CDR3 Sequence Motifs Shared by Oligoclonal Rheumatoid Arthritis Synovial T Cells

Evidence for an Antigen-driven Response

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Abstract

T lymphocytes reactive with as yet undefined joint-localized foreign or autoantigens may be important in the pathogenesis of RA. Molecular studies demonstrating skewed T cell antigen receptor (TCR) variable gene usage and selective expansion of particular T cell clones within the synovial compartment support this view. Based on our recent study documenting selective expansion of Vβ17$^+$ T cells in RA, we have pursued the identification of T cells relevant to the disease process, in an informative patient, by combining molecular analysis of freshly explanted RA synovial tissue Vβ17 TCR transcripts with in vitro expansion of Vβ17$^+$ synovial tissue T cell clones. Peripheral blood Vβ17 cDNA transcripts proved heterogeneous. In contrast, two closely related sequences, not found in the peripheral blood, dominated synovial tissue Vβ17 transcripts, suggesting selective localization and oligoclonal expansion at the site of pathology. CD4$^+$, Vβ17$^+$ synovial tissue–derived T cell clones, isolated and grown in vitro, were found to express TCR β chain transcripts homologous to the dominant Vβ17 synovial tissue sequences. One clone shares with a dominant synovial tissue sequence a conserved cluster of 4/5 amino acids (IGQ-N) in the highly diverse antigen binding CDR3 region, suggesting that the T cells from which these transcripts derive may recognize the same antigen. These findings have permitted a complete characterization of the α/β TCR expressed by putatively pathogenic T cell clones in RA. Functional analysis suggests that the conserved CDR3 sequence may confer specificity for, or restriction by, the MHC class II antigen, DR4. (*J. Clin. Invest.* 1994. 94:2525–2531.) Key words: T cell receptors • rheumatoid arthritis • autoimmunity • polymerase chain reaction • T lymphocytes

Introduction

Many cell types, notably macrophages, synoviocytes, and polymorphonuclear leukocytes, participate in the complex inflammatory response that effects joint destruction in RA. However, a central role for T lymphocytes is suggested by (*a*) the rich infiltration of activated T cells at the primary site of disease activity, the synovial tissue (1, 2); (*b*) genetic studies linking RA disease susceptibility to a defined amino acid sequence in the third hypervariable region of the DRβ chain of the MHC class II molecule (3, 4); (*c*) animal models of chronic arthritis in which antigen-specific T cells are capable of transferring disease to naive recipients (5, 6); and (*d*) amelioration of arthritis, both in murine models of autoimmune disease and in patients with RA, by administration of mAb reactive with the CD4$^+$ T cell subset (7, 8).

While the importance of T cells in RA appears clear, neither the antigen specificity nor the function of disease-inducing T cells has been determined. In an attempt to identify and characterize pathogenic T cells among the vast number present in the inflamed joint, investigators have applied molecular techniques to detect T cells which (*a*) share T cell antigen receptor (TCR)[1] structural features, i.e., restricted usage of particular TCR variable gene elements, or (*b*) are "oligoclonal" with respect to the highly polymorphic antigen binding CDR3 region of the TCR, suggesting antigen-driven expansion at the site of pathology. To date, this approach has yielded conflicting results. Several laboratories have reported evidence of oligoclonality and over-usage of particular TCR V gene products among RA joint-derived T cells (9–11). However, the TCR V gene families implicated vary from study to study and still other investigators find no evidence for TCR skewing in RA (12, 13).

To directly evaluate TCR V gene usage in the RA T cell repertoire, we have used the available panel of mAbs specific for particular human TCR β chain variable region (TCR Vβ) gene products (14). Our results demonstrate selective expansion of the Vβ17$^+$ T cell population, both systemically and in the joint, in a significant percentage of RA patients, as compared to both normals and non-RA arthritis controls. 15 of 49 (31%) RA synovial fluids, but 0 of 19 non-RA control samples, contained > 10% Vβ17$^+$ T cells (14). Moreover, even RA synovial fluid samples which did not contain a numerically expanded Vβ17$^+$ population showed evidence for the preferential activation (IL-2 receptor expression) of CD4$^+$ Vβ17$^+$ T cells. Taken together, these data suggest a role for Vβ17$^+$ T cells in RA.

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1. *Abbreviations used in this paper:* BCL, B cell line; MAM, *Mycoplasma arthriditis* mitogen; MBP, myelin basic protein; MNC, mononuclear cells; PB, peripheral blood; RF, rheumatoid factor; SF, synovial fluid; ST, synovial tissue; TCR, T cell antigen receptor; TCL, T cell line.

To further address this issue, we have analyzed an informative RA patient who exhibits a persistently expanded Vβ17$^+$ peripheral T cell population. While the Vβ17 expression in this patient's peripheral blood T cells proved quite heterogeneous, we have identified a group of closely related joint-derived Vβ17 sequences, including those that dominated the freshly isolated synovial tissue and fluid. These Vβ17 transcripts are highly homologous in the CDR3 antigen binding region, suggesting that T cells from which these transcripts derive may be driven by a common joint-localized antigen. The isolation and in vitro propagation of T cell clones expressing the consensus CDR3 motif has allowed complete characterization of the $\alpha\beta$ TCR expressed by potentially pathogenic T cells in RA.

Methods

RA patient. B.C. is a 74-yr-old white female with a 22-yr history of rheumatoid factor (RF) positive RA. Clinical features of her disease include a history of joint pain and swelling, morning stiffness, fatigue, and dry eyes. Physical examination showed symmetric synovitis involving the left elbow; bilateral wrists, metacarpalphalangeal, and proximal interphalangeal joints; bilateral knees; and right ankle. In addition, ulnar deviation and nodular tenosynovitis of the hands were observed, and a subcutaneous nodule was present on the extensor surface of the left elbow. Laboratory analysis was normal except for an erythrocyte sedimentation rate of 33 and a positive RF. X rays of the hands showed erosions of the distal ulna and radius bilaterally and knee x rays showed osteopenia, joint space narrowing, and lateral osteophytes. Over the course of her disease, the patient had been treated with intramuscular gold, methotrexate, plaquenil, non-steroidal anti-inflammatory drugs, and low dose prednisone. She underwent arthroscopic synovectomies of the left knee in 1987 and 1992 and replacement of the right metacarpalphalangeal joints in 1993. Frequent arthrocenteses of the right and left knees, with injection of corticosteroids, were necessary for control of pain and swelling. Repeated peripheral blood (PB) and synovial fluid (SF) samples, as well as left knee and right hand synovial tissue (ST), were obtained from the patient for research studies from 1992 through 1993. At that time she was being treated with hydroxychloroquine sulfate, ibuprofin, and prednisone 1 mg every other day. HLA DR typing was performed, showing a DR4,7 haplotype.

