Borrelia burgdorferi Activates a T Helper Type 1–like T Cell Subset in Lyme Arthritis

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Summary

18 cloned T cell lines reactive with *Borrelia burgdorferi* proteins, all $CD3^+4^+8^-$TCR-α/β^+ and restricted by HLA class II proteins, were isolated from four patients with chronic Lyme arthritis. Analysis of these T cell clones indicated that the T cell response to the Lyme disease spirochete is not oligoclonally restricted; yet all produced the same pattern of lymphokines, resembling that of murine type 1 T helper cells, after antigen-specific or nonspecific stimulation. Therefore, a subset of human $CD4^+$ T cells, with a distinct profile of lymphokine secretion, is selectively activated by the pathogen inciting this chronic inflammatory disease.

Analysis of experimental animal models (1, 2), as well as more limited data on certain human inflammatory diseases (3, 4), has emphasized the involvement of T cells expressing a restricted set of TCR variable region genes in the pathogenesis of inflammatory disease. The assumption underlying this paradigm is that the selective clonal expansion of T lymphocytes leads to inflammatory disease. An important corollary to this theory is that the TCR expressed on the pathogenic subset of T cells provides a specific target for pharmacologic treatment of the inflammatory disease (reviewed in references 5 and 6). The ability of TCR-specific reagents, including mAbs binding a particular TCR Vβ region (2, 7) or nonstimulatory MHC-binding peptides (8, 9), to prevent the induction of a prototypic T cell–mediated autoimmune disease, murine experimental allergic encephalomyelitis, provides in vivo experimental support for this theory.

In contrast to many models, Lyme disease serves as a unique clinical entity for studying the molecular and cellular mechanisms underlying human chronic inflammatory disease, since the tick-borne spirochete, *Borrelia burgdorferi*, causing Lyme disease has been identified (10) and can be cultured in vitro (11). A subset of patients with Lyme disease develop a chronic inflammatory arthritis that resembles other forms of human inflammatory arthritis, including rheumatoid arthritis (12, 13). Characterization of cloned T cell lines reactive with the inciting spirochetal pathogen may therefore provide insight into the role that T cells play in the pathogenesis of Lyme disease and other inflammatory disorders. Accordingly, we have extensively analyzed the functional properties, including the pattern of lymphokine production of 18 *Borrelia*-reactive T cell clones isolated from four patients with Lyme arthritis.

Materials and Methods

B. burgdorferi Antigen and Purified Proteins. The CA12 isolate of *B. burgdorferi* was cultured in BSKII medium, harvested, and a sonicate was prepared for use in cellular proliferation assays as previously described (14). The proteins in the *B. burgdorferi* lysate were separated by SDS-PAGE and purified proteins, or groups of proteins, were prepared by electroelution from gel slices as previously described (14, 15). *Treponema phagedenis* biotype Reiter was obtained from Dr. James M. Miller (UCLA) and was grown in BBL spirolate broth (Becton Dickinson Microbiology Systems, Cockeysville, MD). A sonicate of this spirochete was prepared for use in cellular proliferation assays, as described for *B. burgdorferi*.

Preparation of Cloned T Cells and B Lymphoblastoid Cell Lines. The clinical histories of the four patients with chronic Lyme arthritis (CR, AP, GN and SS) used in this study have been previously described (14, 15). Cloned T cell lines reactive with *B. burgdorferi* antigens were isolated from PBMC or synovial fluid cells (SFC)[1] obtained from these four patients, as previously described (14, 15). In brief, 10^6 PBMC or SFC were stimulated with 50 μg/ml of spirochetal antigen in 24-well tissue culture plates. After 7–10 d, the growing T cell cultures were cloned by limiting dilution in the presence of a feeder mixture consisting of irradiated (4,000 rad) autologous PBMC, the lymphoblastoid cell line JY, and PHA (Wellcome, Beckenham, UK). Alloreactive and tetanus toxin–specific T cell clones were generated by incubation of 10^6 PBMC from healthy donors or Lyme arthritis patients with 2×10^5 JY cells or 1 μg/ml tetanus toxin (Dr. Bizzini, Institute Pasteur, Paris, France), respectively. After 7–10 d, growing T cells were cloned, as described above. 14–20 d after cloning, growing cultures were transferred, then re-stimulated every 14 d with the feeder cell mixture described above, and expanded with medium containing 20

[1] *Abbreviation used in this paper:* SFC, synovial fluid cells.

U/ml rIL-2. Autologous lymphoblastoid B cell lines were also prepared from each patient by in vitro infection with EBV (14). All cloned T cell lines and lymphoblastoid B cell lines were cultured in Yssel's medium (14) supplemented with 1% AB^+ human serum.

