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14. Brains from 1-year-old AE101 and AE301 transgenic and control mice were fixed with 4% paraformaldehyde. Paraffin sections of brains were cut (8 μm thick) and immunostained by rabbit polyclonal antiserum (90-29) to the amyloid β protein (1:500 dilution) and the avidin-biotin procedure (Vector Labs, Burlingame, CA). The pattern of amyloid β protein immunoreactivity in transgenic mouse brain sections was consistent when four different rabbit polyclonal antisera directed against amyloid β protein were used. Antisera 90-25 and 90-27 were directed against amino acids 1 to 28 of amyloid β protein; antisera 90-28 and 90-29 were directed against amino acids 1 to 42. All four rabbit polyclonal antisera exhibited intense immunoreactivity with senile plaques in sections of postmortem brain from AD patients (B. D. Trapp, unpublished data). Specificity of the immunoreactivity was established by the absence of immunoprecipitate in sections stained by amyloid β protein antiserum that had been absorbed with amyloid β protein and by Western blotting.
15. B. D. Trapp, unpublished data.
16. Brains from 1-year-old AE101 transgenic and control mice were fixed with 2.5% glutaraldehyde and 4% paraformaldehyde. Ultrathin Epon sections of the hippocampus were cut, counterstained with uranyl acetate and lead citrate, and examined in a Hitachi H-600 electron microscope.
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18. The open reading frame for the 42-residue amyloid β protein (A4) was contained within a 148-bp Bgl II–Bam HI restriction fragment and was generated by site-directed mutagenesis [L. Kunkel *et al.*, *Methods Enzymol.* **154**, 367 (1987)] of APP cDNA sequences with a synthetic oligonucleotide primer (5′-GGTGTTGTCATAGCGTAGGATCCGTCATCACCTTGGTG-3′). This Bgl II–Bam HI restriction fragment was ligated into the Bam HI site of pMTI-2307 [D. O. Wirak *et al.*, *EMBO J.* **10**, 289 (1991)] to generate pMTI-2316. An ~2-kb Bam HI restriction fragment, containing APP 695 3′-end cDNA sequences, was inserted into the Bam HI site of pMTI-2316 to generate pMTI-2317. An 0.6-kb Sph I restriction fragment of pMTI-2304, containing SV40 RNA splicing signals [H. Okayama and P. Berg, *Mol. Cell. Biol.* **3**, 280 (1983)] and SV40 polyadenylation signals (Bam HI–Bcl I restriction fragment from SV40 viral DNA) was ligated into a Sph I site of pMTI-2317 to generate PMTI-2318. The full-length cDNA encoding APP695 has been described [J. Kang *et al.*, *Nature* **325**, 733 (1987)].
19. Transgenic mouse lines AE101 and AE301 were generated as described [D. Wirak *et al.*, *EMBO J.* **10**, 289 (1991)]. In both transgenic mouse lines, multiple copies of the transgene have integrated as a head-to-tail tandem array (D. Wirak, unpublished data).
20. RNA was extracted from mouse brain and Hela cells as described [L. G. Davis, M.D. Dibner, J. F. Battey, *Basic Methods in Molecular Biology* (Elsevier, New York, 1986), pp. 130–135]. Synthetic oligonucleotides 29, 5′-GAGATAGAATACATTACTGATGTGTGGATTAATTCAAGTTCAGGCATCTACTTGTGTTACAGCACAGCTGGGCGTCCATA-3′, and 30, 5′-CGCGGGTGGGGCTTAGTTCTGCATTTGCTCAAAGAACTTGTAAGTTGGATAGGTTCCAAG-3′, were labeled with T4 polynucleotide kinase, and their specific activities were 6.04×10^8 and 5.72×10^8 cpm/μg, respectively. S1 nuclease protection analysis [S. Sisodia *et al.*, *Nucleic Acids Res.* **15**, 1999 (1987)] was performed with total RNA (50 μg per sample) and 1 × 10^6 cpm of each ^{32}P-labeled oligonucleotide.
21. We thank C. Kundel and A. Lee for assistance in mini-gene constructions; S. Rocks and T. Garrison for transgenic animal colony management; G. Davis, T. Buckholz, and P. Tamburini for the synthesis and purification of amyloid β proteins; M. Broggi for assistance in polyclonal antibody production; P. Rae and P. Talalay for reviewing the manuscript; R. Graham for editing and typing the manuscript; C. Cootauco and E. Mulrenin for assistance in performing the morphological studies; and K.-H. Büchel for his support.

