

Common Intra-Articular T Cell Expansions in Patients with Reactive Arthritis: Identical β -Chain Junctional Sequences and Cytotoxicity Toward HLA-B27

Nicolas Dulphy, Marie-Alix Peyrat, Vannary Tieng, Corinne Douay, Claire Rabian, Ryad Tamouza, Saddek Laoussadi, Francis Berenbaum, Agnes Chabot, Marc Bonneville, Dominique Charron and Antoine Toubert

J. Immunol. 1999;162;3830-3839

<http://www.jimmunol.org/cgi/content/full/162/7/3830>

References

This article **cites 50 articles**, 18 of which can be accessed free at: <http://www.jimmunol.org/cgi/content/full/162/7/3830#BIBL>

8 online articles that cite this article can be accessed at: <http://www.jimmunol.org/cgi/content/full/162/7/3830#otherarticles>

Subscriptions

Information about subscribing to *The Journal of Immunology* is online at <http://www.jimmunol.org/subscriptions/>

Permissions

Submit copyright permission requests at <http://www.aai.org/ji/copyright.html>

Email Alerts

Receive free email alerts when new articles cite this article. Sign up at <http://www.jimmunol.org/subscriptions/etoc.shtml>

Common Intra-Articular T Cell Expansions in Patients with Reactive Arthritis: Identical β -Chain Junctional Sequences and Cytotoxicity Toward HLA-B27¹

Nicolas Dulphy,* Marie-Alix Peyrat,[†] Vannary Tieng,* Corinne Douay,* Claire Rabian,* Ryad Tamouza,* Saddek Laoussadi,[‡] Francis Berenbaum,[§] Agnes Chabot,[¶] Marc Bonneville,[†] Dominique Charron,* and Antoine Toubert^{2*}

Spondyloarthropathies constitute a group of autoimmune diseases of special interest because of their tight association with the MHC class I molecule HLA-B27 and the bacterial triggering of some clinical forms called reactive arthritis (ReA). One current hypothesis is the presentation by HLA-B27 of a so-called arthritogenic peptide to T cells. To better focus on the relevant T cell populations within the joint, we performed an extensive β -chain T cell repertoire analysis of synovial fluid compared with PBL in seven patients, four of whom were characterized as having ReA triggered by *Yersinia enterocolitica*, *Chlamydia trachomatis*, or *Shigella sonnei*. Analysis of the size diversity of the β -chain complementarity-determining region 3 (CDR3) allowed us to evaluate the degree of T cell clonality in the samples. Oligoclonal T cell expansions were frequently observed in the joint. In one patient, CDR3 amino acid sequences of major expansions using two different BV genes were identical. One dominant T cell expansion and several CDR3 amino acid sequences were identical in two different patients. Furthermore, one sequence was identical with a sequence reported independently in a *Salmonella*-induced ReA patient. Together, these data indicate a surprisingly high degree of conservation in the T cell responses in recent-onset ReA triggered by different micro-organisms. A CD8⁺ synovial line expressing shared clonotypes was established and reacted toward several B*2705 lymphoblastoid cell lines, therefore supporting a molecular mimicry phenomenon at the T cell level in the disease mechanism. *The Journal of Immunology*, 1999, 162: 3830–3839.

Spondyloarthropathies (SA)³ constitute a group of inflammatory rheumatic diseases including ankylosing spondylitis, reactive arthritis (ReA) or Reiter's syndrome, and some clinical forms of psoriatic or inflammatory bowel disease-associated arthritis (1). A key feature in SA pathogenesis is the interplay between genetic factors suggested by the familial aggregation of the disease (2) and environmental factors such as the occurrence of ReA cases following outbreaks of either *Shigella*, *Yersinia*, or *Salmonella* infections. The major predisposing genetic factor that has been identified is the MHC class I molecule HLA-B27. The direct implication of HLA-B27 in the disease process and the role of triggering bacteria have been documented in the animal models of arthritis such as HLA-B27 transgenic rats (3) or HLA-B27 transgenic-murine β_2m knock-out mice (4). However,

despite knowing the three-dimensional structure of HLA-B27 (5), peptide binding rules, and the definition of HLA-B27 subtypes (6), there is still no definitive explanation of the disease mechanism. T cells present at the pathogenic site are thought to play a direct role as in the case of other autoimmune diseases, for instance rheumatoid arthritis or multiple sclerosis. In the case of SA, one main hypothesis, the so-called arthritogenic peptide hypothesis (6), proposes that an antigenic peptide derived from an autoantigen or from a triggering bacteria would be presented in an HLA-B27-restricted fashion to effector CD8⁺ T cells. Evidence for this hypothesis came from the report of HLA-B27-restricted CD8⁺ T cell clones derived from the synovial fluid (SF) of patients with ReA and specific for bacteria or autoantigens (7). The persistence of *Chlamydia* or *Salmonella* inside the joint (8, 9) could explain the continuous triggering of the immune response. Several bacterial proteins are candidate sources of antigenic peptides, including hsp60 or the urease 19-kDa β -subunit from *Yersinia* (10) and hsp57 or Hc1, an 18-kDa histone-like protein from *Chlamydia* (11). Based on the HLA-B27 binding motif, a nonamer peptide from *Yersinia* hsp60 has been identified as a target for HLA-B27-restricted cytolytic responses in *Yersinia*-induced ReA patients (12).

The partner of the HLA-B27/antigenic peptide complex recognition is the $\alpha\beta$ TCR heterodimer. The arthritogenic peptide hypothesis supposes a limited diversity of the T cell repertoire, since both HLA-B27 and the putative antigenic peptide(s) could be shared by different patients, especially in the case of bacteria-triggered ReA. T cell repertoire studies in rheumatoid arthritis (13) revealed more frequent T cell expansions at the pathogenic site than in PBL and that some were shared in different joints of the same patient and persisted over time (14, 15). Overall, these data suggest an in situ Ag-driven immune response. With regard to HLA-B27-related arthritis, lack of a preferential usage of the TCR

*Laboratoire d'Immunologie et d'Histocompatibilité, Institut National de la Santé et de la Recherche Médicale, Unit 396, Université Paris VII, Centre G. Hayem, Hôpital Saint-Louis, Paris, France; [†]Institut National de la Santé et de la Recherche Médicale, Unit 463, Institut de Biologie, Nantes, France; [‡]Service de Rhumatologie A, Hôpital Cochin, Paris, France; [§]Service de Rhumatologie, Hôpital Saint-Antoine, Paris, France; and [¶]Service de Rhumatologie A, Hôpital Lariboisière, Paris, France

Received for publication September 25, 1998. Accepted for publication December 22, 1998.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ This work was supported in part by the Association de Recherche sur la Polyarthrite and BIOMED European Concerted Action EUROAS.

² Address correspondence and reprint requests to Dr. A. Toubert, Centre G. Hayem, Hôpital Saint-Louis, 1 av. C. Vellefaux, 75475 Paris Cedex 10, France. E-mail address: toubert@histo.chu-stlouis.fr

³ Abbreviations used in this paper: SA, spondyloarthropathies; ReA, reactive arthritis; SF, synovial fluid; hsp, heat shock protein; CDR3, complementarity-determining-region 3; aa, amino acid; B-LCL, Epstein-Barr virus-transformed B lymphoblastoid cell line.

Table I. Clinical characteristics and HLA class I typing of the patients

Patient	Sex	Age	Disease Duration	Sacroiliitis (grade)	Serology (titer)	Medications	HLA-A	HLA-B	HLA-C
GM									
Ankylosing Spondylitis	M	62	35 years	+	ND	NSAID ^a	0301 2402	2705 39	02 07
TD									
Ankylosing Spondylitis	M	31	2 years	+(II/III)	ND	NSAID sulphasalazine	0201 1101	2705	01 02
MC									
Ankylosing Spondylitis	F	22	11 years	+	ND	NSAID	02 25	51 2705	01 14
CN									
Reactive Arthritis	F	46	<30 days	—	<i>Y. enterocolitica</i> 0:3 (1/2560)	NSAID	11 24	18 2705	02 1203
PG									
Reactive Arthritis	M	29	<30 days	—	Postdiarrhea	NSAID	0201 3201	61 2705	0202 1501
CF									
Reactive Arthritis	M	30	2 years	—	<i>S. sonnei</i> (1/640)	None	2901 6801	07 2705	0701 0202
CL									
Reactive Arthritis	M	33	14 years	+(III)	<i>C. trachomatis</i> (1/512)	NSAID	29 30	2705 49	02 07

^a NSAID, nonsteroidal anti-inflammatory drugs.