Phenotypic analysis of synovial tissue T cells. PB samples were obtained by venipuncture. SF samples were obtained at the time of therapeutic arthrocentesis. ST specimens were obtained from the Department of Pathology at The Hospital for Special Surgery after therapeutic arthroscopic synovectomy of the left knee. ST was minced under sterile conditions and incubated in 20 ml of an enzyme preparation containing RPMI 1640 (Gibco Laboratories, Grand Island, NY), 20% fetal calf serum (Whittaker M.A. Bioproducts, Inc., Walkersville, MD), 1% penicillin and streptomycin, 1% glutamine (Gibco Laboratories), 0.5 mg/ml collagenase, 0.15 mg/ml DNase and 0.1 mg/ml hyaluronidase (Sigma Chemical Co., St. Louis, MO) at 37°C, 5% CO_2 for 2 h. Partially digested pieces of ST were further disrupted using forces and scalpel, and either pressed through a mesh sieve to prepare mononuclear cells (MNC) for phenotypic analysis, or washed free of enzymes and cultured in fresh media. MNC were isolated from the ST digest on a Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) gradient. T cells were selectively enriched by rosetting of MNC with sheep red blood cells followed by incubation at 4°C for 16 h and subsequent fractionation of rosetted and unrosetted cells over Ficoll-Hypaque. ST T cells were washed in PBS, and stained with a panel of monoclonal antibodies. T cells were incubated with buffer alone or a saturating concentration of mAb at 4°C for 30 min, washed three times with PBS, and incubated with a saturating concentration of fluorescein-labeled F(ab')$_2$ fragments of goat anti-mouse IgG (Tago, Inc., Burlingame, CA) at 4°C for 30 min. After 3 washes in PBS, the cells were analyzed on a cytofluorograph. Two color immunofluorescence analysis was performed using phycoerythrin-labeled anti-CD4 and anti-CD8 mAb obtained from Coulter Immunology (Hialeah, FL). The above procedure was followed by a blocking step, with cells incubated at 4°C for 30 min with an irrelevant murine mAb (anti-trinitrophenol). After three washes, the cells were incubated with a phycoerythrin-labeled murine mAb, washed, and analyzed on an Ortho IIs cytofluorograph, gating on the small, nongranular lymphocyte population. The percentage of cells fluorescent with buffer or irrelevant control murine mAb and fluorescein-labeled goat anti-mouse IgG alone was subtracted.

Isolation of total cellular RNA and cDNA synthesis. Total cellular RNAs were isolated from PB, SF, and ST T cells by the guanidinium/cesium chloride centrifugation method (15) or an acidified guanidinium/phenol/chloroform method as outlined in the RNazol™ kit (Tel-test, Inc., Friendswood, TX). The first-strand cDNAs were reverse transcribed with a cDNA synthesis kit (cDNA Cycle Kit; Invitrogen, San Diego, CA). Briefly, less than 1 μg of total cellular RNA was added to a reaction volume of 20 μl. The reaction mixture was incubated at 42°C for 1 h, heated to 92°C for 3 min, and then chilled on ice. A second aliquot of reverse transcriptase (RT) was added and the incubation at 42°C continued for another hour. After the second incubation, reaction mixtures were passed through a Sephadex 6B spin column to remove oligo(dT) primer and free dNTPs.

PCR, subcloning, and sequencing. α/β TCR gene segments were amplified by PCR. The cDNAs prepared from PB, SF, and ST T cells were used as templates. The 5′ primers used in PCR for Vβ17 and Vβ6.7 were 5′ ACAGCGTCTCTCGGGAGA 3′ and 5′ AGGCAACAGTGCACCAGAC 3′, respectively. The antisense constant Cβ primer was 5′ GGGTGTGGGAGATCTCTGCT 3′. The Vα specific sense primers (except for Vα2) and the antisense Cα primer were those used by Oksenberg et al. (16). The sense 5′ primer for Vα2 was 5′ AAGGTTTACAGCACAGCTC 3′. The PCR was performed 30 cycles each consisting of a 1-min denaturation step at 94°C, a 1-min annealing step at 56°C, and a 1.5-min extension step at 72°C. After 30 cycles the reaction mixtures were prolonged at 72°C for 10 min. The PCR products were subcloned into a T/A cloning vector (Invitrogen) following the instruction manual provided by the company. The ligation mixture was used to transform the competent cells DH5α. The plasmid DNA samples were prepared using a quick protocol (15). The correct inserts in positive clones were confirmed by EcoRI digestion and finally, the sequences were read out by dideoxy chain termination sequencing method (17) using a sequencing kit (Sequenase version 2.0; United States Biochemical Corp., Cleveland, OH).

Propagation in vitro of T cell line (TCL) cells and proliferation assays. Partially digested ST fragments, 1–3 mm^3 each, were cultured with IL-2 alone for 1 wk, then activated with the Vβ17 selective superantigen *Mycoplasma arthritidis* mitogen (MAM) or anti-Vβ17 mAb C1 presented by allogeneic antigen-presenting cells (APC) (18). CD8+ T cells were eliminated by magnetic depletion following treatment with anti-CD8 (OKT 8) and anti-mouse Ig-coated magnetic beads (Dynal Inc., Great Neck, NY). TCL were maintained by weekly retriggering with periodate-treated allogeneic feeders (APC), MAM, or anti-Vβ17 mAb plus APC, and expanded with IL-2. T cell clones were isolated by limiting dilution culture on periodate-treated APC in the presence of IL-2. The T cell clones described in this report have been retriggered over 15 times. In proliferation assays, 5×10^4 in vitro cultured TCL cells were co-cultured with 2.5×10^4 x-irradiated DR homozygous EBV-transformed B cell line cells and IL-2. These cell lines are part of the homozygous HLA typing cell lines compiled at the tenth International Histocompatibility Workshop and kindly provided by Dr. S. Y. Yang (HLA Histocompatibility Typing Laboratory, Memorial Sloan Kettering Cancer Center, New York). After 48 h the cultures were pulsed with [^{3}H]thymidine and incorporation of counts assayed. All measurements were performed in triplicate.