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Evidence for the Effects of a Superantigen in Rheumatoid Arthritis

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While studying the αβ T cell receptor repertoire in rheumatoid arthritis (RA) patients, we found that the frequency of $V_\beta14^+$ T cells was significantly higher in the synovial fluid of affected joints than in the peripheral blood. In fact, $V_\beta14^+$ T cells were virtually undetectable in the peripheral blood of a majority of these RA patients. β-chain sequences indicated that one or a few clones dominated the $V_\beta14^+$ population in the synovial fluid of individual RA patients, whereas oligoclonality was less marked for other Vβ's and for $V_\beta14$ in other types of inflammatory arthritis. These results implicate $V_\beta14$-bearing T cells in the pathology of RA. They also suggest that the etiology of RA may involve initial activation of $V_\beta14^+$ T cells by a $V_\beta14$-specific superantigen with subsequent recruitment of a few activated autoreactive $V_\beta14^+$ T cell clones to the joints while the majority of other $V_\beta14^+$ T cells disappear.

RA IS AN AUTOIMMUNE DISEASE characterized by long-term inflammation of multiple joints. Mononuclear cell infiltration of the synovial membrane eventually can lead to the destruction of articular cartilage and surrounding structures. Because of its high frequency and potentially severe nature, this disease is a major cause of long-term disability in adults. Although the pathogenesis of RA and other similar autoimmune diseases remains unknown, genetic and environmental factors have been implicated. Several lines of evidence suggest that T cells specific for self-antigens may play a critical role in the initiation of these diseases. In the case of RA, the linkage of the disease to the DR4 and DR1 alleles of the class II genes of the major histocompatibility complex (MHC) and the finding of sometimes oligoclonal, activated $CD4^+$ T cells in synovial fluid and tissue of affected joints (*1, 2*) suggest the involvement of $CD4^+$, αβ T cell receptor (TCR)–bearing, class II–restricted T cells in the disease. This view is supported by the finding that partial elimination or inhibition of T cells by a variety of techniques can lead to an amelioration of disease in certain patients (*3*).

Usually, potentially autoreactive T cells are deleted or inactivated by encounter with self-antigen during their development, before they can damage the individual (*4, 5*). To understand autoimmunity one must therefore understand how self-reactive T cells escape these processes to become part of the mature T cell pool and what factors control whether these cells will remain quiescent or become activated to induce autoimmune disease. It is possible that a self-

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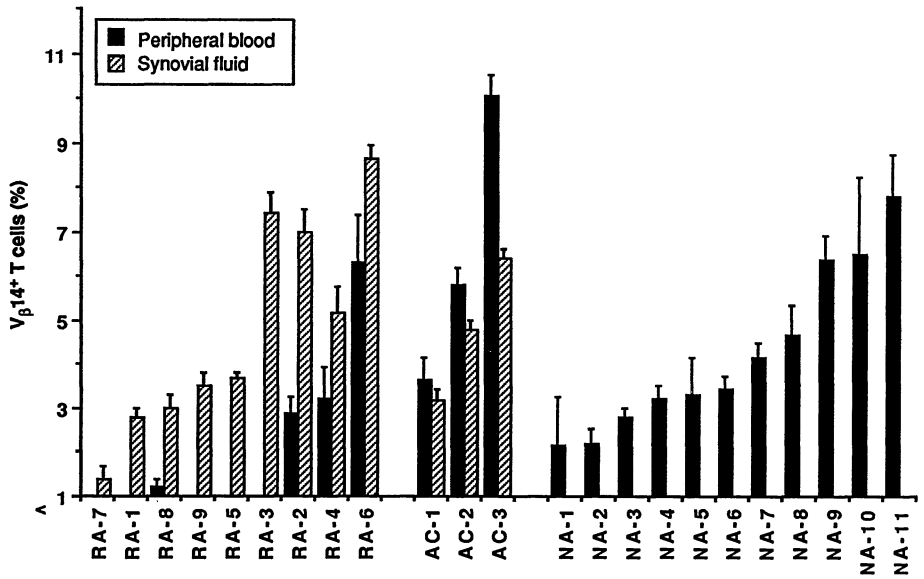

Fig. 1. TCR $V_\beta 14^+$ T cell distribution in RA patients, patients with other forms of arthritis, and nonarthritic donors. The determination of the percentages of T lymphocytes bearing $V_\beta 14$ was performed as described in Table 2. The characteristics and HLA typing of RA patients and controls are presented in Table 1. For the nonarthritic controls, the determination of $V_\beta 14^+$ T cells was only performed for peripheral blood. Results are presented as mean percentage (±SD). RA-1 through RA-9, RA patients; AC-1 through AC-3, arthritic controls; NA-1 through NA-11, nonarthritic controls.

