

Clonal V α 12.1⁺ T Cell Expansions in the Peripheral Blood of Rheumatoid Arthritis Patients

By Harout DerSimonian,* Masahiko Sugita,* David N. Glass,†
Agnes L. Maier,* Michael E. Weinblatt,* Thierry Rème,§
and Michael B. Brenner*

From the *Department of Rheumatology and Immunology, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, 02115; the †Division of Rheumatology, Children's Hospital Medical Center, Cincinnati, Ohio 45229; and §INSERM U 291, 34197 Montpellier Cedex 5, France

Summary

Rheumatoid arthritis (RA) represents a heterogeneous disease characterized by chronic polyarthritis. Most patients with adult RA inherit HLA-DR4 or -DR1 major histocompatibility complex (MHC) genes. While the molecular basis for this genetic predisposition is unknown, the major function of these MHC-encoded molecules is to present peptides to T lymphocytes. It is hypothesized that an endogenous or environmental antigen initiates a MHC-restricted immune response mediated by T lymphocytes, which is followed by a chronic inflammatory reaction involving many cell types. In chronic RA, previous or ongoing antigenic activation might result in detectable skewing of the peripheral α/β T cell receptor (TCR) repertoire. Here we demonstrate a marked expansion of V α 12.1-bearing CD8⁺ T cells in the peripheral blood (mean, 22%; range, 10–43%) of >15% of RA patients. A major proportion of these patients shared HLA-DQ2 in addition to the expected high frequency DR1 and DR4 alleles. Detailed molecular analysis in three of the RA patients with elevated V α 12.1⁺ T cells identified repeated TCR α chain sequences consistent with clonal V α 12.1⁺ CD8⁺ T cell expansion. In addition to shared TCR V α 12.1 germline gene usage among unrelated subjects, a conserved J α motif was also detected. Together, these results suggest an antigen-driven mechanism of T cell expansion in these patients and may offer a new approach in examining specific antigens that stimulate T cells in RA.

Rheumatoid arthritis (RA)¹ is an autoimmune disease that manifests itself in patients with chronic inflammation, synovial proliferation, and progressive joint damage (1). Several lines of evidence point to a role for T cells in the pathogenesis of RA. These include a genetic predisposition associated with MHC class II genes, whose products function to present antigens to T cells (2–4), the presence of activated T cells in inflamed synovial tissues and peripheral blood (5), and the therapeutic utility of treatments that at least partially inhibit T cell function (6, 7).

The major population of T lymphocytes that recognize antigen in the context of self-MHC molecules bear the TCR α/β heterodimer in association with the CD3 complex (8). Each chain is divided into a V region that recognizes the Ag/MHC complex and a C region that anchors the molecule to the cell surface. The TCR α/β chains are assembled by somatic recombination of discontinuous germline gene

segments during T cell development. Polymerization of template-independent N region and template-dependent P nucleotide insertions at the junctions, D β usage, the imprecise joining of the germline gene segments (selected from a large pool), and the combinatorial association of TCR α/β polypeptides help to generate an extensive diversity in the TCR α/β repertoire (9).

During T cell development, only a small fraction of the thymocytes are able to emigrate into the periphery as mature CD4⁺ or CD8⁺ T cells. Negative selection events delete self-reactive TCR α/β -bearing T cells (10, 11), while positive selection events rescue single-positive CD4⁺ or CD8⁺ T cells (12–14). Positive selection yields mature CD4⁺ T cells that are predominantly self-MHC class II restricted or CD8⁺ T cells that are predominantly self-MHC class I restricted (15). The binding of antigen peptides to MHC molecules has provided a molecular explanation for the corecognition of Ag/MHC by the TCR α/β (16). TCR gene sequences and hypothetical modeling based on Ig molecules suggest that the TCR V regions (especially those regions cor-

¹ Abbreviations used in this paper: EAE, experimental allergic encephalomyelitis; iPCR, inverse polymerase chain reaction; RA, rheumatoid arthritis.

responding to CDR1 and CDR2) contact the MHC molecule, while the highly diverse V-(D)-J junctional region (corresponding to CDR3) contacts antigenic peptides bound to the MHC molecule (9, 17).

There are strong correlations between TCR V gene usage and T cell antigen recognition specificity in animal studies (18). Striking T cell repertoire correlations also have been obtained for experimental allergic encephalomyelitis (EAE), an experimental model of autoimmune disease in mice and rats (19). It has been shown, for instance, that induction and maintenance of the disease is dependent on myelin basic protein-specific T cell clones that utilize a restricted pool of V α , J α , and V β gene segments. Since, antigenic specificity in RA is not known, it has been difficult to establish T cell clones from RA patients to study antigen recognition and TCR V gene usage. The identification of a restricted T cell population based on TCR gene usage in RA might be useful in identifying the relevant autoantigens and offer new therapeutic approaches for this disease.

Here, we used a V α 12.1-specific 6D6 mAb (20) in cell surface staining to identify a subset (17%) of RA patients that showed a striking elevation in the number of V α 12.1⁺, CD8⁺ T cells in PBL. Analysis of the V α 12.1⁺, CD8⁺ T cells in three unrelated individuals by nucleotide sequencing revealed evidence for clonal T cell expansion. Most of the patients with TCR V α 12.1-elevated CD8⁺ T cells also shared the HLA-DQ2 phenotype. These results provide evidence suggesting that antigen-driven expansion of T cells may be a component of chronic RA.

