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Limited T-cell receptor β-chain heterogeneity among interleukin 2 receptor-positive synovial T cells suggests a role for superantigen in rheumatoid arthritis

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ABSTRACT **Rheumatoid arthritis (RA) is a disease affecting the synovial membranes of articulating joints that is thought to result from T-cell-mediated autoimmune phenomena. T cells responsible for the pathogenesis of RA are likely present in that fraction of synovial T cells that expresses the interleukin 2 receptor (IL-2R), one marker of T-cell activation. We report herein an analysis of T-cell receptor (TCR) β-chain gene expression by IL-2R-positive synovial T cells. These T cells were isolated from uncultured synovial tissue specimens by using IL-2R-specific monoclonal antibodies and magnetic beads, and TCR β-chain transcription was analyzed by PCR-catalyzed amplification using a panel of primers specific for the human TCR β-chain variable region (Vβ). Multiple Vβ gene families were found to be transcribed in these patient samples; however, three gene families, Vβ3, Vβ14, and Vβ17, were found in a majority of the five synovial samples analyzed, suggesting that T cells bearing these Vβs had been selectively retained in the synovial microenvironment. In many instances, the Vβ3, Vβ14, or Vβ17 repertoires amplified from an individual patient were dominated by a single rearrangement, indicative of clonal expansion in the synovium and supportive of a role for these T cells in RA. Of note is a high sequence similarity between Vβ3, Vβ14, and Vβ17 polypeptides, particularly in the fourth complementarity-determining region (CDR). Given that binding sites for superantigens have been mapped to the CDR4s of TCR β chains, the synovial localization of T cells bearing Vβs with significant CDR4 homology indicates that Vβ-specific T-cell activation by superantigen may play a role in RA.**

T cells, as regulators of immune responses, are thought to be important in the initiation and perpetuation of the pathogenesis of rheumatoid arthritis (RA) (1). The strong association between RA and certain HLA-DR specificities (2–5) is consistent with a role for T cells in RA. More direct evidence is provided by immunohistochemical characterization of the synovial mononuclear cell infiltrate. This infiltrate contains considerable numbers of T cells (6), a fraction of which express markers of activation, such as the interleukin 2 receptor (IL-2R). IL-2R-positive (IL-2R$^+$) T cells make up, on average, 10–15% of the synovial T cells (6), and they often are clustered in foci of blastogenesis that resemble sites of focal transformation observed in immune organs (7–10). Moreover, T cells isolated from RA synovial tissue by *in vitro* cultivation often display dominant T-cell receptor (TCR) gene rearrangement patterns (11–13) which are characteristic of clonal T-cell expansion. These findings are indicative of *in situ* activation of specific T-cell populations by synovial antigen(s) and are supportive of a role for these T cells in the pathogenesis of RA.

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In the present study, we have isolated activated synovial T cells, not by *in vitro* cloning, but by selecting IL-2R$^+$ T cells directly from uncultured synovial tissue. PCR analysis (14) of TCR Vβ gene expression by these T cells reveals the IL-2R$^+$ synovial T-cell repertoire to be limited in heterogeneity and suggests that this limitation is imposed by superantigen-induced, β-chain variable region (Vβ)-specific T-cell activation at one or more stages in the pathogenesis of RA.

MATERIALS AND METHODS

Patient Materials. Synovial tissue specimens were obtained, during joint replacement surgery, from patients conforming to the American Rheumatism Association criteria for the diagnosis of classical or definite RA.

HLA-DR Analysis. HLA-DR analysis was conducted by reverse slot blot PCR hybridization (15). Fragments of HLA-DR genes 75 base pairs (bp) long were PCR amplified, ^{32}P labeled, and hybridized to immobilized allele-specific oligonucleotides (ASOs) encoding amino acids 67–74 of HLA-DR β-chain genes. ASOs corresponding to HLA-DR4w4 and -DR1, -DR4w14, or -DR4w15 (the latter three share DNA sequence identity in this region) were used to assess RA predisposition. ASOs for six other HLA-DRB1 and three HLA-DRB3 genes were included in the analysis. Primer and ASO sequences were deduced from published data (16, 17).