Results

T cell subsets and TCR repertoire in synovial tissue T cells. The RA patient chosen for study has "classic" RF$^+$ polyarticular,

Table I. T Cell Subset and TCR V Gene Analysis of ST T Cells

	CD4	CD8	Vβ3	Vβ5.2/3	Vβ6.7	Vβ8	Vβ12	Vβ17
% mAb$^+$ T cells	72.3	29.2	4.8	2.6	1.5	1.6	3.2	7.9
% mAb$^+$ T cells which are CD4$^+$	100	0	30	31	10	50	50	89

symmetrical joint inflammation, and expresses the RA-associated MHC class II antigen DR4. Over an 18-mo period of study, this patient has maintained a skewed PB T cell repertoire characterized by persistently elevated percentages of Vβ17$^+$ T cells, i.e., 13.2–15.7% as compared to an average normal value of 5.3% (14). At the time this patient underwent arthroscopic synovectomy of the knee, freshly explanted synovial T cells were isolated and used for (*a*) analysis of cell surface antigen expression; (*b*) molecular characterization of α/β TCR rearrangements; and (*c*) in vitro propagation and cloning of Vβ17$^+$ T cells. While the distribution of Vβ17$^+$ T cells in this patient's PB and SF reflected the overall CD4/CD8 ratio, ST TCR repertoire analysis demonstrated a relative abundance of Vβ17$^+$ T cells and their selective representation in the CD4$^+$ subset (Table I).

TCR CDR3 sequence analysis. To assess oligoclonality of Vβ17$^+$ T cells at the site of pathology, we examined sequence diversity in the highly polymorphic antigen binding CDR3 region among Vβ17 transcripts derived from the joint and the peripheral blood. A total of 29 Vβ17 cDNA clones from ST T cells have been sequenced. Among them, 12/29 contain the identical sequence, while 11/29 use a distinct but structurally related sequence. These two dominant synovial tissue sequences (st1 and st2) have been designated Vβ17seq1 and Vβ17seq2, respectively. Their nucleotide and deduced amino acid sequences in the CDR3 region are shown in Fig. 1 *A*. Comparison of Vβ17seq1 and sequences reveals conservation of several amino acids within the CDR3 region, including an I at position 95 and a N at position 99.

To determine if T cells expressing the dominant Vβ17 CDR3 sequences persist over time at the site of pathology, we had the opportunity to study a SF specimen which had been obtained during therapeutic arthrocentesis of the operated knee 4 mo before synovectomy. As shown in Fig. 1 *B*, several of the ST sequences, including Vβ17seq1, were well represented among the SF Vβ17 cDNA transcripts. In contrast to the restricted pattern observed in the joint, Vβ17 transcripts from this patient's PB were quite heterogeneous. 28 cDNA clones sequenced fall into 22 distinct patterns according to their CDR3 sequences (Fig. 2). Moreover, no example of the dominant ST Vβ17 sequences has been identified among PB T cells.

To ensure that the observed clonal dominance did not result from some unusual aspect of PCR amplification of the synovial RNA samples, rearrangements of the Vβ6.7 subfamily from ST T cells were also assessed. Although Vβ6.7$^+$ T cells represented only 1.5% of the synovial T cell pool (Table I), we observed five distinct rearrangements among eight plasmid clones sequenced. These rearrangements are heterogeneous with respect to both CDR3 sequence and Jβ segment usage (Fig. 3). Taken together, these data demonstrate that in the face of a longstanding, polyclonal systemic expansion of Vβ17$^+$ T cells, particular CD4$^+$, Vβ17$^+$ T cell clones expressing structurally related CDR3 sequences localize to and expand in the inflamed synovial compartment.

In vitro propagation of Vβ17$^+$ synovial T cell clones. To examine the possible role played by T cells expressing these dominant Vβ17 sequences in the rheumatoid process, we attempted to isolate and expand these cells in vitro. Freshly isolated ST T cells were cultured in the presence of IL-2 alone for 1 wk, then activated by the Vβ17 selective microbial superantigen

(A)

seq	R*	Jβ	----- V ----	----- N-D-N -----	---------- J ----------
			C A S S	I G Q E N	Y E Q Y F G P G T R L T V
st1 (Vβ17seq1)	12/29	2.7	TGTGCCAGTAGT	ATTGGTCAGGAGAAC	TACGAGCAGTACTTCGGGCCGGGCACCAGGCTCACGGTC
			C A S S	I Q G	Y N E Q F F G P G T R L T V
st2 (Vβ17seq2)	11/29	2.1	TGTGCCAGTAGT	ATACAGGGG	TACAATGAGCAGTTCTTCGGGCCAGGGACACGGCTCACCGTG
			C A S S	L A	S D T Q Y F G P G T R L T V
st3	2/29	2.3	TGTGCCAGCTCA	CTAGCT	TCTGATACGCAGTATTTTGGCCCAGGCACCCGGCTGACAGTG
			C A S S	R L A	Y N E Q F F G P G T R L T V
st4	2/29	2.1	TGTGCCAGCTCC	CGACTAGCT	TACAATGAGCAGTTCTTCGGGCCAGGGACACGGCTCACCGTG
			C A S S	R T S G R G	T D T Q Y F G P G T R L T V
st5	1/29	2.3	TGTGCCAGTAGT	AGGACTAGCGGGAGAGGA	ACAGATACGCAGTATTTTGGCCCAGGCACCCGGCTGACAGTG
			C A S	K S E D	T E A F F G Q G T R L T V
st6	1/29	1.1	TGTGCCAGT	AAGTCAGAAGAC	ACTGAAGCTTTCTTTGGACAAGGCACCAGACTCACAGTT

(B)

sf1	13/23	2.7	identical to st1 (see above)
sf2	7/23	1.1	identical to st6 (see above)
sf3	2/23	2.1	identical to st4 (see above)
			C A S R D R G T E K L F F G S G T Q L S V
sf4	1/23	1.4	TGTGCCAGC CGGGACAGGGGTACT GAAAAACTGTTTTTTGGCAGTGGAACCCAGCTCTCTGTC

R* the number of same rearrangement/total plasmid clones sequenced

Figure 1. (*A*) Vβ17 CDR3 sequences obtained from ST T cells. Two dominant transcripts (st1 and st2) were identified and designated Vβ17seq1 and Vβ17seq2, respectively. (*B*) Vβ17 CDR3 sequences of SF T cells obtained from the same joint 4 mo before synovectomy.