antigen that is sequestered at very low levels or presented on inappropriate cells may fail to remove specific T cells during development and to activate these cells once they mature. Occasionally, however, microbial antigens that are cross-reactive with the self-antigens may lead to activation of these specific T cells. Once activated, these cells may recognize and chronically respond to the previously ignored self-antigen, leading to autoimmune disease. One difficulty with this suggestion is the low probability of a cross-reaction between two infrequently recognized antigens. However, we and others have identified a set of unusual microbial antigens, termed superantigens (*4, 6–10*). Because superantigens engage virtually all T cells which bear a particular TCR V_β, they can affect a much larger number of T cells than can conventional antigenic peptides, which require target T cells to bear the correct versions of all the TCR variable elements (V_α, J_α, V_β, D_β, and J_β). Superantigens are, therefore, statistically more likely than conventional microbial antigens to

Table 1. Donor characteristics. Patients were followed in the rheumatology clinics of Fitzsimons Army Medical Center and were entered into the study consecutively on the basis of diagnosis and availability of synovial fluid. Patients with RA met American Rheumatism Association criteria for classical or definite RA. All arthritis patients had active inflammatory disease at the time of fluid and blood sampling. ND, not determined; Neg, negative.

Donor	HLA–class II type	Age/disease duration (years)	Disease characteristics*	Rheumatoid factor titer	Medications†
			Rheumatoid arthritis		
RA-1	ND	31/2	Destructive, stage 2	1:320	NSAID
RA-2	−DR4,w12; −DRw52,w53; −DQw3	52/11	Destructive, stage 3	1:5120	NSAID, pred, D-pen
RA-3	−DR4; −DRw53; −DQw3	67/11	Nodular, destructive, stage 4	1:320	NSAID
RA-4	−DR4,w14; −DRw53; −DQw1,w7	69/44	Nodular, destructive, stage 2	1:1280	Pred
RA-5	−DR1,4; −DRw53; −DQw1,w3	64/6	Destructive, stage 2	1:5120	NSAID, pred, MTX
RA-6	−DR1,w15; −DQw1	40/7	Destructive, stage 2	Neg	NSAID, pred, gold
RA-7	−DR4,w11; −DRw52,w53; −DQw3	50/7	Destructive, stage 2	1:640	NSAID, pred, gold, HC
RA-8	−DR1,4; −DRw53; −DQw1,w3	69/8	Nodular, destructive, stage 3	1:1280	NSAID, pred, aza
RA-9	−DR3,4; −DRw52,w53; −DQw2,3	73/28	Nodular, destructive, stage 3	1:5120	NSAID, D-pen
			Arthritic controls		
AC-1	−DR4,w11; −DRw52,w53; −DQw3	71/13	Psoriatic arthritis, destructive, stage 3	Neg	NSAID, MTX
AC-2	ND; $-B27^-$	23/<1	Reiter's syndrome 2° chlamydia; arthritis, conjunctivitis, urethritis, balanitis, stage 2	Neg	NSAID
AC-3	ND; $-B27^+$	28/<1	Reiter's syndrome 2° salmonella enteritis; arthritis, diarrhea, conjunctivitis, urethritis, stage 2	Neg	NSAID
			Nonarthritic controls		
NA-1	−DR-4,w11; −DRw52,w53; −DQw7				
NA-2	−DR2,w11; −DRw52,w53; −DQw3,w6				
NA-3	−DR1; −DQ5				
NA-4	−DR3,4; −DRw52,w53; −DQ2,7				
NA-5	−DR3,4; −DRw52,w53; −DQw2,w3				
NA-6	ND				
NA-7	−DR4; −DRw53; −DQw7				
NA-8	−DR2,4; −DQw1,w3				
NA-9	−DR1,4; −DQw1,w3				
NA-10	−DR4,4				
NA-11	−DR4,4				

*"Destructive" refers to the presence of bone erosions on joint radiographs and joint deformities on physical examination. Staging of disability was as follows: stage 1, able to perform all usual activities without handicap; stage 2, able to perform normal activities but with pain; stage 3, unable to accomplish some normal activities, usual occupation, or self-care; stage 4, largely or wholly incapacitated or bedridden and with little ability for self-care. †Medications being taken by patients at the time of blood and synovial fluid sampling included nonsteroidal anti-inflammatory drugs (NSAID), prednisone, (pred), gold compounds given intramuscularly (gold), D-penicillamine (D-pen), methotrexate (MTX), azathioprine (aza), and hydroxychloroquine (HC).

cross-react with self-antigens, and we have suggested that they may play a role in induction of autoimmunity (7).