Isolation of IL-2R$^+$ Synovial T Cells. Synovial tissue was digested with agitation for 4 hr at 37°C in RPMI-1640 medium and 10% fetal bovine serum containing collagenase (Worthington) at 4 mg/ml and DNase (Sigma) at 0.15 mg/ml. Single cells were collected by Ficoll density gradient centrifugation, washed, and precleared by incubation at 10^6 cells per ml for 30 min at 0°C with control mouse IgG (Coulter) at 5 μg/ml in phosphate-buffered saline containing 2% bovine serum albumin. Cells were washed three times and incubated for 30 min at 0°C with magnetic beads conjugated to goat anti-mouse IgG (Advanced Magnetics, Cambridge, MA). After magnetic removal of the beads, the remaining cells were incubated 30 min at 0°C with mouse anti-human IL-2R (Coulter) at 5 μg/ml, washed, and selected with magnetic beads as above. Cell-coated beads were washed three times and immediately resuspended in acidified guanidinium/phenol/chloroform, and RNA was prepared as described (18).

Abbreviations: RA, rheumatoid arthritis; TCR, T-cell antigen receptor; IL-2R, interleukin 2 receptor; V, variable region; C, constant region; J, joining region; CDR, complementarity-determining region.

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Hybridization Analysis of PCR-Amplified TCR β-Chain Genes. TCR β-chain transcripts were amplified in two-stage amplification reactions with Vβ-specific (19) and nested β-chain constant region (Cβ) primers. RNAs were reverse transcribed (1 hr, 42°C) with a Cβ primer (5′-CCA GAA GGT GGC CGA GAC-3′) (800 pmol) in 240 μl of reaction buffer (20). Reaction mixtures were diluted with a master mix (160 μl) containing nucleotides, reaction buffer minus $MgCl_2$ (final Mg^{2+} concentration = 3.6 mM), and *Taq* DNA polymerase (Ampli-taq, Perkin–Elmer/Cetus). Eighteen microliters were added to each of 19 tubes containing the individual Vβ primers (40 pmol of each; final reaction volume = 20 μl). Water blanks were included in all analyses to control against reagent contamination. Samples were denatured (15 min, 95°C) and 20 cycles of PCR were conducted (final *Taq* polymerase concentration = 1 unit per reaction mixture). Each cycle consisted of a 1-min denaturation at 95°C, a 2-min annealing step, and a 2-min extension at 72°C. The first two cycles were annealed at 37°C and 45°C and the remainder at 50°C. One-microliter aliquots of these stage I reaction mixtures were added to 50-μl stage II amplification reaction mixtures (Gene-Amp Kit, Perkin–Elmer/Cetus) containing a second Cβ primer located 5′ to the first (5′-GCG GCT GCT CAG GCA GTA-3′) (50 pmol) and the Vβ primers (50 pmol) used in the corresponding preamplifications. Stage II amplifications were conducted for 20 cycles with a 50°C annealing temperature and without the 37°C and 45°C ramping. Five microliters of each reaction mixture was electrophoresed in 2% agarose gels, transferred to nitrocellulose, and hybridized to a ^{32}P-labeled Cβ probe. Blots were exposed to x-ray film and scored for the presence or absence of Vβ-specific amplification.

Cloning and Sequencing of PCR-Amplified TCR β-Chain Genes. Single, 20-cycle stage I PCRs were conducted as above with each of the patient cDNA samples, the 3′ C primer, and a single Vβ consensus primer (Vβcons) (21) (40 pmol of each primer; final reaction volume = 20 μl). One-microliter aliquots of these stage I reaction mixtures were added to 100-μl stage II reaction mixtures containing Vβ-specific primers, and amplification was conducted as above for 35 cycles. The amplifications of Vβ3 rearrangements from patients 1014, 1015, and 1020, and of Vβ9 rearrangements from patients 1012, 1015, and 1020, were conducted with the respective Vβ-specific primers in place of the consensus primer in stage I reactions. The products of each entire reaction were concentrated and gel-purified by using Gene Clean (Bio 101, San Diego). Most samples were ligated to *Eco*RI adaptors and cloned in Bluescript SK^- (Stratagene), and multiple independent rearrangements were isolated. PCR-amplified Vβ17 rearrangements from patients 1014 and 1015 were base denatured and directly sequenced. All samples were sequenced from a Cβ primer (5′-CGA CCT CGG GTG GGA ACA-3′) with T7 DNA polymerase (Sequenase, United States Biochemical).

