Selective Expansion of Specific T Cell Receptors in the Inflamed Colon of Crohn's Disease

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Abstract

To identify disease-specific T cell changes that occur in Crohn's disease (CD), the T cell receptor BV repertoires of lamina propria lymphocytes (LPL) isolated from both the inflamed and "disease-inactive" colons of seven CD patients were compared by the quantitative PCR and DNA sequence analysis. It was observed that the BV repertoires of LPL isolated from the disease-active and disease-inactive parts of the colon from the same individual were very different. Furthermore, nearly all of the differences occurred in $CD4^+$ LPL, with very few differences in the $CD8^+$ population of LPL. Although the pattern of BV segments that was increased in disease-active tissue relative to disease-inactive tissue was different for all seven CD patients, there were several BV segments that increased uniformly in the disease-active tissue of all seven individuals. CDR3 length analysis and DNA sequencing of these BV segments revealed that in six of the seven CD patients there was a striking degree of oligoclonality that was absent from disease-inactive tissue of the same individual. These observations suggest that at least some of the inflammation in CD is the result of responses by $CD4^+$ T cells to specific antigens. The isolation of such inflammation-specific $CD4^+$ T cells may make it possible to identify the antigens that are responsible for the inflammatory process in CD and provide a better understanding of its pathogenesis. (*J. Clin. Invest.* 1996. 98:1344–1354.) Key words: Crohn's disease • $CD4^+$ lymphocytes • inflammatory bowel disease • T cell receptor • lamina propria lymphocytes

Introduction

Crohn's disease (CD)[1] is a chronic inflammatory disease of the intestine (1) of unknown etiology. Various hypotheses have been proposed to explain the etiology and pathogenesis of CD, ranging from exposure to an infective agent to aberrant T cell regulation (2–9). Despite this lack of basic knowledge regarding the initiating event in CD, there is reason to believe that immunological mechanisms may explain the chronicity and relapse of CD (10, 11). CD is frequently accompanied by alterations in the humoral and/or cellular immune system (12, 13), and there is an increasing amount of evidence that at least part of the inflammation in CD is T cell mediated. The increased number of T cells expressing the activation markers 4F2, transferrin receptor, and IL-2 receptor in the intestinal lesions of CD suggests that T cells are responding to antigenic stimulation and contribute to the local inflammatory process (14–18). This is supported by other studies demonstrating higher levels of IL-2 mRNA and an increased frequency of cells secreting IL-2 and IFN-γ in actively inflamed CD tissue compared to sites of inactive disease (19–23). Finally, recent studies suggest that CD is accompanied by changes in the T cell receptor (TCR) repertoire of LPL that occurs predominantly in the $CD4^+$ population (24).

Despite all of these studies, the nature of the stimulus that leads to activation of T cells in CD still remains undefined. Evidence for a restricted T cell response occurring in the lamina propria of CD patients would have important implications for the pathogenesis and treatment of the disease. Accordingly, we have analyzed the TCR repertoires of LPL from CD patients using several molecular techniques to determine whether there is any evidence of a restricted T cell response that would be suggestive of antigen-specific stimulation.

Methods

Patient specimens. Intestinal surgical resections were obtained from seven patients with CD. The diagnoses of CD were based on clinical, endoscopic, histopathologic, and radiologic criteria (25). All specimens were from the colon and represented the first resection for all individuals. In all cases, tissue that was both grossly and histologically involved, as well as tissue that was histologically uninvolved, as determined independently by a pathologist, was used for analysis. The age, activity, and duration of disease, surgical indications, and medication of all seven patients are shown in Table I.

Cell isolation. Mononuclear cells from lamina propria were isolated by the method of Bull and Bookman (26), modified as previously described (24). Briefly, colonic mucosa was cut into small pieces and incubated with DTT, followed by EDTA treatment. The EDTA treatment was repeated two times or until crypt epithelium was no longer seen under phase microscopy. The remaining mucosal tissue was then incubated in the presence of collagenase for 6–12 h, resulting in the dissociation of the lamina propria and the release of lymphocytes. Cell viability was > 90%. $CD4^+$ and $CD8^+$ cells were isolated using immunomagnetic beads conjugated with anti-CD4 and anti-CD8 mAbs as described previously, and were > 98% pure (27, 28).

