Psoriatic Arthritis Joint Fluids Are Characterized by CD8 and CD4 T Cell Clonal Expansions that Appear Antigen Driven

Patrick J. Costello, Robert J. Winchester, Shane A. Curran, Karin S. Peterson, David J. Kane, Barry Bresnihan and Oliver M. FitzGerald

*J Immunol* 2001; 166:2878-2886; doi: 10.4049/jimmunol.166.4.2878
http://www.jimmunol.org/content/166/4/2878

References

This article cites **53 articles**, 17 of which you can access for free at: http://www.jimmunol.org/content/166/4/2878.full#ref-list-1

Subscription

Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

Permissions

Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts

Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
Psoriatic Arthritis Joint Fluids Are Characterized by CD8 and CD4 T Cell Clonal Expansions that Appear Antigen Driven1,2

Patrick J. Costello,3*† Robert J. Winchester, † Shane A. Curran,*† Karin S. Peterson,† David J. Kane,* Barry Bresnihan,* and Oliver M. FitzGerald*

The CD8 αβ T cell receptor repertoire in joint fluid of individuals with active psoriatic arthritis contained an average of 32 major oligoclonal expansions in many variable genes of the TCR β chain (BV) families, as shown by β-chain CDR3 length analysis. Interestingly, a small number of oligoclonal expansions were shared between simultaneous samples of joint fluid and blood; however, most expansions found in joint fluid were not identifiable in blood emphasizing the immunologic specificity of the clonal events for the inflamed joint at a given point of time. The CD4 T cell joint fluid repertoire contained fewer and smaller oligoclonal expansions also largely restricted to the joint, suggesting that CD4 T cells participate perhaps by interacting cognitively to generate the CD8 clones. The inferred amino acid sequence of a single CD8 oligoclonal expansion revealed that they usually are composed of one or a few structurally related clones at the amino acid sequence level with β-chains that encode identical or highly homologous CDR3 motifs. These were not shared among patients. Moreover, several clones that encoded the same amino acid sequence were found to be structurally distinct at the nucleotide level, strongly implying clonal selection and expansion is operating at the level of specific TCR-peptide interactions. The findings support a model of psoriatic arthritis inflammation involving extensive and selective Ag, likely autoantigen, driven intra-articular CD4, and CD8 T cell clonal expansions. The Journal of Immunology, 2001, 166: 2878–2886.

Psoriatic arthritis is an inflammatory disorder affecting the joints and entheses that develops in ~15% of individuals with psoriasis. Considerable evidence implicates T cells in the pathogenesis of psoriatic arthritis as well as psoriasis. This includes the presence of T cells at sites of inflammation, the response of the disease to therapy directed at T cells, and the association of disease susceptibility with certain HLA alleles (1–6). In particular, the specific importance of CD8 lineage T cells in the pathogenesis of psoriatic arthritis (7) is emphasized by three observations. First, CD8 T cells comprise the majority of T cells in psoriatic arthritis joint fluids and exhibit an activated CD45RO+ HLA-DR+ phenotype (8). Second, the association of disease susceptibility is primarily with class I HLA alleles (9–12). Third, the disorder occurs at an appreciable frequency in individuals with advanced HIV infection and profound CD4 T cell depletion (13, 14).

However, several significant questions remain unanswered regarding the role of the T cell in the pathogenesis of psoriatic arthritis. First, what is the nature of the immunologic drive responsible for the accumulation of T cells in sites of inflammation such as the synovial fluid? Does the joint fluid repertoire have the features of responding to a single or several immune recognition events, to a superantigen, or does it simply reflect secondary non-Ag-specific T cell recruitment mediated by chemokines released by local joint inflammation? Related to this is the question of whether the immunologic processes in the joint are similar to or distinct from those occurring in blood and, reciprocally, the extent to which the two compartments are in equilibrium regarding T cell trafficking. Furthermore, do the immunologically driven alterations involve CD4 as well as CD8 lineage T cells? Although in the minority, CD4 T cells are present in joint fluid (8), leaving open the possibility that they could play a role. Possible support for an immune recognition event involving CD4 T cells is provided by the reports of the association of MHC class II alleles with susceptibility to psoriatic arthritis (15–17), as well as the important participation of CD4 T cells in the cognitive induction of CD8 T cells to effector status in a three cell complex involving dendritic cells (18–20). However, the absence of evidence of involvement of B cells, such as the lack of characteristic autoantibodies, the occurrence of psoriatic arthritis in advanced AIDS patients who lack the ability to mount effective immune responses to most Ags (13, 14), and the potential alternative explanation of linkage disequilibrium with class I alleles considered to drive susceptibility, have directed attention away from the class II associations and from a significant role for CD4 T cells and more toward non-Ag-specific T cell recruitment mechanisms.