Analysis of the Cloned T Cells. Cellular proliferation was measured using a 72-h [^{3}H]thymidine assay, with autologous B lymphoblastoid cells as APC, as described previously (14). Whole *B. burgdorferi* sonicate, gel-purified spirochetal protein fractions, or lysates of *Escherichia coli* expressing recombinant proteins were used as antigen. To study the inhibitory effects of anti-HLA mAbs, the APC were incubated at room temperature with dilutions of anti-HLA-DR (Q5/13) or anti-HLA-DQ (SPVL3) mAbs for 20 min before the assay.

Analysis of cells for the expression of cell surface antigens was performed by flow cytometry using a FACScan® (Becton Dickinson & Co., Mountain View, CA), as previously described (14). mAbs against the following antigens indicated in parentheses were used: 6G4 (CD2), RIV-6 (CD4), SPV-T3b (CD3), SPV-T8 (CD8), BF1 (TCR-α/β; T Cell Sciences, Cambridge, MA), and TCRδ1 (TCR δ chain).

The lytic activity of the T cell clones was measured using a 4-h ^{51}Cr release assay as previously described (14). The target cells were pulsed with antigen for at least 4 h and were washed at least three times with medium before labeling with ^{51}Cr.

HLA Phenotyping. The HLA phenotype of the patients was determined by the Stanford University Hospital tissue typing laboratory. The HLA types of B lymphoblastoid cell lines used to identify the HLA elements involved in the recognition of spirochetal antigen were as follows: GNE, A2/10,B39/(5?15)(W6),DR6/w11 (w52),DQw1,w3; APT, DR3/w6 (w52), DQw1,w2; CAC—A1/x, B8/51, Cw7, DR2/5, DQw1/w3; CNR, DR2/2, w1, w1; SNS, DR2/w6(w52), DQw1/w3; RIG—DR2/4(w53), DQw1/w3; NPR, A2/10,B18/39,DRw11, DQw3; CAD DR7/7, DQw2/2; JY, A2/-,B7/-,DR4/6; and HSY, A1/w19,B8/13, DR3/7.

Lymphokine Assays. Lymphokine-containing supernatants were prepared from cloned T cells stimulated with spirochetal antigen presented by autologous APC or 10 μg/ml Con A for 12–18 h, as described (14). The amount of IL-2 in the culture supernatants was determined using the IL-2-dependent murine CTLL-2 cell line (16). The amount of IL-4 (17), IL-5 (18), granulocyte/macrophage (GM)-CSF (18), IFN-γ (19), and TNF-α (T cell Sciences) in the culture supernatants was determined using cytokine-specific ELISAs. Detectable levels of cytokines were not produced by any of the cloned T cell lines in the absence of stimulation. The values indicated are the average of assays performed on two aliquots harvested from a single supernatant. Similar results were obtained from independently prepared supernatants harvested on different days.

Preparation of Expressed Recombinant B. burgdorferi Proteins. The OspA and OspB genes were isolated from plasmids pTRH44 and pTRH46, respectively, which previously have been shown to encode these two spirochetal outer surface proteins (20, 21), by PCR amplification. Amplifications were performed using the following pairs of oligonucleotide primers: OspA1 GGCCCCGGGATGAAAAAATATTTATTGGGAATA (sense), OspA2 GGCCCCGGGTTATTATTTTAAAGCGTTTTTAATTTC (anti-sense), OspB3 (sense), GGCG*AA TTC*ATGAGATTATTAATAGGATTTGC OspB4 (antisense), GGCG*TC GAC*TTATTATTTTAAAGCGTTTTTAAGCTC. The PCR amplification, cloning into a procaryotic expression plasmid, and preparation of protein lysates was performed as previously described (22). The preparation of expressed recombinant HSP60 (15) and flagellar proteins (22) of *B. burgdorferi* have previously been described.

Immunoblotting. One-dimensional SDS-PAGE separation of proteins, transfer to nitrocellulose, and immunoblotting, using a 1:100 dilution of preabsorbed patient or control sera, was performed as previously described (15). Monoclonal anti-OspA (H5332), anti-OspB (H6831), and anti-flagellin (H9724) antibodies were used as 1:20 dilutions of culture supernatants supplied by Dr. A. Barbour (University of Texas, San Antonio). Control sera were prepared from blood obtained from healthy individuals, 23–45 yr of age, residing in Northern California, without a history of tick bite, skin rash consistent with erythema migrans, arthritis, neurologic disease, or other symptoms of Lyme disease.

TCR Variable Region Gene Utilization. RNA was extracted from 5×10^6 cells of a cloned T cell line and was reversed transcribed using oligo(dT). The resulting cDNA was PCR amplified using a pair of oligonucleotide primers; a 5'-sense oligonucleotide specific for each of the 18 Vα (23) or 20 Vβ (24) subfamilies, and a common Cα or Cβ 3'-antisense oligonucleotide. The Vα or Vβ gene segment expressed was identified by the presence of a 200–400-bp band on an agarose gel visualized by ethidium bromide staining. For each T cell clone analyzed, only one Vα- or Vβ-specific oligonucleotide primer produced a detectable PCR amplified DNA segment. Identical results were obtained with different aliquots of each sample. T cell clone GN12 was the only exception; both Vβ3- and Vβ5.1-specific primers produced amplified products of the appropriate size. Although we can not exclude the possibility that this T cell clone is not of monoclonal origin, the expression of a single Vα gene product makes it is more likely that this is due to amplification of the same Vβ gene segment by two different primers. To confirm that the TCR β chain variable region gene segment was correctly identified by this technique, the cDNA encoding this protein was isolated from one of the T cell clones. Analysis of the sequence of the cDNA prepared from GN30 indicated that its variable region was encoded by the Vβ6.2 gene segment, consistent with the PCR determination that its variable region was Vβ6 (data not shown).