Relative to this hypothesis, some (*2*), but not all (*11*), studies of RA patients have suggested that the T cells in diseased synovia and synovial fluids are relatively oligoclonal. Interpretation of these analyses was complicated, however, by the fact that the diseased joints may have been infiltrated by specific and nonspecific T cells, the latter recruited by the former. With these contradictory results in mind, we used a sensitive quantitative polymerase chain reaction (PCR) technique (*9, 12*) to examine RA patients for the possibility of a skewed β-chain repertoire in affected joints and of the involvement of a superantigen in the disease. The processes of RA occur in synovium; therefore, ideally, an analysis of T cells involved in RA should use T cells isolated from the synovium. Unfortunately, however, synovial tissue is not often obtained from patients with active RA, and, even when it was available, the numbers of T cells isolated were usually too few to allow a quantitative study of their complete repertoire. Therefore, we confined our analysis to T cells isolated from the synovial fluid of affected joints and compared their TCR V_β repertoire to that of peripheral blood T cells from the same patients. We examined the TCR V_β repertoires of T cells in the peripheral blood and synovial fluid of three patients with other inflammatory arthropathies as controls.

The characteristics and human lymphocyte antigen (HLA) types of the patients and normal controls studied are shown in Table 1. All patients had active inflammatory disease at the time of fluid and blood sampling, and all but one of the HLA-typed RA patients expressed HLA-DR4, the DR allele most clearly linked with RA. The estimated percentages of T cells bearing different V_β's in the synovial fluids and peripheral blood of seven of the RA patients are shown in Table 2. Table 2 contains one of the most complete descriptions of V_β expression in different human individuals published so far. Although the data are all derived from patients with RA, the percentages of V_β expression found in peripheral blood T cells reflect some findings that have also been observed in normal individuals (*6, 12*). First, some V_β's are frequently used by T cells in all individuals studied, and others are rarely used. About 10% of peripheral blood T cells bear $V_\beta 2$, for example, and 4 to 13% bear $V_\beta 13.1$. $V_\beta 20$, on the other hand, is usually expressed by less than 2% of such cells. This differential expression does not always seem to be due to different sizes of the V_β family under examination because $V_\beta 13.1$ and $V_\beta 20$ are both members of single-gene or two-gene families. Some unknown factor or factors, perhaps related to those known to exist in mice (*4, 10*), therefore appear to be controlling V_β use by

Table 2. TCR V_β usage in the peripheral blood and synovial fluid of patients with RA. Mononuclear cells from the peripheral blood and the synovial fluid were isolated by Ficoll-Hypaque density gradient centrifugation. Total RNA was prepared from cells stimulated with anti-CD3 for 3 days, then cultured for 24 hours in interleukin-2 (IL-2) as described (*6, 9, 12*). Care was taken to prevent cross-contamination of the samples, and none of the synovial fluids tested demonstrated red blood cell contamination. Under the conditions used, in our experience, stimulation of peripheral blood mononuclear cells with anti-CD3 and IL-2 does not perturb the TCR V_β repertoire or affect the CD4:CD8 ratio of the T cells (*12*). Total RNA (2 μg) was used for the synthesis of the first cDNA strand using reverse transcriptase and random hexanucleotides. The reaction was stopped by heating at 95°C before PCR. The amplification of the cDNA by PCR with a V_β-specific oligomer, an oligomer from the downstream β-chain constant region, and two oligomers from the C_α region, has been described (*9, 12*). The amount of cDNA and the number of cycles used were determined for each cDNA preparation to ensure that the amount of products synthesized was proportional to the amount of V_β mRNA present in the original sample. The quantification of the amplified products was achieved using ^{32}P end-labeled 3′ primers ($\sim 5 \times 10^5$ cpm each) and analysis with an Ambis Radioanalytic Image System (Ambis Systems, San Diego, California) after separation of the synthesized products on a 2% agarose gel. The estimation of the percentage of T cells bearing each V_β was obtained by normalization of the counts per minute in the V_β band to that in the C_α band (the internal control) and transformation of the PCR values into a calculated percentage with a standard curve (*12*). Each V_β determination was repeated at least twice and usually four times. Moreover, for RA-1, RA-3, RA-5, and RA-6, PCR was performed with an increasing number of cycles using Vβ14- and control Vβ2-specific primers to ensure that the differences in percentages of $V_\beta 14$-bearing cells among the samples were reproducible and that the primer efficiencies were comparable (*13*). The results are presented as mean percentage (±SD). PBL, peripheral blood lymphocytes; Sfld, synovial fluid.