RESULTS

Hybridization Analysis of TCR Vβ Gene Expression. IL-$2R^+$ synovial T cells were isolated from five RA patients, who each possessed at least one HLA-DR allele known to predispose to RA (Table 1). RNAs were extracted from IL-$2R^+$ T cells and were reverse transcribed. The resulting first-strand cDNAs were PCR amplified by using 3′ TCR Cβ primers and individual 5′ primers, corresponding to 19 known human Vβ families (Table 1). The total number of Vβ families that were amplified from each patient sample varied from 2 to 12. Four particular Vβ families were found in three or four of the five patient samples analyzed. Vβ17 and Vβ14 transcripts were detected in four of the five patient samples, while Vβ3 and Vβ9 transcripts were found in three of the five patient samples. Three additional Vβs were shared by two of the patient samples, yet 10 or more Vβ families were transcribed in each of these two samples, suggesting that this commonality results only from a more generalized T-cell infiltration. In the three patients with only two or three detectable Vβs, those found were almost exclusively Vβ3, Vβ9, Vβ14, or Vβ17.

Sequence Analysis of TCR Rearrangements. cDNAs from each of the patient samples were preamplified by using a Vβ consensus primer (Vβcons) (21) and reamplified by using Vβ-specific primers, and the products were cloned in plasmids. Sequence analysis of individual plasmid isolates revealed that the Vβ17 repertoire of the patient 1012 IL-$2R^+$ sample contained three distinct rearrangements (Table 2). Two rearrangements predominated, in that each was present in 5 of the 11 plasmid clones that were sequenced. The patient 1013 IL-$2R^+$ sample was composed of only a single rearrangement, found in all 13 of the plasmid clones that were sequenced. Dominance of specific rearrangements was also found in the Vβ17-amplified DNA from patients 1014 and 1015. These PCR products were not cloned in plasmids, but they were alkaline denatured and directly sequenced. In each of these samples, a single DNA sequence was read. As previously demonstrated (21), the ability to directly sequence these PCR products indicates that the rearrangement sequenced made up a majority if not all of the Vβ17 rearrangements amplified from these two samples. Dominance of a particular rearrangement in the patient 1020 IL-$2R^+$ sample was not demonstrated by analysis of the few clones shown in Table 2. J heterogeneity was not limited among these Vβ17 rearrangements, with approximately equal numbers of Jβ1 and Jβ2 elements found. Further, significant homologies were not found among the complementarity-determining region (CDR)3 amino acid sequences, either when different rearrangements from a single patient were compared or when rearrangements from any one patient were compared with those of the other patients.

A similar clonal dominance was observed among Vβ3 rearrangements in three of the patient samples. Five plasmid clones containing Vβ3 rearrangements amplified from 1013 IL-$2R^+$ contained a single, specific Vβ3 sequence. Similarly, a single Vβ3 nucleotide sequence was found in three plasmid clones isolated from 1014 IL-$2R^+$. The 1020 IL-$2R^+$ sample was dominated by one rearrangement, which was present in five of the seven clones sequenced. The 1012 and 1015 IL-$2R^+$ samples were more heterogeneous, with multiple Vβ3 rearrangements found in each of the plasmid libraries. It

Table 1. PCR amplification of TCR β-chain genes from IL-$2R^+$ synovial T cells

Patient	HLA-DR alleles	Amplification of Vβ families																		
		1	2	3	4	5	6	7	8	9	10	11	12	14	15	16	17	18	19	20
1012	*1, 3*			+	+												+			
1013	*1, 7*						+			+				+						
1014	*1, 4w4*													+			+			
1015	*4w4, x*			+			+	+	+	+		+	+	+	+		+	+	+	
1020	*3, 4w4*	+	+	+		+				+				+	+		+	+	+	

Results are boxed for the families found in three or four of the patients.