seq	R*	Jβ	--- V ------	----- N-D-N----------	---------- J ----------
			C A S S	R T G V T	T D T Q Y F G P G T R L T V L
1	1	2.3	TGTGCCAGTAGT	CGAACGGGCGTAACT	ACAGATACGCAGTATTTTGGCCCAGGCACCCGGCTGACAGTGCTC
			C A S S	I T G E A	Y G Y T F G S G T R L T V V
2	5	1.2	TGTGCCAGTAGT	ATCACAGGGGAGGCC	TATGGCTACACCTTCGGTTCGGGGACCAGGTTAACCGTTGTA
			C A S	T K P G T L	N T G E L F F G E G S R L T V L
3	1	2.2	TGTGCCAGT	ACCAAACCCGGGACTCTG	AACACCGGGGAGCTGTTTTTTGGAGAAGGCTCTAGGCTGACCGTACTG
			C A S S	T R D A G G P	E Q Y F G P G T R L T V T
4	1	2.7	TGTGCCAGTAGT	ACCCGGGACGCGGGGGGCCCG	GAGCAGTACTTCGGGCCGGGCACCAGGCTCACGGTCACA
			C A S S	I E V G P	E A F F G Q G T R L T V V
5	1	1.1	TGTGCCAGTAGT	ATAGAGGTGGGACCT	GAAGCTTTCTTTGGACAAGGCACCAGACTCACAGTTGTA
			C A	I R G Q G A F	G Y T F G S G T R L T V V
6	2	1.2	TGTGCC	ATTAGAGGACAGGGGGCTTTC	GGCTACACCTTCGGTTCGGGGACCAGGTTAACCGTTGTA
			C A S S	I G S R G G	Q P Q H F G D G T R L S I L
7	1	1.5	TGTGCCAGTAGT	ATAGGGTCGAGGGGAGGT	CAGCCCCAGCATTTTGGTGATGGGACTCGACTCTCCATCCTA
			C A S	A T G T	N Q P Q H F G D G T R L S I L
8	1	1.5	TGTGCCAGT	GCGACAGGGACC	AATCAGCCCCAGCATTTTGGTGATGGGACTCGACTCTCCATCCTA
			C A S S	L G T S G S Y	E Q F F G P G T R L T V L
9	1	2.1	TGTGCCAGTAGC	CTGGGGACTAGCGGGAGCTAT	GAGCAGTTCTTCGGGCCAGGGACACGGCTCACCGTGCTA
			C A S S	M V G Q G T	L F F G S G T Q L S V L
10	1	1.4	TGTGCCAGTAGT	ATGGTCGGACAGGGGACA	CTGTTTTTTGGCAGTGGAACCCAGCTCTCTGTCTTG
			C A S S	W G S D	T E A F F G Q G T R L T V V
11	1	1.1	TGTGCCAGTAGT	TGGGGGTCCGAC	ACTGAAGCTTTCTTTGGACAAGGCACCAGACTCACAGTTGTA
			C A S S	R W A R T	N Y G Y S F G S G T R L T V V
12	1	1.2	TGTGCCAGTAGT	AGGTGGGCTCGAACT	AACTATGGCTACTCCTTCGGTTCGGGGACCAGGTTAACCGTTGTA
			C A S S	I E V D	Q P Q H F G D G T R L S I L
13	1	1.5	TGTGCCAGTAGT	ATAGAGGTGGAT	CAGCCCCAGCATTTTGGTGATGGGACTCGACTCTCCATCCTA
			C A S S	E G S R N S	E A F F G Q G T R L T V V
14	1	1.1	TGTGCCAGTAGT	GAGGGATCTTGGAACTCT	GAAGCTTTCTTTGGACAAGGCACCAGACTCACAGTTGTA
			C A S S	R T G G	Q E T Q Y F G P G T R L L V L
15	1	2.5	TGTGCCAGTAGT	AGGACAGGGGGA	CAAGAGACCCAGTACTTCGGGCCAGGCACGCGGCTCCTGGTGCTC
			C A S S	P R T G L	N S P L H F G N G T R L T V T
16	1	1.6	TGTGCCAGTAGT	CCACGGACAGGCTTA	AATTCACCCCTCCACTTTGGGAACGGGACCAGGCTCACTGTGACA
			C A S	G L A G G Y P T G	G E L F F G E G S R L T V L
17	1	2.2	TGTGCCAGT	GGCCTAGCGGGGGGCTACCCAACTGGC	GGGGAGCTGTTTTTTGGAGAAGGCTCTAGGCTGACCGTACTG
			C A S S	N G L N R G G	Y N E Q F F G P G T R L T V L
18	2	2.1	TGTGCCAGTAGT	AACGGCCTCAACAGGGGAGGG	TACAATGAGCAGTTCTTCGGGCCAGGGACACGGCTCACCGTGCTA
			C A S S	I S A V S F Y	N E Q F F G P G T R L T V L
19	1	2.1	TGTGCCAGTAGT	ATCTCTGCGGTCTCCTTCTAC	AATGAGCAGTTCTTCGGGCCAGGGACACGGCTCACCGTGCTA
			C A S S	S G Q G V Y R	S P L H F G N G T R L T V T
20	1	1.6	TGTGCCAGTAGT	TCGGGACAGGGGGTGTATCGT	TCACCCCTCCACTTTGGGAACGGGACCAGGCTCACTGTGACA
			C A S S	R T S G R G	T D T Q Y F G P G T R L T V L
21	1	2.3	TGTGCCAGTAGT	AGGACTAGCGGGAGAGGG	ACAGATACGCAGTATTTTGGGCCAGGCACCCGGCTGACAGTGCTC
			C A S S	V F G T R G S G T	Y N E Q F F G P G T R L T V L
22	1	2.1	TGTGCCAGTAGT	GTGTTCGGGACTAGAGGCAGTGGGACC	TACAATGAGCAGTTCTTCGGGCCAGGGACACGGCTCACCGTGCTA

R* the number of same rearrangement/total 28 plasmid clones sequenced

Figure 2. Vβ17 CDR3 sequences from PB T cells. Among a total of 28 cDNA clones examined, 22 different rearrangements have been observed, indicating that the Vβ17$^+$ PB T cell repertoire is quite heterogeneous.