$\alpha\beta$ V regions encoding genes (16) but the occurrence of multiple T cell expansions (17) have been reported in PBL and SF T cells from ReA patients. Analysis of cytolytic CD8⁺ HLA-B27-restricted clones produced in vitro from the synovial fluid of three ReA patients revealed limited use of BV families (BV13, 14, 17) and BJ segments (18) as well as some structural constraints in the β -chain hypervariable complementarity-determining region 3 (CDR3) without sequence homology (19). The TCR β -chain is produced by the combination of V, D, J, and C gene segments. In addition, this combinatorial diversity is increased by the nibbling of germline nucleotides and addition of N and P residues at the V-D-J junction sites. The crucial nature of the CDR3 region encompassing the V-D-J junction in the contact with the central part of the peptide in the MHC-peptide complex was shown by crystallography (20). Two methods called spectratyping (21) and Immunoscope (22) have been developed to determine the size of CDR3 regions in transcripts of whole BV families or in given BV-BJ combinations without any requirement for in vitro cell culture and are therefore particularly suitable for analysis of $\alpha\beta$ T cell clonality in complex clinical situations: tumors, autoimmune diseases, and graft-vs-host disease (23). We took advantage of this approach to compare the $\alpha\beta$ T cell repertoire in paired PBL and SF samples of seven HLA-B27-positive ReA or ankylosing spondylitis patients. Some oligoclonal expansions defined by the BV, BJ gene segments, and the CDR3 size were found in common in different patients. CDR3 amino acid sequence comparisons revealed strikingly conserved patterns. Identical sequences were observed in expansions, firstly using different BV genes in one patient and secondly between two different ReA patients. One sequence was identical with a previously reported sequence of a cytolytic clone derived in vitro from a *Salmonella*-triggered ReA patient (18). Finally, a CD8⁺ SF line from the ReA patient whose SF lymphocytes expressed these sequence identities recognized several B*2705 expressing EBV-transformed lymphoblastoid cell lines (B-LCL). These results strongly support the arthritogenic peptide hypothesis and argue for an Ag-driven skewing of the T cell repertoire common to patients with ReA triggered by different bacteria.

Materials and Methods

Patients

PBL from 19 HLA-B27-positive patients fulfilling the European Spondylarthropathy Study Group diagnostic criteria for SA (1) were included in the study and compared with samples from 10 healthy HLA-B27-positive individuals obtained from the Blood Bank facility of our institution. Among the 19 patients, 12 had classical ankylosing spondylitis, three had psoriatic arthritis, and four had ReA with a bacterial triggering agent defined in three cases (*Chlamydia trachomatis*, *Shigella sonnei*, *Yersinia enterocolitica* 0:3). Patient PG developed ReA after an enteric infection contracted on travels in Peru where *Salmonella* is a frequent cause of ReA (24). SF lymphocytes were obtained under informed consent from knee effusions during SF analysis before steroid administration in three ankylosing spondylitis patients and four ReA patients. Two ReA patients were studied at the onset of the disease (patients PG and CN), while the remaining two (patients CF and CL) had acute relapses. The main clinical features, HLA class I typing, and treatment at the time of sample collection are shown in Table I for these seven patients who were extensively studied.

RNA extraction and cDNA synthesis

PBL were obtained by density gradient centrifugation (Ficoll/Hypaque) and SF cells were isolated directly by three successive washes in PBS. RNA was extracted from cell pellets frozen in liquid nitrogen by lysis in guanidium thiocyanate buffer, and the cDNA was prepared from 5–10 μ g total RNA with AMV reverse transcriptase (cDNA cycle kit, Invitrogen, Leek, The Netherlands) as previously described (25).

Oligonucleotides and CDR3 size analysis

The nomenclature of BV families and the primers used have been previously described (25, 26). Fluorescent primers for BC, BJ, and the BV1-BJ2S3 clonotypic primer (5'-CTGCGTATCTGTGGAATATAGA-3') were labeled at the 5' end with the Fam fluorophore (Applied Biosystems, Foster City, CA). Aliquots of the cDNA synthesis reaction (corresponding to 250 ng total RNA) were amplified in 50- μ l reactions with one of the BV-specific oligonucleotides as the 5'-primer and the BC oligonucleotide as the 3'-primer. The final concentration was 0.5 mM for each primer, 0.2 mM dNTP, and 2 mM MgCl₂ in Taq polymerase buffer (Promega, Madison, WI) in the presence of 1 U of Taq polymerase (Promega) on a DNA thermal cycler (model 9600, Perkin-Elmer, Norwalk, CT). The PCR cycle profile was denaturation at 94°C for 30 s, annealing at 60°C for 45 s, and primer extension at 72°C for 45 s for 40 cycles and a final polymerization step of 5 min at 72°C. Aliquots from each BV-BC PCR product (2 μ l) were copied in six cycle run-off reactions primed with a fluorophore labeled BC, BJ, or clonotypic-specific oligonucleotide. The final concentrations were

0.2 mM dNTP and 3 mM MgCl₂ in the presence of 0.2 U of Taq polymerase. The run-off reactions were migrated on 4.25% acrylamide sequencing gels (377A DNA sequencer, Applied Biosystems) for size (Genescan-500 size marker, Perkin-Elmer) and fluorescence intensity determination. The raw data were analyzed with the help of the Immunoscope software (22). The CDR3 region was defined to include residues 95–106 (23). Since the positions of the BV and the BC primers are fixed, the length distribution observed in the PCR fluorescent BV-BC products depends only on the size of the V-D-J junctions. Statistical analysis was performed to determine whether a profile could be considered Gaussian; a profile was not considered Gaussian if one peak was excluded from the 95% confidence interval of peak level intensities. TCRB subfamilies BV10 and BV19 were omitted from this analysis as they are pseudogenes in most individuals (27).

BV and BJ gene usage

A competitive PCR was used as previously described (25) to quantify the TCR transcripts in each cDNA sample using a deleted (4-bp) CD3 δ chain plasmid. About 3×10^6 copies of cDNA from each sample were then amplified for 30 cycles with the BV primers and an internal fluorescent BC primer. BJ usage was defined after run-off reactions of the unlabeled BV-BC amplification product and is quantitative, since the fluorescent primers have comparable amplification efficiencies (23). The fluorescence intensity in each BV or BJ family was expressed as the percentage of total signals from the 22 BV or 13 BJ subfamilies. Statistical comparisons between samples from healthy donors and patients or between PBL and SF were made using the Mann-Whitney test. Clonotypic sequence usage was defined after run-off reactions with the labeled BJ2S3 and clonotypic primers. The amplification efficiency of the clonotypic primer was assessed beforehand in comparison with the BJ primer on a clonal plasmid DNA.

DNA sequencing

BV-BJ PCR products were cloned into pCR2.1 vector (Invitrogen) and transformed into XL1 Blue supercompetent cells (Stratagene, La Jolla, CA). After blue/white screening of recombinant plasmids on X-galactoside/isopropylthiogalactoside indicator plates, plasmids were purified by alkaline lysis followed by phenol/chloroform/iso-amyl alcohol. Inserts were checked by agarose gel electrophoresis after BV-BJ PCR amplification, and both strands were sequenced with the ABI PRISM Dye Primer Cycle sequencing kit (Perkin-Elmer). Products were loaded on 4.25% acrylamide sequencing gels (377A DNA sequencer, Applied Biosystems) and analyzed with the Sequence Navigator software.

Flow cytometric analysis

Direct immunofluorescence was performed in triple labeling analysis. After washing, SF cells or PBL were incubated for 15 min with one of the BV region-specific labeled mAb (Immunotech, Marseille, France) BL37.2-phycoerythrin (BV1), TAMAYA1.2-FITC (BV16), FIN9-phycoerythrin (BV9), or AF23-phycoerythrin (BV23) together with a labeled CD3, CD4, CD8, or CD45RO mAb (Immunotech, Marseille, France). Events (5×10^3 – 10^4) gated on CD3 or CD8 expression were analyzed using a FACScan flow cytometer (Becton Dickinson, Mountain View, CA), and results were expressed as percentages of cells staining above the background level.

Selection of a CD8-positive cytolytic T cell line from patient PG and 51Cr release assay

Synovial T cells from patient PG were sorted by means of the CD8 mAb (>95% CD8⁺ cells) and cultured in medium supplemented with rIL-2 (Genzyme, Cergy, France) at 150 IU/ml, purified PHA-L at 0.5 μ g/ml (leukoagglutinin, Sigma, St. Louis, MO) and irradiated (50 Gy) allogeneic feeder cells as described previously (28). Cells were maintained by adding IL-2 (150 IU/ml) twice weekly and were restimulated every 2 wk in the presence of allogeneic feeder cells in which HLA-B27-positive cells had been excluded. Cytotoxicity was measured by a standard 4-h ⁵¹Cr release assay, and the percentage of specific lysis was calculated as described previously (28). Targets were B-LCL generated in the laboratory, except ADA (28), HOM-2, COX, MGAR, Sweig, and YAR (homozygous B-LCL from the Tenth International Histocompatibility Workshop), and the T2 and B*2705-transfected T2 cell lines (29). PHA blasts were generated from PBL of individual GC (2×10^6) by three cycles of a 4-day stimulation with PHA-L at 1 μ g/ml. In inhibition experiments, targets were preincubated for 1 h with a 1/100 dilution of ascites of W6/32, an HLA class I monomorphic mAb (30) or B1.23.2, an HLA-B- and -C-specific mAb (31). SF1.111, an anti-H-2 K^d IgG2a isotypic control, was provided by Dr. M. Pla (Hôpital Saint-Louis, Paris, France). Blocking experiments were also performed us-

ing the CD3-specific mAb OKT3 (Ortho Diagnostics, Raritan, NJ). HLA class I typing was determined by standard microcytotoxicity and HLA-B27 subtyping using DNA methods (32).