Materials and Methods

Immunofluorescence and Flow Cytometry. Flow cytometric analyses were performed using directly conjugated antibodies. Two-color analyses on PBL were carried out as described previously using a FACScan[®] (Becton Dickinson & Co., Mountain View, CA) (20). PBMC was isolated from fresh blood of donors using Ficoll-Hypaque and stained with FITC-conjugated anti-CD4 (OKT4) or anti-CD8 (OKT8) versus PE-conjugated anti-V α 12.1 (6D6) mAbs. Viable cells were analyzed by gating on the propidium iodide-excluding lymphocytes. The following formula was used to calculate the percentage value for V α 12.1 expression on CD4⁺ and CD8⁺ subsets: percent V α 12.1⁺, CD4⁺ (or V α 12.1⁺, CD8⁺) cells = 100 × [V α 12.1⁺ cells costained with anti-CD4 (or anti-CD8)/percent of total CD4⁺ (or CD8⁺) T cells].

Tissue Staining. Samples of synovial membranes were obtained as discarded tissue from surgery, snap-frozen immediately in liquid nitrogen, and stored at -80°C. 4- μ m-thick sections were cut and fixed in alcohol-acetone for 10 min at 4°C and stored at -20°C. Sections were rehydrated in PBS for 10 min, and two-color fluorescence staining was performed as described previously (21). Dual-color fluorescence staining was performed using 6D6-biotin (anti-V α 12.1) mAb and developed with tricolor-coupled streptavidin (Caltag, Paris, France). Tricolor is a two-stage PE-Texas red fluorescent dye excited at 488 nm, with an emission peak at 650 nm. As a second step, samples were stained with commercially available FITC-coupled anti-CD4 or anti-CD8 mAb (Immunotech, Marseilles). Slides were mounted with a drop of Fluoprep medium and sealed. Confocal microscope observations were carried out using an Optiphot fluorescence microscope (Nikon Inc., Instr. Group,

Melville, NY) equipped with a confocal head (MRC600; Bio-Rad Laboratories, Richmond, CA) possessing a 488-nm argon laser and two photomultipliers detecting mirror-reflected light (green fluorescence) and the pass-through light (red fluorescence). The two signals were computerized with the Comos software (Bio-Rad Laboratories), and a photograph was taken with a black-and-white camera. After merging both slides in a 256 optimized color bitmap, a photograph was taken with a color screen photographic device. CD8⁺ cells are seen in green and V α 12.1⁺ cells are seen in red. The double-stained V α 12.1⁺, CD8⁺ T cells are seen in yellow.

Polymerase Chain Reaction. Subsets of T cells were enriched using anti-CD4, anti-CD8, or anti-V α 12.1 mAbs with magnetic beads (Dynal, Inc., Great Neck, NY). For example, CD8-enriched T cells were isolated by depletion of CD4⁺ T cells using anti-CD4 mAb and magnetic beads, while CD4-enriched T cells were isolated by depletion of CD8⁺ T cell using anti-CD8 mAb and magnetic beads. Each of these depleted populations was further subjected to enrichment using anti-V α 12.1 mAb and magnetic beads. Purification of RNA from these cells was carried out according to Chomczynski and Sacchi (22). cDNA for the direct PCR method of amplification was synthesized in a reverse transcription reaction using AMV reverse transcriptase, 5 μ g of total RNA, and 500 ng of oligo-(dT)₁₂₋₁₈ primer before amplification with V α 12.1- and C α -specific primer combination. For inverse (i)PCR, double-stranded cDNA was synthesized from total RNA (2 μ g) and circularized using T4 DNA ligase (23). Using a pair of C α or C β primers oriented in an outward direction from one another, ~700-bp PCR products were generated corresponding to the full-length TCR α or β chain transcripts (24). PCR primers were V α 12.1-specific, 5'GGGGTCGACTTGCCAGCCTGTGAGGGCAG; C α -specific, 5'GGGAAGCTTCTGGTACACGGCAGGGTCAGG; C α -forward, 5'GGGTCGACGACCTCATGTCTAGCACAGT; C α -inverse, 5'-GCATGCGGCCCGCCCTGCTATGCTGTGTGTCT; C β -forward, 5'-GGGTCGACACACAGCGACCTCGGGTGGG; C β -inverse, 5'-GCATGCGGCCCGCCATGGTCAAGAGAAAGGA.

Cloning and Sequencing. PCR products were cloned into M13 plasmids and sequenced by the dideoxynucleotide chain termination method using the modified T7 polymerase (Sequenase; United States Biochem. Corp., Cleveland, OH). The sequencing products were resolved on polyacrylamide gels, and autoradiography was carried out according to standard methods.

Serologic HLA Typing. HLA class I and class II antigens were typed serologically by the Brigham and Women's Hospital Tissue Typing Laboratory (Boston, MA). Serotyping of HLA-A, -B, -C, -DR, and -DQ was performed using standard microcytotoxicity techniques.