Results

B. burgdorferi–specific CD4$^+$ T Cell Clones. The T cell clones described in this study have been shown to specifically recognize *B. burgdorferi* antigens; control antigens including tetanus toxin and purified protein derivative (PPD), as well as lysates of other bacteria, including *E. coli* and the related spirochete, *Treponema phagedenis*, failed to trigger proliferative responses (14, 15). The surface phenotype of all of these cloned T cells was CD4$^+$8$^-$TCR-α/β^+. Although CD4$^-$8$^+$ TCR-α/β^+ T cell clones were isolated, none of them reacted with spirochetal antigens (data not shown). However, two CD4$^+$ T cell clones, GN30 (14) and CR238, exhibited HLA-DR-restricted lysis of spirochetal antigen-pulsed B lymphoblastoid cells (Fig. 1). The cytotoxic activities of GN30 and CR238 were restricted by HLA-DRw11 and HLA-DR2, respectively, and were absent if the target cells had not been pulsed with spirochetal antigen.

Identification of Spirochetal Antigens Recognized by B. burgdorferi–specific T Cell Clones. The proteins in a lysate of *B. burgdorferi* were fractionated by SDS-PAGE, and individual proteins or groups of proteins of similar molecular mass were purified by electroelution from polyacrylamide gel slices. The ability of these purified proteins to induce a proliferative re-

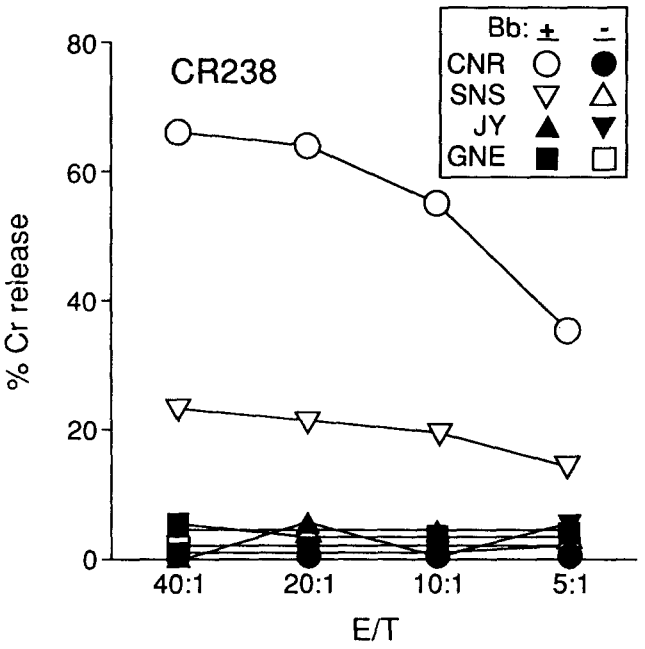

Figure 1. The cytolytic activity of T cell clone CR238. The lysis by CR238 of 10^3 CNR, SNS, JY, or GNE B lymphoblastoid cells pulsed with *B. burgdorferi* antigen or nonantigen-pulsed control cells, was measured in a ^{51}Cr release assay. Each data point represents the mean of triplicate determinations, and the SD for each data point is <10%.

Table 1. *Characterization of Borrelia-reactive T Cell Clones Isolated from Four Patients with Chronic Lyme Arthritis*

Clone	Vα	Vβ	HLA-RE	Bb Ag	Source
GN12	2	3/5.1	DRw11	50–55 kD	SFC
GN30	8	6	DRw11	41 kD	SFC
GN348	15	6	DRw11	Bb lysate*	PBMC
GN351	15	6	DRw11	Bb lysate*	PBMC
CR238	‡	9	DR2	Bb lysate*	PBMC
CR253	1	3	DR2	HSP60§	PBMC
CR329	8	4	DQw1	55–70 kD	PBMC
CR378	6	5.1	DQ§	OspA§	PBMC
CR380	‡	13.1	DR2	OspA§	PBMC
SS7	8	9	DR2	55–70 kD	PBMC
SS10	10	9	DR2	No data	PBMC
SS13	8	9	DR2	55–70 kD	PBMC
SS27	8	9	DR2	55–70 kD	PBMC
AP56	8	20		Bb lysate*	PBMC
AP74	5	2	DR6	30 kD	PBMC
AP75	5	1	DR3	OspA§	PBMC
AP97	3	6		42–55 kD	PBMC
AP141	‡	‡	DR6	OspA§	PBMC