	RA-1		RA-2		RA-3		RA-4		RA-5		RA-6		RA-7	
V_β	PBL	Sfld	PBL	Sfld	PBL	Sfld	PBL	Sfld	PBL	Sfld	PBL	Sfld	PBL	Sfld
1	5.52 (0.76)	6.58 (0.44)	3.32 (0.46)	4.62 (0.51)	2.77 (0.23)	2.97 (0.23)	3.71 (0.73)	4.44 (0.42)	5.78 (0.61)	4.63 (0.47)	5.53 (0.52)	3.28 (0.14)	7.22 (0.97)	3.82 (0.35)
2	10.27 (1.11)	6.92 (0.51)	7.08 (0.42)	6.87 (1.02)	8.44 (0.72)	2.53 (0.31)	10.57 (1.33)	10.90 (0.43)	13.63 (2.09)	10.48 (0.76)	13.35 (2.12)	12.22 (1.52)	11.79 (2.07)	8.79 (1.76)
3	8.91 (0.45)	6.33 (0.20)	9.97 (0.47)	11.29 (0.61)	17.55 (2.59)	5.17 (0.05)	15.01 (2.56)	17.65 (3.24)	9.74 (1.35)	7.64 (0.28)	7.91 (0.78)	7.94 (1.03)	3.00 (0.36)	2.75 (0.40)
4	2.36 (0.32)	1.70 (0.04)	1.52 (0.69)	1.21 (0.32)	3.20 (0.32)	2.97 (0.19)	<1	1.89 (0.36)	2.11 (0.58)	<1	5.08 (0.57)	3.28 (0.17)	<1	<1
5.1	3.57 (0.56)	1.78 (0.17)	2.35 (0.12)	2.51 (0.11)	3.65 (0.53)	2.78 (0.30)	2.25 (0.79)	1.74 (0.32)	4.67 (0.23)	2.62 (0.12)	2.36 (0.14)	1.33 (0.11)	3.28 (0.19)	1.65 (0.28)
5.2-3	2.63 (0.09)	<1	3.36 (0.80)	4.58 (0.06)	3.15 (0.21)	5.12 (0.27)	4.22 (0.36)	3.66 (0.20)	2.27 (0.03)	2.63 (0.91)	4.02 (0.65)	1.51 (0.23)	5.63 (0.99)	1.51 (0.11)
6.1-3	6.74 (0.35)	5.62 (0.04)	8.01 (0.92)	5.21 (0.66)	11.47 (1.17)	13.94 (1.77)	4.93 (0.78)	8.30 (0.65)	3.77 (0.123)	5.58 (0.08)	4.95 (0.27)	5.61 (0.09)	7.03 (0.47)	4.27 (0.49)
7	11.49 (1.37)	5.09 (1.03)	6.49 (0.41)	4.44 (0.22)	2.45 (0.13)	6.07 (0.94)	2.52 (1.12)	5.83 (0.83)	3.21 (0.80)	3.07 (0.26)	6.32 (0.86)	4.82 (0.56)	5.91 (1.31)	6.87 (0.01)
8	5.53 (0.86)	2.45 (0.33)	3.92 (0.43)	2.01 (0.11)	4.62 (0.52)	<1	3.37 (0.14)	6.58 (1.03)	10.66 (0.28)	3.53 (0.66)	11.61 (1.47)	2.89 (0.39)	6.93 (0.95)	3.27 (0.12)
9	<1	2.42 (0.19)	1.09 (0.48)	<1	4.74 (0.37)	2.59 (0.56)	<1	1.35 (0.47)	<1	<1	<1	<1	<1	<1
10	<1	<1	<1	<1	1.04 (0.11)	<1	1.68 (0.53)	1.07 (0.68)	3.05 (0.64)	<1	1.25 (0.12)	<1	<1	<1
11	1.37 (0.07)	<1	1.10 (0.46)	2.14 (0.38)	<1	1.38 (0.15)	1.83 (0.22)	2.02 (0)	1.30 (0.12)	<1	2.45 (0.26)	<1	<1	<1
12	2.66 (0.16)	1.30 (0.28)	<1	<1	7.13 (0.96)	2.83 (0.42)	1.41 (0.17)	4.34 (0.57)	<1	2.24 (0.13)	3.74 (0.08)	<1	2.04 (0.07)	<1
13.1	8.79 (0.63)	6.43 (0.67)	4.36 (0.12)	2.82 (0.80)	4.78 (0.07)	9.98 (0.05)	4.62 (0.38)	6.25 (0.85)	3.74 (0.47)	8.10 (1.36)	12.09 (1.83)	6.81 (0.23)	10.89 (1.73)	5.55 (1.98)
13.2	6.05 (0.59)	4.72 (0.13)	4.96 (0.16)	4.67 (0.37)	3.00 (0.31)	13.54 (0.91)	4.68 (0.62)	1.58 (0.20)	2.60 (1.01)	1.95 (0)	2.60 (0.88)	1.04 (0.10)	1.39 (0.33)	4.84 (0.09)
14	<1	2.77 (0.21)	2.85 (0.42)	6.97 (0.54)	<1	7.42 (0.46)	3.20 (0.71)	5.16 (0.60)	<1	3.69 (0.10)	6.29 (1.09)	8.65 (0.31)	<1	1.40 (0.29)
15	1.15 (0.12)	1.18 (0.06)	1.97 (0.18)	1.87 (0.19)	1.69 (0.42)	<1	2.00 (0.14)	<1	<1	2.36 (0.62)	1.04 (0.06)	<1	1.36 (0.37)	<1
16	2.25 (0.27)	<1	1.04 (0.28)	1.52 (0.25)	2.45 (0.06)	1.53 (0.02)	2.87 (0.22)	1.39 (0.43)	1.85 (0.17)	1.90 (0.52)	<1	<1	<1	<1
17	3.75 (0.03)	3.00 (0.36)	3.30 (0.32)	2.55 (0)	2.10 (0.86)	1.08 (0.21)	4.66 (0.22)	3.19 (0.18)	1.06 (0.07)	1.25 (0.24)	1.97 (0.03)	1.72 (0.15)	2.40 (0.37)	<1
18	1.38 (0.32)	<1	2.06 (0.22)	<1	2.50 (0.21)	2.32 (0.18)	1.39 (0.03)	2.47 (0.46)	<1	1.45 (0.23)	<1	<1	<1	<1
19	<1	<1	<1	1.41 (0.02)	3.90 (0.42)	<1	<1	<1	1.51 (0.45)	1.26 (0.19)	<1	<1	<1	<1
20	<1	<1	1.65 (0.26)	1.60 (0.21)	3.60 (0.03)	<1	<1	<1	1.91 (0.59)	2.67 (0.09)	<1	<1	<1	<1
Total V_β	85.4	63.2	73.6	71.3	92.8	86.3	76.7	90.8	76.0	68.3	93.2	64.9	74.5	51.9