Results

V α 12.1-bearing CD8⁺ T Cells Are Elevated in Patients with RA. The relative percentage of TCR V α 12.1 on CD4⁺ or CD8⁺ T cell subsets in the peripheral blood of RA patients was assessed by two-color fluorescence staining and flow cytometry using the V α 12.1-specific mAb 6D6 (20). Analysis of both RA patient ($n = 46$) and healthy control ($n = 68$) PBL showed similar frequencies of V α 12.1 expression by CD4⁺ T cells (mean, 2.6%; range, 1-4%). Likewise, one group of 38 patients contained percentages of TCR V α 12.1⁺ T cells in the CD8⁺ subset (mean, 3.6%; range, 1-7%) that were similar to those found in healthy controls. However, a second group of RA patients had elevated percentages of V α 12.1⁺ T cells in the CD8⁺ subset that ex-

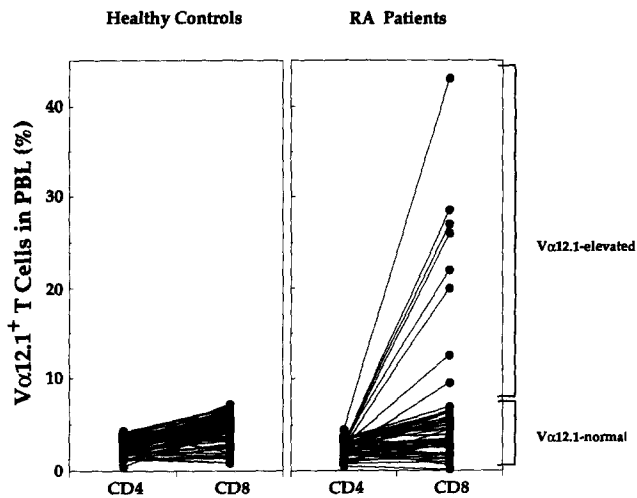


Figure 1. Expansion of $V\alpha 12.1^+$ T cells in RA. Two-color immunofluorescence analysis of TCR $V\alpha 12.1^+$ expression on $CD4^+$ and $CD8^+$ T cell subsets was carried out on PBMC from 68 normal controls (left) and 46 RA patients (right), and the results of each individual were connected by a line. The mean ages (and ranges) for $V\alpha 12.1$ -normal and $V\alpha 12.1$ -elevated individuals were 58.8 ± 13 (29–79) and 56.4 ± 10 (40–69) yr of age, respectively. The mean and proportion of the total $CD4^+$ and $CD8^+$ T cells in the $V\alpha 12.1$ -elevated and $V\alpha 12.1$ -normal groups were similar. 2 of 8 (25%) in the $V\alpha 12.1$ -elevated group and 10 out of 38 (26%) in the $V\alpha 12.1$ -normal group were males.

ceeded 7.4%, 2 SD above the mean of healthy individuals (Fig. 1). $V\alpha 12.1^+$ T cell expansions in the $CD8^+$ population were found in 8 of 46 (17%) RA patients and were absent in all 68 healthy controls (Fisher's exact test, $p = 0.00047$). Among these eight patients $V\alpha 12.1$ was expressed on 43, 29, 27, 26, 22, 20, 12, and 10% (mean, 22%) of the $CD8^+$ T cells, respectively (Fig. 1, $V\alpha 12.1$ -elevated). The elevations of $V\alpha 12.1^+$ T cells was a stable phenomenon since similar values were obtained over a 24-mo period in several of the subjects (data not shown).

Elevated $V\alpha 12.1^+$ T Cells Are Represented as Clonal or Oligoclonal Expansions. The precise molecular structure of the $V\alpha 12.1^+$ TCR α chains was determined by cDNA cloning and sequencing from the $CD8^+$ cells purified from three $V\alpha 12.1$ -elevated patients. PCR products containing $V\alpha 12.1$ rearrangements were generated by direct amplification using $V\alpha 12.1$ - and $C\alpha$ -specific oligonucleotide primers. For each patient a distinct $V\alpha 12.1$ -containing sequence corresponding to a functional TCR α chain transcript was repeated extensively (Fig. 2 A). For example, in patient 1, in whom 43% of the $CD8^+$ T cells were $V\alpha 12.1^+$, all 15 cDNA TCR α sequences obtained were identical. Similarly, 9 of 16 identical sequences were obtained in patient 2, in whom 29% of the $CD8^+$ T cells were $V\alpha 12.1^+$. Analysis of patient 3, in whom 22% of $CD8^+$ T cells were $V\alpha 12.1^+$,