Mycoplasma arthritidis mitogen (MAM) or the anti-Vβ17 specific monoclonal antibody C1 (18) and expanded in the presence of IL-2. Individual T cell clones were isolated by limiting dilution culture. While a large number of distinct Vβ17 TCR sequences are represented among the bulk T cell lines (TCLs) generated, two ST-derived T cell clones, C5-1 and C5-2, attracted our attention. As shown in Fig. 4 *A*, the Vβ17 transcript from clone C5-1 (Vβ17seq3) use Vβ17-Dβ2-Jβ2.1-Cβ2. This sequence, while not identical, is highly homologous to the dominant ST sequences Vβ17seq1 and Vβ17seq2. Homology between Vβ17seq1 and Vβ17seq3 in the CDR3 region at the nucleotide level is 78.5% and is even higher at the amino acid level (86.4%). Most striking, 4/5 amino acids, IGQ-N, numbered as residues 95–99 in the highly diverse V-D junction are conserved (Fig. 5). The Vβ17 transcript from clone C5-2 was named Vβ17seq4 (Fig. 4 *A*). Although the motif IGQ does not appear, the amino acid residue I at position 95 and N at position 99 are shared with Vβ17seq1, 2, and 3 (Fig. 5). Moreover,

R*		Jβ	----- V ----	----- N-D-N -------	---------- J ----------
			C A S S	L D S N	N E Q F F G P G T R L T V
2/8	st	2.1	TGTGCCAGCAGC	TTAGACTCCAAC	AATGAGCAGTTCTTCGGGCCAGGGACACGGCTCACCGTG
			C A S S	F Q G Q A	S Y E Q Y F G P G T R L
2/8	st	2.7	TGTGCCAGCAGC	TTTCAGGGACAGGCC	TCCTACGAGCAGTACTTCGGGCCGGGCACCAGGCTC
			C A S S	F D P S	T G E L F F G E G S R L T
1/8	st	2.2	TGTGCCAGCAGC	TTTGATCCTTCC	ACCGGGGAGCTGTTTTTTGGAGAAGGCTCTAGGCTGACC
			C A S S	L V W G G T R	T Q Y F V P G T R L
1/8	st	2.5	TGTGCCAGCAGC	TTAGTCTGGGGGGGCACAAGG	ACCCAGTACTTCGTTCCAGGCACGCGGCTC
			C A S S	L V W G G I R	T Q Y F G P G T R L
1/8	st	2.5	TGTGCCAGCAGC	TTAGTCTGGGGGGGAATAAGG	ACCCAGTACTTCGGGCCAGGCACGCGGCTC
			C A S S	H S G G A	Y E Q Y F G P G T R L T V
1/8	st	2.7	TGTGCCAGCAGC	CATAGCGGGGGGGCC	TACGAGCAGTACTTCGGGCCGGGCACCAGGCTCACGGTC

R* the number of same rearrangement/total plasmid clones sequenced

Figure 3. The CDR3 sequences of Vβ6.7 among ST T cells. Among the eight cDNA clones examined, five distinct sequences have been identified (the two sequences which differ by a single nucleotide likely reflect a PCR artifact). These five sequences are heterogeneous with respect to Jβ usage, as well as CDR3 amino acid composition.

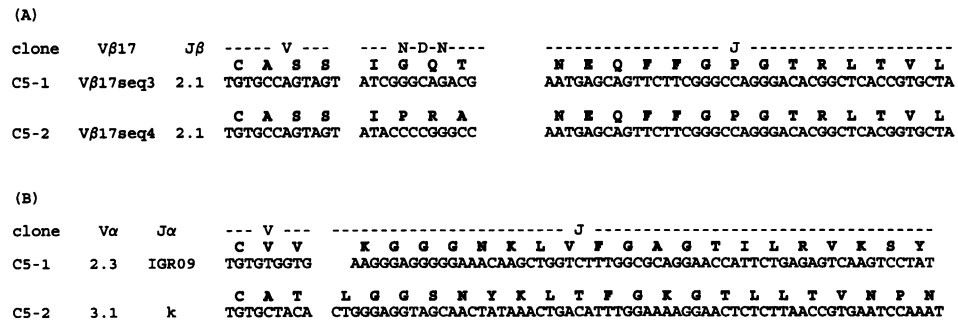

Figure 4. (*A*) The Vβ17 CDR3 sequences from two ST-derived T cell clones C5-1 and C5-2. These sequences were designated Vβ17seq3 and Vβ17seq4. (*B*) The Vα sequences for clone C5-1 and C5-2.

sequenced shares usage of the Jβ2.1 gene segment. In this regard, it should be noted that residue Asn ("N") at position 99 is encoded by the germline Jβ2.1 segment used by Vβ17seq2, 3, and 4. However, the N at the same position in Vβ17seq1, which uses Jβ2.7, is not germline encoded, but results from the process of N region nucleotide addition which generates diversity in the antigen binding VDJ junctional region (19). Our results strongly suggest that the T cells represented by the two dominant ST Vβ17 transcripts and the homologous transcripts from T cell clones, in particular C5-1, recognize the same joint-localized antigen and that conserved CDR3 amino acids of the TCR β chain may prove crucial for antigen recognition.

TCR α expression in Vβ17⁺ synovial T cell clones. As antigen recognition is a function of both the TCR α and β chains, we next characterized α chain usage by synovial tissue derived T cell clones C5-1 and C5-2. TCR α rearrangements have been analyzed by PCR using a panel of Vα specific primers in connection with a TCR α constant region primer (16). This analysis revealed expression of only Vα2.3 by the T cell clone C5-1. Formal sequencing was performed and yielded a sequence consisting of Vα2.3–Jα(IGRJa09)–Cα (Fig. 4 *B*). This Vα designation is consistent with positive staining of this T cell clone by the Vα2.3 specific mAb F1 (20). Expression of Vα2.3 is of interest, as recent reports have shown the selective increase in Vα2.3⁺ T cells in the synovial fluid of some RA patients (21, 22). A similar analysis of T cell clone C5-2 which expresses the Vβ17seq4 has yielded a TCR α rearrangement of Vα3.1–Jαk–Cα (Fig. 4 *B*). Of interest, these two α chains are quite homologous in the CDR3 loop, sharing a pair of G residues at positions 96 and 97, as well as N at position 99 (Fig. 5). Thus, both TCR α and β chains of the T cell clones expressing Vβ17 sequences homologous to the dominant oligoclonal ST Vβ17 transcripts have been characterized.