Results

Oligoclonal T cell expansions in HLA-B27-related arthritis SF lymphocytes

This approach of T cell repertoire analysis was chosen to provide both a qualitative and a quantitative appraisal of the T cell response in terms of clonality and of BV or BJ gene segment usage, respectively. We first compared the TCR BV gene usage in PBL isolated from age-matched HLA-B27-positive individuals who were either healthy ($n = 10$) or were affected by various forms of SA ($n = 19$). The overall picture of BV usage was comparable in the two groups (data not shown). We observed T cell expansions in different BV families without any correlation with disease status. Their frequency increased with age in the group of healthy subjects as well as in the patients as reported previously (33). In line with the hypothesis that a local stimulus could initiate the arthritic process, we thereafter chose to focus on the comparison between the T cell repertoire in PBL and in SF lymphocytes. Sample pairs from seven different HLA-B27 patients were available for analysis. Perturbations of the CDR3 size distribution profiles were the rule in SF T cells with few truly polyclonal families. Oligoclonal expansions were found in three to six BV families in SF and in zero to three families in blood. Some were common at both sites, but the majority were detected in SF lymphocytes only. Major SF expansions and corresponding BV-BC amplifications from PBL are shown in Fig. 1 for the seven patients studied. Expansions were found in different BV families in different patients. However, four of seven patients had expansions in the BV1 or in the BV11 family, and three of seven had expansions in BV23. Patients PG and CN, both affected by an acute form of ReA triggered by an enteric infection, shared T cell expansions in the BV1 and BV23 families. These BV expansions were defined more precisely in most cases with regard to their BJ usage by performing run-off reactions of the BV-BC amplification with BJ fluorescent primers (Table II). At this level of definition some BV expansions apparently common to different patients could be distinguished, for instance the BV16 expansions (11 aa) of patients GM and PG used, respectively, the BJ2S5 and BJ2S7 segments. However, the BV1 expansions (10 aa) of patients PG and CN used the same BJ2S3 segment as did the BV11 expansions (6 aa) of patients CF and CN. As the experiments were also semiquantitative, we could compare the percentage of the T cell expansions in the PBL vs SF. In some cases SF expansions were markedly over-represented compared to blood, for instance the BV1-BJ2S3 expansion, which could be considered the dominant expansion in patient PG accounting for about 4.75% of in situ T cells (Table II).

Dominant T cell expansions are found at different articular sites in patient PG

Patient PG presented at diagnosis an acute right knee arthritis and 15 days later developed a contralateral knee effusion. Fluid was obtained for analysis at both articular sites (SF-R and SF-L). Similar oligoclonal patterns were observed in both knee effusions. The similarity of the BV1, BV9, BV16, and BV23 expansions was confirmed by BJ run-off reactions (Fig. 2). Moreover, the expansions detected in two right knee samples obtained a few days apart were identical (data not shown). These data confirm the relevance of the method and the fixed bias of the T cell response in a given patient during an acute episode of arthritis. The BV1-BJ2S3 expansion at a 10-aa CDR3 size was detected in blood during the

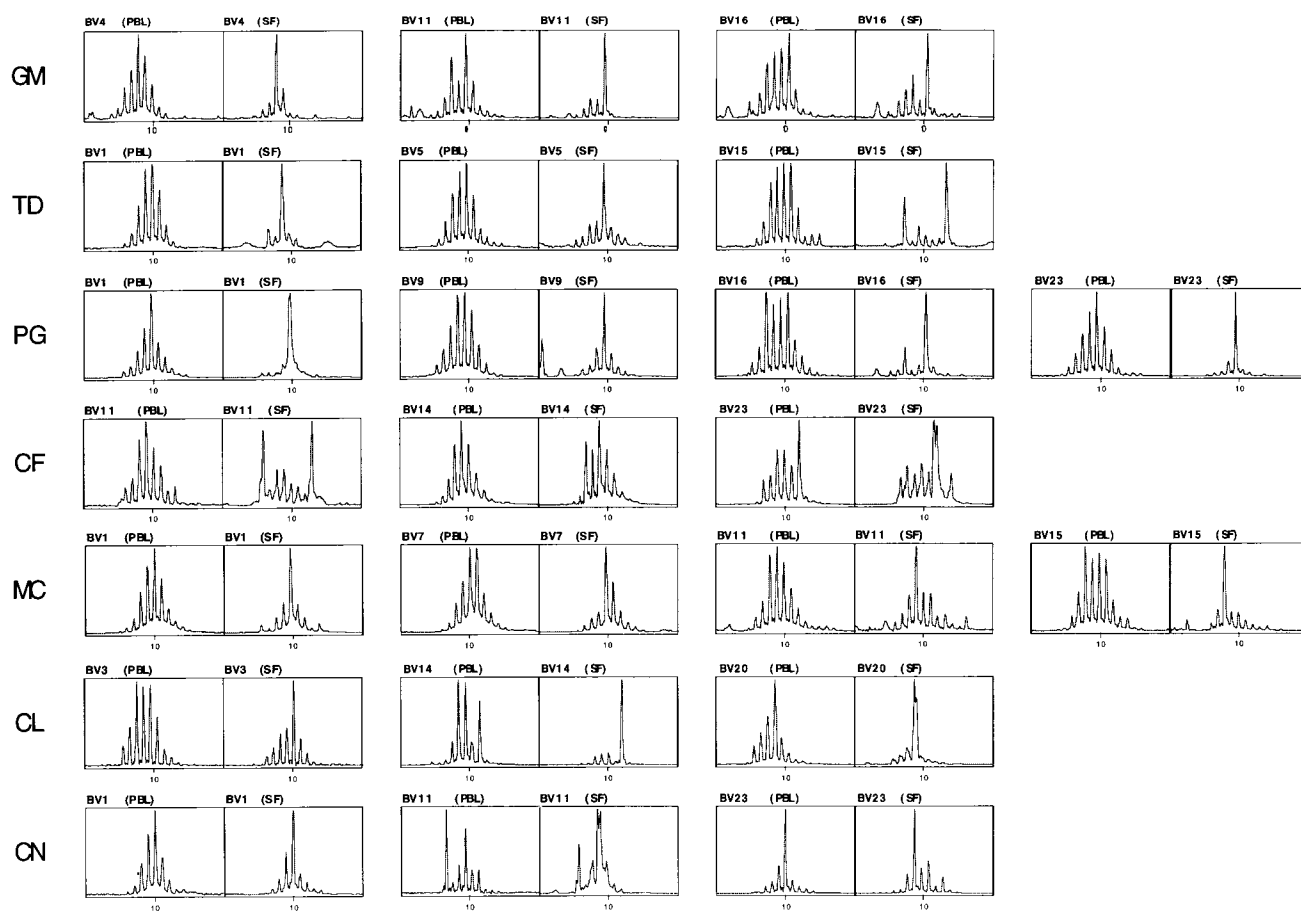


FIGURE 1. CDR3 size distribution of major TCRBV expansions in synovial fluid lymphocytes and PBL from HLA-B27-reactive arthritis and ankylosing spondylitis patients (see Table I). cDNA was amplified in PCR reactions primed by one BV subfamily and a BC-specific fluorescent primer. The 10-aa CDR3 size is indicated.

acute phase of arthritis but no longer in a sample collected 11 mo after recovery (Fig. 2 and data not shown).