Each of the possibilities advanced to explain the presence and immunologic character of T cells in psoriatic arthritis joint fluid implies finding a different pattern in the TCR repertoire of this inflammatory site. Non-Ag-specific recruitment of CD4 and CD8 T cells from blood into the joint in response to chemokines released in sites of synovial inflammation would give a primarily polyclonal repertoire that would mirror the repertoire of blood,
lacking clonal expansions specific to the joint. Alternatively, a superantigen in the joint tissues could induce polyclonal expansion of both CD4 and CD8 T cell populations as suggested by the recognized role of antecedent streptococcal infection in guttate psoriasis (7). Conversely, the stimulation of T cell clones by a specific Ag would result in oligoclonal T cell expansions characterized by related sequence motifs in the CDR3 region.

In recent years there has been considerable progress in understanding the structural and developmental biology of the somatically generated \( \alpha \beta \) TCR repertoire in health and in disease, an approach applicable to advancing understanding of the questions on the pathogenesis of psoriatic arthritis at the level of repertoire analysis. In the case of the \( \beta \)-chain, recombination of different germline V, D, and J gene segments creates clonotypically unique CDR3 regions capable of recognizing the peptide Ag (21–23). As a result of the recombination process, the \( \beta \)-chains are characterized by length differences ranging over 30 nucleotides distributed in a near Gaussian manner (24, 25). The post thymic TCR repertoire of early life is modified by clonal selection through various Ags and subsequent clonal expansion that alter the composition of the repertoire, affecting the distribution of CDR3 lengths, and results in the appearance of occasional CD4 and, more notably, CD8 T cell clonal expansions in healthy individuals (26, 27).

Analysis of the repertoire can be performed at differing levels of resolution, with determination of the bias in usage of variable gene(s) of the TCR \( \beta \) chain; \( \alpha \) elements conferring a low-resolution method for establishing the presence of unusual patterns in the combinatorial usage of the families of BV elements. Intermediate resolution methods take advantage of the process of junctional diversity involved in the joining of the V, D, and J elements that determine the length and amino acid composition of the \( \beta \)-chain CDR3 region. This method provides histograms of the distributions of CDR3 length for each BV family and reveals the presence of oligoclonal expansions (26, 28–30). High-resolution methods involve nucleotide sequencing and, although relatively arduous, offer the best opportunity to gain insights into the clonal structure of the immune recognition motifs underlying the oligoclonal expansions (31–33).

This study on the role of T cells in the pathogenic processes underlying the synovitis of psoriatic arthritis uses intermediate and high resolution techniques to study the synovial fluid T cell repertoire where the fluid phase CD4 and CD8 T cell populations permits assessment of their separate repertoires. A preliminary description of these results has appeared (34).

### Materials and Methods

**Patients**

Paired blood and joint fluid samples were obtained from six patients with active psoriatic arthritis undergoing therapeutic knee aspirations. Three psoriatic arthritis patients had oligoarthritisic disease, whereas the remaining patients had polyarticular disease. All patients had plaque psoriasis and disease duration of 2–10.5 years. Three were not on medication, two were on methotrexate, and one was taking salazopyrine. The HLA locus alleles were

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-B locus</td>
<td>A<em>02:01, A</em>03:01, A<em>04:01, A</em>11:01</td>
</tr>
<tr>
<td>HLA-DR locus</td>
<td>DRB1<em>07:01, DRB1</em>08:01, DRB1*10:01</td>
</tr>
<tr>
<td>HLA-DQ locus</td>
<td>DQB1<em>03:01, DQB1</em>05:01</td>
</tr>
</tbody>
</table>

All the patients were HLA identical. Eight age and ethnically matched controls were characterized by related sequence motifs in the CDR3 region.

### PCR and CDR3 length analysis

DNA was extracted from 1–3 \( \times 10^8 \) positively selected cells using RNAse free reagent according to the manufacturer’s instructions (Tel-Test, Friendswood, TX). CDNA (10–20 ng) was prepared using random priming (35) and adjusted to 50 \( \mu \)L. The PCR nomenclature used is as described (36, 37). The CDR3 length distribution method was performed as described (26, 29, 30, 38) with the following modifications. A fluorochrome-tagged primer for the constant region of the \( \beta \)-chain (BC primer) along with the BV primer was used in the initial PCR. This was performed by adding 1 \( \mu \)L CDNA to the PCR mix (final volume 50 \( \mu \)L) that contained 100 mM KCl, 20 mM Tris-HCl (pH 8.3), 2.0 mM MgCl2, 35 pm BV and BC primer, 0.25 mM of dNTP, and 1.25 U of AmpliTaq gold DNA polymerase (Perkin-Elmer, Norwalk, CT). Initial incubation at 95°C for 15 min was followed by 36 cycles of 96°C for 1 min and 65°C for 1.5 min. The panel of primers recognizing the 23 expressed BV elements was optimized using the DNAstar program Primer Select for reactivity at the above described conditions.

