$). In the current group of RA patients known to express HLA-DR1 or DR4 (as did all of those studied by Paliard et al.) the mean peripheral blood expression of Vβ14 was lower than that of the control donors who expressed these HLA antigens (Table III), but again the difference was not statistically significant ($P = 0.16$). Differences in the study populations may explain the somewhat disparate results obtained in these two studies. In addition, there were a number of methodological differences that might have influenced the results, including stimulation of the cells prior to RNA preparation in the aforementioned study as well as major differences in the PCR technique, means of normalization of the data, and data analysis. In preliminary experiments we have attempted to determine the impact of these variables on the data generated in each study, but have been unable to document that these methodological differences explain the disparate results. Thus, for example, we found that inclusion of an additional pair of oligonucleotides (such as Cα primers) to provide an internal amplification control did not enhance the reproducibility of the Vβ amplifications. In addition, although we found that expression of a number of the Vβ families was significantly altered by stimulation of the cells with anti-CD3 antibodies and IL-2 before analysis, this did not decrease expression of Vβ14 by blood T cells. Since most of the data was generated from triplicate PCR amplifications and the standard error of the mean was quite small, the current data persuasively argue that Vβ14 is not substantially decreased in the blood of patients with RA. One other study of 3 RA patients and 2 normal donors came to a similar conclusion (27).

A number of previous studies have analyzed TCR V gene expression in RA using a variety of techniques, but did not produce a clear picture of the degree of restricted V gene expression in RA synovium or peripheral blood (Table VI). Studies examining RA peripheral blood and synovium for biased V gene expression have also not produced consistent results, perhaps because many of these examined only small numbers of patients. Two studies described greater synovial fluid expression of Vβ14 (14, 29). In the current study Vβ14 expression in synovial fluid was significantly greater than in the simultaneously obtained peripheral blood samples. This bias is consistent with the results of Paliard et al. (14). However, the magnitude of the difference was much less marked in the current study than in the prior study and was not observed in synovial tissue. It is noteworthy that Vβ14 expression has been observed to be enriched in CD8+ T cells (43–45) which are usually increased in RA synovial fluid (46–48). Thus, it is possible that differences in Vβ14 expression are at least partially related to alterations in the proportion of CD8+ cells found in peripheral blood and synovial fluid.

Immune responses to local introduction of an antigen result in infiltration by antigen-specific T cells and by large numbers of nonspecific T cells. Thus, one can assume that T cells responding to pathogenic peptides in rheumatoid synovium represent a small minority of infiltrating T cells. The large proportion of nonspecific T cells may produce variability in the Vβ expression profile of T cell populations with time as was noted in the samples obtained from the same patient at two points in time. These considerations may also partly explain the difference between the present study and those of others. Examination of a large number of patients with a semiquantitative approach comparing synovial and peripheral blood T cell Vβ expression in this study allowed the identification of biased TCR expression despite the presence of nonspecific T cells expressing other TCR Vβ gene products. Many of the previous reports studied an insufficient number of patients for consistent biases to emerge, or did not perform comparisons to peripheral blood rendering conclusions regarding TCR bias somewhat speculative. Moreover, the current study was designed to provide an accurate determination of Vβ expression by T cells as they are found in situ within the synovium or peripheral blood of RA patients. Therefore, stimulation of the cells prior to analysis, as was done in other studies, was avoided. Moreover, since there is no conclusive evidence that one or another of the specific T cell subsets present in the synovium is responsible for disease pathogenesis, no effort was made to isolate indi-

Table VI. Summary of Studies of V Gene Bias in RA

Reference	*n*	Source of material	Synovial T cell expression	
			Restricted	Biased*
24	3	SF and PB	Vβ7 in 2	
			Vα: no	
14	9	SF and PB	Vβ: no	Vβ14 in 9
29	5	SF and PB	Vβ: no	Vβ14 in 3, Vβ18 in 2
58	5	IL-2R+ST T cells	Vβ3 in 3, Vβ9 in 3, Vβ14 in 4, Vβ17 in 4	
30	2	SF	Vα: no	Vα14, Vα15, VαFR1
25	9	tissue	Vβ: no	
			Vα: heterogeneous number detected	
26	10	tissue	Vβ: heterogeneous number detected	
			Vα: heterogeneous number detected	
27	3	SF and PB	Vβ: no	Vβ2 in 2
28	8	SF and PB	Vα: no	Vα10 in 3, Vα15 in 3, Vα18 in 3
			Vβ: no	Vβ4 in 3, Vβ5 in 3, Vβ13 in 3

n, number of patients studied. Abbreviations used in this table: IL-2R, IL-2 receptor; PB, peripheral blood; SF, synovial fluid; ST, synovial tissue.
* Observations from two or more patients of increased synovial expression.

vidual subpopulations before analysis. Finally, the PCR approach we used to analyze Vβ gene expression is sensitive to changes in the activation status of T cells as well as their number because recently activated T cells containing increased amounts of RNA result in an increased PCR product from the appropriate Vβ/Cβ primer pair. These considerations support the conclusion that the biases reported in the current study which have not been reported in previous studies, and are rather small in magnitude, may reflect disease related differences in TCR Vβ gene expression in blood versus synovium.