TCR staining of the BV families expanded in SF (BV1, BV9, BV16, BV23) in the CD4⁺, CD8⁺, and memory CD8⁺CD45RO⁺

lymphocyte populations was directly examined by flow cytometry, thereby avoiding the need for cell culture (Table III). It confirmed the over-representation of the BV1 family in the SF and not in the blood, notably in the CD8 subset. The frequency of CD8⁺CD45RO⁺

Table II. Percentages of TCR BV and BJ usage in the dominant SF compared with peripheral blood T-cell expansions^a

Patient	Dominant SF Expansions	CDR3 Size (aa)	PBL (%)		SF (%)		T cells
			TCRBV	TCRBJ	TCRBV	TCRBJ	
GM	BV4-BJ2S5	8	4.56	ND	3.92	41.52	1.63
	BV11-BJ2S1	10	1.52	8.56	2.54	68.76	1.75
	BV16-BJ2S5	11	2.09	10.37	0.94	25.76	0.24
TD	BV5-BJ2S1	10	7.36	ND	2.96	21.46	0.64
	BV15-BJ2S1	13	2.26	ND	3.71	44.40	1.65
PG	BV1-BJ2S3	10	3.79	19.57	7.02	67.66	4.75
	BV9-BJ2S7	10	4.20	ND	2.96	49.95	1.48
	BV16-BJ2S7	11	3.44	ND	2.40	44.70	1.07
CF	BV23-BJ2S3	10	1.60	9.76	2.50	40.76	1.02
	BV11-BJ2S3	6	0.89	ND	2.19	23.61	0.52
	BV11-BJ2S6	13	0.89	ND	2.19	29.04	0.64
MC	BV1-BJ2S7	10	3.44	ND	3.53	28.31	1.00
	BV15-BJ1S5	8	2.15	ND	2.11	17.78	0.38
CN	BV1-BJ2S3	10	2.06	10.53	1.69	17.65	0.30
	BV11-BJ2S3	6 and 9	1.08	20.56	4.19	28.72	1.20
	BV23-BJ2S1	9	3.30	12.50	2.04	16.98	0.35

^a TCRBV gives the percentage of the BV family used in a given expansion in comparison with all BV families, and TCRBJ the percentage of the BJ segment expressed within a given BV family. The amount of dominant oligoclonal expansions within total SF T-cells are calculated as the product of these two values.

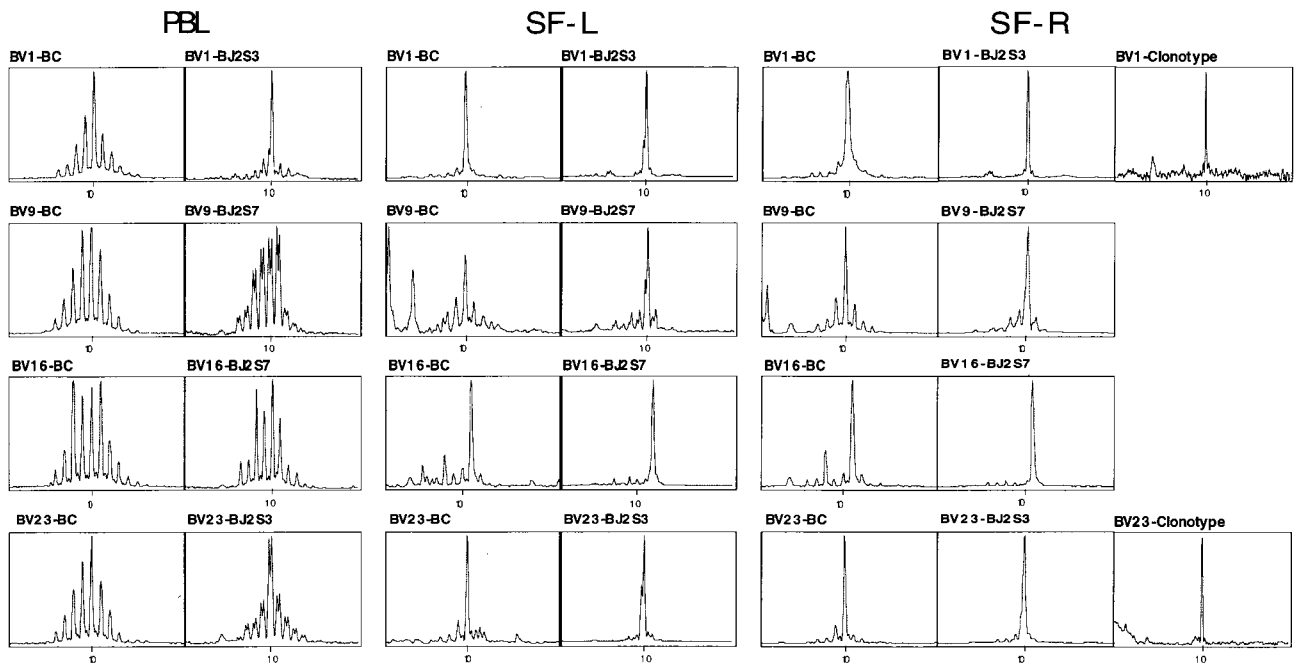


FIGURE 2. CDR3 size distribution of BV1, BV9, BV16, and BV23 subfamily transcripts from PBL, right (SF-R) and left (SF-L) knee effusions of patient PG. BV-BC PCR products were copied in run-off reactions primed with BC, BJ, and clonotype-specific fluorescent primers.

memory cells was markedly increased in the SF, accounting for 40% of the total CD8⁺ cell population. About one-third of these cells (24%) belonged to one of the four expanded BV families, and about 14% belonged to the single BV1 family.

Oligoclonal expansions of patient PG share identical CDR3 β -chain sequences in different BV families

Immunoscope data and cytometric analysis clearly indicated that in the case of patient PG the dominant $\alpha\beta$ T cell expansion found in situ was in the BV1 family and was characterized by the usage of the BJ2S3 gene segment and a CDR3 size of 10 aa. Since such expansions that appear to be monoclonal could in fact reflect the occurrence of many different T cell clones, it was necessary to obtain sequences from this CDR3 junctional region. Oligoclonal T cell expansions in the BV9, BV16, and BV23 families were also sequenced, and the results are shown in Table IV. The sequence SVGLYSTDTQ was present in five of 11 clones and could be considered major in the BV1 expansion. The six other clones provided sequences at the expected CDR3 size and differed from the major sequence by a single amino acid at position 97, 98, or 99 considering the last serine in the CASS motif as position 95. One sequence (SVGYSTDTQ) has even been reported in a T cell

clone derived from a *Salmonella*-triggered ReA patient (18). In the other BV families, the most notable results were obtained in BV23, in which the dominant sequence SVGLYSTDTQ present in eight of 24 clones was the same as that in BV1. Two other sequences, SVGLFSTDTQ and SVGDYSTDTQ, were also common to BV1 and BV23. These sequences had a characteristic BV-encoded residue at position 79, either leucine for BV1 or methionine for BV23, showing that they definitely belonged to different families. The high conservation of the CDR3 within these two families was also demonstrated in 35 sequences by the use of a small (proline, glycine, serine) or aliphatic/hydrophobic (valine, alanine, isoleucine, leucine) residue at position 96 and of an aromatic/hydrophobic residue (tyrosine or phenylalanine) at position 99, while the CDR3 positions 97 and 98 were more variable. A clonotypic primer was designed to estimate the representation of the dominant CDR3 sequence shared in the BV1 and BV23 expansions. It amplified about 0.6% and 0.2% of the total SF T cells in BV1 and BV23, respectively, and was negative in PBL (Fig. 2). However, this clonotype gave a low estimate, since different nucleotide sequences could encode the same CDR3 amino acid sequence, as will be detailed later.

Table III. Immunofluorescence analysis of dominant BV families in peripheral blood and SF T cell subsets of patient PG^a

	CD3 ⁺ (%)		CD3 ⁺ CD4 ⁺ (%)		CD3 ⁺ CD8 ⁺ (%)		CD8 ⁺ CD45RO ⁺ (%)	
	PBL	SF	PBL	SF	PBL	SF	PBL	SF
CD3 ⁺			48.7	38.7	47.9	56.8	4.9	<u>40.2</u>
BV1	5.2	<u>12.4</u>	2.8	1.8	2.6	<u>9.8</u>	0.6	<u>13.7</u>
BV9	5.2	4.5	3.8	4.3	2.9	3	0.9	<u>2.7</u>
BV16	1.7	2.4	0.7	0.7	1.3	1.7	0.4	<u>4.2</u>
BV23	2.6	1.8	1.8	0.5	1.4	1.2	2	<u>3.6</u>

^a Underlined are lymphocytes subsets overexpressed in SF compared to PBL.

Table IV. β -Chain CDR3 amino acid sequences of SF T cell oligoclonal expansions in patient PG

	Junctional region			No. of Colonies
	BV	N-D-N ^a	BJ	
1-CAS	<i>SVGLYSTDTQ</i>	YFGP-2.3		5
1-CAS	<i>SVGDYSTDTQ</i>	YFGP-2.3		1
1-CAS	<i>SVGLFSTDTQ</i>	YFGP-2.3		1
1-CAS	<i>SVGYSTDTQ</i> ^b	YFGP-2.3		1
1-CAS	SVALYSTDTQ	YFGP-2.3		1
1-CAS	SLGRYSTDTQ	YFGP-2.3		1
1-CAS	SVGYSTDTQ	YFGP-2.3		1
9-CAS	SFRTSGFYEQ	YFGPGTRLTV-2.7		1
9-CAS	SQILGLVYEQ	YFGPGTRLTV-2.7		1
9-CAS	SQQQGIVHEQ	YFGPGTRLTV-2.7		1
16-CAS	SQDRNMPAYEQ	YFGPGTRLTV-2.7		1
16-CAS	SPSGMPFGYEQ	YFGPGTRLTV-2.7		1
23-CAS	<i>SVGLYSTDTQ</i>	YFGP-2.3		8
23-CAS	SVGTFSTDTQ	YFGP-2.3		2
23-CAS	<i>SVGLFSTDTQ</i>	YFGP-2.3		1
23-CAS	<i>SPGLYSTDTQ</i>	YFGP-2.3		1
23-CAS	<i>SVGLYSTDTQ</i>	YFGP-2.3		1
23-CAS	SPGLFSTDTQ	YFGP-2.3		1
23-CAS	TAGLFSTDTQ	YFGP-2.3		1
23-CAS	SSATYSTDTQ	YFGP-2.3		1
23-CAS	SVELFSTDTQ	YFGP-2.3		1
23-CAS	SVARYSTDTQ	YFGP-2.3		1
23-CAS	SIMGGSTDTQ	YFGP-2.3		1
23-CAS	SVAVYSTDTQ	YFGP-2.3		1
23-CAS	SAGRYSTDTQ	YFGP-2.3		1
23-CAS	SGGLFSTDTQ	YFGP-2.3		1
23-CAS	SPGTFSTDTQ	YFGP-2.3		1
23-CAS	SALIDQSDTQ	YFGP-2.3		1

^a In italics are sequences shared in BV1 and BV23 expansions and in bold are sequences shared with patient CN (see Table V).