The TCR variable region genes expressed by the cloned T cells, the spirochetal antigens recognized, as well as the HLA class II molecules involved in antigen recognition were identified as described in Materials and Methods.
* Reactive with *Borrelia* lysate, but not with purified protein.
‡ Indicates not amplified using primers for Vα1-18 or Vβ1-20.
§ Reactive with expressed recombinant protein.

sponse by the spirochete-reactive T cell clones was measured. The majority of the cloned T cells recognized one of the following purified spirochetal proteins or protein fractions: 30, 41, 42–55, 50–55, and 55–70 kD (Table 1). However, four T cell clones proliferated in response to an antigen in the *Borrelia* sonicate, but not in response to any of the purified proteins mentioned above. These four clones may recognize antigens whose molecular mass is >70 kD, which are not efficiently isolated using our procedure. These results reveal that there is a diverse array of spirochetal proteins recognized by this panel of cloned human T cells.

When possible, the antigenic specificity of a T cell clone was confirmed by measuring its proliferative activity in response to stimulation with recombinant spirochetal proteins expressed in *E. coli*. Toward this end, procaryotic expression plasmids encoding the *B. burgdorferi* outer surface proteins A and B (OspA and OspB), flagellin, and HSP60 were prepared. The identity of the expressed recombinant OspA and OspB was confirmed by immunoblot analysis using mAbs specific for each protein (Fig. 2). T cell clones AP75, AP141, CR378, and CR380 were found to specifically recognize recombinant OspA (Fig. 3). Although AP74 proliferated in response to a protein fraction with a molecular mass of 30–33 kD, which contains both outer surface proteins, it did not recognize recombinant OspA or OspB (data not shown). T cell clone CR253 has previously been shown to recognize an epitope located between amino acids 260–274 of the recombinant spirochetal 60-kD heat shock protein expressed in *E. coli* (15). None of the other T cell clones was found to recognize any of the available recombinant spirochetal proteins. Taken together, these results reveal that there is a diverse array of spirochetal proteins recognized by this panel of cloned human T cells.

Diversity in the HLA Restriction Elements and TCR Variable Region Gene Usage by the Spirochete-reactive T Cell Clones. The HLA restriction elements involved in the recognition of *B. burgdorferi* antigens by these cloned T cell lines are shown in Table 1. They were determined by measurement of the proliferative responses of the T cell clones to spirochetal antigens either presented by autologous APC in the presence of mAbs against HLA class II proteins, or using APCs that express different HLA class II molecules. As shown in Table 1, different HLA class II molecules are involved in the recognition of spirochetal antigens by the T cell clones isolated from the four patients. It is noteworthy that the proliferative responses of different T cell clones isolated from the same individual were restricted by distinct HLA-DR or DQ specificities. Furthermore, T cell clones AP75 and AP141, isolated from the same patient, recognized the same spirochetal antigen in the context of different HLA class II molecules.

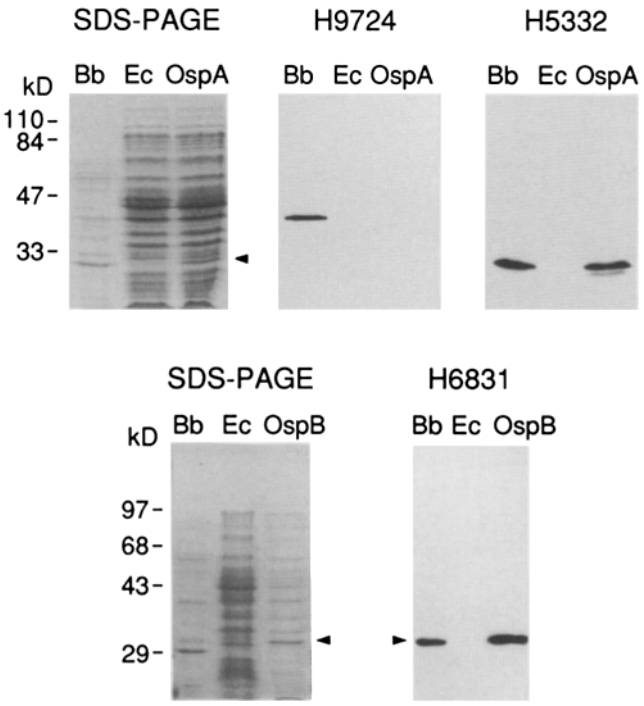

Figure 2. Immunoblot analysis of antibody reactivity with expressed recombinant *B. burgdorferi* OspA and OspB. A coomassie blue–stained 10% SDS-PAGE gel of protein lysates prepared from *E. coli* transformed with a control plasmid (Ec), or with the plasmids encoding OspA and OspB; and *B. burgdorferi* (Bb) is shown. Immunoblotting was performed with murine monoclonal anti-OspA (H5332), anti-OspB (H6831), and anti-flagellin (H9724) antibodies. Arrowheads indicate the position of the expressed recombinant outer surface proteins. Molecular mass markers (in kD) are indicated to the left.