Amplification and quantitation of BV-specific RNA by PCR. BV repertoire analysis was performed by quantitative PCR (qPCR) using a modified procedure as previously described by us (28). Briefly, total cel-

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1. *Abbreviations used in this paper:* CD, Crohn's disease; LPL, lamina propria lymphocytes; qPCR, quantitative PCR; TCR, T cell receptor.

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Table I. Characteristics of CD Patients

Patients	Age	Indications for surgery	Disease activity	Disease duration	Medication
				yr	
A	14	Partial obstruction	Moderate	3	None
B	20	Partial obstruction	Moderate	5	Prednisone
C	24	Intractable disease	Moderate	13	6 MP
D	19	Intractable disease	Moderate	2	5 ASA
E	49	Fistula	Moderate	30	5 ASA
F	15	Partial obstruction	Mild	2	None
G	35	Intractable disease	Moderate	8	None

lular RNA was isolated from positively selected $CD4^+$ and $CD8^+$ cells with RNAzol B (Biotecx Laboratories, Houston, TX) and cDNA was synthesized using reverse BC and AC primers as described (28). BV-specific cDNA was then amplified with BV-specific primers and a ^{32}P-labeled BC nested primer as previously described (28). As controls, a pair of 5′ AC forward and 3′ AC reverse primers was included in each reaction to specifically amplify a portion of AC RNA. The amplified products were electrophoresed on 8% polyacrylamide gels, dried, and exposed to x-ray film. Using the autoradiograms for alignment, the amplified AC and BV bands were excised from the gel and the amount of radioactivity incorporated was determined by liquid scintillation counting. This method of quantitation is as accurate as when a phosphorimager is used for quantitation (data not shown). To correct for differences caused by pipetting errors and efficiency of amplification in each well, the cpm in each BV band was normalized to the AC band. The relative amount of each BV was expressed as a percent of the total of the cpm incorporated in all 22 BV reactions after normalizing for AC cpm. Under these conditions, the amount of radioactivity incorporated into the AC and BV bands is roughly proportional to the amount of input RNA (28). Furthermore, this modified procedure for qPCR analysis is highly reproducible and consistently gives $< 15\%$ variation when the same sample of RNA is repeatedly analyzed (28, 29).

Determination of oligoclonality. Assessment of oligoclonality within individual BV segments was performed as described (30). Total RNA (1–5 μg) from selected $CD4^+$ T cells was converted to cDNA and amplified by qPCR as described above using BV-specific primers. An aliquot (1–2 μl) of this product then served as the template for a second round of PCR using conditions that were identical to those described above, except that primer concentrations were reduced to 3 pmol per 100 μl reaction mixture. After 15 cycles, 2–4 μl of the radioactive product was loaded on a standard 6% acrylamide sequencing gel. After overnight exposure to x-ray film, BV segments of various CDR3 lengths were visualized as a series of bands 3 bp apart and quantitated on a phosphorimager (30).

Cloning and sequencing PCR products. The PCR products of those BV segments that appeared to be oligoclonal were purified using Magic PCR preps DNA purification system (Promega, Madison, WI) and were