^b Identical to the sequence of clone P6.2.22 from Duchmann et al. (18).

Identical β -chain CDR3 sequences are found in different HLA-B27-positive ReA patients

Some oligoclonal expansions were shared between different patients (BV1-BJ2S3 in patients PG and CN, BV11-BJ2S3 in patients CF and CN) or were similar in size or BV usage, for instance the BV16 expansion at 11 aa in patients PG and GM (Fig. 2 and Table II). Although sharing the same size and BV and BJ usage, the major sequences from the BV11-BJ2S3 peaks were different, being SLVDTQ in patient CF and TTGYTQ in patient CN (Table V). In marked contrast, we found identical CDR3 sequences in patients CN (Table V) and PG (Table IV): SVGLFSTDTQ in the BV1 and BV23 oligoclonal peaks of patient PG and in the BV1 peak of patient CN and SPGLYSTDTQ shared by BV23 and BV1 peaks of patients PG and CN, respectively. The most frequent CDR3 sequence in the BV1 expansion of patient CN (SVAHYSTDTQ) differed at positions 97 and 98 only from the main SVGLYSTDTQ sequence found in patient PG. Comparisons at the nucleotide level of identical sequences in patients PG and CN excluded the possibility that these shared sequences could be explained by contamination of samples during PCR amplification, since multiple silent substitutions were found in different clones encoding the same CDR3 sequence (Table VI).

Functional reactivity of a synovial CD8⁺ T cell line from patient PG

A CD8⁺ T cell line was established from SF lymphocytes of patient PG. The BV, BJ, and clonotypic expression was checked by

Table V. β -Chain CDR3 amino acid sequences of SF T cell oligoclonal expansions from reactive arthritis (CN, CF) and ankylosing spondylitis (MC, GM) patients

Patient	Junctional Region ^a			No. of Colonies
	BV	N-D-N	BJ	
CN	1-CAS	SVAHYSTDTQ	YFGPG-2.3	5
	1-CAS	SVEGQSTDTQ	YFGPG-2.3	2
	1-CAS	SPGLYSTDTQ	YFGPG-2.3	1
	1-CAS	SVGLFSTDTQ	YFGPG-2.3	1
	1-CAS	SIGTSGTDTQ	YFGP-2.3	1
	11-CAS	SETGGTDTQ	YFGPG-2.3	17
11-CAS	NETGGTDTQ	YFGP-2.3	1	
11-CAS	TTGYTQ	YFGPG-2.3	6	
11-CAS	TSGYTQ	YFGP-2.3	1	
CF	11-CAS	SLVDTQ	TFGPG-2.3	4
	11-CAS	TIPGHQAPGANVL	TFGAGSR-2.6	2
MC	1-CAS	SAHRGQSYEQ	YFGPGTRLTV-2.7	5
	1-CAS	SAEGGKTYEQ	YFGPGTRLTV-2.7	1
GM	11-CAS	SEWGGNYNEQ	FFGPG-2.1	7
	11-CAS	SGRQGYNEQ	FFGP-2.1	3
	11-CAS	SDGRASYNEQ	FFGPG-2.1	1
16-CAS	SHWTSRSETQ	YFGPGTRLLVLE-2.5	3	

^a In bold are sequences shared between patients PG (see Table IV) and CN.

flow cytometry and immunoscope analysis. The BV1 family was expressed at a similar level as in SF, and the clonotype frequency in BV1 and BV23 families was estimated as 1.2% of T cells (data not shown). This line was tested against a panel of B-LCL, either HLA-B27-positive or -negative (Fig. 3). Several HLA-B27-positive targets were lysed reproducibly, with >15% specific lysis at a 12.5:1 E:T cell ratio. HOM-2, A16, and A19 shared only B*2705 with the CD8⁺ effector line. The cytotoxic activity was blocked by two different HLA class I-specific mAbs, W6/32 and B1.23.2, as well as by a CD3-specific mAb, therefore confirming the HLA-B27 specificity of the T cell recognition (Fig. 4A). The autologous EBV line was not lysed, and this could not be explained by resistance of the target to cytotoxicity, as it was recognized by effector cells from another arthritic patient (M. Bonneville and M. A. Peyrat, unpublished observations). In this panel, several EBV lines from B*2705 individuals were also not recognized (Fig. 3). There was no correlation with disease status, as HOM-2, A16, A19, or GC obtained from healthy individuals and TD or ADA from arthritic patients were lysed. The T2 and T2-B*2705 cell lines, which are deficient in peptide transporters and express empty class I molecules at 37°C, were not lysed (data not shown). The idea that EBV Ags could be recognized by intrasynovial lymphocytes (28) prompted us to test comparatively another kind of target than B-LCL. PHA blasts could be generated from the healthy HLA-B27 individual GC. They were recognized by the CD8⁺ line, and lysis was blocked upon incubation with W6/32 (Fig. 4B), arguing for the recognition of HLA-B27-presented antigenic peptides from endogenous self proteins and not from EBV.

Discussion

These results argue for a strong selective pressure in the setting of the T cell response at the pathogenic site of HLA-B27-associated arthritis and especially in the case of recent-onset ReA. The skewing of the T cell repertoire was demonstrated by the sharing of

Table VI. Nucleotide sequences of the dominant BV23 amino acid CDR3 sequence in patient PG and of identical amino acid β -chain CDR3 sequences in patients PG and CN

	Nucleotide Sequence ^a	Patient
SVGLYSTDTQ	BV23-AGCGTAGG <u>TCTA</u> TATTCACAGATACGCAG-BJ2S3	PG
	BV23-AGCGTAGG <u>ATTAT</u> ATATACACAGATACGCAG-BJ2S3	PG
	BV23-AGCGTAGG <u>GCTTT</u> ACAGCACAGATACGCAG-BJ2S3	PG
	BV23-AGCGTAGG <u>CCTCT</u> ATATACACAGATACGCAG-BJ2S3	PG
	BV23-AGCGTAGG <u>ACTA</u> TACAGCACAGATACGCAG-BJ2S3	PG
SVGLFSTDTQ	BV1-AGCGTAGG <u>ACTTT</u> TCTCCACAGATACGCAG-BJ2S3	PG
	BV1-AGCGTAGG <u>GCTAT</u> TTAGCACAGATACGCAG-BJ2S3	CN
	BV23-AGCGTAGG <u>TCTTT</u> TAGCACAGATACGCAG-BJ2S3	PG
SPGLYSTDTQ	BV23-AGCCCGGACTTTATATACACAGATACGCAG-BJ2S3	PG
	BV1-AGCCCGGACTTTACTCCACAGATACGCAG-BJ2S3	CN

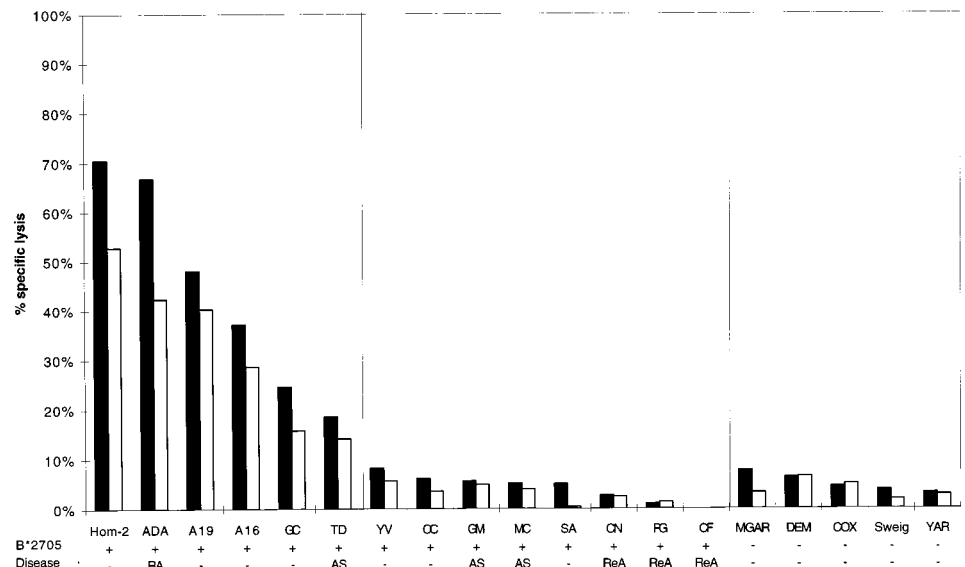
^a Underlined are differences between nucleotide sequences encoding the same amino acid CDR3 sequence.