Primers included those published by Gregersen et al. (39) for BV6 and BV23; by Robinson et al. (40) for BV13A, BV13B, and BV20; and by Puusieux et al. (38) for BV1, BV2, BV3, BV4, BV9, BV13, BV14, BV15, BV16, BV18, BV22, and CB. Additional primers were designed using classified BV genes (37) and included: ctctgagtaaggtagcct, BV3; ctctcacttgaattcccaava, BV7; atgcgagcggcataggtct, BV8; aggaagaagattcctggaat, BV11; ggctggagagctaggeaatge, BV12; gctggattcacaggggagttc, BV17; agagagaaacaggagagagct, BV21; and cattgatccagaagagttg, BV25. The labeled PCR product was run on a model 373 or 377 automated DNA-sequencing apparatus (Perkin-Elmer-Applied Biosystems, Foster City, CA) with internal carboxytetramethylrhodamine (a fluorescent label of molecular weight standards Genescan 500-labeled standards. Genescan gels were analyzed using GeneScan Analysis 2.1 software (Perkin-Elmer-Applied Biosystems). The CDR3 region was defined as the nucleotides 3’ to the conserved C A S motif found in all BV families and 5’ to the F G X G motif of all joining gene (element) of the TCR \( \beta \) chain (BJ) elements. The distribution histogram was exported and analyzed in an Excel spreadsheet.

### Calculation of repertoire distortions

The degree of clonality was calculated in two ways: 1) by using the Hamming distance, a statistic used as an overall measure of the extent that a given repertoire differs from a reference distribution, exactly as outlined by Gorochov et al. (41); and 2) by defining the number and size of oligoclonal expansions. The Hamming distance ranges from 0 (if the two distributions are identical) to 100 (if they are completely different). Reference distributions for the Hamming distance determinations were prepared for each BV family by averaging the values for the CD4 T cell repertoire obtained from age and ethnicity matched individuals from the same geographic area. The CD4 profiles from the control population were used for the analysis of both CD4 and CD8 because the CD8 T cell repertoires from healthy controls more frequently contain oligoclonal expansions (39). However, the Hamming distance does not enumerate all individual oligoclonal expansions and provides less insight into the biological significance of alterations in the repertoire than does enumeration of oligoclonal expansions. In addition, Hamming distances may underestimates the repertoire distortion when multiple oligoclonal expansions are symmetrically distributed about the mean. Therefore, both enumeration of oligoclonal expansions and Hamming distances have been used as parallel measures of repertoire aberration.

The number of oligoclonal expansions present in the sample for each BV family was calculated from a composite reference profile. This composite profile was generated from the 140 length probability distributions of all BV families in controls that had a Hamming distance of <10. The 95th percentile at a particular CDR3 length was obtained by adding 2 SD to the mean of that CDR3 length, forming the upper limit of the expected. This 95th percentile level for each peak was normalized against the median peak after giving it a value of 100%. The CDR3 length of the median was an average of 66.5% of the total area of the two surrounding peaks with the +2 SD value being 88.7%. Hence expansion at the median could be detected if the area of the median was >88.7% of the sum of the areas of the CDR3 lengths on either side of the median. Major expansions were defined as those peaks with an area 5% greater than the expected area for the given BV family at the 95th percentile cutoff. An oligoclonal expansion was classified as putatively shared if it was found at the same CDR3 length of a particular BV family in both blood and joint fluid and if both expansions exceeded the 5% cutoff for that CDR3 length. If a significant expansion was found in only one compartment it was termed restricted. Statistical
significance was calculated using the Mann-Whitney U Wilcoxon rank sum test in SPSS 6.1 (SPSS, Chicago, IL). χ² analysis was performed to compare differences between individual pairs of blood and joint fluid at each CDR3 length. The length distributions were also tested for closeness of fit to a normal distribution using the Kolmogorov-Smirnov (K-S) statistic.

For sequencing, the appropriate PCRs were repeated, and amplified products were then subcloned using a Topo TA Cloning Kit (Invitrogen, Carlsbad CA) according to the manufacturer’s instructions. Plasmids mini-preps, sequencing, and analyses were performed as previously described (31). Plasmids were prepared in a separate area to PCR preparations to prevent cross-contamination. The CD4 and CD8 populations of joint fluid and blood in two patients for three BV families were selected for sequencing, with 90–200 clones sequenced for each condition.

Results
Predominance of oligoclonal expansions in synovial fluid CD8 T cell repertoire

Fig. 1 contains representative examples of patterns illustrating that the CDR3 length distribution of the CD8 and CD4 αβ T cell repertoire in psoriatic arthritis joint fluid differs considerably from blood due to the presence of increased numbers of oligoclonal expansions as marked by arrows in Fig. 1. The CD8 BV13 repertoire distribution of joint fluid in patient 2, Fig. 1B, contains three distinctive oligoclonal expansions at CDR3 lengths of 8, 9, and 12 that are restricted to joint fluid and not evident in blood. The percent area occupied by each CDR3 length in a distribution was calculated for each BV family. Subtraction of the values of the composite reference distribution from the observed values yields the number and quantitates the size of putative oligoclonal expansions at each CDR3 length. The three expansions in this example account for 44.6% of the BV13 repertoire in joint fluid. The Hamming distance of 28 and K-S statistic of \( p < 0.0001 \) also reflect that this profile departs considerably from the BV family-specific reference distribution and a theoretical Gaussian distribution.