Table 2. CDR3 sequences of TCR rearrangements amplified from IL-2R$^+$ synovial T cells

Patient	Ratio*	Sequence		
		Vβ	Dβ and/or N-diversity	Jβ
		Vβ17 family		
1012	5/11	YLCASS	ISSG	SYNEQ (Jβ2.1)
	5/11	YLCASS	PIGTGIH	GYTFG (Jβ1.2)
	1/11	YLCASS	IIEDSAG	EKLFF (Jβ1.4)
1013	13/13	YLCAS	KGRDEPFF	YNEQF (Jβ2.1)
1014	Direct	YLCAS	VRDRR	NYGYT (Jβ1.2)
1015	Direct	YLCASS	SIDS	SYEQY (Jβ2.7)
1020	2/3	YLCASS	IVTGTT	YNEQF (Jβ2.1)
	1/3	YLCAS	TAAGGA	DTQYF (Jβ2.3)
		Vβ3family		
1012	2/6	YLCASS	RM	NTEAF (Jβ1.1)
	2/6	YLCASS	HLGHP	NTEAF (Jβ1.1)
	1/6	YLCASS	FRTTI	GYTFG (Jβ1.2)
	1/6	YLCASS	QDRTTL	TGELF (Jβ2.2)
1013	5/5	YLCASS	LGGE	ETQYF (Jβ2.5)
1014†	3/3	YLCASS	NRLR	QFFGP (Jβ2.1)
1015†	1/3	YLCASS	FSRGGTRI	TQYFG (Jβ2.3)
	1/3	YLCAS	TDS	STDTQ (Jβ2.3)
	1/3	YLCAS	VVGIPPRTSD	NEQFF (Jβ2.1)
1020†	5/7	YLCASS	LGLAGRP	DTQYF (Jβ2.3)
	1/7	YLCASS	LSGQGR	YNEQF (Jβ2.1)
	1/7	YL	SSGSFG	SPLHF (Jβ1.6)
		Vβ14family		
1012	2/7	YFCASS	LGGAV	SYNEQ (Jβ2.1)
	2/7	YFCASS	LASGGAV	SYNEQ (Jβ2.1)
	1/7	YFCASS	RFGGDG	SYEQY (Jβ2.7)
	1/7	YFCASS	PLAGV	NTGEL (Jβ2.2)
	1/7	YFCASS	LSQATPLR	ND
1015	6/6	YFCASS	LSFGGRMA	NEQFF (Jβ2.1)
1020	1/3	YFCAS	RDFKGGR	NTEAF (Jβ1.1)
	1/3	YFCASS	FGEV	TEAFF (Jβ1.1)
	1/3	YFCA	THPRDRH	TEAFF (Jβ1.1)
		Vβ9family		
1013†	4/4	Non-TCR		
1015†	1/19	YFCASS	QDTLGG	ND
	18/19	Non-TCR		
1020†	1/11	YFCASS	QDLVGVG	NTEAF (Jβ1.1)
	10/11	Non-TCR		

Contiguous sequences have been divided into segments for clarity; Jβ, β-chain joining region. ND, not determined.
*Number of plasmid clones containing the indicated rearrangement/total number of plasmids sequenced for that patient sample. "Direct" denotes those PCR products that were directly sequenced.
†Samples amplified with specific primers in stage I and II reactions.

is noteworthy that two of the three samples amplified without the use of Vβcons (1014 and 1020) show clonal dominance, arguing that the dominance seen in other samples is not the result of Vβcons amplification bias. As with the Vβ17 rearrangements, neither significant CDR3 homology nor preferential Jβ gene usage was observed among any of the Vβ3 rearrangements that were sequenced.

Clonal dominance among Vβ14 rearrangements of patient 1015 also was found. Each of the six plasmid clones that was sequenced contained the rearrangement shown in Table 2. In contrast, the IL-2R$^+$ sample from patient 1012 contained five rearrangements among the seven plasmid clones sequenced. Despite this lack of clearcut clonal dominance in the 1012 sample, four of the seven rearrangements were related by significant CDR3 homology and Jβ2.1 usage. Similarly, while the sequences of the three Vβ14 rearrangements cloned from patient 1020 were all different, each individual rearrangement utilized the Jβ1.1 element. The cloned and sequenced products of Vβ14-specific amplifications from 1013 and 1014 IL-2R$^+$ that were detected in the original hybridization analysis (Table 1) proved to be Vβ3 rearrangements apparently resulting from misamplification by the Vβ14 primer. This primer infidelity likely results from the 25 of 30 nucleotide identity between the Vβ14 primer (19) and Vβ3 genes.