identical or highly homologous sequences in the same patient in two different BV families, BV1 and BV23, which each include a single gene and are the most closely related members within the BV subgroup I (34). Even more interestingly, sequence sharing was detected in different patients. The arthritis-triggering organism was *Yersinia enterocolitica* in one patient (CN) and was unknown in the other patient (PG). One sequence of the dominant BV1-BJ2S3 expansion in patient PG was identical with a sequence previously reported in a *Salmonella typhimurium*-induced ReA patient (18). Therefore, it appears that in ReA a public T cell response could be driven by an Ag shared by different bacterial strains or by a common self-antigenic peptide presented by HLA-B27. Since bacterial infection could play a role not only in ReA but also in the far more frequent so-called undifferentiated arthritis (35), such a public T cell clonotype could be of potential diagnostic value.

Evidence is now accumulating for the occurrence of a limited heterogeneity in the T cell response at the pathogenic site of autoimmune diseases, much of which has been provided by animal models, e.g. experimental autoimmune encephalitis in rodents (36) or the NOD mouse model of spontaneous autoimmune diabetes (37). T cell repertoire studies have also been conducted in human autoimmune diseases with the aim of defining public T cell responses and potential targets for immunotherapy. In the case of rheumatoid arthritis, TCR β -chain CDR3 motifs shared by different patients could be defined (13), and sequence identity between

different patients has been occasionally found (15, 38). Other examples of identical CDR3 sequences have been reported in brain lesions in multiple sclerosis (39), in CD4⁺ T cell expansions in coeliac disease patients (40), and in a pair of homozygous twins with Crohn's disease (41). HLA matching for the disease susceptibility allele and a recent disease onset seem to be crucial points in these observations, as was the case in HLA-DR2 multiple sclerosis patients (42). The importance of homogeneous HLA-typed patients to pinpoint such TCR skewing is not surprising if the structural data concerning MHC peptide presentation to T cells are taken into account (20). In that respect, HLA-B27-associated arthritis could be a privileged example of public β -chain CDR3 sequences, especially in bacteria-triggered cases of ReA. We did not observe overall differences in the frequency of BV transcripts in SF lymphocytes compared with PBL. This is expected in view of the complexity of the T cell response and is in agreement with a previous report (16). A higher degree of resolution was needed, which was achieved using this CDR3 size analysis approach. In these conditions, we were able to select step by step the most relevant T cell populations from the picture of the global T cell repertoire ex vivo. More generally speaking, the conservation of β -chain junctional regions has been shown during HLA-B27-restricted cytotoxic responses against the influenza A virus nucleoprotein 383–391 peptide (43) and to a lesser extent in anti-HLA-B27 alloresponses in which multiple antigenic peptides could be

FIGURE 3. Cytotoxic activity of the CD8⁺ line from patient PG toward B*2705-positive or -negative B-LCL. HOM-2, A16, and A19 share B*2705 only as HLA class I molecule with the effector cell line. Results are expressed as the percent specific lysis at E:T cell ratios of 25:1 (black boxes) and 12.5:1 (open boxes). Disease status is indicated as – (healthy), ReA (reactive arthritis), AS (ankylosing spondylitis), or RA (rheumatoid arthritis).



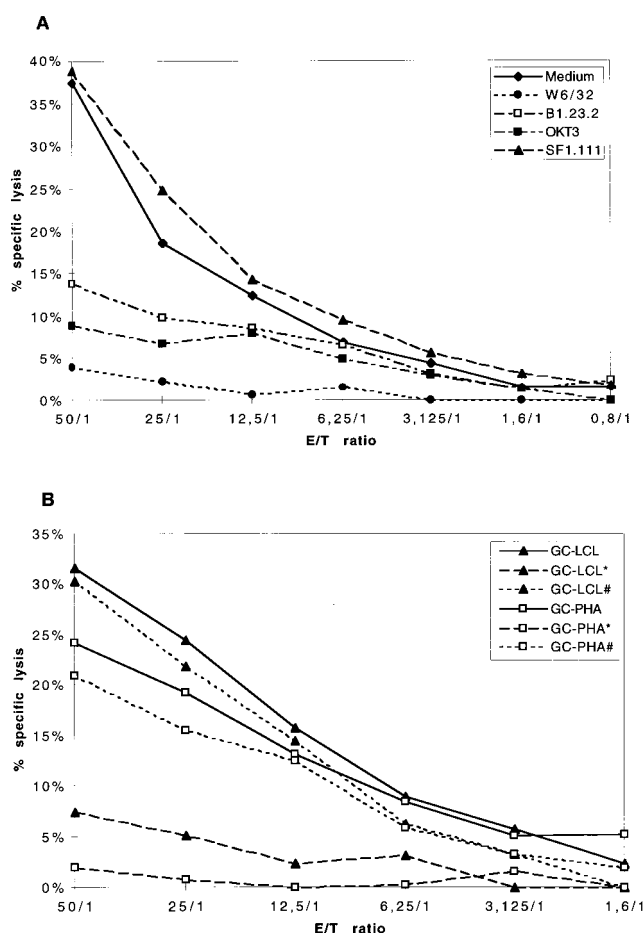


FIGURE 4. A, Inhibition of the cytotoxic activity of the synovial CD8⁺ line toward the B*2705 B-LCL A19. Before addition of the effector cells, targets were incubated for 1 h with W6/32, an HLA class I monomorphic mAb; B1.23.2, HLA-B and -C specific; SF1.111, an IgG2a isotypic control; or medium alone. The T cell recognition was also blocked with OKT3, a CD3-specific mAb. B, Lysis of B-LCL target cells compared with PHA blasts from individual GC. W6/32 (*) and SF1.111 (#) were used as in A.

recognized (44). The comparison of TCRBV heterogeneity in various class I-restricted responses suggested that a highly restricted pattern could reflect a selection of the response toward peptides that were cross-reactive with self Ags (45). In line with this hypothesis, our data would support the idea of molecular mimicry between bacterial and endogenous HLA-B27 ligands. The fact that the dominant clonotype SVGLYSTDTQ is homologous to the sequence SVGTSGLTDTQ of an anti-HLA-B27 alloreactive clone (clone 18DLH) reported by Barber et al. (46) also using the same BV1 and BJ2S3 gene segments is consistent with this assumption. The β -chain CDR3 mainly contacts the COOH-terminal part of the antigenic peptide (20). The conserved aromatic residue at position 99 in the public clonotype could be critical for peptide contact, similarly to the key residue at position 98 in another TCR/MHC/peptide complex (47).

One aim of this work was to take advantage of the T cell repertoire data to select the best candidates for further functional analysis and possibly the definition of the peptide(s) recognized. In the present study it was crucial to focus on the T cell populations expanded in the SF of patient PG, which expressed T cell clono-

types shared by other ReA patients. The major TCRBV1 expansion was no longer detected in blood after recovery. This observation is in contrast with the persistence of T cell expansions observed in other autoimmune diseases or even in healthy individuals, especially in the CD8⁺ subset (33). This could be explained by the acute course of the disease in this patient compared with more chronic diseases in other patients in this study and in the literature and could provide an opportunity to study more closely the disease triggering. A CD8⁺ T cell line was established that expressed a clonotypic frequency similar to the initial SF lymphocytes. This frequency of about 1% is expected for autoantigen-specific T cells at the pathogenic site of autoimmune diseases (48). This CD8⁺ line had cytotoxic activity toward HLA-B27 molecules expressed in B-LCL without any prior in vitro stimulation. There was no correlation with disease status and the patient's own B-LCL was not lysed. The lysis of different targets sharing B*2705 only with the effector cell, and the blockade by HLA class I and CD3-specific mAbs argue for a classical B27-restricted CD8⁺ T cell response. The fact that the transporter-deficient T2 cell line transfected with B*2705 was not lysed also suggests recognition of HLA-B27/peptide complexes. Although the triggering organism was unknown for patient PG, because of both the CDR3 sequence identity with a *Salmonella*-induced ReA patient (18) and of epidemiological data (24), we tested the cytotoxic activity of the CD8⁺ line against *Salmonella typhimurium*-infected target cells. Infection did not induce specific lysis of PG B-LCL (data not shown). Our data therefore indicate that a cytotoxic T cell response triggered during the bacterial infection could be directed toward HLA-B27 molecules bound to endogenous peptides and supports the concept of molecular mimicry at the T cell level. The lack of cytolytic activity on some B*2705 B-LCL, including the patient's own line, could be simply due to inadequate avidity of the effector CD8⁺ line (49). Otherwise, the different pattern of recognition among B*2705 B-LCL is reminiscent of a previous report in which the inability of HLA-B*2702 viral peptide presentation was attributed to a defect in Ag processing (50). Differences in endogenous peptide presentation could be explained by many factors, such as genetic differences in the source protein of the peptide as in the case of minor histocompatibility Ags (51) or in genes involved in the processing machinery and peptide presentation, the description of which is ever increasing (52). Notably, genetic susceptibility markers in addition to HLA-B27 have been recently defined in the MHC region (53). The final explanation of this observation could be provided by direct characterization of the peptides recognized among acid-eluted peptides from these targets.

