CD8+ T Cells Oligoclonally Expanded in Synovial Fluid at Onset of Spondyloarthropathy Selectively Proliferate in Response to Self-Antigens: Characterization of Cell Specificities in Nonclonal Populations

MIGUEL A. TREVIÑO, EMMA TEIXEIRO, and RAFAEL BRAGADO

ABSTRACT. ***Objective.*** To characterize putative T cells responsible for the pathogenesis of spondyloarthropathies (SpA).

Methods. T cells from synovial fluid (SF) and peripheral blood lymphocytes from a patient with chronic ankylosing spondylitis and a patient at the onset of SpA were analyzed for the size of the ß-chain complementarity-determining region 3 to evaluate the degree of clonality. To assess their putative role in triggering disease, immortalized local T cells were tested in lymphocyte proliferation assays against a restricted panel of cell lines.

Results. At disease onset, expansions were detected only in the SF CD8+ T cell subset. As well, SF CD8+ T cells sharing an expanded clonotype (TCR-BV17–J2S1) selectively proliferated when stimulated with autologous-presenting cells. The search for sequence similarities with the expanded clonotype revealed a high homology with the major clonotype in response to influenza A matrix peptide M58-66.

Conclusion. A CD8+ T cell-mediated antigen-driven mechanism seems to be responsible in the pathogenesis of SpA. Immune response to viral antigens (e.g., from influenza) could be the initiating event in seronegative arthropathies. The combination of spectratyping with RT-PCR and specific Southern blot for the expanded clonotypes on cells derived from mixed lymphocyte cultures was useful to evaluate the proliferative responses of *in vivo*-expanded cells and to assess T cells involved in the pathogenesis of SpA. (J Rheumatol 2004;31:1962–72)

Key Indexing Terms:
SPONDYLOARTHROPATHY T LYMPHOCYTES T CELL RECEPTOR AUTOIMMUNITY

Spondyloarthropathies (SpA) are a group of interrelated rheumatic diseases that include ankylosing spondylitis (AS), psoriatic arthritis, reactive arthritis (ReA), inflammatory bowel disease, and undifferentiated SpA. In addition to the interplay between genetic and environmental factors, several features suggest T cells present at the pathogenic site may play a direct role in the development of these disorders: the presence of proinflammatory cytokines in the affected joints[1-4], the high prevalence of the HLA-B27 antigen in patients with some of these syndromes[5,6], and the fact that roughly 50% of the cells infiltrated in the affected joints are memory T lymphocytes[7]. However, due to the failure in identifying local T cells potentially responsible for the disease and the local antigens recognized by them, the mechanisms involved in the development of these diseases are still unclear.

Many groups have based their investigations on the so-called arthritogenic peptide hypothesis. First described for HLA-B27-related SpA[8], it states that peptides derived from triggering bacteria or viruses are presented to peripheral CD8+ specific T cells, which start an immunological response. A cross-reaction mediated by self-antigen peptides from joint tissues provokes the development of the autoimmune disease[8]. Evidence for this hypothesis comes from reports of HLA-B27-restricted CD8+ T cells derived from the synovial fluid (SF) and peripheral blood of patients with ReA and AS and specific for bacteria or autoantigens[9-13]. Historically, these studies used *in vitro* stimulation procedures, which could selectively expand some T cells while underrating others; together with the low frequency of isolated clones, this precluded establishment of an unequivocal relationship between those cells and the disease.

The counterpart of the HLA/antigenic peptide complex recognition is the αß heterodimeric T cell receptor (TCR). A

From the Department of Immunology, Fundación Jiménez Díaz, Madrid, Spain.

Supported by grants from Fondo de Investigaciones Sanitarias and Ministerio de Ciencia y Tecnología (01/0272 and BMC 2002-02644, respectively, to Dr. Bragado); and from Fundación Conchita Rábago (Dr. Treviño) and Comunidad de Madrid (Dr. Teixeiro).

M.A. Treviño, PhD; E. Teixeiro, PhD; R. Bragado, PhD.

Address reprint requests to Dr. R. Bragado, Department of Immunology, Fundación Jiménez Díaz, Avda. Reyes Católicos 2, 28040 Madrid, Spain. E-mail:rbragado@fjd.es

Submitted November 13, 2003; revision accepted April 14, 2004.

Personal, non-commercial use only. The Journal of Rheumatology. Copyright © 2004. All rights reserved.

Downloaded on April 23, 2024 from www.jrheum.org

diverse TCR repertoire results from combinatorial rearrangement of V, D (ß-chains only), J and C gene segments, and addition or deletion of nucleotides at the resultant junctions to form the highly variable complementarity-determining region 3 (CDR3), which is thought to be involved in the fine specificity of antigen recognition. With regard to HLA-B27-related arthritis, lack of preferential usage of the TCR-αß V regions encoding genes[14], but occurrence of multiple T cell expansions[15], is described in peripheral blood lymphocytes (PBL) and SF T cells from patients with ReA. Analysis of HLA-B27-restricted cytolytic CD8+ T cell clones produced *in vitro* from the SF of 3 patients with ReA revealed limited use of BV families (BV13, 14, and 17) and BJ segments[11], as well as some structural constraints in the ß-chain CDR3 region without sequence homology[16]. In these scarce and controversial results, biases due to the type of sample, time of sample collection, lack of paired PBL and SF samples, and small sample numbers cannot be ruled out.

Spectratyping has been developed to determine the size of CDR3 regions in transcripts of whole BV families or in given BV-BJ combinations, to define overrepresented T cells *in vivo* in affected places. Due to the lack of requirement for *in vitro* stimulation, this approach is particularly suitable for direct analysis of T cell clonality in various clinical situations[17]. Thus, some oligoclonal expansions, defined by BV/BJ gene segments and CDR3 size, were reported to be common in the SF of HLA-B27 positive patients with ReA and AS. Further, in one patient with ReA, identical CDR3 sequences were observed in oligoclonal expansions that used different BV genes[18]. However, although a CD8+ T cell line from the SF of this patient recognized several HLA-B27-expressing Epstein-Barr virus (EBV)-transformed lymphoblastoid cell lines (B-LCL), the autologous B-LCL was not recognized by the presumably CD8+ effector cell line. Although it could simply be due to inadequate avidity, this result makes it difficult to assign a clear role of oligoclonally expanded cells in the pathogenesis of the disease. To overcome this potential problem in testing autoimmune reactivity in SpA, we designed an alternative strategy that combines spectratyping, mixed lymphocyte cultures, and semiquantitative polymerase chain reaction (PCR) to evaluate the proliferative response of *in vivo* expanded T cells.

MATERIALS AND METHODS

Patients and samples. Patient 1, a 56-year-old woman (HLA-A2; –B27, 44; –Cw2, 6; –DR1, 11), presented with peripheral arthritis and synovitis affecting both knees. She was diagnosed with AS according to the modified New York criteria[19]. She was treated with nonsteroidal antiinflammatory drugs (NSAID). There were no relapses during the following 3 years. In the 4th year she had a second inflammatory episode of the right knee, and the disease became chronic. At this time, with informed consent, SF from the right knee (Patient 1 SFR1) and peripheral blood (Patient 1 PBL1) samples were collected. Six months later, SF from both knees (Patient 1 SFR2 and Patient 1 SFL2) and peripheral blood (Patient 1 PBL2) specimens were obtained.

Patient 2 was a 22-year-old man (HLA-A2, 24; –B50, 51; –Cw2, 6; –DR7, 11) with symptoms of peripheral arthritis, dactylitis, and synovitis of the right knee characteristic of SpA[5,6]. SF (Patient 2 SF1) and peripheral blood (Patient 2 PBL1) samples were extracted during the episode. He was finally diagnosed as having undifferentiated SpA and 2 more peripheral blood samples (Patient 2 PBL2 and PBL3) were taken, after NSAID treatment, 6 and 10 months, respectively, after disease onset.

Mononuclear cells from SF and PB samples were obtained after Ficoll-Hypaque density gradient centrifugation. In samples from Patient 2, additional fractionations were performed with magnetic beads (Dynal) to isolate pure CD4+ and CD8+ T cell subpopulations. In both cases, purity was higher than 95% as ascertained by flow cytometry with monoclonal antibodies (mAb) anti-CD4 and anti-CD8.

