An Autoantigen-Specific, Highly Restricted T Cell Repertoire Infiltrates the Arthritic Joints of Mice in an HLA-DR1 Humanized Mouse Model of Autoimmune Arthritis

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An Autoantigen-Specific, Highly Restricted T Cell Repertoire Infiltrates the Arthritic Joints of Mice in an HLA-DR1 Humanized Mouse Model of Autoimmune Arthritis

Zhaohui Qian,* Kary A. Latham,* Karen B. Whittington,† David C. Miller,∗‡ and Edward F. Rosloniec∗†§

Although it is clear that CD4+ T cells play a major role in mediating the pathogenesis of autoimmunity, they often represent only a minor population at the site of inflammation in autoimmune diseases. To investigate the migration and specificity of autoimmune T cells to the inflammatory site, we used the collagen-induced arthritis model to determine the frequency, clonotype, and specificity of T cells that infiltrate arthritic joints. We demonstrate that despite the fact that CD4+ T cells are a minor population of the synovial infiltrate, the CD4+ T cells present are a highly selective subset of the TCR repertoire and, based on CDR3 length specificity of T cells that infiltrate arthritic joints. We demonstrate that although the number of CD4+ T cells that are present at the site of inflammation in autoimmune diseases, it has been difficult to identify these T cells or to determine their function and how they promote the pathology. One example of the difficulty of determining the role of CD4+ T cells in an autoimmune disease is rheumatoid arthritis (RA). Susceptibility to RA is clearly linked to the expression of specific HLA-DRB1 alleles (4–7), and this relationship with class II expression and the presence of T cells in the synovium suggests that CD4+ T cells play a direct role in the pathogenesis. Several studies have examined these T cells, but conflicting reports of their TCR gene expression and their function have left major questions as to whether they are specifically attracted to the joint and what their specificity and contribution is to the pathogenesis. Indeed, some have even proposed that the T cell infiltration of the joint in RA is a consequence of the disease and not the cause (8). Although there is some evidence that there is a selective migration of cytokine producing T cells to arthritic joints in RA (9–12), it is also clear that cytokines derived from fibroblast or monocytes are abundantly present in RA synovium (13).

Several humanized animal models have been developed to study the pathogenic mechanisms of T cells that lead to the development of autoimmune arthritis, including proteoglycan-induced arthritis (14) and collagen-induced arthritis (CIA) (15–17). Both of these animal models of autoimmunity share several pathogenic features with RA, including susceptibility being associated with the expression of specific MHC class II alleles and the subsequent participation of CD4+ T cells. Although there is clearly a CD4+ T cell component to the autoimmune response in proteoglycan-induced arthritis and CIA, like RA, T cells constitute only a small percentage of the arthritic joint infiltration in both of these models (18, 19). For example, in CIA at the peak of arthritis severity, <10% of the cellular infiltrate is composed of CD4+ T cells (19), and little is known regarding the specificity or the functional phenotype of these cells. In the studies described in this paper, we investigate the role of T cells that infiltrate the arthritic limbs in CIA by analyzing the clonality and specificity of these cells using a humanized HLA-DR1-Aβ transgenic (Tg) mouse model. These mice express an RA susceptibility class II allele, DRB1*0101, but no murine class II. Upon immunization with human type II collagen (hCII) or bovine CII (bCII), they develop a severe arthritis with a high incidence, and the CD4+ T cell response in the periphery is heavily skewed toward the expression of T cells expressing TCR-BV8 or BV14 (17). Using this model, we demonstrate that although the number of CD4+ T cells that are present in the arthritic joints are small, they are highly clonal T cells that preferentially migrate to these arthritic joints. In addition to their limited clonality, these T cells also appear to have specificity for the autoantigen, supporting the hypothesis that a localized immune response may play a role in driving the pathogenicity of autoimmune arthritis in this model.