A

$V\alpha / J\alpha$:	3' $V\alpha 12.1$	$J\alpha$ Sequence	# of Repeated Clones	
			Direct	Inverse (PCR)
Patient 1:				
$V\alpha 12.1/J\alpha A1$	CALS	DGYGQNFVFGPGTRLSVLPY	15/15	9/9
Patient 2:				
$V\alpha 12.1/J\alpha A12$	CALS	DYQGGSEKLVFGKGMKLTVNPY	9/16	ND
$V\alpha 12.1/J\alpha A24$	CALSE	GAYNTNAGKSTFGDGTTLTKPN	1/16	ND
$V\alpha 12.1/J\alpha A24$	CAL	TQTNAGKSTFGDGTTLTKPN	1/16	ND
$V\alpha 12.1/J\alpha A20$	CALSE	VGGANNLFFGTGTRLTVPY	1/16	ND
$V\alpha 12.1/J\alpha A15$	CALSE	PGSNNDMRFAGTRLTVKPN	1/16	ND
$V\alpha 12.1/J\alpha P$	CALS	GIDDKIIFGKGTSLHLPN	1/16	ND
$V\alpha 12.1/J\alpha A16$	CAL	LRSGGYNKLIIFGAGTRLAHPY	1/16	ND
$V\alpha 12.1/J\alpha A9$	CALSEA	WGGSQGNLIFGKGTSLVKPN	1/16	ND
Patient 3:				
$V\alpha 12.1/J\alpha A6$	CAL	RGGGADGLTFGKGTSLIHPY	16/24	21/27
$V\alpha 12.1/J\alpha A6$	CALSE	PYSGGADGLTFGKGTSLIHPY	3/24	5/27
$V\alpha 12.1/J\alpha A14$	CALS	GLDSGNTPLVFGKGTSLVIAN	1/24	0/27
$V\alpha 12.1/J\alpha A14$	CALSEA	QDSGNTPLVFGKGTSLVIAN	0/24	1/27
$V\alpha 12.1/J\alpha A26$	CALSE	PNTGRRALTFGSGTRLQVQPN	1/24	0/27
$V\alpha 12.1/J\alpha A$	CALSEA	YSSASKLIIFGSGTRLSIRPN	1/24	0/27
$V\alpha 12.1/J\alpha A10$	CAL	RVGKSISNFGNEKLTFTGTSLIIPN	1/24	0/27
$V\alpha 12.1/J\alpha N$	CALSE	GANDYKLSFGAGTTVTVRAN	1/24	0/27

B

$V\beta / D\beta / J\beta$:	3' $V\beta$	$D\beta / J\beta$ Sequence	# of Repeated Clones
			Inverse PCR
Patient 1:			
5.1/1.1/2.7	CASS	SKQGLMHEQYFPGPTRLTVT	18/18
Patient 3:			
8.1/1.1/1.2	CASS	LATPAYGYTFGSGTRLTVV	12/20
17/1.1/1.1	CASS	WTGNTFAFFGQGTSLTIV	1/20
17/2.1/2.6	CASS	TFRSGANVLTFFGASRLTIV	1/20
22/1.1/2.1	CASS	QCGNNEQFFGPGTRLTIVL	1/20
22/1.1/2.7	CASS	EDVSFYEQYFPGPTRLTVT	1/20
2.1/2.1/2.3	CSA	RVPGLAGGTTNTDTQYFPGPTRLTIVL	1/20
3.1/2.1/2.3	CASS	ATGTSGGGVDTQYFPGPTRLTIVL	1/20
3.3/2.1/2.1	CASS	WTLHNEQFFGPGTRLTIVL	1/20
15/1.1/2.5	CATS	SLGQGREETQYFPGPTRLTIVL	1/20

C

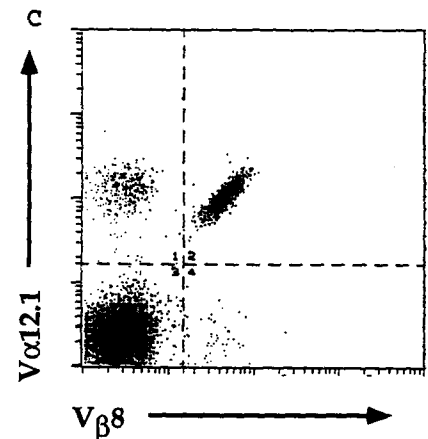


Figure 2. Repeated TCR α and β chain sequences in $V\alpha 12.1^+$, $CD8^+$ T cells. (A) Amino acid sequences were deduced from nucleic acid sequencing of cDNA clones generated by direct PCR or iPCR amplification. TCR $V\alpha 12.1$ sequences were obtained by direct PCR using $V\alpha 12.1$ - and $C\alpha$ -specific oligonucleotide primers on highly enriched $CD8^+$ T cells and by iPCR using highly enriched $V\alpha 12.1^+$, $CD8^+$ T cells of patients 1 and 3 were generated by iPCR. (C) Two-color FACS[®] analysis with anti- $V\alpha 12.1$ (6D6) and anti- $V\beta 8$ (16G8/29) mAbs was performed on $CD4$ -depleted PBL from patient 3.

identified two distinct repeated sequences; of these, one sequence accounted for 16 of 24 clones, and the second repeated sequence was identified in 4 of 24 clones. Although the junctional sequences were different in the two repeated $V\alpha 12.1$ sequences in patient 3, both used the same $J\alpha 6$ gene segment, $J\alpha A6$ (Fig. 2 A).

These results were confirmed using the iPCR methodology, which utilizes circularized DNA and a pair of α chain C region oligonucleotide primers oriented away from one another (23, 24). TCR $V\alpha$ -containing transcripts were cloned and sequenced from $V\alpha 12.1^+$, $CD8^+$ T cells purified from PBL. This analysis of patients 1 and 3 revealed the same repeated TCR α chain sequences that were found with the direct PCR amplifications (Fig. 2 A). A similar analysis of healthy subjects revealed diverse TCR α chain sequences with no occurrence of repeated sequences (data not shown). Among the repeated sequences found in the three patients examined, the $V\alpha 12.1$ was rearranged to distinct $J\alpha$ gene segments: $J\alpha A1$, $J\alpha A12$, and $J\alpha A6$ in patients 1, 2, and 3, respectively. Interestingly, all of these $J\alpha$ gene segments encode the same amino acid dipeptide sequence (pro-tyr) at their 3' ends (Fig. 2 A). Notably, only 6 of the 80 known $J\alpha$ genes encode this

dipeptide sequence, and may constitute a family of functionally related $J\alpha$ gene segments (25–28; DerSimonian, H., and M. B. Brenner, manuscript in preparation).