In contrast to joint fluid, the paired blood repertoire distribution (Fig. 1A) more closely approaches a Gaussian pattern, although the K-S statistic of \( p = 0.0008 \) indicates that it still departs significantly from an ideal Gaussian distribution. The Hamming distance of 13 also reflects a smaller departure from the BV family reference distribution compared with 28 for the joint fluid. In addition to the differences in Hamming distance and number of oligoclonal expansions, comparison of the proportion of the paired blood and

**FIGURE 1.** Examples of common patterns illustrating the differences in CDR3 length distribution of the CD8 (A–D) and CD4 (E–H) αβ T cell repertoire between psoriatic arthritis blood and joint fluid. A and B, Paired blood and synovial fluid CD8⁺ T cell repertoire for BV13 in patient 2. C and D, BV16 of CD8⁺ T cells in patient 1. Bold arrows mark expansions that account for >5% of a BV family repertoire. In B the bold arrows point to major expansions in joint fluid that are not evident in blood and are accordingly considered restricted to the joint fluid. A small peak at a CDR3 length of 7 is restricted to the blood. C and D, Very large oligoclonal expansions shared between joint fluid and peripheral blood at a CDR length of 9 and a smaller one at the unusual CDR3 length of 17. A polyclonal background is evident in the blood (C) but not the joint fluid (D). Peripheral blood-derived CD4⁺ T cells tended to be more polyclonal in nature (E and G), whereas major oligoclonal expansions were mainly restricted to joint fluid (F and H). Some panels contain various carboxytetramethylrhodamine, a fluorescent label of molecular weight standards (in this case Genescan 500 standards), labeled m.w. markers evident by a peak in the baseline that are used to identify fragment size, but are otherwise irrelevant. Varying degrees of double peaks within one CDR3 length are the adenosine overhang added during PCR due to the lack of proofreading by the DNA polymerase.
The sequence analysis of this sample is presented subsequently. The unusual CDR3 length of 17 is also seen in both blood and joint fluid. A minor expansion at the unshared 18% of the repertoire, with the Hamming distance also highly elevated to 67. A minor expansion at the unshared expansions, compared with 17% of the repertoire in paired blood and joint fluid. The background expansion of the BV16 CD8 T cell repertoire at a CDR3 length of 18 years was shared by the blood and joint fluid CD8 T cell populations, with quantification of the divergence of the patterns from blood summarized in Table I. Notable is the presence of a major oligoclonal expansion accounting for 76.3% of the repertoire, with the Hamming distance also highly elevated to 67. A minor expansion at the unusual CDR3 length of 17 is also seen in both blood and joint fluid. The sequence analysis of this sample is presented subsequently.

Occurrence of CD4 oligoclonal expansions in blood and synovial fluid

Fig. 1, C and D, shows an instance where the oligoclonal expansion is shared by the blood and joint fluid CD8+ T cells, with quantification of the divergence of the patterns from blood summarized in Table I. Notable is the presence of a major oligoclonal expansion of the BV16 CD8 T cell repertoire at a CDR3 length of 9 that is present in both joint fluid and blood. The background polyclonal repertoire is low in joint fluid with the oligoclonal expansion accounting for 76.3% of the repertoire, with the Hamming distance also highly elevated to 67. A minor expansion at the unusual CDR3 length of 17 is also seen in both blood and joint fluid. The sequence analysis of this sample is presented subsequently.

Summarized repertoire characterization for all individuals using number of oligoclonal expansions

The CDR3 length distributions of the separated CD8 T cell population of six pairs of blood and joint fluid were determined for all 23 expressed BV families. This revealed an average of 32 major CD8 oligoclonal expansions found in the joint fluids of individuals with psoriatic arthritis (Table I). Twenty-four (75%) of these are restricted to the joint, whereas the remaining eight are shared by blood and joint fluid. A mean of 21 oligoclonal expansions are found in the blood of the patient, p = 0.042. This number is greater than, but not significantly different from, the number of oligoclonal expansions (18) in healthy controls. For each BV family, 29% of the joint fluid CD8 T cell repertoire is present in oligoclonal expansions, compared with 17% of the repertoire in paired blood samples. This latter value is identical with that of the healthy controls.