Antibodies and cell lines. The following antibodies were used: mAb anti-CD4 (HP2/6), anti-CD8 (B9/4), and anti-CD25 (MAR108) were generous gifts of Drs. F. Sanchez-Madrid, Hospital de la Princesa, Madrid, and B. Malissen, Centre d'Immunologie, Marseille. mAb UCHT-1, anti-CD3e, and mAb MCA1509, anti-TCRVb17, were from Caltag (Burlingame, CA, USA) and Serotec (Oxford, UK), respectively.

Epstein-Barr virus (EBV)-lymphoblastoid B cell lines LG-2 (HLA-A2, –B27, –Cw1, –DR1) and LG15 (HLA-A32; –B7, 27; –DR1) were routinely grown in RPMI supplemented with 10% fetal calf serum (FCS) and antibiotics.

RNA extraction, cDNA synthesis, and PCR-spectratyping. Total RNA was routinely isolated from about 5×10^6 mononuclear cells as described[20]. cDNA was generated with AMV-Reverse Transcriptase (Promega, Madison, WI, USA) from 1–2 µg of total RNA, using oligo$(dT)_{15}$ as primer, as recommended by the manufacturer. Previous studies[18,20,21] and our preliminary experiments showed that this amount of total RNA was clearly sufficient to detect TCR-B-derived products after PCR with specific TCR-B-derived oligonucleotides, avoiding, as far as possible, artifactual expansions.

For each sample, 2 independent spectratyping experiments were performed according to a modification of the protocol defined by Gregersen, *et al*[22]. Briefly, TCR-BV genes were amplified by PCR using a set of 5'-specific primers for each TCR-BV subfamily (TCR-BVnE) together with a 3'-primer specific for a sequence shared by the constant loci TCR-BC1 and TCR-BC2 (TCR-BCE, 5'-TTTTGGGTGTGGGA-GATCTC-3')[20]. Aliquots (1/20) of cDNA were used for each 50 µl amplification reaction in 30 conventional PCR cycles (1 min at 95°C, 1 min at 49°C, 1 min at 72°C), followed by a final extension cycle of 7 min at 72°C. An aliquot 1/100 of each amplified material was reamplified by 25 cycles of PCR in the same conditions as above, but substituting the cold oligonucleotide TCR-BCE with a ^{32}P-labeled TCR-BCI oligonucleotide (5'-TTCT-GATGGCTCAAACAC-3')[20], located in the 5' position to the TCR-BCE sequence, which increased the specificity and sensitivity of PCR reactions. Amplifications were subjected to high resolution denaturing 5% polyacrylamide gel electrophoresis, and the ensuing bands were quantified in a Phospor-Imager (Molecular Dynamics, Sunnyvale, CA, USA). Band number is a measure of repertoire complexity. Band intensity can reflect both complexity (many different T cells with TCR of the same CDR3 length) and TCR quantity (expansion of one or more T cells with TCR of the same CDR3 length). An oligoclonal expansion was considered to occur when a band accounted for at least 50% of total radioactivity in each subfamily amplification[22].

The intensity of bands, corresponding to PCR amplifications of TCR-BV12, 15, 17, and 20 from PBL1, SFR1, and PBL2 samples from Patient 2, was comparatively quantified in a laser densitometer (Molecular Dynamics) using ImageQuant software. TCR-B real expansions, or TCR-B apparent expansions with identical CDR3 size, cannot be differentiated from this analysis. Ambiguities in this respect were resolved by sequencing.

PCR product cloning, sequencing, and colony counting. The amplified material, corresponding to those TCR-BV subfamilies showing T cell oligoclonal expansions exclusively in SF, was cloned into the PCR vector (Stratagene, La Jolla, CA, USA) following the manufacturer's recommendations. Colonies were picked and grown in nitrocellulose membranes.

Personal, non-commercial use only. The Journal of Rheumatology. Copyright © 2004. All rights reserved.

Downloaded on April 23, 2024 from www.jrheum.org

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2004. All rights reserved

After hybridization with the ^{32}P-labeled TCR-BCI oligonucleotide, to test which colonies had incorporated TCR-BV amplified material, inserts from 10 to 12 transformed colonies were sequenced for each TCR-BV expansion using T3 or T7 oligonucleotides as primers.

Upon sequencing, statistical analysis was performed for TCR-BV and J usages, CDR3 size, and for the use of different amino acids both in CDR3 as a whole and in different positions. In the absence of values reported for the repertoire in normal SF, individual frequencies were compared with those reported for control PB[23,24]. For statistical values we used the chi-square test with Yates' correction.

The sequence-based oligoclonality in the expansion of TCR-BV17 observed in CD8+ SF T cells was additionally confirmed using a clonotypic oligonucleotide, OET(N+D), with the sequence 5'-AGTAGTATAA-GATCGGGC-3', which includes part of the N+D region of the oligoclonally expanded transcript (OET). The specificity of this probe was tested against mismatched sequences in Southern blot experiments. The analysis allowed us to define optimal restrictive conditions for the hybridization (56°C), which discriminated DNA sequences that differed from the probe in as little as one nucleotide. Once the procedure was set up, 225 colonies transformed with the amplified DNA for TCR-BV17 from both Patient 2 PBL1 and Patient 2 SF1 samples were screened sequentially by direct Southern blot using the ^{32}P-labeled common TCR-BCI oligonucleotide and the specific OET(N+D) oligonucleotide as probes.

EBV transformation of B cells and herpesvirus saimiri (HVS) transformation of T cells. B cells were infected and immortalized by incubation with EBV-containing supernatants generated by culture of EBV-infected marmoset cells, as described[25]. B-LCL were grown in RPMI-1640 culture medium supplemented with 10% FCS and antibiotics.

T cells were infected and immortalized with HVS in the presence of phytohemagglutinin (1 µg/ml) and interleukin 2 (IL-2) (40 units/ml) as described[26,27]. They were maintained in culture in complete RPMI-1640/CG medium [45% RPMI-1640, 45% CG medium (Serotec), 10% FCS, Gln 200 µM] in the presence of 40 units/ml of IL-2. This procedure exclusively yields transformed CD8+ T cells[28], which only survive in culture in the presence of IL-2 or when they are stimulated with their specific antigens.

Cell surface expression of phenotypic markers (TCR/CD3, CD4, CD8, CD25, and TCR-Vß17) by HVS-transformed T cells was ascertained by flow cytometry analysis with specific mAb. The expression of the markers was compared by flow cytometry with that determined before the transforming procedure.

Proliferative response to self-antigens. HVS-transformed T cells (10^6 cells/ml) were cocultured and stimulated with irradiated (60 Gy) B-LCL (autologous EBV-Patient 2 PBL, LG2, or LG15, 5×10^5 cells/ml) in complete RPMI/CG medium without IL-2. As control, HVS-transformed T cells were cultured without B-LCL but in the presence of 40 units/ml IL-2 (basal conditions). After several days of culture, cell proliferation was confirmed by optical microscope visualizations. Then total RNA was extracted from cultured cells and subjected to specific reverse transcription (RT)-PCR for TCR-BV17 transcripts. Among these transcripts, the putative increase of OET(N+D)-positive transcripts was assumed to be an indirect measure of the proliferative response to specific antigens presented by the stimulatory B-LCL. To quantify that increase, PCR amplifications were subjected to 1% agarose electrophoresis, transferred to nitrocellulose membranes, and subjected to sequential Southern blot hybridization with ^{32}P-labeled TCR-BCI and OET(N+D) probes, as detailed above. Bands were then detected by autoradiography.