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Abbreviations used in this paper: bCII, bovine type II collagen; CIA, collagen-induced arthritis; CIL, type II collagen; mCII, mouse type II collagen; hCII, human type II collagen; PMN, polymorphonuclear neutrophil; RA, rheumatoid arthritis; RT, reverse transcription; Tg, transgenic.
Materials and Methods

Mice and immunization

The generation of DRB1*0101 Tg mice (DR1) has been described previously (17). The DRA1 and DRB1 transgenes were established in (C57BL/6 × SJL/J)F2 mice and backcrossed to the B10.M background. In addition, the I-Af molecule expressed by B10.M mice was genetically deleted by backcrossing the B6.129-H2d(−/−) strain (stock number 003584; The Jackson Laboratory, Bar Harbor, ME) onto the B10.M-DR1 strain for six generations. Mice were maintained in microisolators in a pathogen-free environment. All animal studies were approved by the Institutional Animal Care and Use Committee. For stimulation of CII-specific T cells and arthritis induction, 8- to 10-wk-old mice were immunized s.c. at the base of the tail with 100 μg bCII emulsified in CFA (Difco, Detroit, MI) containing 4 mg/ml heat-killed Mycobacterium. Synthetic peptides representing the CII(257–274) immunodominant determinant of bCII, hCII, and mouse CII (mCII) were used for in vitro T cell stimulation assays. The bCII and hCII sequences are identical (EPGIAFGKQGQPKEPG), and the mCII peptide differs by one amino acid at position 266, Glu for bCII and hCII, and Asp for mCII.

Flow cytometry analysis of TCR repertoire

To determine the frequency of individual Vβ subfamilies expressed by CD4+ T cells in DR1 mice, spleen cells were incubated with anti-CD4-PE, anti–αβ-TCR-PerCP (BD Biosciences, San Jose, CA), and an anti–TCR-BV-specific Ab conjugated with FITC for 30 min at 4°C (Mouse Vβ TCR Screening Panel; BD Biosciences). The percentage of T cells expressing each TCR-BV gene subfamily was determined after gating on CD4+ T cells and using FlowJo software (Tree Star, Ashland, OR).

RNA isolation and reverse transcription

RNA was isolated from T cells obtained from spleens, lymph nodes, or PBls using RNAqueous (Ambion, Austin, TX), and RNA was isolated from arthritic limbs using the RNAqueous-Micro (Ambion). For analysis of arthritic joints, synovial tissues were dissected from arthritic paws and incubated in lysis solution, and all RNA was processed according to the manufacturer’s guidelines. cDNA was synthesized using SuperScript II reverse transcription (RT) (Invitrogen, Carlsbad, CA). Briefly, 1 μl 50 ng/μl random hexamers and 1 μl of a 10 mM 2’-deoxyribonucleoside 5’-triphosphate mixture were added to 8 μl RNA, and the sample was incubated at 65°C for 5 min. Following the incubation, 2 μl 10× RT buffer, 4 μl 25 mM MgCl2, 2 μl 0.1 M DTT, and 1 μl RNase inhibitor were added, and the sample was incubated at 25°C for 2 min. The SuperScript II RT was then added, and the sample was incubated at 25°C for 10 min and then transferred to 42°C for 50 min.

PCR and CDR3 length analysis

CDR3 length analysis was performed by two rounds of nested PCR as described previously (20). The initial TCR-BV gene family-specific PCR was carried out in separate reactions for each BV in 25 μl total volumes consisting of 0.5 μl cDNA, 2.5 μl 10× PCR buffer, 0.5 μl 10 mM 2’-deoxyribonucleoside 5’-triphosphates, 1 μl 10 μM TCR-BV forward primer (Table I), 1 μl 10 μM BV reverse primer (Table I), and 19.5 μl H2O. The PCR was performed as follows: 3 min of initial denaturation at 95°C, 30 cycles of 45 s at 95°C, 1 min at 55°C, and 72°C for 1 min, and a final 7 min at 72°C to ensure completion of all chains. Each of the first-round PCR products served as a template for a second round of nested PCR (Table I) with the nested TCR-BC reverse primer labeled with FAM on its 5’ end. The nested PCR was performed as follows: 3 min at 95°C, 20 cycles of 30 s at 95°C, 30 s at 55°C, 30 s at 72°C, and a final 7 min at 72°C. The fluorescent PCR products along with a size marker were separated using an ABI 310 Genetic Analyzer (GeneScan-500, Applied Biosystems, Foster City, CA), and the data were analyzed using ABIPRISM GeneScan software. Using “spiked” samples where decreasing numbers of TCR+ cells were added to TCR- cells, the sensitivity of this assay was determined to be at least two TCR+ cells in 105 total cells.