We also analyzed the TCR β chain transcripts in the purified $V\alpha 12.1^+$, $CD8^+$ T cells using the iPCR methodology. Hence, all potential $V\beta$ transcripts in $V\alpha 12.1^+$, $CD8^+$ T cells from patients 1 and 3 were cloned and sequenced (Fig. 2 B). All 18 $V\beta$ cDNA clones in the purified $V\alpha 12.1^+$, $CD8^+$ T cells were identical in patient 1. Moreover, 12 of 20 cDNA clones from patient 3 were identical but differed from the repeated $V\beta$ sequence identified in patient 1. Since the repeated sequence in patient 3 predicted a $V\beta 8$ -encoded TCR, cell surface coexpression of $V\alpha 12.1$ and $V\beta 8$ gene products on $CD8^+$ T cells was demonstrated by flow cytometry using the $V\alpha 12.1$ -specific mAb (6D6) and the $V\beta 8$ -specific mAb (16G8; 29), confirming the molecular cloning results (Fig. 2 C).

Expanded $V\alpha 12.1^+$ T Cells Carry the "Memory" Phenotype. Staining of $V\alpha 12.1^+$ T cells with mAb specific to various activation antigens was carried out in two-color flow cytometry analysis as shown for $V\alpha 12.1$ -elevated patient 1 (Fig. 3 A). Such analyses for patients 1–3 are summarized in histo-