Sequence analysis of the PCR products of Vβ9 amplification revealed even more significant primer infidelity. The majority of the plasmid clones obtained from the three patient samples that were hybridization positive for Vβ9 amplification were aberrantly rearranged β chains or were not β chains at all. Only two plasmid clones containing functional Vβ9 rearrangements were isolated, one each from patients 1015 and 1020. The low frequency of legitimate TCR rearrangements in the products of Vβ9 primer amplification minimizes the significance of the hybridization reported in Table 1.

In summary, Vβ17 rearrangements were isolated from all five of the patient samples analyzed, and in four of these five patients particular Vβ17 rearrangements predominated. Vβ3 rearrangements also were cloned from all five patient samples, and, in three of those, a single Vβ3 rearrangement predominated. Vβ14 rearrangements were isolated from three of the patients and in one patient a dominant Vβ14 sequence was detected. In addition, each patient displayed clonal dominance in one or more of these three gene families. Patient 1012 shows clonal dominance among Vβ17 T cells, patient 1013 and 1014 among Vβ3 and Vβ17 T cells, patient 1015 among Vβ14 and Vβ17, and patient 1020 among Vβ3.

To ensure that the observed clonal dominance did not result from some unusual aspect of PCR amplification of these synovial RNA samples, rearrangements of other Vβ families were amplified and sequenced from synovial IL-2R$^+$ RNAs (Table 3). Four distinct Vβ8 rearrangements were found in five plasmid clones that were sequenced, and five different Vβ12 rearrangements were represented in 5 sequenced plasmids. Heterogeneity also was found among the Vβ3, Vβ14, and Vβ17 rearrangements amplified from peripheral blood lymphocytes (PBLs) (Table 3). Vβ3, Vβ14, and Vβ17 rearrangements were amplified and cloned, using RNA extracted from 1012 PBLs that were cultured for 3 days with phytohemagglutinin and phorbol 12-myristate 13-acetate. The diversity within each of these Vβ families approximated the number of clones that were analyzed. Further, none of the rearrangements that were found in the 1012 synovial sample (Table 2) were present among the β-chain genes amplified from 1012 PBLs. Thus, the restricted heterogeneity of Vβ3, Vβ14, and Vβ17 rearrangements in the synovium does not reflect the heterogeneity in peripheral blood, nor is it characteristic of other synovial Vβ gene families. This suggests that T cells bearing Vβ3, Vβ14, and/or Vβ17 polypeptides are selectively retained and expanded in the synovial microenvironment.

Sequence Similarity among Vβ3, Vβ14, and Vβ17 Polypeptides. To assess the relatedness of the polypeptides encoded by the Vβ gene families found in the present analysis, GenBank (release 66.0) was searched for homology to the

Table 3. TCR β-chain heterogeneity in patient 1012

Cells	Vβ	No. of clones sequenced	No. of rearrangements
IL-2R$^+$ synovial cells	Vβ8	5	4
	Vβ12	5	5
PBL	Vβ3	7	7
	Vβ14	5	4
	Vβ17	10	9

Heterogeneity of Vβ8 and Vβ12 rearrangements amplified from 1012 IL-2R$^+$ synovial cell RNA is indicated, along with heterogeneity of Vβ3, Vβ14, and Vβ17 rearrangements amplified from 1012 peripheral blood lymphocyte (PBL) RNA.