Cloning and sequencing

As described for the CDR3 length analyses, two rounds of PCR were performed with the exception that the high-fidelity enzyme Pfu (Stratagene, La Jolla, CA) polymerase was used, and the nested TCR-BC reverse primer was used without the fluorescent label. The PCR products were purified and incubated with Tag to adenylate the PCR product to facilitate cloning. The final PCR products were gel purified using GeneClean (Bio101) and cloned into the pCR2.1 vector via TA cloning (Invitrogen). Individual clones were subjected to DNA sequencing using an ABI 3100 sequencer (Applied Biosystems).

Soluble HLA-DR1 protein production and tetramer formation

The production and tetramerization of soluble HLA-DR1 covalently linked to the immunodominant peptide from CII (HLA-DR1-CII) has been described previously (19). The CII peptide sequence in the DR1 molecule is identical to bCII(259–273) and hCII(259–273). Briefly, soluble HLA-DR1-CII proteins were biotinylated using the BirA enzyme (Avidity, Aurora, CO) at a ratio of 15 μg Bir A to 38 nmol HLA-DR1-CII. To form the tetramers, the biotinylated proteins were incubated serially with increasing

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Table I. Primers used for PCR amplification of TCR-BV genes

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amounts of PE-conjugated streptavidin (Rockland, Gilbertsville, PA) reaching a final ratio of 10 μg PE-conjugated streptavidin to every 66 μg protein.

**Tetramer staining and cell sorting**

Cells were harvested from the draining lymph nodes of DR1 mice 10 d after immunization with CII, and enriched CD4+ T cells were recovered using the AutoMacs (Miltenyi Biotec, Auburn, CA). Cells were washed and resuspended at a density of 2.5 × 10^7 cells/ml in HL-1 medium (BioWhittaker, Walkersville, MD) supplemented with 0.05 mM 2-ME, 2 mM l-glutamine, 50 U/ml penicillin, and 50 μg/ml streptomycin. DR1 tetramers were added to cell suspensions at a concentration of 1 μg per 2 × 10^6 cells and incubated for 2.5 h at 37˚C. After washing, cells were incubated with anti-BV8–FITC, BV14–FITC, CD4–PerCP, and CD8a–APC (BD Biosciences) at 4˚C for another 30 min. Cells were analyzed using a FACSCalibur or LRS II (BD Biosciences) or sorted into tetramer^+^ and tetramer^−^ populations using a MoFlo FACS (DakoCytomation, Fort Collins, CO).

**Ag presentation assay**

Ag presentation experiments were performed in 96-well microtiter plates in a total volume of 0.3 ml containing 4 × 10^5 syngeneic spleen cells as APCs, 10^6 T hybridoma cells, and 100 μl of the CII(257–274) peptide at various concentrations in DMEM complete (DMEM supplemented with 10% FBS, 100 U/ml penicillin G sodium, 100 μg/ml streptomycin sulfate, 0.05 mM 2-ME, and 2 mM l-glutamine). Cell cultures were maintained at 37˚C in 5% humidified CO2 for 24 h, after which 80 μl supernatant was removed from each well, and 2-fold serial dilutions were made through each row of the plate. IL-2-dependent HT-2 cells (6 × 10^4) were then added to each well of the 96-well plate, and following an 18-h incubation, HT-2 cell viability was assessed by cleavage of MTT and quantitation at 690 nm (4). HT-2 cells were obtained from the splenic lymph node of DR1 mice 10 d after the CII immunization and were stained with Abs specific for CD4 and the TCR-BV chains as indicated. Data shown are based on sequentially gated populations of DAPI^−^ cells, forward versus side scatter, CD19^−^ and CD8^−^ cells, and CD4^+^ T cells. One percent of the CD4^+^ and BV8^+^ or BV14^+^ T cells bound the DR1-CII tetramer (A) compared with 0.08% with the DR1-HA tetramer (B). Of the tetramer^+^ T cells, 85% expressed either BV8 or BV14.

**TCR clonality of the CII-specific, DR1-restricted T cell response**

Given the selective repertoire responding to the CII immunization, we sought to determine the clonality of the T cell response, and how the clonality may differ between the T cells in the periphery and those that infiltrate the arthritic limbs. As shown in Fig. 3A, CII-specific CD4^+^ T cells using TCR-BV14 and BV8 were isolated from peripheral lymph nodes by cell sorting using an HLA-DR-CII tetramer and Abs specific for CD markers. Cells were sorted into two populations: 1) tetramer^+^CD4^+^/BV14^+^ or BV8^+^ and 2) tetramer^−^/CD4^+^BV14^+^ or BV8^+^, and the clonality of the TCR expression was analyzed by measurement of CDR3 length.