DR4 reactivity of Vβ17⁺ synovial T cell clones. As an initial approach to the question of antigen specificity, we have assayed the proliferative response of synovial tissue–derived T cell clones, C5-1 and C5-2, to a panel of EBV-transformed DR homozygous lymphoblastoid B cell lines (BCL). As a control, an uncloned synovial tissue Vβ17⁺, CD4⁺ TCL designated culture 10 was assayed simultaneously. Clone C5-1 expressing Vα2.3/Vβ17seq3 is remarkable for low levels of proliferation, assessed by [³H]thymidine incorporation, in response to all stimuli, including superantigens and anti-TCR mAb. However, as shown in Fig. 6 *A*, this clone proliferates selectively to BCL cells expressing RA-associated alleles of DR4, Dw4, and Dw14. Somewhat surprisingly, clone C5-2 expressing Vα3.1/Vβ17seq4 is highly responsive to DR4, Dw10 bearing BCL cells (Fig. 6 *B*). The response can be selectively inhibited by anti-DR mAb (data not shown). This preliminary evidence of DR4 recognition by synovial T cells expressing the conserved CDR3 sequences is intriguing. Additional studies will be needed to determine if these T cell clones are specific for the DR4 alleles themselves, or for joint-derived or EBV-encoded peptides presented on the DR4 molecules. In this regard, a five amino acid region of homology between the EBV gp110 protein and the RA MHC class II disease susceptibility epitope has been noted and proposed to be potentially relevant to the pathogenesis of RA(23).

Discussion

Previous studies designed to correlate TCR structure with antigen-MHC molecular complex recognition have emphasized the importance of critical amino acid residues in each of the three polymorphic CDR regions of both α and β chains, with the N–D–N region of CDR3 playing a dominant role. In both the murine and human systems, T cells specific for a particular peptide–MHC complex often use a restricted group of TCR V

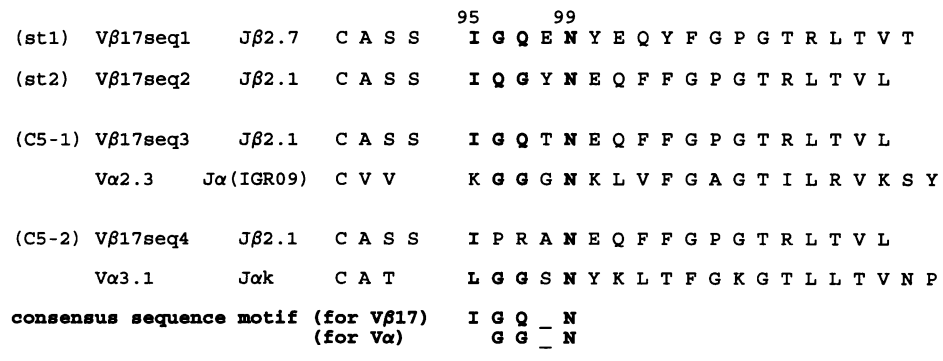

Figure 5. The CDR3 sequence alignment for Vβ17 and Vα obtained from ST T cells as well as ST-derived T cell clones C5-1 and C5-2. The conserved amino acid residues at the position 95–99 are presented in boldface. The consensus sequence motifs in the CDR3 region of Vβ17 and Vα are listed at the bottom.

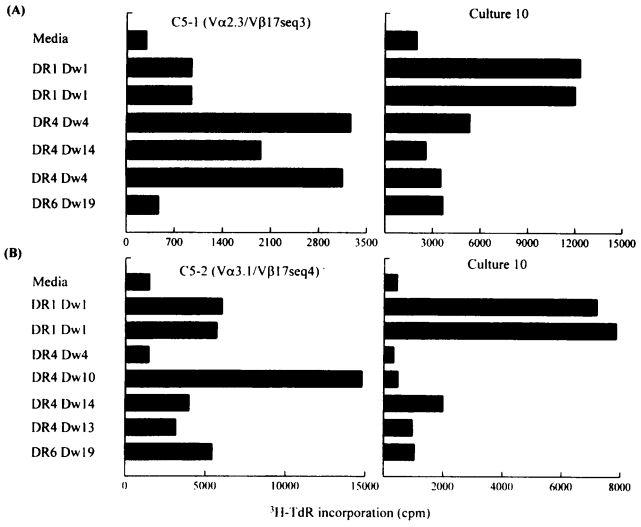

Figure 6. Proliferation of ST-derived T cell clones C5-1 (*A*) and C5-2 (*B*) induced by EBV-transformed B cell lines. Culture 10, an uncloned $CD4^+$, $V\beta17^+$ ST-derived T cell line, was included as control. 2×10^4 T cell line cells were cultured, in triplicate, in 96-well round bottom tissue cultures plates with medium alone or with 5×10^4 EBV-transformed HLA DR homozygous B cell line cells x-irradiated with 4000 rads from a cesium source. Cultures were supplemented with 5% IL-2 and after 96 h, proliferation assayed by [^{3}H]thymidine incorporation.

gene products and a characteristic amino acid residue in the N–D–N region (24). Recent studies demonstrate that the introduction of charge-altering amino acids in a well defined antigenic peptide results in a T cell response characterized by antigen-specific TCRs which have incorporated reciprocal charge changes in the N–D–N region of CDR3 (amino acid residues 95–99) of both the α and β chains (25). This result suggests that these TCR residues bind directly to the antigenic peptide. In a related study, it was found that the murine TCR repertoire recognizing foreign peptides which are highly homologous to self is markedly constrained with respect to TCR Vα and Vβ gene usage, CDR3 length, and the presence of canonical amino acid residues in the CDR3 domain (26, 27).

These data suggest that antigen-driven pathogenic T cells mediating autoimmune disease might be expected to express TCR which share crucial structural characteristics. This situation obtains for the myelin basic protein (MBP) specific T_h cells which induce experimental allergic encephalomyelitis (28–30). Encephalitogenic T cell clones are strongly biased with respect to Vβ and Vα gene usage as well as CDR3 region structure (31). Recently, it has been shown that TCR Vβ transcripts isolated from central nervous system lesions of patients with multiple sclerosis exhibit sequence motifs in the N–D–N region homologous with those expressed by encephalitogenic MBP reactive murine T cell clones (32, 33).