TCR Vα and Vβ gene segments expressed by the *Borrelia*-reactive T cell clones were examined by PCR amplification using Vα- and Vβ-specific oligonucleotide primers. This allowed us to determine if there was restricted TCR V region gene usage among the T cells responding to spirochetal antigens. This analysis indicates that a wide array of Vα and Vβ gene segments were used to form the TCRs of the *Borrelia*-reactive T cell clones (Fig. 4, Table 1). For three of the four patients examined, the TCR of different T cell clones, though isolated from the same patient, utilized different V region gene segments. T cell clones obtained from patient SS were the exception; three of the four T cell clones appeared to be daughter colonies derived from the same parental T cell.

Spirochete-reactive T Cells Produce a Specific Pattern of Lymphokines. To analyze their profile of cytokine production, the *B. burgdorferi*–reactive T cell clones were stimulated with Con A or spirochetal antigen in the presence of APC. Despite the fact that they recognized different spirochetal antigens and expressed distinct TCRs, all of the cloned *Borrelia*-reactive T cell lines had a pattern of lymphokine secretion resembling that of murine Th1 cells. All T cell clones produced IL-2 (not shown), GM-CSF, TNF-α, and IFN-γ upon stimulation with spirochetal antigen or Con A (Table 2). However, either mode of activation repeatedly failed to induce the production of detectable levels of IL-3 (not shown), IL-4, or IL-5, indicating that this lymphokine production profile is an intrinsic property of these T cell clones. To determine whether the Th1-like pattern of lymphokine secretion was specific for the spirochete-reactive T cell clones, we analyzed the cytokine production levels by tetanus toxoid–specific T cell clones derived from Lyme arthritis patient CR. Upon

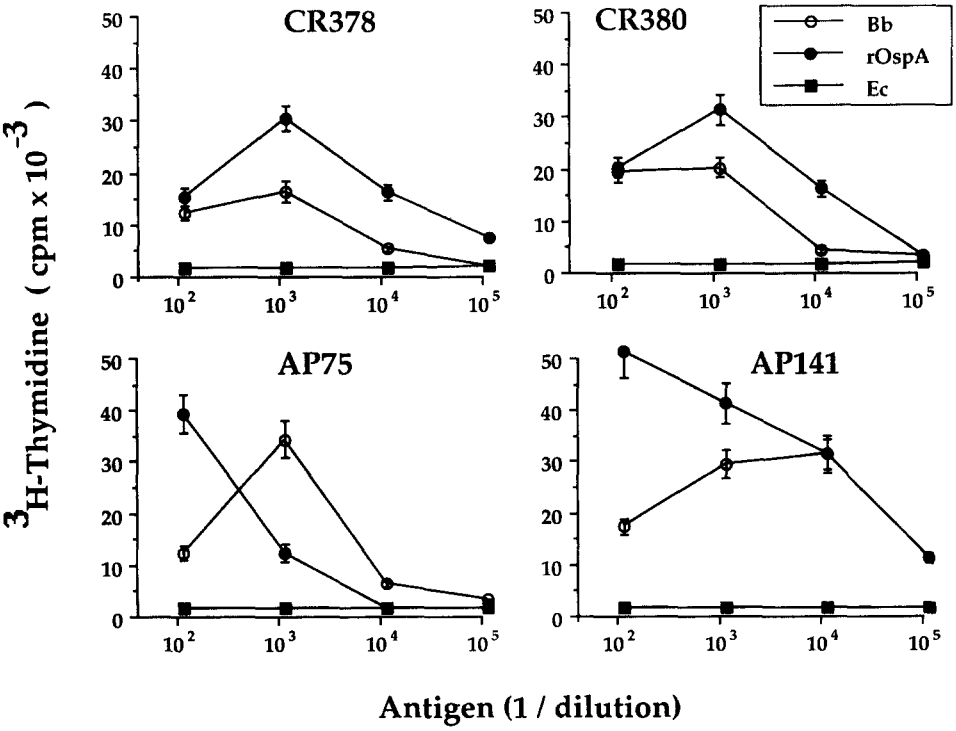

Figure 3. The proliferative responses of T cell clones AP75, AP141, CR378, and CR380 to serial dilutions of protein lysates of *B. burgdorferi* (Bb), and *E. coli* transformed with control plasmid (Ec) or with the plasmid encoding spirochetal OspA (OspA). Each data point represents the mean of triplicate determinations, and the SD for each data point is <10%.