**Results**

**Selective use of TCR-BV in an HLA-DR1–restricted autoimmune T cell response**

Previously, we determined that the arthritogenic HLA-DR1–restricted T cell response to the immunodominant determinant of bCII and hCII in HLA-DR1 Tg mice made up <0.5% of the total CD4^+^ population and is predominantly composed of T cells expressing TCR-BV8 and BV14 gene segments (19). As shown in Fig. 1, at the peak of the T cell response (day 10), >90% of the DR1-restricted T cells that recognize the immunodominant determinant of CII, CII(263–270), preferentially use either the TCR-BV14 or BV8 gene segments, with BV14 used nearly two times more frequently than BV8 (19). Despite this selective use of BV8- and BV14-expressing CD4 T cells for the CII autoimmune response, the DR1 molecule does not preferentially select for T cells expressing these receptors during development in the thymus (Fig. 2). Although T cells expressing BV8 (8.1, 8.2, and 8.3) and BV14 gene segments combined make up 35% of the CD4^+^ TCR repertoire in the DR1 mice, statistically significant differences in TCR repertoire expression between the B10.M-DR1^+/I-A^0 mice and the B10.M mice were found only with BV6, BV10 (both positively selected), BV11, and BV12 (both negatively selected). These data indicate that the Tg plays a significant role in shaping the overall repertoire in these mice but does not appear to specifically select for a pathogenic repertoire in terms of the BV8 and BV14 gene segments expressed by the CII-specific T cells.

**FIGURE 1.** CII-specific T cells predominantly express TCR-BV8 or BV14 gene segments. Lymph node cells were recovered from B10.M-DR1 mice immunized 10 d prior with bCII and were stained with either a DR1-CII (A) or DR1-HA (B) tetramer for 2 h, followed by a 30-min incubation with Abs specific for CD4, CD8, CD19, BV8, and BV14. Data shown are based on sequentially gated populations of DAPI^−^ cells, forward versus side scatter, CD19^−^ and CD8^−^ cells, and CD4^+^ T cells. One percent of the CD4^+^ and BV8^+^ or BV14^+^ T cells bound the DR1-CII tetramer (A) compared with 0.08% with the DR1-HA tetramer (B). Of the tetramer^+^ T cells, 85% expressed either BV8 or BV14.

**FIGURE 2.** Comparison of TCR repertoires expressed in the periphery of B10.M-DR1 and B10.M mice. Spleen cells were recovered from B10. M-DR1 (DR1^+/I-A^0) and B10.M mice (I-A^0) and were stained with Abs specific for CD4 and the TCR-BV chains as indicated. Data shown are gated sequentially on forward versus side scatter and on CD4^+^ T cells. Error bars indicate SEM; *p < 0.05 by Student t test.
polymorphism. That this approach clearly identified and selected for the CII-specific T cells is demonstrated in Fig. 4. When placed in culture and restimulated with CII(257–274), the tetramer+/CD4+/BV8 or BV14+ T cells proliferated vigorously when restimulated with CII peptide, whereas the tetramer− cells did not respond.

Analysis of the polymorphism of the TCR CDR3 regions from these sorted populations indicated a clear difference in the clonal expansion of BV8 and BV14 subsets. The lengths of the CDR3 regions from the tetramer+ T cells were distributed in a Gaussian manner, a pattern identical to that of naive CD4+ T cells from DR1 Tg mice (data not shown), indicating the absence of significant clonal expansion of any of these T cells. In contrast the polymorphism of the TCR CDR3 regions from the tetramer− T cells was clearly skewed, especially from T cells expressing BV8.3 or BV14 (Fig. 3B). In each case, fewer variations in CDR3 lengths were observed, and in most cases, a significant skewing toward one CDR3 length had occurred. These data indicate that select clones of these T cells have been amplified during the autoimmune response.