peripheral blood T cells in humans. Second, some V_β's are expressed by widely different percentages of T cells in different individuals. $V_\beta 12$, for example, was found on less than 1% of peripheral T cells in some patients (RA-2 and RA-5) but up to 7% in others (RA-3) (Table 2). These individual variations were conserved from test to test and over time. They appear to be genetically controlled, at least in part, because we have found much less variation between identical twins (*13*).

The TCR V_β repertoires of the T cells in the synovial fluids of RA patients were also variable and on the whole reflected the TCR V_β usage seen on peripheral T cells of the same patient (Table 2). Some idiosyncratic mismatches were present, however. In patient RA-5, for example, the percentages of T cells bearing V_β's 12, 13.1, 14, and 15 were higher in synovial fluid than in peripheral blood, whereas the converse was true for T cells bearing V_β's 5.1, 8, and 10. These observations indicate that infiltration of the synovial fluid by T cells in RA is not totally "random" or "passive."

In spite of these individual variations, a consistent finding in all seven patients was the higher frequency of $V_\beta 14^+$ T cells in synovial fluid than in peripheral blood, in most cases due to strikingly low numbers of $V_\beta 14^+$ T cells in peripheral blood (Table 2 and Fig. 1). The patient with the highest number $V_\beta 14^+$ T cells in peripheral blood and least skewing toward the synovial fluid (RA-6) was the one whose disease characteristics were different compared to the other RA patients; that is, this individual was $DR4^-$ and lacked detectable rheumatoid factor (Table 1). This association between skewing of $V_\beta 14^+$ T cells and RA was further confirmed when two additional patients (RA-8 and RA-9) were analyzed (Fig. 1). The difference between the numbers of $V_\beta 14^+$ T cells in synovial fluid and peripheral blood of RA patients was highly significant ($P < 0.001$ by t test on the differences). The specificity of this finding for RA was evident when three patients with other arthropathies were studied as controls. V_β usage by T cells in the synovial fluids of these patients was very heterogeneous and similar to that seen in peripheral blood (Table 3). Importantly, no enrichment of $V_\beta 14^+$ T cells in the synovial fluid was seen (Table 3 and Fig. 1).

Our most unexpected finding was the remarkably low number of $V_\beta 14^+$ T cells in the peripheral blood of a majority of the RA patients. To confirm that this phenomenon was linked to RA rather than the DR type of the RA patients, we estimated the percentages of $V_\beta 14^+$ T cells in the peripheral blood of normal individuals, many of whom shared DR4 expression with the RA patients. These are shown, together with the patient data, in Fig. 1. Five of the RA patients had less than 1% or undetectable $V_\beta 14^+$ T cells in their peripheral blood. None of the normal individuals had $V_\beta 14^+$ T cells at this low number. This difference was highly significant ($P < 0.01$ for all RA patients compared to normal individuals, and $P < 0.001$ if the comparison omitted patient RA-6, by Mann-Whitney U test). These results show that $V_\beta 14^+$ T cells were somehow perturbed in individuals with RA and that this perturbation was not caused exclusively by expression of DR4.