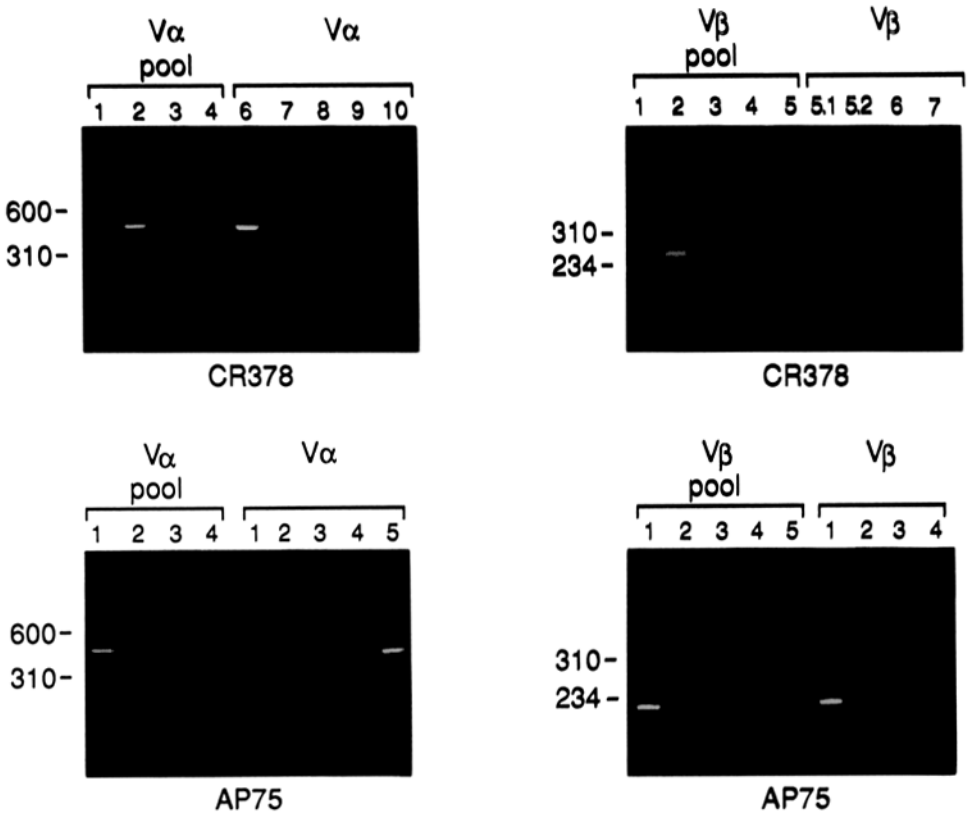

Figure 4. Identification of TCR variable region genes expressed by T cell clones CR378 and AP75. cDNA prepared from T cell clones CR378 and AP75 was amplified by PCR using TCR Vα- and Vβ-specific oligonucleotide primers. PCR amplifications were initially performed using pooled primers, each pool containing four or five different Vα- or Vβ-specific 5′-sense oligonucleotides and a single Cβ antisense oligonucleotide (pools *1–4*). To identify the TCR variable region gene segments utilized by the T cell clones, PCR amplifications were performed using the Vα- or Vβ-specific oligonucleotide primers found in the positive pool. The expressed Vα or Vβ gene segment was identified by the presence of a 200–400-bp band on the agarose gel visualized by ethidium bromide staining.

Table 2. *Lymphokine Production by Borrelia-reactive T Cell*

	IL-4		IL-5		IFN-γ		GM-CSF		TNF-α	
Clone	Bb	Con A	Bb	Con A	Bb	Con A	Bb	Con A	Bb	Con A
	pg/ml		*pg/ml*		*pg/ml*		*ng/ml*		*pg/ml*	
CR238	<50	<50	<50	<50	12.4	17.3	1.2	4.3	260	5,675
CR253	<50	<50	<50	<50	<0.4	5.7	2.0	4.6	630	1,535
CR329	<50	<50	<50	<50	3.6	20.4	2.1	7.7	495	3,460
CR378	<50	<50	<50	<50	4.2	12.3	6.3	14.4	4,425	4,155
CR380	<50	<50	<50	<50	7.1	25.5	1.9	18.7	5,085	5,770
AP56	<50	<50	<50	<50	4.7	15.9	3.1	7.8	ND	1,810
AP74	<50	<50	<50	<50	49.5	52.7	4.3	5.0	2,565	2,780
AP75	<50	<50	<50	<50	5.0	12.5	2.1	5.6	20	3,040
AP97	<50	<50	<50	<50	2.0	3.3	ND	ND	20	500
AP141	<50	<50	<50	<50	42.6	>>60	3.6	3.1	1,750	1,955
GN12	<50	<50	<50	<50	8.0	>60	1.0	>60	2,217	>3,000
GN30	<50	<50	<50	<50	14.2	>>60	2.2	>60	>3,000	>3,000
GN348	<50	<50	<50	<50	1.0	>60	0.5	12.5	606	2,302
GN351	<50	<50	<50	<50	2.0	50	0.6	14.0	986	2,143