Extension of this concept to T cells mediating other autoimmune diseases, including RA, remains controversial. Oligoclonal expansions of Vβ3, 14, and 17 bearing synovial T cells in RA (9–11), as well as Vβ14 in juvenile rheumatoid arthritis (34) have been described. In this study, we have identified the RA joint specific localization of several closely related $V\beta17^+$ T cells, including those expressing the oligoclonal sequences which dominated fresh ST and SF. The TCR Vβ17 expressed by these T cells preferentially use two Jβ gene segments (Jβ2.1 and 2.7), and also are highly homologous in their antigen-binding N–D–N region of CDR3. Of particular interest, the sequence motif "IQG-N" in the N–D–N region of our Vβ17seq2 has been independently identified among the expanded, oligoclonal Vβ14 TCR transcripts isolated from SF and ST T cells of a $DR4^+$, RF^+ patient suffering from polyarticular juvenile rheumatoid arthritis (34). Taken together, these results provide strong experimental evidence that oligoclonal synovial T cells in RA represent a joint-localized, antigen-driven process and may, therefore, be of pathogenic importance.

The isolation and in vitro growth of $CD4^+$ T cell clones expressing conserved CDR3 motifs has allowed elucidation of the complete structure of the $\alpha\beta$ TCR expressed by potentially pathogenic RA T cells. Of interest, preliminary functional analysis of these clones suggests that they are specific for, or restricted by, alleles of the DR4 molecule. The availability of these clones will permit us to assess reactivity against a panel of potentially important self antigens, including joint-restricted antigens postulated to be targets of autoimmune attack, e.g., type II collagen, proteoglycans, heat shock proteins, as well as an array of synthetic peptides containing the sequence shared by RA-associated DR molecules, QKRAA (3). The identification of specificity for a self antigen among the oligoclonal synovial $V\beta17^+$ T cell clones expressing a common CDR3 motif may suggest a more specific immunotherapy for RA based on "blocking" antigenic peptides or mAb-mediated deletion of T cells bearing the pathogenic TCR.

The nucleotide sequences in the CDR3 region for Vβ17seq1-seq4 as well as for Vα2.3 and Vα3.1 reported in this paper have been deposited in the GenBank data base (accession Nos. U07134-35, 37-38, and U07636-37).

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References

1. van Boxel, J. A., and S. A. Paget. 1975. Predominantly T-cell infiltrate in rheumatoid synovial membranes. *N. Engl. J. Med.* 293:517–520.

2. Panayi, G. S., J. S. Lanchbury, and G. H. Kingsley. 1992. The importance of the T cell in initiating and maintaining the chronic synovitis of rheumatoid arthritis. *Arthritis Rheum.* 35:729–735.

3. Gregersen, P., J. Silver, and R. J. Winchester. 1987. The shared epitope hypothesis: an approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. *Arthritis Rheum.* 30:1205–1213.

4. Nepom, G. T., P. Byers, C. Seyfried, L. A. Healey, K. R. Wilske, D. Stage, and B. S. Nepom. 1989. HLA genes associated with rheumatoid arthritis: identification of susceptibility alleles using specific oligonucleotide probes. *Arthritis Rheum.* 32:15–21.

5. Holmdahl, R., L. Klareskog, K. Rubin, E. Larsson, and H. Wigzell. 1985. T lymphocytes in collagen II-induced arthritis in mice. Characterization of arthritogenic collagen II-specific T-cell lines and clones. *Scand. J. Immunol.* 22:295–306.

6. van Eden, W., J. Holoshitz, Z. Nero, A. Frenkel, A. Klajman, and I. R. Cohen. 1985. Arthritis induced by a T-lymphocyte clone that responds to mycobacterium tuberculosis and to cartilage proteoglycans. *Proc. Natl. Acad. Sci. USA.* 82:5117–5120.

7. Ranges, G. E., S. Sriram, and S. M. Cooper. 1985. Prevention of type II collagen-induced arthritis by in vivo treatment with anti-L3T4. *J. Exp. Med.* 162:1105–1110.

8. Horneff, G., G. R. Burmester, F. Emmrich, and J. R. Kalden. 1991. Treatment of rheumatoid arthritis with an anti-CD4 monoclonal antibody. *Arthritis Rheum.* 34:129–140.

9. Howell, M. D., J. P. Diveley, K. A. Lundeen, A. Esty, S. T. Winters, D. J. Carlo, and S. W. Brostoff. 1991. Limited T-cell receptor β-chain heterogeneity among interleukin 2 receptor-positive synovial T cells suggests a role for superantigen in rheumatoid arthritis. *Proc. Natl. Acad. Sci. USA.* 88:10921–10925.

10. Paliard, X., S. G. West, J. A. Lafferty, J. R. Clements, J. W. Kappler, P. Marrack, and B. L. Kotzin. 1991. Evidence for the effects of a superantigen in rheumatoid arthritis. *Science (Wash. DC).* 253:325–329.

11. Williams, W. V., Q. Fang, D. Demarco, J. VonFeldt, R. B. Zurier, and D. B. Weiner. 1992. Restricted heterogeneity of T cell receptor transcripts in rheumatoid synovium. *J. Clin. Invest.* 90:326–333.

12. Uematsu, Y., H. Wedge, A. Straus, M. Ott, W. Bannnwarth, J. Lanchbury, G. Panayi, and M. Steinmetz. 1991. The T-cell receptor repertoire in the synovial fluid of a patient with rheumatoid arthritis is polyclonal. *Proc. Natl. Acad. Sci. USA.* 88:8534–8538.

13. van Laar, J. M., M. M. Miltenburg, M. J. A. Verdonk, M. R. Daha, R. R. P. de Vries, P. J. van den Elsen, and F. C. Breedveld. 1991. Lack of T cell oligoclonality in enzyme-digested synovial tissue and in synovial fluid in most patients with rheumatoid arthritis. *Clin. Exp. Immunol.* 83:352–358.

14. Zagon, G., J. R. Tumang, Y. Li, S. M. Friedman, and M. K. Crow. 1994. Increased frequency of Vβ17-positive T cells in patients with rheumatoid arthritis. *Arthritis Rheum.* 10:1431–1440.

15. Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

16. Oksenberg, J. R., S. Stuart, A. B. Begovich, R. B. Bell, H. A. Erlich, L. Steinman, and C. C. A. Bernard. 1990. Limited heterogeneity of rearranged T-cell receptor Vα transcripts in brains of multiple sclerosis patients. *Nature (Lond.).* 345:344–346.

17. Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA.* 74:5463–5467.

18. Friedman, S. M., M. K. Crow, J. R. Tumang, M. Tumang, Y. Xu, A. S. Hodtsev, B. C. Cole, and D. N. Posnett. 1991. Characterization of human T cells reactive with the *Mycoplasma arthritidis*-derived superantigen (MAM): Generation of a monoclonal antibody against Vβ17, the T cell receptor gene product expressed by a large fraction of MAM-reactive human T cells. *J. Exp. Med.* 174:891–900.

19. Lieber, M. R., J. E. Hesse, K. Mizuuchi, and M. Gellert. 1988. Lymphoid V(D)J recombination: nucleotide insertion at signal joints as well as coding joints. *Proc. Natl. Acad. Sci. USA.* 85:8588–8592.

20. Janson, C. H., M. J. Tehrani, H. Mellstedt, and H. Wigzell. 1989. Anti-idiotypic monoclonal antibody to a T cell chronic lymophatic leukemia: characterization of the antibody, in vitro effector functionas and results of therapy. *Cancer Immunol. Immunother.* 28:225–232.

21. Pluschke, G., G. Ricken, H. Taube, S. Kroninger, I. Melchers, H. H. Peter, K. Eichmann, and U. Krawinkel. 1991. Biased T cell receptor Vα region repertoire in the synovial fluid of rheumatoid arthritis patients. *Eur. J. Immunol.* 21:2749–2754.

22. Bröker, B. M., U. Korthäuer, P. Heppt, G. Weseloh, R. de la Camp, R. A. Kroczek, and F. Emmrich. 1993. Biased T cell receptor V gene usage in rheumatoid arthritis: Oligoclonal expansion of T cells expressing Vα2 genes in synovial fluid but not in peripheral blood. *Arthritis Rheum.* 9:1234–1243.

23. Roudier, J., J. Petersen, G. Rhodes, J. Luka, and D. A. Carson. 1989. Susceptibility to rheumatoid arthritis maps to a T-cell epitope shared by the HLA-Dw4 DRβ-1 chain and the Epstein-Barr virus glycoprotein gp110. *Proc. Natl. Acad. Sci. USA.* 86:5104–5108.

24. Hedrick, S. M., I. Engel, D. L. McElligott, P. J. Fink, M.-L. Hsu, D. Hansburg, and L. A. Matis. 1988. Selection of amino acid sequences in the beta chain of the T cell antigen receptor. *Science (Wash. DC).* 239:1541–1544.

25. Jorgensen, J. L., U. Esser, B. F. Groth, P. A. Reay, and M. M. Davis. 1992. Mapping T-cell receptor-peptide contacts by variant peptide immunization of single-chain transgenics. *Nature (Lond.).* 355:224–230.

26. Casanova, J.-L., P. Romero, C. Widmann, P. Kourilsky, and J. L. Maryanski. 1991. T cell receptor genes in a series of class I major histocompatibility complex-restricted cytotoxic T lymphocyte clones specific for a *Plasmodium berghei* nonapeptide: implications for T cell allelic exclusion and antigen-specific repertoire. *J. Exp. Med.* 174:1371–1383.

27. Casanova, J.-L., J.-C. Cerottini, M. Matthes, A. Necker, H. Gournier, C. Barra, C. Widmann, H. R. MacDonald, F. Lemonnier, B. Malissen, and J. L. Maryanski. 1992. H-2-restricted cytolytic T lymphocytes specific for HLA display T cell receptors of limited diversity. *J. Exp. Med.* 176:439–447.

28. Zamvil, S. S., D. J. Mitchell, N. E. Lee, A. C. Moore, M. K. Waldor, K. Sakai, J. B. Rothbard, H. O. McDevitt, L. Steinman, and H. Acha-Orbea. 1988. Predominant expression of a T cell receptor V_β gene subfamily in autoimmune encephalomyelitis. *J. Exp. Med.* 167:1586–1596.

29. Urban, J. L., V. Kumar, D. H. Kono, C. Gomez, S. J. Horvath, J. Clayton, D. G. Ando, E. E. Sercarz, and L. Hood. 1988. Restricted use of T cell receptor V genes in murine autoimmune encephalomyelitis raises possibilities for antibody therapy. *Cell.* 54:577–583.

30. Burns, F. R., X. Li, N. Shen, H. Offner, Y. K. Chou, A. A. Vandenbark, and E. Heber-Katz. 1989. Both rat and mouse T cell receptors specific for the encephalitogenic determinant of myelin basic protein use similar Vα and Vβ chain genes even though the major histocompatibility complex and encephalitogenic determinants being recognized are different. *J. Exp. Med.* 169:27–39.

31. Gold, D. P., M. Vainiene, B. Celnik, S. Wiley, C. Gibbs, G. A. Hashim, A. A. Vandenbark, and H. Offner. 1992. Characterization of the immune response to a secondary encephalitogenic epitope of basic protein in lewis rats: II. Biased T cell receptor Vβ expression predominates in spinal cord infiltrating T cells. *J. Immunol.* 148:1712–1717.

32. Martin, R., M. D. Howell, D. Jaraquemada, M. Flerlage, J. Richert, S. Brostoff, E. O. Long, D. E. McFarlin, and H. F. McFarland. 1991. A myelin basic protein peptide is recognized by cytotoxic T cells in the context of four HLA-DR types associated with multiple sclerosis. *J. Exp. Med.* 173:19–24.

33. Oksenberg, J. R., M. A. Panzara, A. B. Begovich, D. Mitchell, H. A. Erlich, R. S. Murray, R. Shimonkevitz, M. Sherritt, J. Rothbard, C. C. A. Bernard, and L. Steinman. 1993. Selection for T-cell receptor Vβ–Dβ–Jβ gene rearrangements with specificity of a myelin basic protein peptide in brain lesion of multiple sclerosis. *Nature (Lond.).* 362:68–70.

34. Grom, A. A., S. D. Thompson, L. Luyrink, M. Passo, E. Choi, and D. N. Glass. 1993. Dominant T-cell-receptor b chain variable region Vβ14$^+$ clones in juvenile rheumatoid arthritis. *Proc. Natl. Acad. Sci. USA.* 90:11104–11108.