To find out whether the $V_\beta 14^+$ T cells in RA synovial fluids were oligoclonal, random cDNA clones encoding TCR $V_\beta 14^+$ β chains from synovial fluid T cells were sequenced. We used $V_\beta 13.2$, a V_β related in sequence to $V_\beta 14$, and $V_\beta 2$, one of the V_β's most distantly related to $V_\beta 14$ (*14*), as controls. For comparison, we sequenced $V_\beta 14^+$ cDNA clones from the peripheral blood of one RA patient and from one normal individual, as well as from the synovial fluid of a non-RA arthritic control. In RA patients, the $V_\beta 14^+$ T cell population was dominated by a few clones (Table 4). In each RA patient, two clones, as indicated by their V_β-$ND_\beta N$-J_β sequence, accounted for 46 to 72% of the $V_\beta 14^+$ T cells. These dominant clonotypes were different in each patient, although they showed evidence for restricted J_β usage. Nearly all the $V_\beta 14^+$ dominant clones used either $J_\beta 1.1$ or $J_\beta 2.3$, whereas these J_β's accounted for just 39% of the other RA synovial fluid $V_\beta 14^+$ clones and only 14% of peripheral blood $V_\beta 14^+$ clones. Some evidence of $V_\beta 14$ oligoclonality was also seen in the synovial fluid of the arthritic control patient, although the effect was less dramatic and the clones used a different set of J_β elements.

$V_\beta 14^+$ β chains from peripheral blood T cells of an RA patient and a normal individual showed no evidence of clonality; that is, none of the 35 sequences examined were repeated. This result rules out the possibility that our finding of dominant clones in the synovial fluid was somehow an artifact in-

Table 3. TCR V_β usage in peripheral blood and synovial fluid of patients with other types of inflammatory arthritis. The determination of the percentages of T cells bearing a particular V_β was performed as described in Table 2. The results are presented as mean percentage (±SD).

	AC-1		AC-2		AC-3	
V_β	PBL	Sfld	PBL	Sfld	PBL	Sfld
1	5.21 (0.9)	4.63 (0.17)	2.53 (0.35)	4.29 (0.57)	4.23 (0.69)	5.47 (0.54)
2	8.42 (0.75)	7.44 (0.06)	5.27 (0.79)	7.05 (0.74)	8.06 (1.20)	10.79 (0.44)
3	12.11 (0.64)	11.96 (0.55)	9.81 (1.13)	13.77 (1.73)	8.63 (0.78)	2.78 (0.11)
4	2.03 (0.09)	2.99 (0.28)	<1	2.21 (0.78)	<1	<1
5.1	1.72 (0.11)	1.63 (0.45)	<1	<1	2.06 (0.23)	<1
5.2-3	4.77 (0.05)	2.98 (0.12)	3.79 (0.18)	2.67 (0.77)	2.07 (0.28)	2.47 (0.54)
6.1-3	6.53 (0.47)	11.83 (0.89)	10.34 (1.20)	8.09 (0.39)	<1	<1
7	6.34 (1.14)	8.57 (0.91)	10.87 (0.93)	4.68 (0.63)	4.49 (0.01)	4.58 (0.69)
8	4.24 (0.02)	3.61 (0.06)	4.15 (1.02)	3.87 (0.30)	1.01 (0.30)	<1
9	<1	1.06 (0.10)	1.92 (0.07)	1.74 (0.45)	3.00 (0.33)	4.30 (0.58)
10	1.02 (0.31)	<1	1.40 (0.16)	<1	<1	<1
11	1.87 (0.18)	1.95 (0.17)	1.03 (0.25)	1.35 (0.06)	1.43 (0.17)	1.45 (0.15)
12	1.53 (0.31)	1.86 (0.40)	1.32 (0.38)	2.43 (0.74)	1.32 (0.85)	2.89 (0.24)
13.1	4.76 (0.44)	5.17 (0.61)	8.72 (0.94)	5.63 (0.32)	7.01 (1.05)	5.02 (0.36)
13.2	3.31 (0.16)	2.06 (0.04)	2.38 (0.54)	2.23 (0.27)	5.58 (0.23)	3.81 (0.45)
14	3.65 (0.5)	3.17 (0.27)	5.80 (0.38)	4.79 (0.21)	10.04 (0.48)	6.38 (0.22)
15	1.26 (0.34)	2.06 (0.06)	1.27 (0.05)	1.02 (0.06)	5.08 (0.36)	2.83 (1.01)
16	<1	<1	1.12 (0.31)	<1	1.87 (0.08)	2.12 (0.26)
17	3.50 (0.03)	2.83 (0.27)	4.18 (0.02)	2.97 (0.25)	<1	<1
18	1.01 (0.46)	<1	<1	<1	<1	<1
19	1.47 (0.10)	1.54 (0.37)	<1	<1	<1	<1
20	1.48 (0.08)	1.02 (0.14)	<1	<1	<1	<1
Total V_β	77.4	79.6	79.2	73.0	70.9	60.7

troduced during the PCR amplification of the β-chain cDNA. However, we did find one example in the peripheral blood T cell clones from the RA patient of a $V_\beta14^+$ sequence that was identical to the sequence of the major clone found in synovial fluid of the same patient (Table 4).