The availability of a paired joint fluid and blood sample taken 3 years after the initial analysis afforded the opportunity to begin to gain insight into the behavior of the repertoire at two points in time. A total of 22 informative major CD8 T cell oligoclonal expansions (seven restricted to peripheral blood, 13 restricted to joint fluid, and two shared by both compartments) were found at the initial time, and 34 oligoclonal expansions (10 restricted to peripheral blood, 20 restricted to joint fluid, and four shared by both compartments) were found at the follow-up time. All but five of these 56 oligoclonal expansions occurred at different CDR3 length positions and in different BV families emphasizing that the major portion of the oligoclonal expansions characterizing each repertoire differed markedly over this time of observation. Of the five oligoclonal expansions appearing to be in common between the two time points, one was found restricted to the joint fluid at both time points, and one was shared by peripheral blood and joint fluid at both time points. Interestingly, the three remaining oligoclonal expansions were initially restricted to blood at the first time point, but at the second time point were found either shared by blood and joint fluid or restricted to joint fluid.

Analysis of the CD4 populations obtained from these six joint fluids shows that there is an average of 22 major CD4 oligoclonal expansions present per patient, of which 20 (91%) are restricted to that compartment; two clones are putatively shared with blood. In contrast, an average of eight CD4 oligoclonal expansions are found in blood, p = 0.0001. Excluding the two oligoclonal expansions shared with joint fluid, the remaining number of six oligoclonal expansions restricted to blood is identical with the number of oligoclonal expansions found in blood of healthy controls. Within each BV family 13% of the joint fluid CD4 repertoire is composed of oligoclonal expansions, 12% of which are restricted to the joint fluid, whereas the remaining 1% are shared with blood and joint fluid.

Summarized repertoire characterization for all individuals using Hamming distances

Using Hamming distance as a measure of the departure of the distribution of CDR3 lengths from reference values, CD8 T cell repertoire departures are greater than that found in CD4 T cells. Similarly, the joint fluid of all patients have a greater derangement of both the CD4 and CD8 T cell repertoires than is found in paired...
The BV16 CDR3 length 9 repertoire in peripheral blood and synovial fluid of patient 1 is made up of multiple clones characterized by a Hamming distance of the paired blood sample, 22.4. The CD8 T cell repertoire is 29, which is significantly greater than the blood samples. The average Hamming distance of the joint fluid CD4 T cell repertoire is 57%, which is significantly greater than that of the paired blood sample (13, p = 0.0002). In turn, this value is also significantly greater than that of the blood CD4 T cell in healthy controls (8, p = 0.00001). However, in contrast to the situation in the CD8 T cell repertoire of joint fluid, a large proportion of the BV families in the joint fluid CD4 T cell repertoire have Hamming distances in the range of the healthy control reference population. This suggests that relatively more polyclonal CD4 T cell clones are recruited into the joint fluid than polyclonal CD8 T cells. The distortions in repertoire distributions due to oligoclonal expansions in the CD4 and CD8 joint fluid T cells in different patients are scattered across all BV families and not selectively found in any given BV family. However, the repertoires of certain BV families, most notably BV25 in CD4 and CD8 populations, were more likely to have higher Hamming distances in both reference controls and patients.

Sequence analysis of the αβ TCR repertoire

Sequence analysis performed on three BV families, BV7, BV16, and BV21 in two patients for both CD4 and CD8 lineages in blood and joint fluid, generally supports the subdivision of CD8 oligoclonal expansions into those shared by joint fluid and blood or restricted to either compartment. However, a few instances of small clonal expansions identifiable only on sequencing were encountered.

Table II illustrates that the CD8 T cell BV16 oligoclonal expansion at a CDR3 length of 9 shared between blood and joint fluid (Fig. 1, C and D) is composed of multiple homologous clones. In blood, the 29 nucleotide sequences of CDR3 length 9 comprise eight clones. The first three clones listed differ from one another by two to five nucleotides only in the CDR3 region and use BJ2S3, but notably all three encode the same inferred amino acid sequence motif SQSPPGGT. The fourth sequence, comprising a single sequence clone, contains the homologous sequence SQSPDPGT and also uses BJ2S3. A fifth clone containing four sequences and accounting for 17% of the oligoclonal expansion at a CDR3 length of 9 is characterized by the closely related amino acid CD3 sequence motif SQSMGGGT involving only a proline-to-methionine interchange but uses a different BJ element. The remaining three sequences have no structural homology with the CD3 sequence motif and likely reflect the polyclonal background in blood present at that CDR3 length. Sequencing of the paired joint fluid revealed that all 73 sequences of CDR3 length 9 in the joint fluid BV16 repertoire are made up of four clones that share the same SQS/P/ MGGT motif. Two of these are identical in nucleotide sequence to those found in blood. The dominant joint fluid clone, accounting for 57% of the repertoire in the joint fluid of CDR3 length 9, was similar to the dominant clone in blood. The fourth expanded clone