TCR-BV clonotypes in arthritic joints

Because our data indicated that there was an oligoclonal expansion of CII-specific T cells in the periphery, we sought to determine whether these clonotypes were present at the site of pathogenesis in the arthritic joints. Arthritic hind or forepaws were dissected to recover the T cells from the synovial fluid and the synovial tissue, avoiding contamination from peripheral blood. A total of 17 arthritic paws ranging in a severity score of 1–4 were analyzed, including two with a severity score of 1, three with a severity score of 2, four with a severity score of 3, and eight with a severity score of 4. For comparison controls, T cells were recovered from the lymph nodes of the same mouse and were analyzed in tandem with the corresponding synovial T cells. The total T cell populations infiltrating the joints were analyzed; tetramer staining and cell sorting were not performed prior to these analyses because of the small numbers of T cells. As shown in Fig. 5A,
80% of the CD4+ T cells recovered from these arthritic joints expressed either BV8 or BV14. This is a >2-fold increase in the percentage of T cells expressing these gene segments as compared with the TCR-BV repertoire of naive or CFA-stimulated peripheral T cells (%BV8.1, 8.2, 8.3 + %BV14 = 35%) (Fig. 2).

Analysis of the repertoire and clonality of these T cells by measurement of CDR3 length revealed that only a very select population of T cells migrates to the arthritic joints based on the small number of BV genes detected and their limited CDR3 polymorphism (Figs. 5B, 6). This is in contrast to the data obtained using peripheral T cells that revealed a focused but broader clonotypic response (Fig. 3B). Using the same TCR-BV-based CDR3 PCR amplification, only seven different BV genes were identified in the synovium among all of the arthritic paws analyzed, and 76% (15 of 17) of the BV genes identified were either BV8 or BV14 (Fig. 6). In comparison, all BV gene families were detected by PCR using T cells from lymph nodes of the same mice, with the exception of BV19, which does not appear to be expressed in these mice (data not shown).

The complete T cell repertoire analysis of the 17 arthritic paws is summarized in Fig. 6. Although there was a trend for the presence of a more diverse set of TCR-BV genes present in the arthritic paws with a higher severity of disease (Fig. 6A), the small sample sizes prevented these differences from reaching statistical significance.

Of the seven TCR-BV genes identified in the 17 arthritic paws, BV8S1 T cells were found in 5 of the paws, BV8S2 and BV8S3 were present among 9 paws each, and BV14 was found in 7 paws (Fig. 6B). Indeed, every individual arthritic paw analyzed contained either a BV8- or BV14-expressing T cell, suggesting that these make up the major pathogenic T cells in this model. Although the clonality of these T cells varied from paw to paw, the CDR3 analyses all indicated expansion of clonal subsets of the respective BV family. More than half of the TCR-BV amplifications using joint-derived cells were found to be composed of a single CDR3 length (e.g., profiles in Fig. 5B). This is in contrast to the CDR3 lengths identified in the control analyses that demonstrate a typical Gaussian distribution (Fig. 3B), with the exception of the BV12 amplification, which always yielded several major peaks. Although the remaining amplifications revealed multiple CDR3 lengths, all of them were clearly skewed in comparison with peripheral T cell CDR3 profiles, indicating that the T cells that were present in these arthritic joints were the likely result of select clonal expansions. In addition, as shown in the representative CDR3 analysis in Fig. 5B, the size of the amplicons for BV genes frequently differed significantly from the size of the predominant peak of the corresponding controls. Clearly T cells expressing BV8 and BV14 dominated in these arthritic paws. BV1, BV12, and BV18 were the only other TCR-BV genes detected in all of the arthritic paws. BVV, BV12, and BV18 were the only other TCR-BV genes detected in all of the arthritic paws, and they were found in only 3 of the 17 arthritic paws (Fig. 6B). In all, these data suggest that a highly selective subset of T cells are recruited and/or retained in the arthritic joints in this autoimmune model.

Sequence analysis of BV8 and BV14 genes expressed by T cells from arthritic joints