There was evidence for some dominant T cell clones bearing other V_β's in synovial fluids, although in general the results were less dramatic than those seen with $V_\beta14$. For example, from patient RA-1, 7 of 17 sequences that included $V_\beta13.2$ had the same junctional sequences and 3 of 18 $V_\beta2$-expressing clones from patient RA-2 were identical. Overall, the data support the idea that the T cells in synovial fluids of patients with arthropathies are made up of a collection of expanded clones, presumably with some specificity for the disease, and a large number of nonspecifically recruited cells. In RA, the population of T cells bearing $V_\beta14$ are particularly clonal, suggesting a specific role for these cells in the disease. In patient RA-2, for example, it can be estimated that more than 4% of all the T cells in the joint bore the same β-chain sequence.

What could account for the low percentages of $V_\beta14^+$ T cells in the peripheral blood, but the presence of a few dominant clones of $V_\beta14^+$ T cells in the synovial fluids of RA patients? Clearly the disease does not involve recruitment of all $V_\beta14^+$ T cells from the blood into the joints of these patients by a joint-expressed, $V_\beta14$-specific superantigen. If this were to occur, we would expect a much higher percentage of such cells in RA joints, and we would not expect dominant clones. We would like to propose an alternative explanation based on recent experiments studying the fate of T cells in adult mice confronted with viral or bacterial V_β-specific superantigens in vivo. These studies (*15*) have revealed that, although initially inducing a powerful response from T cells bearing the appropriate V_β elements, these superantigens often lead to the eventual disappearance or inactivation of these T cells. Therefore, we would like to suggest that patients with RA have encountered a microbial superantigen specific for $V_\beta14$. This has led to the activation of most cells bearing $V_\beta14$, a few of which, because of their cross-reaction with particular self-antigens, have homed to the joints, where they initiated disease and have been maintained by long-term stimulation with self-antigen presented in the joint tissue. The inflammatory process has led to recruitment to the joint of additional T cells bearing various V_β elements. The superantigen has subsequently caused the elimination or anergy of most of the remaining $V_\beta14^+$ T cells in the periphery of the majority of patients.

We realize that this is only one of several possible explanations for the results reported in this paper, and that a disease as complex as RA is unlikely to have a single cause and course. The data presented here, however, indicate evidence for a $V_\beta14$-specific superantigen at work in the majority of RA patients studied suggesting that these powerful immune stimulants may explain in part the often reported connection between microbial agents and the onset of human autoimmune diseases.

Table 4. Analysis of β-chain sequences from synovial fluid and peripheral blood T cells. All PCR-generated β-chain fragments were cloned into a pTZ18R cut with Eco RI–Bam HI. Double-stranded plasmid DNAs prepared with acid phenol (*16*) were sequenced directly with the Sequenase kit (U.S. Biochemical). Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

				Sequences occurring more than once						Sequences occurring once			
						Junctional sequence						$J_{\beta1}$	$J_{\beta2}$
Patient	Tissue	V_β	No. of seq.	No. of occur.	% of total	V_β	$ND_\beta N$	J_β		No.	% of total	123456	1234567
RA-2	Synovial fluid	14	18	11	61	CASSL	SRP	DTQY	(2.3)	5	28	001001	1020000
				2	11	CASSL	YGTRN	TQY	(2.3)				
		13.2	18	2	11	CAS	KRVSD	NEQF	(2.1)	14	78	100011	5111102
				2	11	CAS	MGLAG	QETQY	(2.5)				
		2	18	3	17	CSA	IDRA	SYEQY	(2.7)	15	83	000110	5031004
	Peripheral blood	14	18	0	0					18*	100	010011	2330115
RA-1	Synovial fluid	14	21	13	62	CASS	TG	NTEAF	(1.1)	6	29	000010	0030011
				2	10	CAS	RLAS	NEQF	(2.1)				
		13.2	17	7	41	CASSL	VTSG	YNEQF	(2.1)	8	47	020010	4000100
				2	12	CASS	YPTAG	NQPQH	(1.5)				
RA-5	Synovial fluid	14	22	7	32	CAS	NPGGV	NTEAF	(1.1)	12	54	310000	4010102
				3	14	CASS	PRGAY	TQY	(2.3)				
AC-3	Synovial fluid	14	17	3	18	CAS	RSDRVG	NYGYT	(1.2)	9	53	010000	2110301
				3	18	CA	NLRAA	TNEKLF	(1.4)				
				2	12	CASS	GRGDD	NQPQH	(1.5)				
NC-7	Peripheral blood	14	17	0	0					17	100	120020	5220003

*Although all clones sequenced from the PBL of this patient were unique, one was identical to the most frequent $V_\beta14$ clone found in synovial fluid.

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