<table>
<thead>
<tr>
<th>CDR3 Length</th>
<th>No. of Sequences in Clone</th>
<th>CAS</th>
<th>CDR3 Region Sequence</th>
<th>BJ Gene Element</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>13</td>
<td>TGTGCCAGC</td>
<td>AGCCAgtccccagcggTACGGAGTAT</td>
<td>BJ2S3</td>
</tr>
<tr>
<td>9</td>
<td>5</td>
<td>TGTGCCAGC</td>
<td>CASSQSPGGTQY</td>
<td>BJ2S3</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>TGTGCCAGC</td>
<td>CASQSPGGTQY</td>
<td>BJ2S3</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>TGTGCCAGC</td>
<td>CASQSPGGTQY</td>
<td>BJ2S3</td>
</tr>
<tr>
<td>9</td>
<td>4</td>
<td>TGTGCCAGC</td>
<td>CASQSMGGTQY</td>
<td>BJ2S5</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
<td>TGTGCCAGC</td>
<td>CASRSTVGYT</td>
<td>BJ1S2</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>TGTGCCAGC</td>
<td>CASGPRSAQY</td>
<td>BJ2S7</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
<td>TGTGCCAGC</td>
<td>CASGRLSPEQY</td>
<td>BJ2S7</td>
</tr>
<tr>
<td>17</td>
<td>3</td>
<td>TGTGCCAGC</td>
<td>CASQSPGGTGSTNTGELF</td>
<td>BJ2S2</td>
</tr>
<tr>
<td>17</td>
<td>5</td>
<td>TGTGCCAGC</td>
<td>CASHLPGGTGSTNTGELF</td>
<td>BJ2S2</td>
</tr>
<tr>
<td>Synovial fluid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>42</td>
<td>TGTGCCAGC</td>
<td>AGCCAgctccccagcggTACGGAGTAT</td>
<td>BJ2S3</td>
</tr>
<tr>
<td>9</td>
<td>6</td>
<td>TGTGCCAGC</td>
<td>CASQSPGGTQY</td>
<td>BJ2S3</td>
</tr>
<tr>
<td>9</td>
<td>9</td>
<td>TGTGCCAGC</td>
<td>CASQSPGGTQY</td>
<td>BJ2S3</td>
</tr>
<tr>
<td>9</td>
<td>16</td>
<td>TGTGCCAGC</td>
<td>CASQSMGGTQY</td>
<td>BJ2S5</td>
</tr>
<tr>
<td>17</td>
<td>1</td>
<td>TGTGCCAGC</td>
<td>CASQSPGGTGSTNTGELF</td>
<td>BJ2S2</td>
</tr>
<tr>
<td>17</td>
<td>3</td>
<td>TGTGCCAGC</td>
<td>CASHLPGGTGSTNTGELF</td>
<td>BJ2S2</td>
</tr>
</tbody>
</table>

* This motif is shared by clones of BV16 CDR3 length 17. Lower case letters mark the CDR3 region of these clones.
The BV16 CDR3 length 9 synovial fluid repertoire in patient 2 is characterized by the presence of multiple restricted clones containing the same somatically encoded motif, LG. The eight clones with the LG motif differ at CDR3 position 2 where Q, L, or P are found and in the use of different BJ elements. The two largest clonal expansions contain Q at position 2, suggesting the presence of some degree of fine structural preference in the drive for the expansion, with the greatest preference also shown for the clone using the BJ1S5 element. However, there is no evident homology of any structural feature with the motifs in patient 1; moreover, among all sequences obtained, no identical or homologous sequence motifs were identified as shared between different patients.

**Discussion**

One principal finding of this study is that the CD8 αβT cell receptor repertoire in joint fluids of individuals with active psoriatic arthritis is characterized by a striking predominance of oligoclonal expansions identified in a variety of BV families as shown by β-chain CDR3 length analysis. The preponderance of these CD8 T cell oligoclonal expansions were relatively restricted to joint fluid in that they were not identified in sequencing samples of 90–200 clones from blood. The nature of the immunologic drive responsible for the accumulation of T cells in sites of synovial fluid inflammation exhibits features of being a response to a limited number of distinct immune recognition events. It lacks features suggesting a response to a superantigen, or to non-Ag-specific T cell recruitment mediated by chemo- kines released by local joint inflammation. These data imply that entry of T cells into the joint fluid is a highly specific process that is regulated at the clonal level by the recognition of certain peptides, with evidence of relatively little nonspecific interchange or rapid equilibrium between the repertoires of blood and joint fluid evident at a given time point.

Higher resolution analysis of the composition of the CD8 T cell-oligoclonal expansions by determining the TCR β-chain amino acid sequence in selected BV families revealed a single

---

Table III. The BV16 CDR3 length 9 synovial fluid repertoire in patient 2 is characterized by the presence of multiple restricted clones containing the same somatically encoded motif LG, with only one clone shared with peripheral blood