In conclusion, these data provide evidence for shared T cell responses in bacteria-triggered ReA and favor the arthritogenic peptide hypothesis by showing bias in the T cell repertoire as well as HLA-B27-directed T cell responses during the course of the immune process. They also provide tools for further defining the nature and the origin of such peptide(s) before further attempts of therapy.

Acknowledgments

We thank Drs. P. Kourilsky, C. Pannetier, and J. Even for providing us the Immunoscope software; Dr. P. Cresswell for the T2 and T2-B*2705 cell lines and Dr. N. Mooney for a critical reading of the manuscript. The excellent technical assistance of J. Treton, C. Dehay, R. Chantome, J. C. Poirier, and O. Flinois is gratefully acknowledged.

References

- Dougados, M., S. van der Linden, R. Juhlin, B. Huitfeldt, B. Amor, A. Calin, A. Cats, B. Dijkmans, I. Olivieri, G. Passero, et al. 1991. The European Spondyloarthritis Study Group preliminary criteria for the classification of spondyloarthritis. *Arthritis Rheum.* 34:1218.
- Brown, M., and P. Wordsworth. 1997. Predisposition factors to spondyloarthropathies. *Curr. Opin. Rheumatol.* 9:308.
- Taugros, J., J. A. Richardson, J. T. Croft, W. A. Simmons, M. Zhou, J. L. Fernandez-Sueiro, E. Balish, and R. E. Hammer. 1994. The germfree state prevents development of gut and joint inflammatory disease in HLA-B27 transgenic rats. *J. Exp. Med.* 180:2359.
- Khare, S. D., H. S. Luthra, and C. David. 1995. Spontaneous inflammatory arthritis in HLA-B27 transgenic mice lacking β_2 -microglobulin: a model of human spondyloarthropathies. *J. Exp. Med.* 182:1153.
- Madden, D. R., J. C. Gorga, J. L. Strominger, and D. C. Wiley. 1991. The structure of HLA-B27 reveals nonamer self-peptides bound in an extended conformation. *Nature* 353:321.
- Lopez de Castro, J. A. 1998. The pathogenic role of HLA-B27 in chronic arthritis. *Curr. Opin. Immunol.* 10:59.
- Hermann, E., D. T. Y. Yu, K. H. Meyer zum Büschenfelde, and B. Fleischer. 1993. HLA-B27 restricted CD8 T cells derived from synovial fluids of patients with reactive arthritis and ankylosing spondylitis. *Lancet* 342:646.
- Branigan, P. J., H. C. Gérard, A. P. Hudson, and H. R. Schumacher, Jr. 1996. Comparison of synovial tissue and synovial fluid as the source of nucleic acids for the detection of *Chlamydia trachomatis* by polymerase chain reaction. *Arthritis Rheum.* 39:1740.
- Nikkari, S., T. Möttönen, R. Saario, U. Yli-Kerttula, M. Leirisalo-Repo, P. Laitio, and P. Toivanen. 1996. Demonstration of *Salmonella* DNA amplification in the synovial fluid in reactive arthritis. *Arthritis Rheum.* 39:S185 (Abstr.).
- Mertz, A. K. H., S. Ugrinovic, R. Lauster, P. Wu, M. Grolms, U. Böttcher, H. Appel, Z. Yin, E. Schiltz, S. Batsford, et al. 1998. Characterization of the synovial T cell response to various recombinant *Yersinia* antigens in *Yersinia enterocolitica*-triggered reactive arthritis. *Arthritis Rheum.* 41:315.
- Gaston, J. S., K. H. Deane, R. M. Jecock, and J. H. Pearce. 1996. Identification of two *Chlamydia trachomatis* antigens recognized by synovial fluid T cells from patients with *Chlamydia* induced reactive arthritis. *J. Rheumatol.* 23:130.
- Ugrinovic, S., A. Mertz, P. Wu, J. Braun, and J. Sieper. 1997. 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.* 159:5715.
- Struyk, L., G. E. Hawes, M. K. Chatila, F. C. Breedveld, J. T. Kurnick, and P. van den Elsen. 1995. T-cell receptors in rheumatoid arthritis. *Arthritis Rheum.* 5:577.
- Lim, A., A. Toubert, C. Pannetier, M. Dougados, D. Charron, P. Kourilsky, and J. Even. 1996. Spread of clonal T-cell expansions in rheumatoid arthritis patients. *Hum. Immunol.* 48:77.
- Kato, T., M. Kurokawa, K. Masuko-Hongo, H. Sasakawa, T. Sekine, S. Ueda, K. Yamamoto, and K. Nishioka. 1997. T cell clonality in synovial fluid of a patient with rheumatoid arthritis. *J. Immunol.* 159:5143.
- Verjans, G. M. G. M., U. N. A. Klaren, M. Leirisalo-Repo, J. H. Ringrose, H. Repo, A. Steinle, C. E. M. van Doornik, and T. E. W. Felkamp. 1996. Heterogeneity of rearranged T cell receptors V α and V β gene transcripts in synovial fluid T cells of HLA-B27 positive reactive arthritis patients. *Clin. Rheumatol.* 15:91.
- Allen, R. L., G. M. Gillespie, F. Hall, S. Edmonds, M. A. Hall, B. P. Wordsworth, A. J. McMichael, and P. Bowness. 1997. Multiple T cell expansions are found in the blood and synovial fluid of patients with reactive arthritis. *J. Rheumatol.* 24:1750.
- Duchmann, R., E. May, B. Ackermann, B. Goergen, K. H. Meyer zum Büschenfelde, and E. Märker-Hermann. 1996. HLA-B27-restricted cytotoxic T lymphocyte responses to arthritogenic enterobacteria of self-antigens are dominated by closely related TCRBV gene segments: a study in patients with reactive arthritis. *Scand. J. Immunol.* 43:101.
- May, E., R. Duchmann, B. Ackermann, K. H. Meyer zum Büschenfelde, and E. Märker-Hermann. 1996. TCRB junctional regions from HLA-B27-restricted T cells and HLA-B27 binding peptides display conserved hydrophobic profiles in the absence of primary sequence homology. *Int. Immunol.* 11:1815.
- Garboczi, D., P. Ghost, U. Utz, Q. R. Fan, W. E. Biddison, and D. C. Wiley. 1996. Structure of the complex between human T-cell receptor, viral peptide and HLA-A2. *Nature* 384:134.
- Maslanka, K., T. Piatek, J. Gorski, M. Yassai, and J. Gorski. 1995. Molecular analysis of T cell repertoires. Spectratypes generated by multiplex polymerase chain reaction and evaluated by radioactivity or fluorescence. *Hum. Immunol.* 44:28.
- Pannetier, C., M. Cochet, S. Darche, A. Casrouge, M. Zöller, and P. Kourilsky. 1993. The sizes of the CDR3 hypervariable regions of the murine T-cell receptor β chains vary as a function of the recombined germ-line segments. *Proc. Natl. Acad. Sci. USA* 90:4319.
- Even, J., A. Lim, I. Puisieux, L. Ferradini, P. Y. Dietrich, A. Toubert, T. Hercend, F. Triebel, C. Pannetier, and P. Kourilsky. 1995. T-cell repertoires in healthy and diseased human tissues analysed by T-cell receptor β -chain CDR3 size determination: evidence for oligoclonal expansions in tumours and inflammatory diseases. *Res. Immunol.* 146:65.
- Garcia, C. O., S. Paira, R. Burgos, J. Molina, J. F. Molina, C. Calvo, L. Vega, L. J. Lara, A. Garcia-Kutzbach, M. L. Cuellar, et al. 1996. Detection of *Salmonella* DNA in synovial membrane and synovial fluid from latin american patients with reactive arthritis using the polymerase chain reaction. *Arthritis Rheum.* 39:S185 (Abstr.).
- Garderet, L., N. Dulphy, C. Douay, N. Chalumeau, V. Schaeffer, M. T. Zilber, A. Lim, J. Even, N. Mooney, C. Gelin, et al. 1998. The umbilical cord blood $\alpha\beta$ T-cell repertoire: characteristics of a polyclonal and naive but completely formed repertoire. *Blood* 91:340.
- Puisieux, I., J. Even, C. Pannetier, F. Jottreau, M. Favrot, and P. Kourilsky. 1994. Oligoclonality of tumor-infiltrating lymphocytes from human melanomas. *J. Immunol.* 153:2807.
- Currier, J. R., M. Yassai, M. A. Robinson, and J. Gorski. 1996. Molecular defects in TCRBV genes preclude thymic selection and limit the expressed TCR repertoire. *J. Immunol.* 157:170.
- Scotet, E., J. David-Ameline, M. A. Peyrat, A. Moreau-Aubry, D. Pinczon, A. Lim, J. Even, G. Semana, J. M. Berthelot, R. Breathnach, et al. 1996. T cell response to Epstein-Barr virus transactivators in chronic rheumatoid arthritis. *J. Exp. Med.* 184:1791.
- Cerundolo, V., K. J. Alexander, K. Anderson, C. Lamb, P. Cresswell, A. McMichael, F. Gotch, and A. Townsend. 1990. Presentation of a viral antigen controlled by a gene in the major histocompatibility complex. *Nature* 345:449.
- Barnstable, C. J., W. F. Bodmer, G. Brown, G. Galfre, C. Milstein, A. F. Williams, and A. Ziegler. 1978. Production of monoclonal antibodies to group A erythrocytes, HLA and other human cell surface antigens. New tools for genetic analysis. *Cell* 14:9.
- Layet, C., P. LeBouteiller, C. N'Guyen, P. Mercier, F. Rosa, M. Fellous, D. Caillol, B. R. Jordan, and F. A. Lemonnier. 1984. Transformation of LMTK-cells with purified HLA class I gene. VI. Serological characterization of HLA-B7 and Aw24 molecules. *Hum. Immunol.* 11:31.
- Tamouza, R., F. Marzais, R. Krishnamoorthy, C. Besmond, C. Raffoux, and D. Charron. 1997. Rapid subtyping for HLA B27 by denaturing gradient gel electrophoresis (DGGE). In *HLA: Genetic Diversity of HLA, Functional and Medical Implication*, D. Charron, ed. EDK, Paris, p. 345.
- Hingorani, R., I. H. Choi, P. Akolkar, B. Gulwani-Akolkar, R. Pergolizzi, J. Silver, and P. K. Gregersen. 1993. Clonal predominance of T cell receptors within the CD8⁺CD45RO⁺ subset in normal human subjects. *J. Immunol.* 151:5762.
- Arden, B., S. P. Clark, D. Kabelitz, and T. W. Mak. 1995. Human T-cell receptor variable gene segment families. *Immunogenetics* 42:455.
- Wilbrink, B., I. M. van der Heijden, L. M. Schouls, J. D. A. van Embden, J. M. W. Hazes, F. C. Breedveld, and P. P. Tak. 1998. Detection of bacterial DNA in joint samples from patients with undifferentiated arthritis and reactive arthritis, using polymerase chain reaction with universal 16S ribosomal RNA primers. *Arthritis Rheum.* 41:535.
- Kim, G., N. Tanuma, T. Kojima, K. Kohyama, Y. Suzuki, Y. Kawazoe, and Y. Matsumoto. 1998. CDR3 size spectratyping and sequencing of spectratype-derived TCR of spinal cord T cells in autoimmune encephalomyelitis. *J. Immunol.* 160:509.
- Yang, Y., B. Charlton, A. Shimada, R. Dal Canto, and C. G. Fathman. 1996. Monoclonal B T cells identified in early NOD islet infiltrates. *Immunity* 4:189.
- Gonzales-Quintal, R., R. Baccañal, R. M. Pope, and A. N. Theofilopoulos. 1996. Identification of clonally expanded T cells in rheumatoid arthritis using a sequence enrichment nuclease assay. *J. Clin. Invest.* 97:1335.
- Oksenberg, J. R., M. A. Panzara, A. B. Begovich, D. Mitchell, H. A. Erlich, R. S. Murray, R. Shimonkevitz, M. Sherritt, J. Rothbard, C. C. A. Bernard, et al. 1993. Selection for T-cell receptor V β -D β -J β gene rearrangements with specificity for a myelin basic protein peptide in brain lesions of multiple sclerosis. *Nature* 362:68.
- Prisco, A., R. Troncone, G. Mazzarella, C. Gianfrani, S. Auricchio, J. Even, C. Tiberio, J. Guardiola, and P. De Berardinis. 1997. Identical T-cell receptor β chain rearrangements are present in T cells infiltrating the jejunal mucosa of untreated celiac patients. *Hum. Immunol.* 55:22.
- Probert, C. S., A. Chott, J. R. Turner, L. J. Saubermann, A. C. Stevens, K. Bodinaku, C. O. Elson, S. P. Balk, and R. S. Blumberg. 1996. Persistent clonal expansions of peripheral blood CD4⁺ lymphocytes in chronic inflammatory bowel disease. *J. Immunol.* 157:3183.
- Musette, P., D. Bequet, C. Delarbre, G. Gachelin, P. Kourilsky, and D. Dormont. 1996. Expansion of a recurrent V β 5.3⁺ T-cell population in newly diagnosed and untreated HLA-DR2 multiple sclerosis patients. *Proc. Natl. Acad. Sci. USA* 93:12461.
- Bowness, P., P. A. H. Moss, S. Rowland-Jones, J. I. Bell, and A. J. McMichael. 1993. Conservation of T cell receptor usage by HLA-B27-restricted influenza-specific cytotoxic T lymphocytes suggests a general pattern for antigen-specific major histocompatibility complex class I-restricted responses. *Eur. J. Immunol.* 23:1417.
- Barber, D. F., D. Lopez, and J. A. Lopez de Castro. 1995. T cell receptor diversity in alloreactive responses against HLA-B27 (B*2705) is limited by multiple-level restrictions in both α and β chains. *Eur. J. Immunol.* 25:2479.
- Maryanski, J. L., J. L. Casanova, K. Falk, H. Gournier, C. Jaulin, P. Kourilsky, F. A. Lemonnier, R. Lüthy, H. G. Rammensee, O. Röttschke, et al. 1997. The diversity of antigen-specific TCR repertoires reflects the relative complexity of epitopes recognized. *Hum. Immunol.* 54:117.
- Barber, D. F., D. Obeso, R. Garcia-Hoyo, A. Villadangos, and J. A. Lopez de Castro. 1996. T-cell receptor usage in alloreactivity against HLA-B*2703 reveals