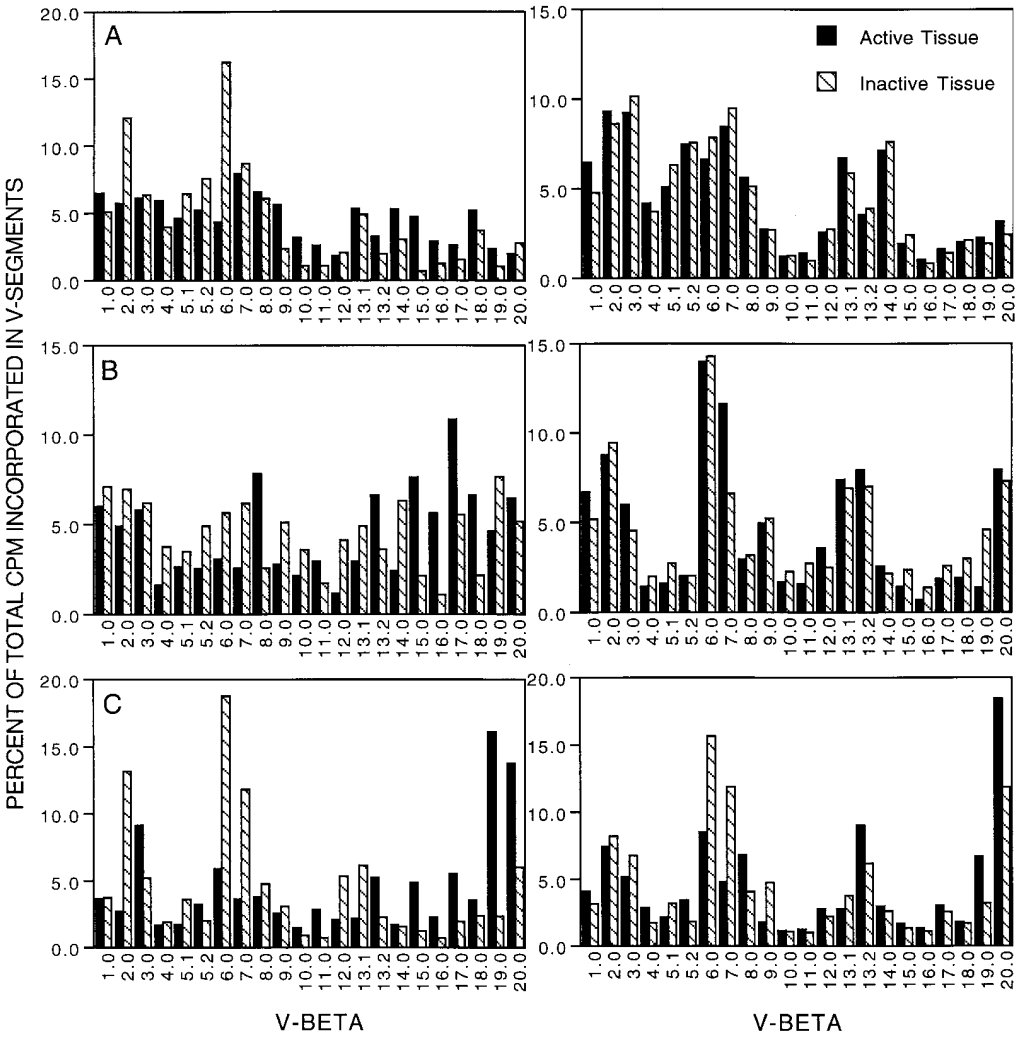

Figure 1. Comparisons of LPL TCRBV repertoires of disease-active and disease-inactive tissue by qPCR in three patients. In each case, the LPL repertoires within the same individual are compared for both $CD4^+$ (*left panel*) and $CD8^+$ (*right panel*) cells.

cloned into the TA cloning vector (Novagen, Madison, WI). 30–50 colonies containing inserts were randomly selected, and the inserts were sequenced using an automated sequencer (Applied Biosystems, Inc., Foster City, CA). The sequences were analyzed using the Mac-Vector sequence analysis program (IBI-A Kodak Co., New Haven, CT).

Results

Comparisons of TCR repertoires of LPL from disease-active and "disease-inactive" tissue in CD. Previous studies from this laboratory in which the TCR repertoires of PBL and LPL within the same individual were compared had suggested that CD is accompanied by an alteration in the TCR repertoire of LPL that occurs predominantly in $CD4^+$ cells (24). To confirm this observation in a more definitive way, the TCR repertoires of LPL from both the disease-active and disease-inactive portions of the colons of seven CD patients were compared to each other. Because of the limited number of T cells that could be obtained from these tissues, and the fact that mAb specific for many TCR BV families are not yet available, semiquantitative PCR using BV-specific oligonucleotide was the only method by which a comprehensive analysis of the TCR repertoire for both $CD4^+$ and $CD8^+$ cells could be accomplished. A comparison of the BV-specific TCR repertoires of LPL from the disease-active and adjacent disease-inactive portions of the colons of three of the seven individuals is shown in Fig. 1; the relative increase or decrease in frequency of the various BV segments in disease-active tissue vs. disease-inactive tissue is shown in schematic form for all seven patients in Table II. As can be seen, the TCR repertoires of LPL isolated from the disease-active and disease-inactive portions of the colons differed extensively for all seven patients. Indeed, in all seven patients, there were many instances in which there were two-fold or greater differences in the levels of specific BV segments between the disease-active and disease-inactive portions of the colon (indicated by double and triple arrows, Table II). These extensive differences in repertoire were observed primarily in the $CD4^+$ population. Especially striking were the relative increases in BV11, 15, and 17 in the disease-active tissue of all seven patients, and in BV16 in six of the seven patients; in many cases, the increases were two- and three-fold. In addition, all seven patients showed significant (> 50%) relative increases in the levels of BV13S2. In contrast, there was a striking similarity in the repertoires of $CD8^+$ cells, and even when there were differences between the disease-active and disease-inactive tissues, they did not parallel those observed in the $CD4^+$ population.