<table>
<thead>
<tr>
<th>CDR3 Length</th>
<th>No. of Sequences in Clone</th>
<th>CAS</th>
<th>CDR3 Region Sequence</th>
<th>BJ Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral blood</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>39</td>
<td>TGTGCAAGC</td>
<td>AGCCAAccttgCAATCGACCCAGCAGAT</td>
<td>BJ1S5</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
<td>TGTGCAAGC</td>
<td>AGCCAAagtttaATAGCACAGGTTCC</td>
<td>BJ1S1</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>TGTGCAAGC</td>
<td>AGCCCTggagatattTATGCTACACC</td>
<td>BJ1S2</td>
</tr>
<tr>
<td>Synovial fluid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>60</td>
<td>TGTGCAAGC</td>
<td>AGCCAAccttgCAATCGACCCAGCAGAT</td>
<td>BJ1S5</td>
</tr>
<tr>
<td>9</td>
<td>11</td>
<td>TGTGCAAGC</td>
<td>AGCCAAccttgCAATCGACCCAGCAGAT</td>
<td>BJ1S5</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
<td>TGTGCAAGC</td>
<td>AGCCAAagtttaATAGCACAGGTTCC</td>
<td>BJ1S1</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
<td>TGTGCAAGC</td>
<td>AGCCAAagtttaATAGCACAGGTTCC</td>
<td>BJ1S1</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>TGTGCAAGC</td>
<td>AGCCCTggagatattTATGCTACACC</td>
<td>BJ1S2</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>TGTGCAAGC</td>
<td>AGCCCTggagatattTATGCTACACC</td>
<td>BJ1S2</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
<td>TGTGCAAGC</td>
<td>AGCCCTggagatattTATGCTACACC</td>
<td>BJ1S2</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
<td>TGTGCAAGC</td>
<td>AGCCCTggagatattTATGCTACACC</td>
<td>BJ1S2</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>TGTGCAAGC</td>
<td>AGCCCTggagatattTATGCTACACC</td>
<td>BJ1S2</td>
</tr>
</tbody>
</table>

* Lower case letters mark the CDR3 region of these clones.
CD8 oligoclonal expansion to be usually composed of one or a few structurally related clones with $\beta$-chains that encode identical or highly homologous CDR3 motifs. However, importantly, when the sequence results were analyzed at the nucleotide level it was often found that several structurally distinct clones defined at the level of primary nucleotide sequence degenerately encoded the same sequence of amino acids, as illustrated in Tables II and III. These data argue strongly that clonal selection and expansion is occurring at the level of the Ag recognition function of the expressed receptor and is not due to the alternative explanation of the random entry of a small number of founder T cells. Taken together, these findings strongly implicate a role for Ag-, and likely autoantigen-, driven clonal expansion underlying the accumulation of the CD8 T cells. Based on the preferential accumulation of clones in the joint fluid, the inciting peptide Ag is likely to be selectively expressed in the joint or related tissues such as the entheses. In view of the major HLA associations of psoriatic arthritis with certain class I alleles and the activation and differentiation state of the T cell phenotype (8), the finding of CD8 clonal expansions supports the hypothetical class I MHC molecule-autologous peptide-CR3 T cell immune recognition event paradigm that has been proposed to underlie psoriatic arthritis (7).

Recently, oligoclonal expansions have been reported in the joint fluids of individuals with Reiter’s syndrome (42). The similarity of those observations to the present findings in psoriatic arthritis provides additional evidence emphasizing the analogy of Reiter’s syndrome and psoriatic arthritis (7). However, the present findings differed from those reported in Reiter’s syndrome (42) in that the considerable sharing of CDR3 motifs between different patients reported in Reiter’s syndrome was not observed in psoriatic arthritis, despite a fairly extensive sequencing endeavor. It is possible that the greater number of HLA susceptibility alleles for psoriatic arthritis and the fact that none of the patients sequenced were HLA identical might account for the lack of common $\beta$-chain motifs. Alternatively, such sharing may not be a feature of the immune response that engenders psoriatic arthritis.

In contrast to the striking alterations of the joint fluid CD8 T cell repertoire, the blood CD8 T cell repertoire is much less distorted by oligoclonal expansions. The presence of a small number of oligoclonal expansions that were shared with joint fluid is the most striking feature of the blood T cell repertoire. However, if these are excluded, the remaining repertoire is essentially equivalent to the repertoire of matched normal individuals in terms of the number and size of oligoclonal expansions as reflected in Hamming distance analyses. It is possible that the small number of additional clones in the blood of individuals with psoriatic arthritis reflects a response related to the skin disease. Preliminary evidence was also obtained suggesting the possibility that the sequence of clonal traffic may be from the blood to the joint, based on the appearance of a small number of oligoclonal expansions in the joint fluid that at an earlier time point were found restricted to the blood. Conversely, no clones that were present in the joint fluid at the earlier time point were identified in the blood, emphasizing that the joint may be populated by clones that are first expanded in the blood. This would be consistent with the clinical observation that psoriatic skin lesions usually antedate the development of arthritis by many years and that the immune response to the skin Ags in some way initiates the joint response. The dynamics of the oligoclonal expansions as a function of time are currently being studied in greater detail to better define the temporal relationships of the events and characterize the precise differences at a sequence level that distinguishes recrudescent arthritis.