RESULTS

TCR-BV expansions in samples collected from a patient with chronic AS change along the course of the disease. SpA are considered to be the consequence of specific local reactions that after an acute episode tend to be chronic. We wished to characterize putative T cell expansions in inflammatory lesions that could be responsible for the pathogenesis of the disease; a major point was to evaluate whether the status of the disease is critical for deciding the time for sample collection. Thus we performed spectratyping analysis of samples collected at 2 different times from Patient 1, who had chronic AS. Analysis of the TCR-BV PCR corresponding to the first samples (denoted with 1) revealed the existence of several TCR-BV expansions in SF (Patient 1 SFR1) and, although in much lower quantities, also in blood (Patient 1 PBL1). Expansions in subfamilies TCR-BV1, 2, 6, 9, 11, 15, and 16 were selectively detected in SF but not in peripheral blood (Figure 1). Notably, comparison of the above spectratyping results with those corresponding to a second extraction of both SF and blood (denoted with 2) revealed that TCR-BV expansions changed throughout the course of the disease and even among joints with presumably similar inflammation processes [right (R) vs left (L) knee]. Thus, for example, the TCR-BV15 expansion in Patient 1 SFR1 was not detected in Patient 1 SFR2 and, instead, expansions in TCR-BV3, 4, 7, 10, and 14 that were not detected in Patient 1 SFR1 appeared in Patient 1 SFR2. The sample from the other joint, Patient 1 SFL2, shared some expansions with Patient 1 SFR2, but also showed different ones, such as BV9. Similarly to Patient 1 PBL1, expansions in Patient 1 PBL2 were very scarce. Similar patterns were obtained in the 2 independent experiments performed in each sample. Together, the variations observed concomitantly with the time of the sample collection, and even among different joints, hindered further analysis of the etiopathological significance of each expansion.

Absence of local oligoclonal expansions in the SF CD4+ T cell subpopulation from a patient experiencing a first acute inflammatory episode. Considering the difficulties in determining the significance of the persistence of some expansions, while others change with time and joint (in light of the results in chronic samples it was not possible to determine which expansions were present at disease onset, or whether persistent expansions were irrelevant to the onset of disease, or whether the original expansions shifted with time), we decided to focus on an individual (Patient 2) at the time he was experiencing the first inflammatory episode. In this regard, we considered that only those putative expansions that were exclusively detected in SF should be the focus of the study. Additionally, we decided to isolate CD4+ and CD8+ subpopulations because at present the influence of each subset in the pathogenesis of SpA is not well defined[21,29]. To this purpose, CD4+ and CD8+ T cells were separated by magnetic beads and purity was confirmed by flow cytometry (data not shown), and sufficient cells from each population were isolated to be able to assume the existence of a high number of transcripts to avoid artifactual oligoclonalities.

Spectratyping analysis of CD4+ T cells from peripheral blood (Patient 2 CD4+ PBL1) showed "apparent" oligo-

Personal, non-commercial use only. The Journal of Rheumatology. Copyright © 2004. All rights reserved.

Downloaded on April 23, 2024 from www.jrheum.org

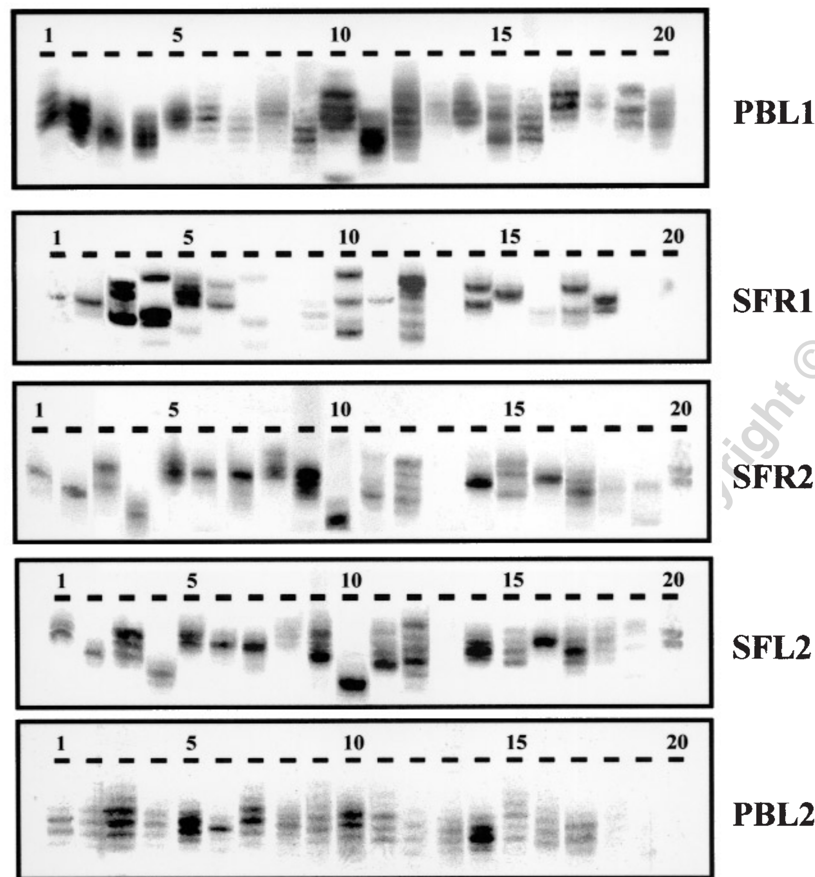

Figure 1. T cell repertoire analysis based on TCRB CDR3 length heterogeneity (spectratyping). cDNA from PBL and SF samples from Patient 1, with AS, were subjected to 2-step PCR using TCR-BV1 to BV20-subfamily-specific upstream primers, then processed in high resolution gels and by autoradiography. Samples denoted "1" were derived from blood (PBL1) and right knee synovial fluid (SFR1) collected at the first chronic inflammatory episode. SFR2, SFL2, and PBL2: samples collected 6 months later from right knee, left knee, and blood, respectively. Results are representative of the 2 spectratyping procedures performed independently in each sample.

clonal expansions in TCR-BV10 and V16 (Figure 2, left, top panel), which were not detected in the SF compartment. Indeed, no expansions were evident by densitometric analysis (data not shown) in this sample (Figure 2, left, SFR1, middle panel), suggesting that expansions detected in PBL were not related to the development of the local inflammation and that CD4+ T cells do not participate in the initial stages of the disease. As expected from the results illustrated in Figure 1, the repertoire of amplified transcripts from a sample of blood collected later, after disease onset (PBL2) and after NSAID treatment, differed from those observed in SFR1- and in PBL1-derived samples (Figure 2, left, bottom panel).

SF-specific oligoclonal expansions within the CD8+ T cell subset. Spectratyping analysis and densitometric quantitation of PCR bands in SF CD8+ T cells from Patient 2 (Patient 2 CD8+ SFR1) revealed expansions in TCR-BV12, 15, 17, and 20 subfamilies (Figure 2, right, middle panel, and data not shown). By the criteria for oligoclonality[22], the above expansions were exclusive of Patient 2 CD8+ SFR1 and they were not detected in the CD8+ subsets of blood samples collected at the same time (PBL1) or later (PBL2) than the SFR1. To define these expansions more accurately, we cloned the corresponding PCR-amplified materials. We then determined the TCR-BV transcript sequences in 10–12 transformed colonies per each expanded subfamily. The sequences indicated that the expansions detected in the TCR-BV12, 15, and 20 subfamilies were caused by the growth of more than one cellular clone, although most of the transcripts in each expansion had the same CDR3 size (Table 1). However, the expansion in TCR-BV17 corresponded to the local proliferation of one cellular clone (denoted oligoclonal expanded transcript of BV17, OETBV17), confirmed by the fact that 7 out of 11 analyzed DNA sequences, and consequently their amino acid sequences, were identical (Table 1). Interestingly, 3 more transcripts of this subfamily showed not only the same CDR3 size of the expanded transcript but also common structural motifs in their CDR3 regions (see below).

The validity of the results from sequencing was

Personal, non-commercial use only. The Journal of Rheumatology. Copyright © 2004. All rights reserved.