To determine whether the TCR-BV8 and BV14 receptors present in the arthritic joints were derived from a mono- or oligoclonal T cell population, the amplicons from several of the CDR3 analyses were cloned and sequenced (Table II). As a comparison control, the BV8 and BV14 PCR products from lymph node-derived T cells were used. As expected, the BV and CDR3 sequences from the control samples were very diverse, varying widely in both CDR3 length and diversity region sequence (data not shown). In contrast, the TCR-BV genes detected in the synovium of the arthritic paws were derived from a very limited number of T cell clones present in each paw. Of the 17 CDR3 amplifications that were sequenced, 6 of them appear to derive from clonal populations of T cells present in their respective arthritic joints (K11 BV8.1, K10 BV8.2, H8-1 BV8.2, A1 BV8.3, AHS BV8.3, and H8-2 BV14) (Table II). The remaining CDR3 amplifications contained only two or three different BV sequences. For example, mouse paw K10 contained T cells expressing BV8.1, BV8.2, and BV14, and the BV8 T cells were all derived from a single clone, whereas the BV14 T cells were composed of two clones. Although T cell infiltrates within a single joint were highly clonal, there were also distinct variations in the BV sequences identified among multiple arthritic joints from a single mouse. Paws H8-1, H8-2, and H8-3 were all obtained from the same mouse, yet the T cells that infiltrated each paw were from a different clonotype. Interestingly, the H8-2 paw gave a monoclonal profile in the CDR3 analysis but contained two different CDR3 sequences of the same length. In addition to the highly clonal nature of the T cells from individual mice, two redundant uses of TCR BV genes were observed. Mouse AH13 and AH3 both expressed the identical BV8S3-JB2S6 clone in their arthritic paws, and it was the only BV8S3 clone detected in the AH3 mouse paw. In addition, the BV8S3 clone identified in the H8-2 paw is identical to a previously described TCR-BV8 chain expressed by a CIA-specific T cell hybridoma that was derived from peripheral lymphoid tissue.

![Figure 6](http://www.jimmunol.org/)
of a DR1 Tg mouse immunized with CII (25). Thus, these data indicate that a highly restricted set of T cells migrate to the arthritic joints in these mice, although the same clone may not predominate in each joint from the same mouse. These result strongly suggested that CII-specific T cells preferentially infiltrate the arthritic paws.

Ag specificity of T cells found in the arthritic joints

Even though an identical BV8S3 sequence is expressed by both a joint-derived T cell and a CII-specific T cell hybridoma, there is no way to know for certain whether any of these BV genes identified form an HLA-DR1–restricted TCR specific for the CII immunodominant peptide. To address this question, T cell hybridomas were produced from T cells isolated from arthritic joints and tested for their ability to recognize the hCII(257–274) or mCII(257–274) peptide presented by HLA-DR1. Mononuclear cells were purified from arthritic joint infiltrates, stimulated with Con A or plate-bound anti-CD3/CD28, and fused with BW5147 cells to produce the T cell hybridomas. This pan-stimulus approach for T cells was used to avoid any bias in selection for CII-specific T cells when producing the hybridomas. From four separate fusions, a total of 46 hybridomas were produced with 14 (30%) of the hybrids found to have specificity for the CII (257–274) peptide presented by DR1. Nine of these hybridomas were selected on the basis of their growth stability for further analysis (Table III). Seven of the T hybridomas expressed either BV8 or BV14 as would be predicted by the CDR3 analysis of the synovial T cells (Figs. 3, 5) and flow cytometry data (Fig. 4). Although all nine of these hybridomas responded to stimulation with the bCII peptide, only seven responded to the corresponding mCII peptide presented by HLA-DR1 (Table III). In addition, as demonstrated in Fig. 7, the response of these hybridomas to the mCII peptide is significantly lower in comparison with the CII that was used for immunization. These data indicating that changing the Asp (bCII or hCII) at residue 266 to Glu (mCII) make a significant difference in the recognition of the these DR1-CII complexes by the CII(263–270) determinant-specific TCR. Sequence analysis of the CDR3 regions of the TCR-BV chains expressed by these T cell hybridomas (Table IV) indicated that they have very similar N-D-N regions in comparison with the CDR3s identified in Table II, with arginine and negatively charged amino acids (Glu and Asp acids) frequently observed. In one instance, the CDR3 was nearly identical between T hybridoma DR1-J10 (ERTGG) (Table IV) and the BV8.2 receptor identified in the arthritic paw H8-1 (DRTGG) (Table II). In all, these data demonstrate that, although only small numbers of T cells are present in the arthritic joints in this model, the cells that migrate to these joints appear to be selectively recruited and are likely specific for the primary autoantigenic determinant of CII(263–270).