Table 4. Amino acid sequence comparison between human Vβ17 and other Vβ polypeptides

Polypeptide	Amino acid residues	% identity to Vβ17
Human Vβ17	94	100
Murine Vβ6	94	69.1
Human Vβ3	94	58.5
Human Vβ12.1	94	53.2
Human Vβ14	94	52.1
Murine Vβ7	89	51.7
Human Vβ9	94	33.0

Rankings through murine Vβ7 represent unedited results of TFASTA analysis. Similarity of Vβ9 is shown for comparison.

complete (less the leader peptide) 94-amino acid sequence of Vβ17, using the sequence similarity program TFASTA (22). As shown in Table 4, mouse Vβ6 was found to be most similar to Vβ17, followed by human Vβ3, Vβ12.1, and Vβ14, and mouse Vβ7. Significantly, of the three human Vβs that are most similar to Vβ17, two (Vβ3 and Vβ14), like Vβ17, are present among activated synovial T cells in RA patients. The third, Vβ12.1, was detected in only one of the five synovial samples. Vβ9, which is shown for comparison, is only 33% identical to Vβ17.

The greatest similarity found among this group of related Vβ polypeptides is in a contiguous stretch of 15 amino acids located carboxyl to the CDR2 region (Table 5). This corresponds to a segment of the TCR β-chain recently appreciated to be a fourth β-chain hypervariable region, or CDR4 (24, 25). The similarities in the CDR4 regions of Vβ3, Vβ14, and Vβ17 polypeptides are greater than the similarities shown by the complete V amino acid sequences (Table 4) and greater still than similarities in the respective CDR1 and CDR2 regions (data not shown). This is particularly of note since CDR4 is in the region of the β chain that is implicated in binding to both exogenous and endogenous superantigens (Table 5). For example, staphylococcal enterotoxin SEC_2 binds human Vβ13.2 polypeptides at a site contained in the underlined sequence of Table 5 (26). In addition, a variant Vβ17 polypeptide (Vβ17a2), identified in wild mice, displays altered reactivity with endogenous superantigen as assessed by deletion of murine $Vβ17^+$ T cells (27). Vβ17a2 differs from Vβ17a1 of laboratory mice by two amino acid substitutions, one of which is a glutamine → arginine substitution at residue 71. Similarly, the importance of residues 70 and 71 in binding of the self superantigen Mls-1a has been demonstrated through the analysis of Vβ8.2a and Vβ8.2c polypeptides (23), Mls-1a nonresponder and responder β chains, respectively.

Table 5. TCR β-chain sequences involved in superantigen binding

Poly-peptide	Sequence	% identity to hVβ17
	Proposed superantigen-binding site	
hVβ17	EGYSVSREKKESFPL	
hVβ3	EGYSVSREKKERFSL	86.7
hVβ14	EGYKVSRKEKRNFPL	66.7
hVβ12.1	DGYSVSRSKTEDFLL	66.7
hVβ9	NRFSPKSPDKAHLNL	≤30
mVβ6	EGYDASREKKSSFSL	73.3
mVβ7	KGYRVSRKKREHFSL	64.3
	Previously identified superantigen-binding sites	
hVβ13.2	DGYNVSRLKKQNFLLGLE	
	60 70	
mVβ17a1	KRFSAKCSSNSQCIL	
mVβ17a2	.....R...	
mVβ8.2a	DGYKASRPSQENFSL	
mVβ8.2c	.....KE...	

Proposed superantigen-binding site in RA-associated β-chain polypeptides and previously identified sites of superantigen binding in human and murine TCR β-chain polypeptides are shown; h-, human; m-, murine. Numbering is based on the lysine and glutamic acid residues in murine Vβ8.2c, previously designated (23) as positions 70 and 71, respectively. Dots indicate identity with the sequence above.

DISCUSSION

We have analyzed TCR β-chain gene expression among synovial T cells isolated from RA patients. Rather than analyze the entire synovial T-cell compartment, however, we have preselected $IL\text{-}2R^+$ T cells as those most likely to be relevant to the disease pathogenesis. Using PCR amplification, we find that three Vβ gene families, Vβ3, Vβ14, and Vβ17, are present in this T-cell compartment in at least three of the five patient samples that were analyzed. Sequencing of the individual rearrangements within each of these gene families revealed the dominance of certain rearrangements in different patients and in different combinations of these gene families within individual patients. This is in sharp contrast to the heterogeneity of those same Vβ families in the periphery and to that of other Vβ families in the synovium. These findings are consistent with the preferential expansion of specific T cells bearing these β chains in the synovial microenvironment and, by inference, implicate these T cells in synovial inflammation.