Downloaded on April 23, 2024 from www.jrheum.org

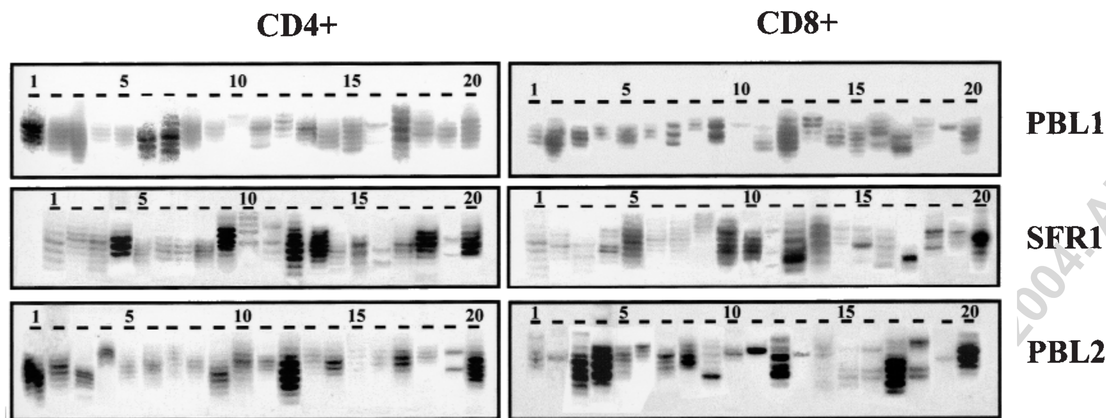

Figure 2. Spectratyping of CD4+ and CD8+ T cells from Patient 2. CD4+ and CD8+ subpopulations were isolated from samples collected at disease onset (PBL1 and SFR1) or 6 months later (PBL2). Spectratyping for each purified subset was then performed as described in the legend to Figure 1. Results are representative of 2 spectratyping procedures performed independently in each sample.

confirmed by comparative Southern blot using oligonucleotides that were specific to the N+D region of OETBV17 or to the TCR-BC. These results showed that 53.7% of the TCR-BV17+ colonies derived from SF were positive for the specific probe, while only 13% of the TCR-BV17+ from PBL-derived colonies were positive for the same probe (data not shown). Moreover, these results confirm that a remarkable band observed in the densitometric analysis profile of BV17 in PBL1 is not related to the oligoclonal expansion in SFR1.

Common structural motifs among the CDR3 regions of SF CD8+ T cell expanded transcripts. Although the oligoclonality in TCR-BV12, 15, and 20 expansions was not strictly genuine (Table 1), it was striking that such cell expansions, expressing TCR with the same CDR3ß size, were only found in the SF sample. We then evaluated whether each TCR-BV expansion was associated with particular diversity elements in the CDR3 region, and if so, whether the transcripts of other expansions, including those of TCR-BV17, shared some motifs. This analysis could reveal whether the corresponding TCR are recognizing related antigens.

A simple analysis of the sequences showed no obvious structural motifs shared by the expanded transcripts (Table 1). However, some values with statistical significance were identified after comparison with the frequencies reported for control PBL. Thus, the majority (7 out of 8, 87.5%) of TCR-BV12 transcripts with the predominant size (7 amino acids in the CDR3 region) used TCRBJ2S7, which is used by just 17.6% of TCR-BV12 transcripts in controls[23]. The frequencies of amino acids in the N+D region showed a high use of Ser (28.6%) and Ile or Leu (14.3%) compared with 10.5% and 3.5%, respectively, in controls[23]. A comparative study of the amino acids in each position in CDR3ß as defined by Chothia, *et al*[30] revealed a preferential usage of polar amino acids at position 3 (62.5%), Gly at 4 (75.0%), and Tyr at 5 (75.0%) compared with 22.3%, 26.8%, and 2.3%, respectively, in controls[24].

Similar comparative analysis of TCR-BV15 transcripts of the majority size indicated high frequency of Asp (20.8%) in the N+D region with respect to controls (6.0%). Usage in different positions in CDR3ß showed preferential use of acidic residues in position 1 (75% vs 9.8% in controls) and polar amino acids in position 2 (75% vs 27.3% in controls). Although there are some ambiguities in sequencing, TCR-BV20 transcripts of the predominant size (7 amino acids in CDR3 region) revealed a high frequency of Thr in the N+D region (28% vs 4.4% in controls).

All transcripts of the predominant size (7 amino acids in CDR3) in TCR-BV17 expansions used TCR-BJ2 segments containing the Glu-Gln motif (100% vs 36.2% in controls). This TCR-BJ2 element is also present in most (7 out of 8) of the TCB-V12 expanded transcripts. The frequencies of amino acids in the N+D region revealed a high use of Ser (23.7% vs 8.1% in controls), Ile (21.1% vs 5.8% in controls), and Arg (23.7% vs 8.3% in controls). Positions within CDR3 revealed a preferential usage of hydrophobic amino acids at position 1 (100%), basic at position 2 (90%), polar at position 3 (90%), Gly at 4 (70%), and Tyr at 5 (30%) compared with 34.1%, 12.5%, 22.3%, 26.8%, and 3.2%, respectively, in controls. Seven out of the 10 TCR-BV17 transcripts of predominant size have the same sequence (corresponding to the OETBV17), and its CDR3 region (...-CASSIRSGNEQ-...) included the majority of motifs found in the global analysis of TCR-BV17 expanded transcripts and shared some motifs with the CDR3 regions belonging to expanded transcripts of the other TCR-BV subfamilies (see below).

The simultaneous comparison of uses in SF expansions, including those corresponding to TCR-BV17, revealed that 58.1% of transcripts had the same size (7 amino acids in CDR3). This size is scarcely found in the TCR pool of control individuals (6%)[24]. The importance of this increased proportion is difficult to evaluate because predominant sizes of CDR3 have been more clearly related with the activity of TdT than with the response to a given antigen[24]. Other

Personal, non-commercial use only. The Journal of Rheumatology. Copyright © 2004. All rights reserved.

Downloaded on April 23, 2024 from www.jrheum.org

Table 1. TCR-ß CDR3 amino acid sequences of transcripts corresponding to oligoclonal expansions detected in CD8+ T cell subsets of SF from Patient 2 at the first inflammatory episode. Bold type indicates N+D regions. Sequences within boxes represent amino acids in positions 1 to 5 in CDR3ß as defined[30], in those sequences that contain the predominant CDR3 size within each subfamily. CDR3 size is defined by the 4th amino acid after the constant cysteine of the BV segment and constant XGF motif of the BJ segment. Experimental nucleotide sequences corresponding to amino acid sequences shown here are Genbank data base accession numbers AY145751 to AY145780.

TCRBV	CDR3β	TCRBJ	Nº of sequences	CDR3 size	%	% by size
TCRBV12	CASS**YDEN**EQFFG	JB2S1	1	6	8.3	8.3
	CASS**FGQG**YEQYFG	JB2S7	1	7	8.3	66.6
	CAS**GHRAGH**EQYFG	JB2S7	1	7	8.3	
	CA**ITGGP**SYEQYFG	JB2S7	1	7	8.3	
	CASS**SSSG**YEQYFG	JB2S7	2	7	16.7	
	CASS**SLSG**YEQYFG	JB2S7	2	7	16.7	
	CAS**QGLI**NQPQHFG	JB1S5	1	7	8.3	
	CASS**LLGGDL**YEQYFG	JB2S7	1	9	8.3	25.0
	CASS**YSNGVS**YGYTFG	JB1S2	2	9	16.7	
TCRBV15	CATS**DRAQ**QPQHFG	JB1S1	1	7	10.0	20.0
	CAT**GEG**SNQPQHFG	JB1S5	1	7	10.0	
	CATS**GGEGGHG**AFFG	JB1S1	2	8	20.0	80.0
	CAT**IDTTT**NQPQHFG	JB1S5	2	8	20.0	
	CATS**DSGP**NQPQHFG	JB1S5	2	8	20.0	
	CATS**DNRGDTED**FFG	JB1S1	2	8	20.0	
TCRBV17	CASS**TGG**TEAFFG	JB1S1	1	6	9.1	9.1
	CASS**VQGA**YEQYFG	JB2S7	1	7	9.1	90.9
	CASS**VRS**SYEQYFG	JB2S7	1	7	9.1	
	CASS**IRS**SYEQYFG	JB2S7	1	7	9.1	
	CASS**IRSG**NEQFFG	JB2S1	7	7	63.6	
TCRBV20	CAWS**PWGN**EAFFG	JB1S1	1	6	10.0	10.0
	CAWS**WQGFGVQ**HFG	JB1S5	1	7	10.0	50.0
	CAWS**VRGTDT**QYFG	JB2S7	1	7	10.0	
	CAWS**TRTGXX**LHFG	JB1S6	1	7	10.0	
	CAWS**KDXDTXX**FFG	JB1S1	1	7	10.0	
	CAWS**TGTG**SPLHFG	JB1S6	1	7	10.0	
	CAWS**DQTGD**YEQYFG	JB2S7	1	8	10.0	30.0
	CAWS**TGTG**SYEQYFG	JB2S7	1	8	10.0	
	CAWS**VGVG**SYEQYFG	JB2S7	1	8	10.0	
	CA**SSIRIRSXXQNEXX**FFG	JB1S4	1	10	10.0	10.0