Discussion

The role of T cells at the site of inflammation in both RA and animal models of RA is incompletely understood. The number of T cells that are found at these sites of inflammation make up a small percentage of the total numbers of inflammatory cells present, yet...
especially relevant because the T cells recovered from the joint were not stimulated with CII prior to fusion but with a pan-specific agent prior to fusion. Thus, other than their frequency in the synovial tissue and fluid, no selective advantage for the generation of CII-specific T cell hybridomas was used, yet a high percentage of the resulting hybridomas was found to be CII-specific. Although the majority of the T cell hybridomas expressed TCR that recognized both the bCII and the corresponding mCII peptide, T cell stimulation by the mCII peptide was weaker than that of the bCII peptide. If these T cells were being selectively retained in the arthritic joints, it would seem logical that their specificity for mCII was a basis for their migration and/or retention. On the basis of this concept, two possible explanations for these data are offered. First, T cells capable of recognizing the low-affinity complex of DR1-mCII may be the only mCII-specific T cells that escape selection in the thymus, with the high-affinity mCII-specific TCR being deleted by negative selection. Second, the mCII peptide used in these studies may not precisely represent the natural peptide presented in the joint. Studies by others have indicated that lysine residues in the Y position of the repeating Gly-X-Y motif of CII can be glycosylated (29), and there is evidence that this glycosylation can play a role in the TCR recognition of the CII peptide (30–32). The CII peptide used in these studies, CII(257–274), contains two such lysine residues within the determinant core, one at position 264 and one at 270. At this time we cannot rule out the possibility that glycosylation of one or both of these residues in the mCII peptide is preferred or required by the TCR expressed by the T cell hybridomas that were derived from the arthritic joint T cells.

How these T cells that localize to the joint function in the overall pathogenesis of autoimmune arthritis is yet to be determined, and several possibilities exist. They may interact with APCs or B cells in situ to promote a localized autoimmune response that drives the production of autoantibody to CII at the site of inflammation, or through cytokine secretion they may activate the proinflammatory response of monocytes, synoviocytes, or fibroblast in the joint, culminating in the recruitment of polymorphonuclear neutrophils (PMNs). Even though selective clonotypes are retained in the arthritic joints, T cells in general make up <10% of the cellular infiltrate during the afferent phase of arthritis in this model. It is not clear which cells arrive first, T cells, PMNs, or monocytes, but it is likely that the selective migration and retention of these autoantigen-specific T cells in the joint is an important component of the pathogenesis of this model. Although it is clear that passive transfer of CII-specific Ab induces an inflammatory arthritis in the

![FIGURE 7. Recognition of bCII and mCII (257–274) peptides by T cell hybridomas derived from T cells recovered from arthritic joints.](image)

There is some evidence that these T cells are selectively attracted to the site (9, 10, 19, 26–28). The CIA model studied in this paper, we have clearly demonstrated that a highly restricted T cell population migrates to the site of autoimmune arthritis and that many of these cells have specificity for mCII. In peripheral lymphoid organs, the autoimmune CD4+ T cell response to CII is composed primarily of cells that express either BV14 or BV8 TCR gene family members, and this expression pattern of select TCR gene family members, and this expression pattern of select TCR genes extends to the T cells found in the arthritic joints. Although T cells expressing these TCR-BV gene segments make up ~35% of the total CD4+ T cell repertoire in naive DR1 mice, similar percentages were observed in backgrounds matched non-Tg mice, indicating that the DR1 class II molecule does not preferentially select for these BV8 or BV14 pathogenic T cells during thymic development. Their preponderance as CII-specific T cells appears to be based solely on the ability of these gene segments to form α/β TCR that are capable of recognizing the CII(257–274) peptide presented by DR1 and, in turn, promote the development of autoimmunity.

Although no Ag specificity of the corresponding α/β TCR can be inferred from the BV sequence analysis, three pieces of data indicate that the BV8- and BV14-expressing T cells in the joint are likely specific for the CII immunodominant determinant. First, the majority of these BV8 and BV14 T cells bind a DR1 tetramer containing the CII dominant peptide (19). Second, one of the BV8 CDR3 TCR sequences found in an arthritic joint matches precisely with a BV8 sequence derived from a previously described CII-specific TCR (25). Third, the majority of T cell hybridomas produced from the synovial T cells was found to have specificity for both bCII and mCII, the autoantigen in situ. This last point is

<table>
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<th>Table IV. CDR3 amino acid sequence used by CII-specific T hybridomas derived from T cells infiltrating arthritic joints</th>
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aArthritic paws were harvested from mice when the paw reached a grade 3 in severity. Cellular infiltrates were recovered and stimulated for 2 d with anti-CD3 and anti-CD28. Stimulated T cells were fused with BW5147 (TCR αβ negative) and screened for recognition of CII(259–273) presented by HLA-DR1.