- significant conservation of the antigenic structure of B*2705. *Tissue Antigens* 47:478.
47. Wang, F., T. Ono, A. M. Kalergis, W. Zhang, T. P. DiLorenzo, K. Lim, and S. Nathenson. 1998. On defining the rules for interactions between the T cell receptor and its ligand: a critical role for a specific amino acid residue of the T cell receptor β chain. *Proc. Natl. Acad. Sci. USA* 95:5217.
 48. Londei, M., G. F. Bottazzo, and M. Feldmann. 1985. Human T-cell clones from autoimmune thyroid glands: specific recognition of autologous thyroid cells. *Science* 228:85.
 49. Hill, A. B., S. P. Lee, J. S. Haurum, N. Murray, Q.-Y. Yao, M. Rowe, N. Signoret, A. B. Rickinson, and A. J. McMichael. 1995. Class I major histocompatibility complex-restricted cytotoxic T lymphocytes specific for Epstein-Barr virus (EBV) nuclear antigens fail to lyse the EBV-transformed B lymphoblastoid cell lines against which they were raised. *J. Exp. Med.* 181:2221.
 50. Pazmany, L., S. Rowland-Jones, S. Huet, A. Hill, J. Sutton, R. Murray, J. Brooks, and A. McMichael. 1992. Genetic modulation of antigen presentation by HLA-B27 molecules. *J. Exp. Med.* 175:361.
 51. Den Haan, J. M. M., L. M. Meadows, W. Wang, J. Pool, E. Blokland, T. L. Bishop, C. Reinhardus, J. Shabanowitz, R. Offringa, D. F. Hunt, et al. 1998. The minor histocompatibility antigen HA-1: a diallelic gene with a single amino acid polymorphism. *Science* 279:1054.
 52. Simmons, W. A., D. C. Roopenian, S. G. Summerfield, R. C. Jones, B. Galocha, G. J. Christianson, S. D. Maika, M. Zhou, S. J. Gaskell, R. S. Bordoli, et al. 1997. A new MHC locus that influences class I peptide presentation. *Immunity* 7:641.
 53. Brown, M. A., K. D. Pile, L. G. Kennedy, D. Campbell, L. Andrew, R. March, J. L. Shatford, D. E. Weeks, A. Calin, and B. P. Wordsworth. 1998. A genome-wide screen for susceptibility loci in ankylosing spondylitis. *Arthritis Rheum.* 41:588.