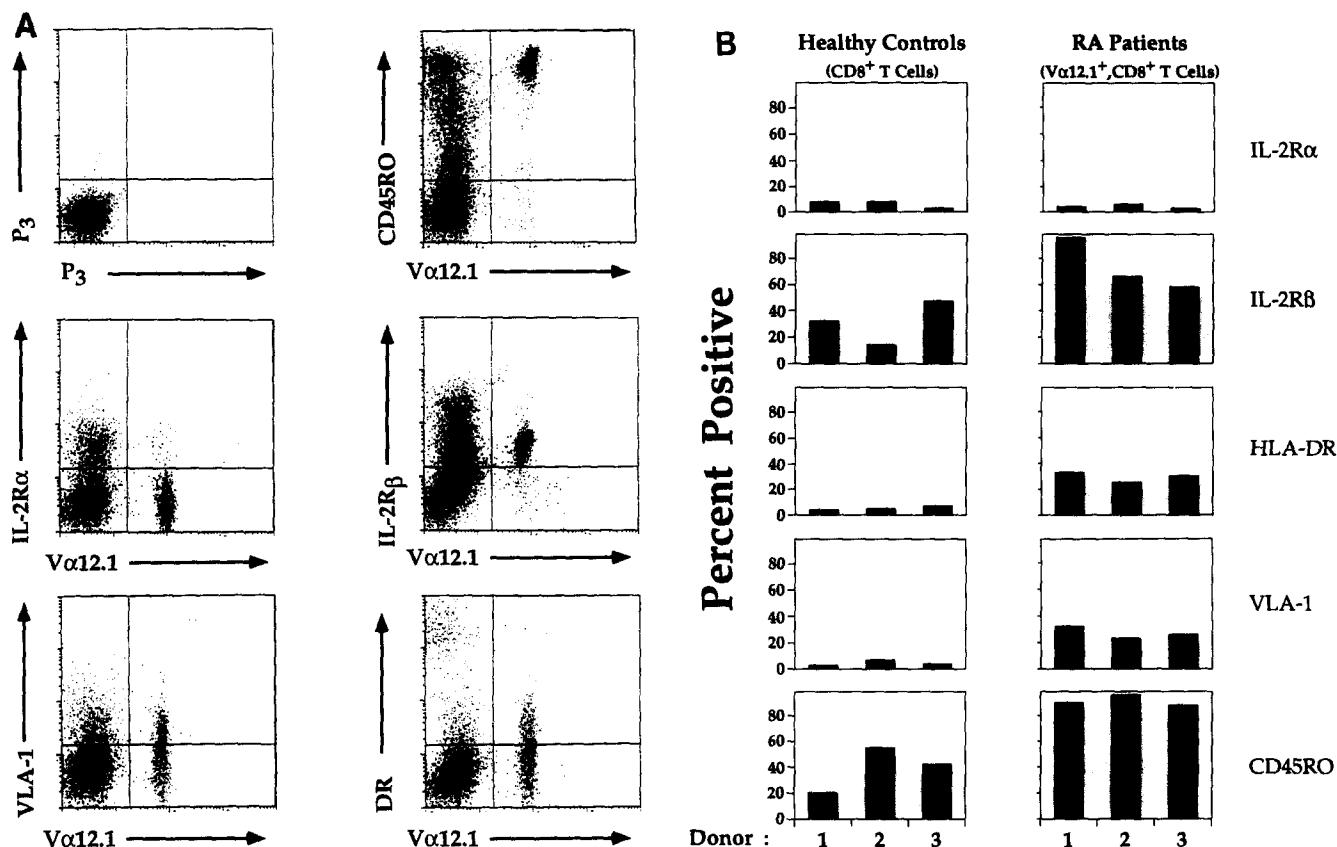


Figure 3. $V\alpha 12.1^+$ T cells activation phenotype. Two-color staining of $V\alpha 12.1^+$ T cells from fresh PBMC of one $V\alpha 12.1$ -elevated patient is displayed by dot plot analysis. (A) PBL from patient 1 shows costaining with FITC-coupled anti- $V\alpha 12.1$ mAb on the x-axis vs. PE-coupled anti-CD45RO (top right) (UCHL1; 30), anti-IL-2R α (middle left) (B1.49.9; 31), anti-IL-2R β (middle right) (TU27; 32), anti-VLA-1 (bottom left) (TS2/7; 33), or anti-HLA-DR (bottom right) (LB3.1; 34) mAb staining on the y-axis. (B) Bar graph representation of percent positive for the two-color staining using the anti-IL-2R α , anti-IL-2R β , anti-HLA-DR, anti-VLA-1, and anti-CD45RO mAbs for $CD8^+$ T cells in healthy donors 1–3 (left) and for $V\alpha 12.1^+$, $CD8^+$ T cells in patients 1–3 (right).

gram format (Fig. 3 B). A majority (>85%) of the expanded peripheral $V\alpha 12.1^+, CD8^+$ T cells expressed the memory marker $CD45RO^+$. In addition, 10–30% of these cells expressed detectable levels of VLA-1, an antigen expressed on long-term or chronically activated T cells (35, 36), HLA-DR antigens, and IL-2R β chain. These results are consistent with previous antigen activation and reveal ongoing activation by a fraction of the $V\alpha 12.1^+, CD8^+$ peripheral T cells in the $V\alpha 12.1$ -elevated RA patients. They stand in contrast to the lower percentages of $CD8^+$ T cells from healthy subjects expressing these activation markers (Fig. 3 B). Only percentages of IL-2R α chain-bearing cells are similar in normal $CD8^+$ T cells from healthy donors and $V\alpha 12.1^+, CD8^+$ T cells from the RA patients.

Distribution of $V\alpha 12.1^+, CD8^+$ T Cells in the Synovium. The primary finding in this study is the expansion of a $V\alpha 12.1$ -bearing $CD8^+$ T cell population in the peripheral blood of a subset of RA patients. Two of these $V\alpha 12.1$ -elevated patients received a medically indicated joint operation during the course of study allowing analysis of their synovial tissues. Frozen sections were stained for two-color confocal fluorescence microscopy with anti- $V\alpha 12.1$ mAb 6D6 (red) and anti- $CD8$ or anti- $CD4$ mAb (green). Each two-color fluorescent slide was screened for at least 50 cells containing at least five lymphocyte-rich areas (at 240 \times). The black-and-white bitmap pictures from each photomultiplier were simultaneously recorded and merged after recoloring. An example of such an evaluation of double-positive ($V\alpha 12.1^+, CD8^+$) cell staining is shown as the merged product of the $CD8^+$ (top left) and $V\alpha 12.1^+$ (top right) by scanning of the same field (Fig. 4). Each double-stained cell is seen in yellow ($V\alpha 12.1^+, CD8^+$), composed of green ($CD8^+$) and red ($V\alpha 12.1^+$) color overlap. Synovial membrane staining of the two $V\alpha 12.1$ -elevated patients (nos. 2 and 4) revealed that 8.5 and 10% of $CD8^+$ T cells in their synovial tissue expressed $V\alpha 12.1^+$, compared with 29 and 27% of their peripheral blood $CD8^+$ T cells, respectively (Table 1). Thus, the $V\alpha 12.1^+, CD8^+$ T cells appear to represent a substantial number of the synovial tissue $CD8^+$ T cells, but not as large a fraction as of the peripheral $CD8^+$ T cells. Two $V\alpha 12.1$ -normal patients (nos. 25 and 46) were also examined and they expressed 3.6 and 6.2% $V\alpha 12.1^+, CD8^+$ cells in their synovial tissues compared with 4.9 and 5.3% in their peripheral blood samples, respectively. Too few synovial tissue analyses from (peripheral) $V\alpha 12.1$ -evaluated donors were made to allow a statistical assessment.

Patients with Expanded $V\alpha 12.1^+$ T Cells Share HLA-DQ2. The MHC alleles present among the RA patients were analyzed and compared in both the $V\alpha 12.1$ -elevated and $V\alpha 12.1$ -normal groups. As expected, DR1 and DR4 alleles were prominently represented in both groups. Interestingly, the proportion of HLA-DQ2 (which is in strong linkage disequilibrium with DR3 and DR7) was substantially higher among the $V\alpha 12.1$ -elevated versus the $V\alpha 12.1$ -normal group (Fisher's exact test, $p = 0.002$), as it was found in 7 of 8 (88%) (Table 2) compared with 10 of 38 (26%) patients, respectively. This difference was similar to that of $V\alpha 12.1$ -elevated patients when