Lymphokine-containing supernatants were prepared from cloned T cells stimulated with spirochetal antigen presented by autologous APC or 10 μg/ml Con A for 12–18 h. Lymphokine production was quantitated using the IL-2-dependent murine CTLL-2 cell line for IL-2, or cytokine-specific ELISAs for IL-4, IL-5, GM-CSF, IFN-γ, and TNF-α, as described in Materials and Methods. None of the cloned T cell lines produced any of the cytokines in the absence of stimulation. Each data point is the average of duplicate determinations performed on single supernatant. Similar results were obtained using three independently prepared supernatants.

activation, these T cell clones were found to produce IL-4 and IL-5 (Table 3). In addition, identical procedures have been used to generate cloned T cell lines from healthy individuals reactive with tetanus toxin or alloantigen. These T cell clones were found to produce both IL-4 and IFN-γ upon activation at levels ranging from 0.1–9 ng/ml and 1–60 ng/ml, respectively (Table 3). None of these T cell clones failed to produce either of these cytokines.

Discussion

In this study we describe the functional characterization of cloned T cell lines specific for *B. burgdorferi* isolated from the peripheral blood and synovial fluid of patients with chronic Lyme arthritis. In contrast to results obtained from experimental animal models of inflammatory diseases, where it was found that the pathogenic T cells were oligoclonally derived (1, 2), our data indicate that there is diversity in T cells responding to spirochetal antigens in Lyme arthritis patients. This diversity is reflected in the many spirochetal antigens recognized by *Borrelia*-reactive T cell clones, their utilization of TCR variable region gene segments, as well as the multiple HLA class II alleles involved in the recognition of spirochetal antigens. Detailed analysis of the cloned $CD4^+$ T cells reactive with myelin basic protein (25) and the acetylcholine receptor (26) isolated from patients with multiple sclerosis or myasthenia gravis, respectively, have also indicated that there is heterogeneity in the responding T cells. The increased heterogeneity noted in the human T cell response to antigen, relative to that of the inbred animal strains, may partially result from the increased number of distinct HLA class II proteins found on human cells. There are six, and in certain haplotypes eight, distinct HLA class II antigens expressed on human APC, while the APC of inbred mouse or rat strains have at most two distinct alleles (27). The demonstration that multiple distinct HLA class II proteins can present spirochetal antigens to human T cells is consistent with the absence of an immunogenetic association with susceptibility to development of arthritis among patients with Lyme disease (28). The chronicity of antigen exposure may also be another factor contributing to the diversity of the human T cell response. Experimental animals are often evaluated a short time after disease initiation, while human diseases may become clinically apparent many months to years after the inciting event.

Despite the lack of clonal restriction and their recognition of distinct antigens, all of the spirochete-reactive T cell clones exhibited the same restricted profile of lymphokine production. $CD4^+$ T cells in the mouse have been divided into two distinct subsets, based on the pattern of lymphokines secreted (reviewed in reference 29). One subset, referred to as Th1 cells, produces IL-2 and IFN-γ upon activation and mediates delayed type hypersensitivity. A second subset, type 2 helper T cells (Th2) secretes IL-4 and IL-5, and supports antibody production by B cells. Initially, it did not appear that human $CD4^+$ T cells could be separated into Th1 and Th2 cell types. Two studies, examining large panels of cloned human T cells isolated from peripheral blood and lymphoid organs

Table 3. *Lymphokine Production by Human T Cell Clones Reactive with B. burgdorferi Antigens, Tetanus Toxoid, Alloantigen, or of Undetermined Antigenic Specificity.*

Donor		No. of clones	Specificity	IFN-γ	IL-4	IL-5
				ng/ml	*pg/ml*	*pg/ml*
Control	1	5	Alloreactive	3.4 ± 1 (2–5)	260 ± 195 (200–550)	–
	2	23	Unknown	5.3 ± 3 (1–10)	1,255 ± 1,515 (<50–9,600)	–
	3	5	Tetanus toxoid	54 ± 45 (4–124)	815 ± 850 (200–2,500)	–
	4	5	Tetanus toxoid	25 ± 24 (6–68)	3,250 ± 3,605 (220–7,800)	627 ± 667 (149–1,742)
Lyme arthritis	CR	5	*B. burdorferi*	16 ± 7 (5.7–25)	<50	<50
	CR	5	Tetanus toxoid	–	1,825 ± 1,668 (148–4,198)	995 ± 831 (129–2,374)

Lymphokine-containing supernatants were prepared from cloned T cells stimulated with 10 μg/ml Con A for 12–18 h. The amount of IL-4, IL-5, or IFN-γ in the supernatants was determined using cytokine-specific ELISAs as described in Table 2. The numbers indicate the mean and SD for the amount of each lymphokine produced by T cell clones prepared from the indicated donor. The range of values obtained are indicated by the numbers in parenthesis. A dash indicates data that are not available.

of normal individuals without allergic or chronic inflammatory diseases, failed to demonstrate clonal segregation in lymphokine gene expression similar to that of murine T cell clones (30, 31). The majority of mature human T cells produced effector lymphokines characteristic of both Th1 and Th2 cell subsets. In addition, it had been demonstrated that after activation, human T lymphocytes undergo a functional differentiation, acquiring the ability to produce effector cytokines characteristic of both Th1 and Th2 cells (32, 33).