In the current report, the possibility of skewing of Vβ expression in rheumatoid synovium compared to peripheral blood was examined in 21 samples from 19 patients. Whereas we found skewed expression of each Vβ family in at least two comparisons, the current experimental results provide evidence for consistently increased expression of Vβ6 and Vβ15 in rheumatoid synovium. Expression of Vβ6.1-3 in the 8 synovial tissue samples, in the 13 synovial fluid samples, and in the group including all 21 synovial samples was significantly higher than in the corresponding peripheral blood samples (Table III). Furthermore, in 10 of 19 comparisons done with triplicate amplifications synovial Vβ6.1-3 expression was significantly higher than in peripheral blood (Table V), although the magnitude of increased expression was sometimes less than 50%. The expression of Vβ15 in the synovial tissue samples, in the group of 21 synovial samples, and in the group of synovial samples from HLA-DR1/DR4+ patients was significantly greater than that in the simultaneously obtained peripheral blood samples (Table III). The bias toward synovial T cell expression of Vβ15 was also apparent in synovial fluid samples in which 6 of 13 cases exhibited at least a 50% greater expression of Vβ15 (Table IV). Thus, increased Vβ6.1-3 and increased Vβ15 expression in rheumatoid synovium were established by a number of analytic methods.

Evidence of biased Vβ expression in rheumatoid synovium was also revealed by decreased expression of certain Vβ families. Vβ1, Vβ4, Vβ5.1, Vβ10, Vβ16, and Vβ19 expression by synovial T cells was significantly lower than in the corresponding blood samples. It has been reported that Vβ5.1 expression was skewed toward the CD4+ subset (43, 45, 49). This subset bias may contribute to the relative decrease in Vβ5.1 expression in synovial fluid, but is probably less of a factor in synovial tissue where the relative proportion of CD4 and CD8 single positive cells is usually the same as in peripheral blood (37, 50). Evidence of similar bias in the expression of Vβ1, Vβ4, Vβ10, Vβ16, or Vβ19 by CD4+ vs. CD8+ cells has not been reported and therefore alterations in expression of these are likely unrelated to biases of subset distribution in various compartments.

The analyses we performed did not suggest that the presence of RF, the expression of HLA-DR1 or DR4, the duration of disease, or the medications used had a substantial impact on the likelihood of detecting biased Vβ expression in rheumatoid synovial T cells. Thus, the subset of synovial samples from seropositive patients, from patients known to express HLA-DR1 or DR4, from patients with RA for 2 yr or less, from patients with RA for more than 2 yr, from those medicated with NSAIDs alone, and from those also being treated with DMARDs all exhibited similarly biased TCR Vβ expression in comparison to the corresponding peripheral blood samples. For example, all of the biases present in the HLA-DR1/4+ synovia were also present in the analysis of all 21 synovial samples (Tables III and IV). However, greater numbers of seronegative RA patients, and longitudinal study of several patients, would be required to determine definitively the impact of RF, disease duration, or medications on Vβ expression by synovial T cells.

The high level of expression of the Vβ6 family in the synovial and peripheral blood T cells of RA patients, and in the peripheral blood of the control subjects is likely to be related to the size of the Vβ6 family which contains at least nine members. However it is evident from the data in Tables II and III that levels of expression are not strictly dependent on the number of genes in a gene family, and, therefore, are likely to be influenced by thymic selection and antigen stimulation. Within rheumatoid synovium, skewed expression of Vβ gene families among T cells might result from altered migration into and retention in the synovium, or from selective proliferation or activation (or inactivation) of cells utilizing these V gene families.

Superantigen stimulation, characterized by Vβ specific activation of T cells that is often followed by depletion or inactivation of cells bearing the appropriate Vβ (51–54), could account for selective enrichment or depletion of selected Vβ families in RA. Although several superantigens interact with one or more of the relevant Vβ gene families (55), no known superantigen could explain all of the observed biases in the peripheral blood of RA patients who express HLA-DR1 or DR4. Similarly, the current data do not implicate a known superantigen as being responsible for simultaneous expansion of Vβ6 and Vβ15 bearing T cells in rheumatoid synovium. Vβ6 is stimulated by staphylococcal enterotoxin (SE) A (R. Jenkins and D. Karp, unpublished results) and SEE (55), whereas Vβ15 is stimulated by SEB, SEC2 (56), and streptococcal pyrogenic exotoxin (SPE) A (57). However, none of these superantigens interacts with both Vβ6 and Vβ15 bearing T cells. Finally, no known superantigen could explain the observed depletion of several Vβ families in rheumatoid synovium. Nevertheless, it remains possible that the actions of as yet unidentified superantigens account for the biases observed in RA synovium and peripheral blood.

The present study of blood samples from 24 RA patients, 4 control patients, and 15 normal donors, and of synovial tissue from 8 RA patients and 13 RA synovial fluid samples, represents the most extensive examination of Vβ gene utilization in RA to date. The results clearly indicate that the number of Vβ families expressed by RA peripheral blood and synovial T cells is not significantly restricted. More importantly, several lines of evidence indicate biased Vβ gene utilization in different peripheral compartments of rheumatoid patients can be observed in unselected T cell populations. These analyses revealed several statistically significant trends, with either enrichment or depletion of particular Vβ genes noted in the synovium. Increased expression of Vβ6 and Vβ15 was evident in a majority of patients. In addition, Vβ4, Vβ5.1, Vβ10, Vβ16, and Vβ19 expression was decreased in rheumatoid synovium in a majority of the comparisons. Because the PCR assay is sensitive to changes in activation status, alterations in either the number of cells expressing these Vβ families or the activation state of these cells could explain the differences observed in the Vβ-specific PCR products from RA T cells. Therefore, the data are consistent with the postulate that antigen or superantigen driven processes may cause Vβ-specific enrichment or depletion of T cells in RA.

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