The CD8 T cell oligoclonal expansions shared by blood and joint fluid were of particular interest because they were almost invariably very large in size in the joint fluid and commonly accounted for the major proportion of the joint fluid repertoire for the particular class I MHC molecule as shown in Figs. 1, B and C. The distribution of the individual clones comprising the oligoclonal expansions as characterized by sequence analysis (Tables II and III) revealed that the relative ratio of each individual clone between blood and joint fluid was not necessarily uniform, suggesting separate Ag-driven accumulation in each compartment, rather than extensive interchange of the entire oligoclonal expansion. As opposed to the primarily articular location of one putative driving antigenic peptide that accounts for clones restricted to the joint fluid and suggests that the responsible protein is expressed in the joint and entheses, the presence of a clonal expansion shared between blood and synovial fluid suggests that a second class of driving peptides may exist. The protein giving rise to these peptides would be likely found in both the joint and the other tissues of the body, such as the skin. The finding of sharing of certain CD8 T cell oligoclonal expansions between blood and joint fluid would provide a potential explanation for the recent report of Tassiulas et al. (43) that demonstrated the same $\beta$-chain sequences in both skin lesions and synovium in psoriatic arthritis. Although those authors did not identify the T cell lineage of the clone bearing the TCR sequence shared between the two sites, in light of the present results these cells may have been CD8 T cells.

The second principal finding of this study was that CD4 T cell oligoclonal expansions were also present in the joint fluid, but smaller in number and size compared with the CD8 expansions. The CD4 T cell expansions were present at about two-thirds the number of CD8 clones and in the aggregate occupied only 13% of the BV repertoire compared with 29% for the CD8 T cell clonal expansions. Nearly all were restricted to the joint fluid. It is possible that more sensitive analyses, using methods such as sequence-specific conformation polymorphism (SSCP) or Hamming distance analysis, would demonstrate additional CD4 T cell clones, especially because CD4 clones do not expand to the same extent as CD8 T cell clones. The findings in the psoriatic arthritis joint fluids are opposite to the situation in rheumatoid arthritis, where a preponderance of clonal expansions in the CD4 T cell repertoire are reported in both joint fluid and blood (44–49), with a considerably smaller number of CD8 T cell clonal expansions also identifiable (50–52). Indeed, Striebich et al. (48) also demonstrated sets of related, but different CDR3 nucleotide sequences that encoded identical or highly homologous $\beta$-chain amino acid sequences within the synovial fluid-derived CD4 T cell clones of rheumatoid arthritis patients. The identification of CD4 T cell oligoclonal expansions in psoriatic arthritis raises the possibility that the long recognized, but poorly understood, association of psoriatic arthritis susceptibility with particular MHC class II alleles (15–17) in fact reflects a significant event in the disease involving recognition of a peptide by CD4 T cells in the context of class II MHC molecules as has been postulated to occur in this disease (53). The MHC class II allele associations with psoriatic arthritis have usually been ascribed to linkage disequilibrium with class I susceptibility alleles. This potentially could involve dual recognition of different peptides derived from the same putative antigenic protein.
However, the observation of the CD4 clonal expansions in psoriatic arthritis is seemingly inconsistent with the earlier reports on the development of psoriatic arthritis and Reiter’s syndrome in individuals with advanced HIV infection who are nearly devoid of any CD4 T cells (13, 14). The report by Ridge et al. (19) potentially reconciles this apparent inconsistency by showing that viral infection of dendritic cells may bypass the requirement for the presence of CD4 T cells and allows the dendritic cell to directly activate the CD8 T cells. This may occur with HIV infection where it has been suggested that with relaxation of the genetic constraint for MHC class II complementation, HIV-positive dendritic cells present particular Ags that induce CD8 T cells to both break tolerance and be induced to CD8 effector status (53).

This study underlines the importance of identifying the nature of the peptides driving the immune response responsible for psoriatic arthritis. The finding of multiple clones in a given BV family characterized by highly homologous CD3 motifs restricted to the same CD3 length argn for considerable structural fidelity in the selection by a given peptide of different T cell clones involved in disease pathogenesis, and it supports an important role for the β-chain CD3 in defining the peptide recognition region of the αβTCR. This observation suggests that MHC peptide binding degeneracy and TCR plasticity that are evident in some autoreactive CD4 T cell clones (54) may not be highly relevant to the CD8 T cell clonal repertoire of psoriatic arthritis. The average of 32 oligoclonal expansions places a rough upper limit on the number of peptides recognized in the psoriatic arthritis CD8 immune response. This is a large number of distinct peptides and at face value suggests the recognition of multiple protein targets. However, part of this complexity could represent presentation of multiple peptides from the same protein by more than one MHC molecule involving a spread to subdominant and cryptic determinants (55) or even recognition by different clones of the same peptide presented by the same MHC molecule. Additional high-resolution repertoire analysis and biological cloning of these T cells currently under way should allow additional implications on similarities or differences of recognition motifs to be drawn, the basis of MHC restriction, and how the process changes with time.

References