The significance of our finding that the *Borrelia*-reactive T cell clones produce a Th1-like pattern of cytokine production is underscored by the above data. Presumably, the spirochete-reactive T cell clones represent T cells that have been repetitively stimulated by antigen in vivo, a process that may be required before the T cells acquire a restricted profile of lymphokine secretion. In support of this, allergen-specific human T cell clones isolated from atopic patients, apparently repeatedly exposed to the allergen, have also been shown to exhibit a restricted profile of lymphokine secretion (34; H. Yssel, et al., manuscript submitted for publication).

It should also be emphasized that a substantial number of control experiments were performed to ensure that these results are not due to a bias in the methodology used to generate the T cell clones. Identical procedures have been used to produce cloned T cell lines reactive with tetanus toxin, from the Lyme arthritis patients as well as healthy individuals, which produce IL-4, IL-5, and IFN-γ, as well as other cytokines. In addition, allergen-specific T cell clones isolated from atopic patients using the same methodology produced exceedingly high levels of IL-4 and IL-5 upon activation (H. Yssel et al., manuscript submitted for publication). Therefore, the finding that a relatively large number of *Borrelia*-reactive cloned T cells (recognizing a diverse array of spirochetal antigens and obtained from four different Lyme arthritis patients) were all unable to secrete IL-4 or IL-5 suggests that this specific pattern of lymphokine production may be spirochete induced. The mechanism through which the spirochete selectively activates Th1-like T cells is unknown at present. The fact that the spirochete-reactive T cells express different TCRs and recognize different antigens indicates that their pattern of lymphokine production is not the result of selection in the thymus, nor is it determined by a particular epitope.

These findings demonstrate that a human $CD4^+$ T cell subset with a specific pattern of lymphokine secretion exists, and can be selectively activated by a pathogen inciting a chronic inflammatory disease. The selective activation of this T cell subset is likely to play a role in the pathogenesis of chronic inflammation in this and other inflammatory diseases. The importance of the production of distinct lymphokines through selective activation of $CD4^+$ T cell subsets in the immune response to infectious agents has been demonstrated in experimental murine leishmaniasis (35). Although it may be advantageous to mount a Th1-like T cell response in controlling infection by an intracellular pathogen such as *L. major* or a virus, this seems not to be effective against an extracellular pathogen like *B. burgdorferi* (reviewed in reference 29). It has previously been demonstrated in mice that protection against *B. burgdorferi* infection requires the generation of complement-fixing antibodies directed against certain spirochetal antigens (36). Therefore, we propose that the selective activation of Th1-like T cells, which do not efficiently stimulate antibody production by B cells, may be a mechanism by which the spirochete can avoid elimination by the host immune response. The continuous production of Th1-like cytokines by *Borrelia*-reactive T cells, unable to eliminate the pathogen, is likely to induce and maintain a state of inflammation within the target tissue. TNF-α has been shown to stimulate cartilage and bone resorption (reviewed in reference 37), while IFN-γ and GM-CSF induce expression of HLA class II molecules on human monocytes (38). However, additional studies will have to be performed to directly demonstrate that the selective activation of Th1-like T cells by spirochetal antigen plays a role in the pathogenesis of human Lyme disease.

It is likely that the arthritic potential of *B. burgdorferi* in humans results from both its ability to selectively activate Th1-like T cells, as well as its tropism for articular tissue. With time, an infected individual will develop a cellular immune response against a number of different spirochetal antigens. The presence of spirochetal antigens in articular tissue, one or more of which may have affinity for connective tissue components (39), activates Th1-like T cells leading to an inflammatory synovitis. Each of the $CD4^+$ T cell subsets produce factors that inhibit the growth or differentiation of the other (reviewed in reference 40). Therefore, the selective activation of Th1 cells by spirochetal antigen, in the absence of the regulatory signals from Th2 cells, contributes to the development of synovial inflammation. Although this model may explain some of the early events in the pathogenesis of inflammatory synovitis, it does not explain why a minority of individuals infected with with *B. burgdorferi* will develop a chronic arthritis (12, 13). Other factors such as the HLA haplotype (28) or the status of the immune system (41, 42) were thought to determine whether the immune response of an infected individual will control the infection with little inflammatory tissue injury, or contribute to the development of a chronic destructive arthritis. We propose that the probability that an individual will develop an inflammatory or allergic disease in response to exposure to a pathogen or allergen may be determined by the type of $CD4^+$ T cell that dominates the response to the antigen. Immune-mediated disease may develop if the cellular response becomes pathologically fixed in a Th1 or Th2 mode, while a balanced response, or a response dominated by the appropriate subset, will be protective and not have pathologic consequences.

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