significant common traits among the expanded transcripts were as follows: (1) TCR-BV12, 15, and 17 are members of the homology subgroup 4 defined by Chothia, *et al*[30]; (2) there was a biased usage of TCR-BJ2S1 and TCR-BJ2S7 (58.1% vs 36.2% in controls); and (3) a significant proportion of transcripts used basic amino acids in position 2 (38.7% vs 12.5% in controls), polar in position 3 (60.0% vs 23.3%), Gly in position 4 (61.3% vs 26.8%), and Tyr in position 5 (30.0% vs 3.2%). All these motifs, except Tyr in position 5, are present in OETBV17, the oligoclonally expanded transcript in Patient 2 SF CD8+ T cells.

OETBV17+ T cells specifically proliferate in response to

Personal, non-commercial use only. The Journal of Rheumatology. Copyright © 2004. All rights reserved.

Downloaded on April 23, 2024 from www.jrheum.org

autologous antigen-presenting cells. Identification of clonally expanded OETBV17+ cells in the SF of Patient 2 at disease onset prompted us to investigate the ability of the cells bearing this ß-clonotype to respond to self-antigens. Conventional analysis of proliferative or cytotoxic responses does not allow characterization of responder cells unless the cellular line is clonal. In the absence of a known antigen, and as all our attempts to obtain clones of the expanded cells failed, we designed an alternative approach. The rationale was that, if relevant to the autoimmune response, the proportion of the expanded transcript in a culture should increase with respect to the basal conditions when an appropriate antigen is presented by autologous stimulatory cells. With this premise, we followed this procedure: First, we immortalized SF CD8+ cells with herpesvirus saimiri (HVS). Transformed cells grew, although very slowly, in IL-2 cultures with no need for antigenic stimulation and preserved their response capacity via TCR triggering[31,32]. Indeed, flow cytometry analysis after infection (data not shown) revealed that all were immortalized (CD3+CD8+CD25+), and that the proportion of TCR-Vß17+ cells (about 1%) was similar to that observed prior to the infection and consistent with that measured in control T cells[33]. Second, we evaluated the proliferative response of transformed SF CD8+ cells after culture in the presence of different targets and compared the values with those obtained after incubation with IL-2 (basal conditions). In addition to EBV-transformed autologous PBL, Patient 2 EBV, we selected 2 additional B-LCL: LG-2 (HLA-A2-positive) and LG-15 (HLA-A2-negative, but sharing the same HLA-B27 allele with LG-2), according to the following considerations: (1) Patient 2 was HLA-A2+; (2) the high sequence similarity between the expanded transcript described here and that previously found in CD8+ cytotoxic clones restricted by HLA-A2 (see Discussion); and (3) as both LG-2 and LG-15 are DR1+, the putative minority class II–driven responses sometimes observed in CD8+ T cells would be similarly observed in the response to those cells. Therefore, after stimulation, total RNA was extracted from each cell culture and subjected to RT-PCR specific for the amplification of TCR-BV17 gene products. Southern blots were then serially hybridized with the common TCR-BCI (CßI) and the selective OETBV17 ^{32}P-labeled probes, and radioactive signals were compared.

As shown in Figure 3, stimulation of transformed T cells with autologous B cells resulted in a strong signal after hybridization with the OET(N+D)+ probe (middle panel), revealing a proliferation of OETBV17+ cells. However, upon stimulation with the other presenting cells (LG-2 and LG-15), a comparative signal was not observed. Indeed, in these cases, the OET(N+D)+ transcripts were negligible and they were only detected after very long exposures (Figure 3, bottom panel), similar to what was observed after basal culture conditions. These results were repetitive even when using both autologous B cells collected at different times and immortalized T cells derived from independent HVS infections.

DISCUSSION

We investigated expanded T cell clones in the inflammatory lesions associated with spondyloarthropathies with the aim of establishing a relationship between the role of these cells and the pathogenesis of the disease. We observed (1) The status of the disease is critical in terms of sample selection. In the chronic stage, expansions change concomitantly with the time of sample collection, and even among different joints. (2) At disease onset, we observed (a) absence of intraarticular oligoclonal expansions in SF CD4+ cells but presence of different TCR-BV expansions in SF CD8+ cells; (b) expansions in the SFCD8+ T cell subset share common structural motifs; and (c) CD8+ T cells sharing a TCR-B expanded clonotype selectively proliferate when they are stimulated with autologous presenting cells.

In vivo, T cell responses to cognate antigens are mediated by clonal proliferation. Therefore, clonally expanded T cells within a pathologic situation, as in SpA, may indicate the existence of pathogenic relevant antigens and may then be used as tools to characterize crucial epitopes. It is therefore of substantial interest to identify selectively expanded T cell clones at the onset of inflammatory diseases, particularly when the triggering antigens are unknown. Historically, however, the role of T cells in human SpA has been based on analysis of T cell clones generated after *in vitro* stimulation with antigens putatively involved in the disease pathogenesis. Thus, focused on the role of HLA-B27, it has been shown that among SF and peripheral blood-derived T cells from patients with enterobacteria-induced ReA, several bacteria-specific[10,13] or autoreactive[10,11] CD8+ T cell clones use HLA-B27 as restriction molecule. Most of these clones had rearranged a subset of TCR-BV segments (VB13, 14, and 17) and cloning of both bacteria-specific and autoreactive T cells suggested that such cells had undergone clonal expansion *in vivo* and had been activated by a limited number of antigens.

However, these and other studies in human SpA could be limited by several conditions: (1) samples were usually collected from chronic patients; (2) compared with the total number of cells at sites of inflammation or within a whole organism, only a very small selection of T cell clones could be isolated and characterized *in vitro*; (3) statistical biases due to small sample numbers and biases during the culture procedures cannot be completely ruled out. Additionally, even in those cases in which local activated T cells are polyclonally activated *in vitro* with allogeneic cells and then tested against autologous cells, the putative autoreactivity should be evaluated cautiously because autoreactive T cells have been described in healthy individuals[34-36].

To avoid these problems, we performed TCR spec-

Personal, non-commercial use only. The Journal of Rheumatology. Copyright © 2004. All rights reserved.