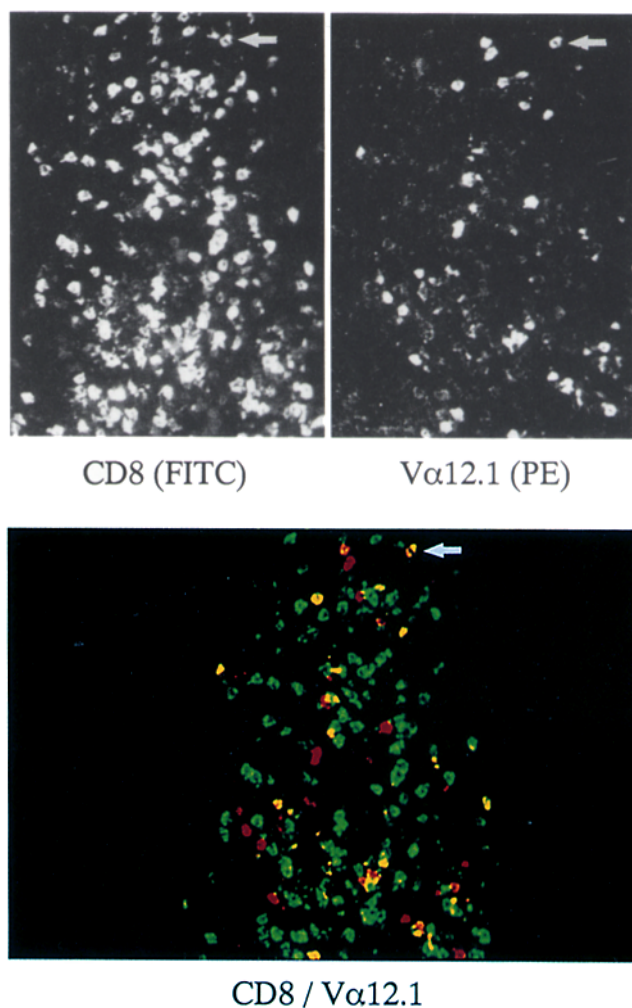


Figure 4. Analysis of $V\alpha 12.1^+, CD8^+$ T cells in synovial tissue by confocal microscopy. Double-fluorescence staining (see Materials and Methods) of RA synovial membrane with FITC-coupled anti- $CD8$ mAb (top left) and PE-coupled anti- $V\alpha 12.1$ (6D6) mAb (top right) are shown in black and white. Fluorescence of the same field is recorded at the two emission wavelengths in a confocal microscope. Computerized 256 optimized color side merge of black and white bitmaps is shown where single-positive $CD8^+$ (green) or $V\alpha 12.1^+$ (red) cells are visualized (bottom). Double-stained ($V\alpha 12.1^+, CD8^+$) cells are seen in yellow. An example of a double-stained cell is marked with the arrow ($\times 240$).

compared to the expected positivity of 33% in an outbred North American population ($\chi^2 5.05$, $p < 0.025$) (37). While the significance of increased DQ2 frequency for the risk of developing RA is unknown, the clonal (or oligoclonal) expansions of $V\alpha 12.1^+, CD8^+$ T cells appear to be more likely in DQ2-positive individuals. Patients in the $V\alpha 12.1$ -elevated group did not differ clinically, however, from the $V\alpha 12.1$ -normal group with respect to age, sex, duration of disease, rheumatoid factor positivity, erythrocyte sedimentation rates, or arthritis activity measured by number of painful joints and global assessments.

Table 1. Relative Frequency of V α 12.1⁺ T Cells in the CD4 and CD8 Subsets

	Patients			
	2	4	25	46
Peripheral blood				
V α 12.1/CD4	2.8%*	2.6%	1.5%	1.8%
V α 12.1/CD8	29%	27%	4.9%	5.3%
CD4/CD8	63:38	61:41	71:29	48:52
Synovial tissue				
V α 12.1/CD4	3.7%	6.7%	0.0%	0.3%
V α 12.1/CD8	8.5%	10.0%	3.6%	6.2%
CD4/CD8	55:45	58:42	44:56	61:39

* Percentage of V α 12.1⁺ T cells in the CD4⁺ or CD8⁺ T cell subset was calculated according to the formula in Materials and Methods: percent of V α 12.1⁺,CD4⁺ (or V α 12.1⁺,CD8⁺)/percent of total CD4⁺ (or CD8⁺) T cells.

Discussion

In this study, staining with V α 12.1-specific mAb and flow cytometry identified a subset of RA patients with elevated numbers of V α 12.1⁺,CD8⁺ T cells in the peripheral blood. The molecular analysis revealed that these T cells were clonally (patients 1 and 2) or oligoclonally (patient 3) expanded, evidenced by TCRs encoded by repeated V α 12.1 gene sequences. The marked expansion in V α 12.1⁺,CD8⁺ T cells reported here was detected because fluorescence staining with the V α 12.1-specific mAb could be readily performed on a large number of subjects with CD4⁺ and CD8⁺ T cells analyzed separately. Such screening approaches may be necessary to detect a particular TCR usage phenomenon when it occurs in only a subset of patients. The V α 12.1-elevated patients described here may correspond to an immunologically defined subset even though clinically these patients were similar to the V α 12.1-normal group. This study, examining the TCR repertoire in RA, differs from other analyses in several aspects (38). Other studies have predominantly focused on the V β repertoire (39–41), performed analyses on the synovial fluid or tissue and on mixtures of both CD4⁺ and CD8⁺ T cells (24, 39–44), and generally have examined only a few donors (24, 41–43). Nevertheless, previous examples of repeated TCRs V β 14 and V β 17 (as well as V β 2, V β 3, and V β 13.2 sequences) in synovial fluid or tissue have suggested that clonal T cell expansion maybe an important aspect of this disease (40, 41).