These differences between the repertoires of LPL from disease-active and disease-inactive colonic tissue, and the restriction of these differences, for the most part, to $CD4^+$ LPL cannot be attributed to either experimental error or sampling location; when a similar analysis and comparison was performed for LPL isolated from adjacent colonic sections derived from the same active lesion, no such extensive differences were observed in either the $CD4^+$ or $CD8^+$ populations (Table III).

Determination of oligoclonality. To determine whether the increased levels in some of the BV segments in the disease-active tissue might be caused by expansion or activation of a restricted number of T cells, a method that we and others have

Table II. Repertoire Comparisons of LPL Isolated from Inflamed and Normal Colon of the Same Individual for Seven Individuals

	CD4							CD8						
BV	A	B	C	D	E	F	G	A	B	C	D	E	F	G
1	—	—	—	↓	—	—	—	—	—	—	—	—	—	—
2	↓↓	—	↓↓↓	↓	—	—	↑↑↑	—	—	—	—	↓	—	—
3	—	—	↑	—	—	—	↑↑	—	—	—	—	—	—	—
4	—	↓↓	—	—	—	—	—	—	—	↑	—	—	—	—
5.1	—	—	↓↓	—	↑↑↑	↑	—	—	↓	—	—	↑↑	—	—
5.2	—	↓	↑	—	—	—	—	—	—	↑	—	—	—	↑↑
6	↓↓↓	↓	↓↓↓	—	—	—	↓	—	—	↓	—	—	—	—
7	—	↓↓	↓↓↓	—	—	↓	↓	—	↑	↓↓	—	↓	—	—
8	—	↑↑↑	—	—	↑↑	—	↓	—	—	↑	—	—	—	—
9	↑↑	↓	—	—	—	—	—	—	—	↓↓	↑	—	—	—
10	↑↑	↓	↑	—	↓↓↓	↑↑	↑↑	—	—	—	—	—	—	↑
11	↑↑	↑	↑↑↑	↑	↑	↑↑	↑↑↑	—	↓	—	—	—	—	—
12	—	↓↓↓	↓↓	—	↑↑↑	↑	—	—	—	—	—	—	—	—
13S1	—	↓	↓↓	—	—	—	↓↓	—	—	—	↓	—	—	—
13S2	↑	↑	↑↑	↑	↑	↑	↑	—	—	—	—	↑	—	↑↑↑
14	↑	↓	—	—	—	↓↓↓	—	—	—	—	—	—	—	↑↑↑
15	↑↑↑	↑↑↑	↑↑↑	↑	↑	↑	↑	—	↓	—	↑	—	—	—
16	↑↑	↑↑↑	↑↑↑	↑	↑	↑↑	—	—	↓	—	↓	—	—	↑
17	↑	↑	↑↑	↑	↑	↑↑	↑	—	—	—	—	—	—	—
18	—	↑↑↑	—	↓↓↓	—	—	↓↓	—	↓	—	—	—	—	↓↓
19	↑↑	↓	↑↑↑	—	—	↑	↓	—	↓↓↓	↑↑	—	—	—	—
20	—	—	↑↑	↑↑↑	—	—	—	—	—	↑	—	—	—	↓↓

↑ 50–100% increase diseased over normal. ↑↑ 100–200% increase diseased over normal. ↑↑↑ > 200% increase diseased over normal. ↓ 50–100% decrease diseased from normal. ↓↓ 100–200% decrease diseased from normal. ↓↓↓ > 200% decrease diseased from normal. — < 50% change.