Downloaded on April 23, 2024 from www.jrheum.org

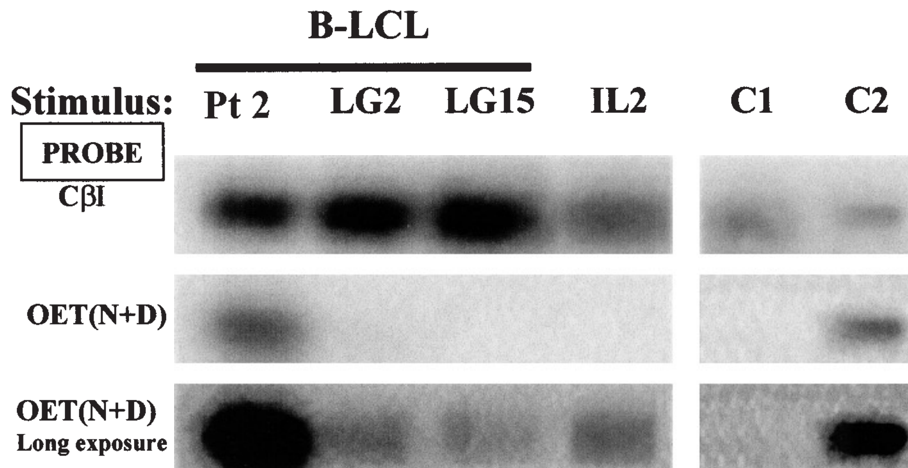

Figure 3. Proliferative response of OETBV17+ T cells to autologous antigen-presenting cells. Herpesvirus saimiri-transformed SF CD8+ T cells from Patient 2 were stimulated with an EBV-transformed autologous B cell line, with LG-2 and LG-15 B-LCL or just with IL-2. Proliferative response to stimulation was estimated by evaluating the hybridization signal obtained with the ^{32}P-OET(N+D) probe on PCR products derived from amplification of TCR-BV17 transcripts in each culture. Only stimulation with autologous antigen-presenting cells results in a significant hybridization band with the specific probe for OETBV17+ cells (middle panel). A very long exposure (bottom panel) illustrates that the putative proliferations induced by LG-2 and LG-15 were not greater than the one observed in basal culture (IL-2). Reprobing of membrane with the common ^{32}P-TCR-CßI oligonucleotide (top panel) is shown for comparison of total TCR-BV17 amplifications. Lines C1 and C2: PCR products amplified from 2 different control colonies transformed with TCR-BV17-containing plasmids that were negative (C1) or positive (C2) for hybridization with OET(N+D) probe. This experiment is representative of 3 more assays performed with Patient 2 autologous cells collected at different times and with cells derived from independent herpesvirus saimiri transforma-

tratyping on samples collected at disease onset. Thus, without any cloning procedure, it is possible to reliably detect and characterize preactivated and oligoclonally expanded cells *in vivo*. Although the method used to define expansions[22] does not consider the likelihood that there may be more than one expansion within a TCR-BV subset, this approach (together with the possibility of growing local T cells in culture, maintaining their original phenotype and specificity after infection with HVS[31]) provides a powerful tool to test these cells directly for autoreactivity in order to characterize cells involved in triggering the autoimmune disease. Further, the study of an HLA-B27-negative patient, a feature not selected in advance but representative of a high percentage of patients with SpA (5–90% depending on the disease), could provide additional information on general features of these diseases and not only those related to the HLA-B27.

Although it is clear that the spectratypes from the different joints and time points in samples from a patient with chronic disease show some similarities (Figure 1), suggesting the persistence of several clonal expansions, the status of the disease *per se* makes it impossible to determine which of these putative expansions were present at disease onset. Further, the significant variations in T cell expansions depending on time and joint (Figure 1) would suggest that antigenic stimulatory complexes are generated during the disease course that are probably different from those initially recognized at disease onset. These results suggest the observed differences are most probably caused by an ongoing inflammatory process due to continuous stimulation of T cells bearing different TCR-BV subfamilies, and that antigen spreading, by revealing new autoantigens, is a primary mechanism in perpetuation of the disease. Such a mechanism has been postulated by the epitope spreading theory[37-39] to explain the maintenance of autoimmune reactions. More important, the data stress the necessity of working with local samples collected at disease onset when studies to identify relevant cells in initiating the disease are performed. Such requirements dramatically reduce the availability of relevant samples but assure a higher potential regarding the quality of the data.

TCR expansions have been described in PBL of controls, and variation with time and age seems to be common even in healthy individuals[40]. Although we are aware that common expansions in both blood and SF could be relevant to the disease, we focused our study exclusively on expansions detected in local samples in the first inflammatory episode that, *a priori*, must be a direct consequence of the immunological response taking place only in the affected joints. In this way, assignment of expansions to the pathogenic process, although probably incomplete, is more unequivocal.

The first observation from spectratyping of samples collected from Patient 2 was the abundance of oligoclonal T cell expansions in synovial CD8+ T cells and the absence of

Personal, non-commercial use only. The Journal of Rheumatology. Copyright © 2004. All rights reserved.

Downloaded on April 23, 2024 from www.jrheum.org

such expansions in SF CD4+ T cells (Figure 2). Although the relative importance of the CD4+ and CD8+ T cells has yet to be determined, our data would support that CD8+ T cells play an important role in the pathogenesis of SpA, and rule out a similar role for the CD4+ subset. A major role for CD8+ T cells has been previously suggested on the basis of the high percentage of these cells infiltrated in the affected joints[7,41]; the high frequency of expansions in the synovial CD8+ compartment, where the cells appeared to express both activation and memory cell surface markers[15]; and by the finding that the classical genetic marker for SpA, HLA-B27[5,6,42], encodes a class I restriction molecule presenting antigenic peptides to CD8+ T cells. Since both class II-restricted CD4+ clones[43-45] and class I-restricted CD8+ clones[10,11] with specificity for arthritogenic bacteria have been characterized from the SF of patients with chronic disease, our data tend to confirm that the priming of CD8+ cytotoxic T lymphocyte (CTL) responses results in target-cell destruction, which then leads to cytokine release and attraction of CD4+ inflammatory T cells that finally contribute to the induction, maintenance, and extension of synovitis rather than initiating the disease.

In the same line of argument, the existence of oligoclonal expansions in SF and PBL of patients with both chronic and acute disease seems to be derived from activation of conventional antigenic complexes because superantigen (SAg) activation would induce all cells with the same TCR-BV to proliferate and it would not be reflected as an increase of specific TCR-BV in the repertoire, as shown here. That expansions were found only in the CD8+ subpopulation at the disease onset also apparently rules out the possibility of activation by SAg, which are incapable of discriminating between CD8+ and CD4+ subpopulations.

A second finding of this study concerns the usage of diversity elements in the sequenced transcripts derived from those expansions showing a transcript with a predominant size. The biased usage of TCR-BV segments from homology subgroup 4 (TCR-BV12, 15, 17) and the high proportion of transcripts using TCR-BJ2 segments (mainly JB2S7) are remarkable. Even more significant is the conservation of amino acids in the CDR3ß region (basic residue in position 2, polar in 3, Gly in 4, and Tyr in position 5; Table 1). Considered together, the similar size, the common structural motifs, and the presumably similar orientation of their CDR3ß over antigenic complexes[46-48] would suggest that those T cells could be participating in a restricted response by recognizing highly homologous antigenic complexes or peptides with similar hydropathic profiles[16].

A third, unexpected result is the sequence of the clonally expanded transcript (OETBV17) within the SF of Patient 2 and its sharing of diversity elements with other non-oligoclonally expanded transcripts within the same subfamily (Table 1). Using these sequences as search terms, database searching revealed that the OETBV17 sequence, and 2 more among the other 4 remaining sequences, matched sequences reported within the highly restricted TCR-ß repertoire to influenza A matrix peptide M58-66 — GILGFVFTL — in the context of HLA-A2. Although highly polyclonal regarding CDR3 sequences, the repertoire of memory CD8+ T cells specific for this antigenic system is constrained by the use of BV17, the I/V-RS motif in the CDR3 region, and the use of JB2[49-51]. To our knowledge, these characteristics have not been reported for any other antigenic system, including clonal T cell expansions associated to SpA or rheumatoid arthritis. Of note, a junctional region with partial homology to the one reported here, derived from SF CD8+ T cells from an HLA-B27+ patient with ReA, appears in the GenBank (unpublished, accession number AJ296352).

Notably, in an extensive work performed in individuals with different rheumatic diseases and HLA-B27+ controls, May, *et al* recently described a canonical TCRB motif, mainly prevalent in early acute ReA and associated to the TCR-BV1–J2S3 rearrangement, that could be a diagnostic marker in HLA-B27-associated "undifferentiated arthritis"[52]. As the study analyzed just the frequency of the above rearrangement (comparative analysis with other collections of TCR was not performed), these results do not exclude the participation of other CD8+ T cells in the development of SpA, and therefore our data are compatible with them. Due to the presence of such a motif not only in T cells derived from HLA-B27-positive patients with ReA associated with different bacteria but also in "*in vitro*"-derived HLA-B27-alloreactive CTL clones, our results could be viewed as complementary to these new findings. Indeed, besides supporting the role of CD8+ T cells, our findings tentatively support the interpretation of the arthritogenic peptide model[8] for HLA class I antigens other than HLA-B27 and provide a view that could explain why arthritis occurs in HLA-B27-negative individuals.