Several examples of restricted V α , J α , and V β usage in murine T cells have been reported that characterize a particular T cell response to known foreign antigens, for example, V α 11 and V β 3 in response to cytochrome *c* (45–47), V α 2 and V β 1 in response to λ repressor cI protein (48), V α 4 and V β 10 in response to lymphocytic choriomeningitis virus glycoprotein (49, 50), and V α 2.3 or V α 4 rearranged to the same J α gene segment, together with V β 8.2 in the myelin basic

Table 2. Patients with Expanded V α 12.1⁺ T Cells in the CD8 Subset

RA patient	CD8 ⁺ T cells expressing V α 12.1 ⁺	HLA class II	
		HLA class I	HLA class II
	%		
1	43*	A α , B44, A32, B60, Cw3	DR7, DQ2 DR4, DQ3
2	29	A1, B8, A2, B α ,	DR3, DQ2 DR4 DQ3
3	22	A2, B52, A11, B48	DR3, DQ2 DR10, DQ α
4	27	A2, B35, Cw4, A11, B57, Cw3	DR1, DQ1 DR2,
5	26	A30, B14, A23, B13,	DR7, DQ2 DR1, DQ1
6	20	A23, B27, Cw2, A24, B42,	DR4, DQ2 \dagger DR5, DQ α
7	12	A2, B7, A29, B44,	DR3, DQ2 DR7, DQ4
8	9	A30, B7, A11, B40,	DR3, DQ2 DR10, DQ1

Typing for HLA class I and II was carried out serologically using standard microcytotoxicity techniques.

* mAb 6D6 surface staining.

\dagger Confirmed by PCR sequence-specific probe analysis.

protein-induced EAE animal model of autoimmune disease (51–53). Thus, a strong antigen-driven activation may cause proliferation of T cell clones that utilize specific V α , J α , and V β sequences that ultimately dominate as clonal (or oligoclonal) populations of circulating T cells in patients with chronic RA. While the mechanism responsible for the clonal V α 12.1⁺ T cell expansion in this subset of RA patients is not known, the restricted V α /J α usage in unrelated patients and the CD45RO expression by the elevated V α 12.1⁺ T cells provide support for an antigen-driven activation mechanism.

The additional occurrence of the P-Y residues in the J α -encoded sequences of these TCRs is another interesting feature of their structure. Preliminary TCR modeling suggests this segment of the J α is near the switch or hinge region that joins the V and C domains. Residues at this site would be unlikely to participate in antigen-MHC recognition, but are predicted to be solvent exposed and might interact with an accessory cellular ligand or impose an effect on TCR α chain conformation if this region of the TCR is analogous to the counterpart region in Ig (A. R. Rees, personal communication, University of Bath, UK).

A relationship between HLA-DQ2 and the TCR V α 12.1⁺ clonal expansion can only be inferred at present. One hy-

pothesis would indicate that within the thymus HLA-DQ2 or a closely linked gene might act to select for the individual clone, or alternatively HLA-DQ2 might act as the restriction element in presenting antigen to V α 12.1⁺ T cells in the periphery. It is surprising that the expansion of a CD8⁺ T cell subset would be associated with the expression of a MHC class II allele since CD8⁺ T cells generally recognize peptide antigens presented by MHC class I molecules (15). However, a role for MHC class II and HLA-DQ-encoded molecules in particular in the activation and expansion of CD8⁺ T cells has been suggested in a number of experimental and clinical conditions where these cells function as regulatory or cytolytic lymphocytes (54–57). It is notable that the human antigen-specific T cell clones so far characterized show a bias for HLA-DQ restriction. For example, CD8⁺ T cell clones derived from blood or lesions of patients with lepromatous leprosy were restricted to HLA-DQ (54). Alternatively, peptides derived from HLA-DQ molecules may be relevant antigens presented by other HLA molecules. For instance, it has been shown that HLA-B7-derived peptides can be presented by DR11.1 (58), that HLA-DR3-

derived peptides can be presented by HLA-DPw3 (59), and in a cross-species system HLA-Cw3-derived peptides were presented on murine H-2K^d (60). Further, recent peptide elution experiments from human and mouse MHC molecules have identified numerous peptide sequences encoded by other MHC alleles (61, 62).

Whether the V α 12.1⁺ T cell population or the antigen to which it responds are critical to the pathology of the rheumatoid disease process itself remains to be determined. Nevertheless, clonal expansions that account for a mean of 22% of the CD8⁺ T cells in the peripheral blood are such large populations numerically that it seems likely that they may have an important functional impact. Since the expansion is in the CD8⁺ pool of peripheral blood T cells, these may be a regulatory T cell subset, rather than the predominant proinflammatory T cell in the synovium. Defining this aberration in a subset of patients may have relevance to the diagnosis and treatment of this disease. These clonal T cell populations together with associated MHC molecules offer newly identified tools to examine specific T cell antigens in this autoimmune disorder of unknown etiology.

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Address correspondence to Michael Brenner, Seeley Mudd Building, Room 504, 250 Longwood Avenue, Boston, MA 02115.

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