The β chains associated with RA by the present study are members of a family of related Vβ polypeptides, displaying 52–59% overall V identity. Vβ3, Vβ14, and Vβ17 polypeptides are markedly divergent in their germline-encoded CDR1 and CDR2 sequences, yet they display considerable similarity in their CDR4 regions. The retention of T cells bearing Vβ polypeptides with related CDR4 sequences is of note, since this portion of the β chain has been shown to directly contribute to superantigen reactivity. This strongly suggests that Vβ-specific T-cell activation by superantigen plays a role in RA.

Several scenarios may be envisioned to explain the involvement of superantigen in RA. Superantigen expression in the periphery (e.g., enterotoxin production during the course of a bacterial infection), may result in Vβ-specific activation of otherwise tolerant T cells, some of which may subsequently react with synovial targets (28). These synovial targets may be complexes of major histocompatibility complex (MHC) and synovial peptides, a hypothesis consistent with the clonal dominance that we have observed. Were conventional recognition of MHC and peptide antigen operative in the synovium, the observed clonal dominance among Vβ3, Vβ14, and Vβ17 T cells would indicate that different synovial peptide antigens are recognized by T cells bearing these respective Vβs or that all three of these β-chain polypeptides are permissive for binding a single MHC–synovial antigen complex. The latter seems unlikely, however, given the known divergence in the germline-encoded CDR1 and CDR2 sequences and in the combinatorial CDR3 sequences described in this report. Conversely, the synovial target recognized by T cells previously activated by exogenous superantigen may, itself, be a superantigen. Endogenous superantigens, or coligands (29), which have been proposed to exist in humans (though not yet found), are potential candidates. Reactivity with an endogenous synovial superantigen, by T cells previously activated in the periphery by exogenous superantigen, is an appealing variation on molecular mimicry models, which often are invoked to explain the induction of RA. Alternatively, superantigen expression by a pathogen, trophic for cells in the synovial membrane, may directly stimulate local expansion of Vβ3, Vβ14, or Vβ17 T cells. *In situ* expression of superantigen, as a product either of a synovial pathogen or of an endogenous

gene, is consistent with the synovial localization of T cells bearing Vβ polypeptides related in the superantigen-binding site (Vβ3, Vβ14, and Vβ17). However, it is inconsistent with the observed clonal dominance of T cells bearing these β chains, since studies of inbred mouse strains have shown that virtually all T cells bearing a reactive Vβ polypeptide are expanded upon exposure to the appropriate superantigen (30). In an outbred RA patient population, however, superantigen reactivity of T cells may be influenced by other factors such as TCR α chains (31) or MHC molecules (32, 33) that serve to limit synovial expansion to only a fraction of the relevant Vβ repertoire.

A number of bacterial superantigens are known to stimulate T cells bearing the β chains associated with RA in the present report (28). Of particular interest is *Mycoplasma arthritidis* mitogen (MAM), a superantigen recently found to specifically stimulate human Vβ3- and Vβ17-bearing T cells (ref. 45; A. Theofilopoulos and B. Cole, personal communication). *M. arthritidis* induces inflammation and degeneration of articular synovia, which are clinically and histologically similar to RA, in murine (34, 35) models. Moreover, MAM is the only known bacterial superantigen that stimulates mouse T cells bearing Vβ6 (36), which, as shown in Table 4, is the murine homologue of human Vβ17. Collectively, these findings, in conjunction with those of the present report, suggest that the superantigen of *M. arthritidis* (or a functionally similar molecule produced by a related organism) plays an important role in the pathogenic mechanisms of RA.

The restricted TCR heterogeneity observed among IL-2R$^+$ T cells in the rheumatoid synovium strongly parallels previous findings from studies of T-cell-mediated autoimmunity in animal models. For example, T cells that mediate central nervous system inflammation in experimental allergic encephalomyelitis (EAE) preferentially use only one (37, 38) or two (39) Vβ gene families. This limited TCR heterogeneity has allowed the successful treatment of EAE, using immunotherapeutic strategies directed at TCRs conserved among disease-inducing T cells (37, 39–44). The similar limitation on TCR heterogeneity found among activated synovial T cells indicates that RA also may be amenable to TCR-specific immunotherapies.

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