It is tempting to speculate about a relationship between an influenza infection and the triggering of the disease. In this regard, the frequent presence of virus-specific CD8+ T cells, mainly reactive to EBV or cytomegalovirus, within inflamed lesions of patients with autoimmune inflammatory disease, has been reported. This phenomenon has been associated with chronic inflammation rather than with the initiating cause of the process[53]. In a computer search for putative arthritogenic peptides sharing high homology with the M58-66 matrix peptide (GILGFVFTL), we found that the peptide GVIGFLFAI from syndecan-2, a proteoglycan expressed on fibroblasts, endothelial cells[54], and activated macrophages[55], displayed a noteworthy similarity. Notably, the central residues, those being recognized by the TCR, are identical. An interesting alternative is the peptide SLLGFVYKL of IFI-56K (IFIT1), a protein inducible by interferons and viral infections[56].

Finally, a question remains concerning the fine specificity and the differential proliferative capacity of cells

Personal, non-commercial use only. The Journal of Rheumatology. Copyright © 2004. All rights reserved.

Downloaded on April 23, 2024 from www.jrheum.org

bearing the expanded clonotype. The approach we described here solves the inherent limitations of the use of standard techniques (e.g., tritium uptake) to evaluate specific T cell proliferation when the stimulatory antigen is unknown and the responder cell line is not clonal. Although it is possible that the responses to LG-2 and LG-15 could predominantly select some clones and, consequently, dilute the putative response mediated by OETBV17 clonotypic cells, the comparison of band intensities between autologous and the alloreactive responses (Figure 3) is very conclusive. The response to autologous B cells and the lack of this response to the LG-2, both sharing the HLA-A2 antigen, would suggest that the response is specific for an antigenic complex selectively expressed by autologous cells. In addition, it is conceivable that processes such as EBV transformation alter the origins and the amounts of self-peptides bound to the cell-surface class I molecules. These or similar cryptic self-determinants could be upregulated and expressed more densely at the articular compartment, thus triggering the proliferation of local cells and, consequently, the arthritis. From our data, we cannot rule out that our observations could be derived from an epiphenomenon and that OETBV17 cells could be virus-specific HLA-A2-restricted and not strictly autoreactive. It seems unlikely due to the lymphotrophic features of influenza virus and because the stimulation with different autologous cells, coming from different collection times, rendered the same results. These issues, the extent to which our observations apply to patients with different SpA, and whether the mechanism we propose is a general mechanism or just operates under certain restrictions (i.e., HLA typing, susceptibility genes, etc.), will require further investigation.

Our data show that particular CD8+ intraarticular T cells collected at the onset of a spondyloarthropathy share structural motifs in their T cell receptors. We have designed an innovative approach that allows collection of information on cell specificity without prior cloning. Using this method, we show that cells expressing a TCR-BV expanded clonotype proliferate specifically in response to autologous presenting cells, suggesting they might be responsible, in part, for the autoimmune process. These results suggest that, in addition to the well known role of bacterial infections, common viral infections (i.e., influenza) could be triggering agents of spondyloarthropathies.

ACKNOWLEDGMENT

We thank Dr. A. Bañares (Servicio de Reumatología, Hospital Clínico Universitario San Carlos, Madrid, Spain) for providing samples, Drs. F. Sánchez-Madrid and B. Malissen for providing reagents, and Dr. J.A. López de Castro (Centro de Biología Molecular CBM-CSIC, Madrid) for helpful discussion.

REFERENCES

1. Simon AK, Seipelt E, Sieper J. Divergent T-cell cytokine patterns in inflammatory arthritis. Proc Natl Acad Sci USA 1994;91:8562-6.
2. Grom AA, Murray KJ, Luyrink L, et al. Patterns of expression of tumor necrosis factor alpha, tumor necrosis factor beta, and their receptors in synovia of patients with juvenile rheumatoid arthritis and juvenile spondylarthropathy. Arthritis Rheum 1996;39:1703-10.
3. Wong WM, Howell WM, Coy SD, Cawley MI, Smith JL. Interleukin-2 is found in the synovium of psoriatic arthritis and spondyloarthritis, not in rheumatoid arthritis. Scand J Rheumatol 1996;25:239-45.
4. Murray KJ, Grom AA, Thompson SD, Lieuwen D, Passo MH, Glass DN. Contrasting cytokine profiles in the synovium of different forms of juvenile rheumatoid arthritis and juvenile spondyloarthropathy: prominence of interleukin 4 in restricted disease. J Rheumatol 1998;25:1388-98.
5. Dougados M, van der Linden S, Juhlin R, et al. The European Spondylarthropathy Study Group preliminary criteria for the classification of spondylarthropathy. Arthritis Rheum 1991;34:1218-27.
6. Amor B, Dougados M, Mijiyawa M. Criteria of the classification of spondylarthropathies [French]. Rev Rhum Mal Osteoartic 1990;57:85-9.
7. Braun J, Bollow M, Neure L, et al. Use of immunohistologic and in situ hybridization techniques in the examination of sacroiliac joint biopsy specimens from patients with ankylosing spondylitis. Arthritis Rheum 1995;38:499-505.
8. Benjamin R, Parham P. Guilt by association: HLA-B27 and ankylosing spondylitis. Immunol Today 1990;11:137-42.
9. Hermann E, Mayet WJ, Meyer zum Buschenfelde KH, Fleischer B. MHC-unrestricted recognition of bacteria-infected target cells by human CD8+ cytotoxic T lymphocytes. Cell Immunol 1992;143:253-60.
10. Hermann E, Yu DT, Meyer zum Buschenfelde KH, Fleischer B. HLA-B27-restricted CD8 T cells derived from synovial fluids of patients with reactive arthritis and ankylosing spondylitis. Lancet 1993;342:646-50.
11. Duchmann R, May E, Ackermann B, Goergen B, Meyer zum Buschenfelde KH, Marker-Hermann E. HLA-B27-restricted cytotoxic T lymphocyte responses to arthritogenic enterobacteria or self-antigens are dominated by closely related TCRBV gene segments. A study in patients with reactive arthritis. Scand J Immunol 1996;43:101-8.
12. Huang F, Hermann E, Wang J, et al. A patient-derived cytotoxic T-lymphocyte clone and two peptide-dependent monoclonal antibodies recognize HLA-B27-peptide complexes with low stringency for peptide sequences. Infect Immunol 1996;64:120-7.
13. Ugrinovic S, Mertz A, Wu P, Braun J, Sieper JJ. A single nonamer from the Yersinia 60-kDa heat shock protein is the target of HLA-B27-restricted CTL response in Yersinia-induced reactive arthritis. J Immunol 1997;159:5715-23.
14. Verjans GM, Klaren VN, Leirisalo-Repo M, et al. Heterogeneity of rearranged T cell receptor V alpha and V beta gene transcripts in synovial fluid T cells of HLA-B27 positive reactive arthritis patients. Clin Rheumatol 1996;15 Suppl 1:91-6.
15. Allen RL, Gillespie GM, Hall F, et al. Multiple T cell expansions are found in the blood and synovial fluid of patients with reactive arthritis. J Rheumatol 1997;24:1750-7.
16. May E, Duchmann R, Ackermann B, Meyer zum Buschenfelde KH, Marker-Hermann E. TCRB junctional regions from HLA-B27-restricted T cells and HLA-B27 binding peptides display conserved hydropathy profiles in the absence of primary sequence homology. Int Immunol 1996;8:1815-23.
17. Even J, Lim A, Puisieux I, et al. T-cell repertoires in healthy and diseased human tissues analysed by T-cell receptor ß-chain CDR3 size determination: evidence for oligoclonal expansions in tumours and inflammatory diseases. Res Immunol 1995;146:65-80.
18. Dulphy N, Peyrat MA, Tieng V, et al. Common intra-articular T cell expansions in patients with reactive arthritis: identical ß-chain junctional sequences and cytotoxicity toward HLA-B27. J Immunol

Personal, non-commercial use only. The Journal of Rheumatology. Copyright © 2004. All rights reserved.