Table III. Repertoire Comparisons of LPL Isolated from Two Inflamed Portions of Colon of the Same Individual for Three Individuals, AA, BB, and CC

	CD4			CD8		
BV	AA	BB	CC	AA	BB	CC
1	—	—	—	↑	↓	—
2	—	—	—	—	—	—
3	—	—	—	—	—	—
4	—	—	—	↑	—	—
5S1	—	—	—	↑	—	—
5S2	—	—	—	—	—	—
6	—	—	—	—	—	—
7	↓	—	—	—	—	—
8	—	—	—	—	—	—
9	—	—	—	↑	↑	↑
10	—	—	—	↑↑↑	—	—
11	—	—	—	—	—	—
12	—	—	—	—	—	—
13S1	—	—	—	—	—	—
13S2	—	—	—	—	—	—
14	—	—	—	—	—	—
15	—	—	↓	—	—	—
16	↑↑	—	—	↑	—	↓
17	—	—	—	↑	↓	—
18	—	—	—	—	—	—
19	—	—	—	—	↑	—
20	—	↑↑↑	—	—	—	—

↑ 50–100% increase of diseased sample 1 over diseased sample 2. ↑↑ 100–200% increase of diseased sample 1 over diseased sample 2. ↑↑↑ > 200% increase of diseased sample 1 over diseased sample 2. ↓ 50–100% decrease of diseased sample 1 over diseased sample 2. ↓↓ 100-200% decrease of diseased sample 1 over diseased sample 2. — > 50% change.

previously described for the rapid detection of oligoclonality was used to compare LPL from the disease-active and disease-inactive tissues of all seven CD patients (30–32). This method, which separates PCR-amplified BV segments on the basis of their CDR3 size and is capable of detecting clonal populations at the 1–10% level, was used to analyze the joining region diversity and heterogeneity of all of the BV segments that were increased in disease-active tissue relative to disease-inactive tissue (see Methods). In all, 53 BV segments were analyzed in this fashion (Fig. 2). Patterns that were characteristic of restricted heterogeneity and were unique to disease-active tissue were observed in at least 11 instances; in BV11 and BV16 of patient B, BV11 and BV15 of patient C, BV13S2 of patient D, BV16 of patient E, BV13S2, 15, and 17 of patient F, and BV2 and 16 of patient G (Fig. 2). Although additional examples of apparent oligoclonality could be observed, these appeared, in general, to be the same for both disease-active and disease-inactive tissue (e.g., BV14 in patient A and BV20 in patient C).

In some cases, the decrease in heterogeneity and indications of oligoclonality in the disease-active tissue were very striking. For example, in patient C, LPL isolated from the disease-active colon, showed only two bands in BV11 compared to LPL isolated from disease-inactive colon which showed the characteristic six or more bands consistent with extreme heterogeneity. An even more striking example of restricted heterogeneity was observed in BV15 of patient C, where essentially only a single intense band was observed in the disease-active tissue, suggesting a high degree of oligoclonality. In other cases, the decreases in heterogeneity were more modest (e.g., BV11 and BV16 of patient B, and BV13S2, 15, 17 of patient F). In one individual, patient A, there was no evidence of restricted heterogeneity unique to the disease-active tissue.

Sequences of expanded TCRBV segments. The differences in the CDR3 length patterns observed for LPL from disease-active and disease-inactive tissue suggested that their TCR repertoires might be different with respect to CDR3 size and sequence. Furthermore, as suggested by the limited number of bands in BV11 and BV15 in the disease-active tissue of patient C, expansion of a limited number of T cells appeared to have occurred in some cases. Accordingly, BV segments that displayed restricted heterogeneity in disease-active tissue and where an apparent oligoclonal band representing at least 45% of the radioactivity in that particular BV segment was observed (see Methods) were amplified by PCR and cloned. As controls, the same BV