b|^CDR3^ sequence was determined by DNA sequencing of TCR-BV cDNA amplified by PCR using TCR-C region and TCR-BV14- or TCR-BV8-specific primers. Spaces in the amino acid sequences indicate the end of the BV gene (left) and the beginning of the J gene (right).
CIA model (33), this arthritis is short-lived and completely resolves. The simplest explanation of the short disease duration is that the passively transferred Ab has a short $t_{1/2}$, although it is intriguing to speculate that a localized CII-specific T cell response is required to perpetuate the inflammatory response once it is initiated by the Ab. It is clear that CII-specific T cells are required to initiate a full course of disease in CIA by promoting the development of the autoantibody response (34, 35), but the presence of CII-specific T cells in the arthritic joint may also be required for full disease expression (36). The T cells that infiltrate the joints in this model are not only highly clonotypic but appear to be a subset of the CII-specific T cells found in the periphery of the arthritic mice. Whether or not specificity for self-Ag mCII is required for migration to the joint is unclear. Although the majority of the T cell hybridomas derived from the joint T cells were mCII specific, two clones only recognized the heterologous CII used for immunization. However, we cannot presently rule out that these clones recognize mCII peptides containing posttranslational modifications. Regardless, on the basis of our, data it appears that CII-specific T cells, or at least T cells expressing TCR-BV genes known to be expressed by CII-specific T cells, are overrepresented in the arthritic joints of these mice compared with those found in the periphery.

The limited repertoire of the T cells that infiltrate the joints appears to be a primary event as no significant difference in the clonality of the T cells present were observed between early arthritids (severity of 1) and late arthritis (severity of 4). Although there was a significant increase in the percentage of cells expressing the BV8 or BV14 gene segments among the T cells found in the arthritic joints, ~30% of the T cells did not express either of these genes, and they were not CD8+ T cells. It is possible that this minor population represents T cells with specificities for CII peptides other than CII(257–274) because these cells do not bind the DR1-CII tetramer (19). In addition, they may represent a subpopulation of T cells that are in constant flux, migrating in to the affected tissue and are not retained or clonally expanded in situ because of lack of Ag-specific activation. Although our assay conditions cannot account for every T cell present in the arthritic joint, the sensitivity of the PCR assay designed for detecting TCR genes was found to be two cells per $10^6$ or less, thus giving us a high degree of confidence in these repertoire analyses.

The role of T cells in the pathogenesis of RA is controversial with some studies concluding that the T cells in the arthritic joint are there as a by-product of the inflammatory response initiated by the synoviocytes and monocytes. These observations and questions are similar in the CIA mouse model, where T cells are the minority in comparison with the numbers of PMNs and monocytes present. Yet our data indicate that these T cells are not the result of a nonspecific influx of PBLs. In all, these data have several potential implications for the study of the pathogenesis of RA. Although we have not formally demonstrated that CII-specific T cells in the arthritic joints have a direct pathogenic function, it is clear that there is a selective attraction of these T cells that appear to have specificity for the autoantigen, and it seems likely that once there, they participate directly by either promoting the inflammation via soluble mediators or driving the production of autoantibody in situ. In RA, not only is there evidence of select T cell populations migrating to the inflamed joints (9, 10, 26, 27, 37), but the presence of organized lymphoid tissue resembling germinal centers has been also been demonstrated (38, 39). It is interesting to consider that these in situ organizations of lymphocytes are responsible for the continual propagation of the inflammation in the affected joints. Unlike our model, studies examining the TCR expression by T cells infiltrating RA joints have resulted in a number of different observations regarding TCR-BV or AV usage, although many have indicated a limited or restricted repertoire present (9, 10, 26, 27, 37). Our model is based on a single known autoantigen that drives a restricted immune response primarily composed of T cells expressing BV8 and BV14. Because the studies in RA indicate overrepresented populations of T cells based on BV and AV expression, but a wider range of these families among studies, it is possible that the Ags that drive RA are either diverse from patient to patient or the T cells are driven by different autoantigens in the joint that become exposed to the immune system following inflammation-mediated degradation of the joint tissue. In all, the data from the studies described in this paper suggest that this model may be very useful in studying the mechanism by which autoimmune T cells are recruited to the site of inflammation in autoimmune arthritis and their pathogenic function in situ.

Disclosures
The authors have no financial conflicts of interest.

References