Downloaded on April 23, 2024 from www.jrheum.org

1999;162:3830-9.
19. van der Linden S, Valkenburg HA, Cats A. Evaluation of diagnostic criteria for ankylosing spondylitis. A proposal for modification of the New York criteria. Arthritis Rheum 1984;27:361-8.
20. Bragado R, Lauzurica P, Lopez D, Lopez de Castro JA. T cell receptor V beta gene usage in a human alloreactive response. Shared structural features among HLA-B27-specific T cell clones. J Exp Med 1990;171:1189-204.
21. Duchmann R, Lambert C, May E, Höhler T, Märker-Hermann E. CD4+ and CD8+ clonal T cell expansions indicate a role of antigens in ankylosing spondylitis. Clin Exp Immunol 2001;123:315-22.
22. Gregersen PK, Hingorani R, Monteiro J. Oligoclonality in the CD8+ T-cell population. Analysis using a multiplex PCR assay for CDR3 length. Ann NY Acad Sci 1995;756:19-27.
23. Quiros RE, Sottini A, Bettinardi A, Albertini A, Imberti L, Primi D. Different TCRBV genes generate biased patterns of V-D-J diversity in human T cells. Immunogenetics 1995;41:91-100.
24. Moss PA, Bell JI. Sequence analysis of the human αß T-cell receptor CDR3 region. Immunogenetics 1995;42:10-8.
25. Miller G, Lisco H, Kohn HI, Stitt D, Enders JF. Establishment of cell lines from normal adult human blood leukocytes by exposure to Epstein-Barr virus and neutralization by human sera with Epstein-Barr virus antibody. Proc Soc Exp Biol Med 1971;137:1459-65.
26. Biesinger B, Muller-Fleckenstein I, Simmer B, et al. Stable growth transformation of human T lymphocytes by herpesvirus saimiri. Proc Natl Acad Sci USA 1992;89:3116-9.
27. Weber F, Meinl E, Drexler K, et al. Transformation of human T-cell clones by Herpesvirus saimiri: intact antigen recognition by autonomously growing myelin basic protein-specific T cells. Proc Natl Acad Sci USA 1993;90:11049-53.
28. Rivero-Carmena M, Porras O, Pelaez B, Pacheco-Castro A, Gatti RA, Regueiro JR. Membrane and transmembrane signaling in Herpesvirus saimiri-transformed human CD4(+) and CD8(+) T lymphocytes is ATM-independent. Int Immunol 2000;12:927-35.
29. David CS. The mystery of HLA-B27 and disease. Immunogenetics 1997;46:73-7.
30. Chothia C, Boswell DR, Lesk AM. The outline structure of the T-cell alpha beta receptor. EMBO J 1988;7:3745-55.
31. Mittrucker HW, Muller-Fleckenstein I, Fleckenstein B, Fleischer B. Herpes virus saimiri-transformed human T lymphocytes: normal functional phenotype and preserved T cell receptor signalling. Int Immunol 1993;5:985-90.
32. Berend KR, Jung JU, Boyle TJ, et al. Phenotypic and functional consequences of herpesvirus saimiri infection of human CD8+ cytotoxic T lymphocytes. J Virol 1993;67:6317-21.
33. Rosenberg WM, Moss PA, Bell JI. Variation in human T cell receptor V ß and J ß repertoire: analysis using anchor polymerase chain reaction. Eur J Immunol 1992;22:541-9.
34. Ota K, Matsui M, Milford EL, Mackin GA, Weiner HL, Hafler DA. T-cell recognition of an immunodominant myelin basic protein epitope in multiple sclerosis. Nature 1990;346:183-7.
35. Pette M, Fujita K, Kitze B, et al. Myelin basic protein-specific T lymphocyte lines from MS patients and healthy individuals. Neurology 1990;40:1770-6.
36. Wekerle H, Bradl M, Linington C, Kaab G, Kojima K. The shaping of the brain-specific T lymphocyte repertoire in the thymus. Immunol Rev 1996;149:231-43.
37. Vanderlugt CJ, Miller SD. Epitope spreading. Curr Opin Immunol 1996;8:831-6.
38. Craft J, Fatenejad S. Self antigens and epitope spreading in systemic autoimmunity. Arthritis Rheum 1997;40:1374-82.
39. Mamula MJ. Epitope spreading: the role of self peptides and autoantigen processing by B lymphocytes. Immunol Rev 1998;164:231-9.
40. Batliwalla F, Monteiro J, Serrano D, Gregersen PK. Oligoclonality of CD8+ T cells in health and disease: aging, infection, or immune regulation? Hum Immunol 1996;48:68-76.
41. Kidd BL, Moore K, Walters MT, Smith JL, Cawley MI. Immunohistological features of synovitis in ankylosing spondylitis: a comparison with rheumatoid arthritis. Ann Rheum Dis 1989;48:92-8.
42. Braun J, Sieper J. The sacroiliac joint in the spondyloarthropathies. Curr Opin Rheumatol 1996;8:275-87.
43. Hermann E, Lohse AW, van der Zee R, et al. Synovial fluid-derived Yersinia-reactive T cells responding to human 65-kDa heat-shock protein and heat-stressed antigen-presenting cells. Eur J Immunol 1991;21:2139-43.
44. Hermann E, Fleischer B, Mayet WJ, Poralla T, Meyer zum Buschenfelde KH. Response of synovial fluid T cell clones to Yersinia enterocolitica antigens in patients with reactive Yersinia arthritis. Clin Exp Immunol 1989;75:365-70.
45. Probst P, Hermann E, Meyer zum Buschenfelde KH, Fleischer B. Identification of the Yersinia enterocolitica urease ß subunit as a target antigen for human synovial T lymphocytes in reactive arthritis. Infect Immun 1993;61:4507-9.
46. Garcia KC, Degano M, Stanfield RL, et al. An αß T cell receptor structure at 2.5 Å and its orientation in the TCR-MHC complex. Science 1996;274:209-19.
47. Garboczi DN, Ghosh P, Utz U, Fan QR, Biddison WE, Wiley DC. Structure of the complex between human T-cell receptor, viral peptide and HLA-A2. Nature 1996;384:134-41.
48. Speir JA, Garcia KC, Brunmark A, et al. Structural basis of 2C TCR allorecognition of H-2Ld peptide complexes. Immunity 1998;8:553-62.
49. Naumov YN, Hogan KT, Naumova EN, Pagel JT, Gorski J. A class I MHC-restricted recall response to a viral peptide is highly polyclonal despite stringent CDR3 selection: implications for establishing memory T cell repertoires in "real-world" conditions. J Immunol 1998;160:2842-52.
50. Moss PA, Moots RJ, Rosenberg WM, et al. Extensive conservation of α and ß chains of the human T-cell antigen receptor recognizing HLA-A2 and influenza A matrix peptide. Proc Natl Acad Sci USA 1991;88:8987-90.
51. Lehner PJ, Wang EC, Moss PA, et al. Human HLA-A0201-restricted cytotoxic T lymphocyte recognition of influenza A is dominated by T cells bearing the V ß 17 gene segment. J Exp Med 1995;181:79-91.
52. May E, Dulphy N, Frauendorf E, et al. Conserved TCR ß chain usage in reactive arthritis; evidence for selection by a putative HLA-B27-associated autoantigen. Tissue Antigens 2002;60:299-308.
53. Scotet E, Peyrat MA, Saulquin X, et al. Frequent enrichment for CD8 T cells reactive against common herpes viruses in chronic inflammatory lesions: towards a reassessment of the physiopathological significance of T cell clonal expansions found in autoimmune inflammatory processes. Eur J Immunol 1999;29:973-85.
54. Kim CW, Goldberger OA, Gallo RL, Bernfield M. Members of the syndecan family of heparan sulfate proteoglycans are expressed in distinct cell-, tissue-, and development-specific patterns. Mol Biol Cell 1994;5:797-805.
55. Clasper S, Vekemans S, Fiore M, et al. Inducible expression of the cell surface heparan sulfate proteoglycan syndecan-2 (fibroglycan) on human activated macrophages can regulate fibroblast growth factor action. J Biol Chem 1999;274:24113-23.
56. Wathelet MG, Clauss IM, Content J, Huez GA. Regulation of two interferon-inducible human genes by interferon, poly(rI).poly(rC) and viruses. Eur J Biochem 1988;174:323-9.

Personal, non-commercial use only. The Journal of Rheumatology. Copyright © 2004. All rights reserved.

Downloaded on April 23, 2024 from www.jrheum.org