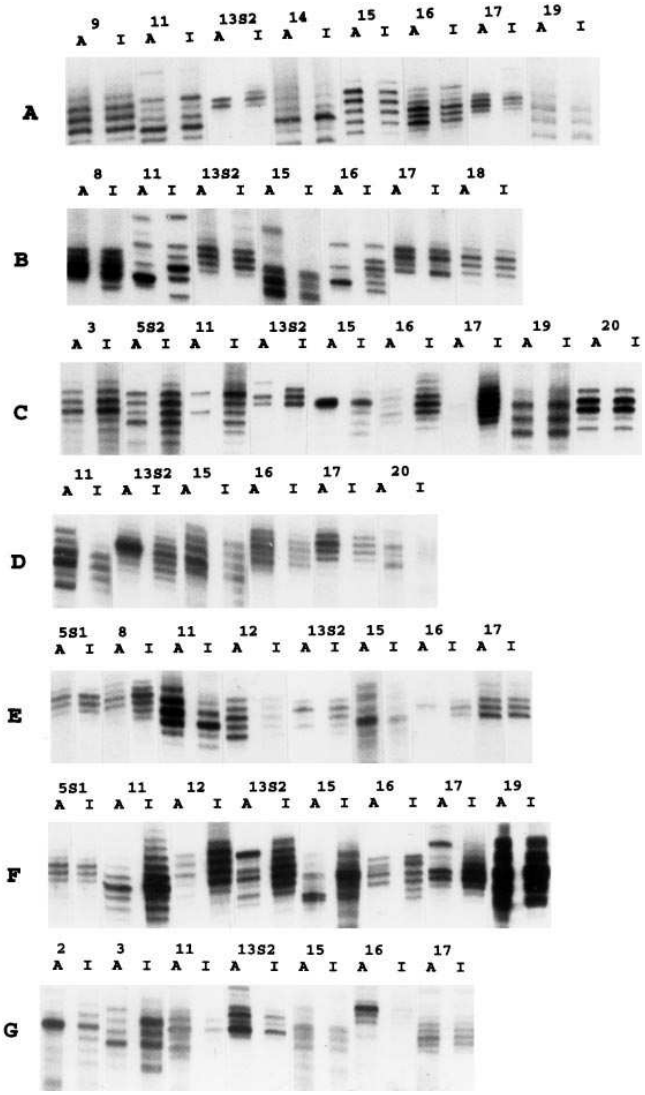

Figure 2. CDR3 length analysis of TCRBV segments from disease-active (*A*) and disease-inactive (*I*) tissue from the colons of seven patients.

segment from the disease-inactive tissue was also amplified and cloned. Thus, for example, for patient B, PCR-amplified BV11 and BV16 segments from LPL isolated from both the disease-active and disease-inactive tissue were cloned, and a large number of clones from each were sequenced. Similarly, the BV11 and BV15 segments of patient C were cloned and sequenced. No BV segments were sequenced for patients A and F because the former did not display any evidence of restricted heterogeneity by CDR3 length analysis (Fig. 2) and the latter did not display sufficient evidence of oligoclonality to warrant sequence analysis at this time. In all, of the 11 BV segments that showed restricted heterogeneity unique to the disease-active tissue, 7 were analyzed by DNA sequencing.

The results of sequence analysis of these seven BV segments from five individuals (B, C, D, E, and G) are shown in Tables IV–VIII. This analysis revealed expansion of a limited number of TCR in all seven cases. In some cases, this expansion was very striking. For example, in patient C, two clones represented 87% of the entire BV11 repertoire in the disease-active tissue, but only 2% in the "disease-inactive" tissue, and

*Table IV. CDR3 Sequences in Patient B**

	No.	BV Family	BV	BD	BJ	BJ family	BC
Active							
	19/31 (61%)	11	CASS	GGGG	NEKLFFGSG	1.4	1
	3/31	11	CASS	ESE	NSPLHFGNG	1.6	1
	2/31	11	CASS	EDRLAGSRG	EQYFGPG	2.7	2
	2/31	11	CASS	EDGN	YEQYFGPG	2.7	2
	2/31	11	CASS	EPGTSL	DTQYFGPG	2.3	2
	1/31	11	CASS	ELSFE	ETQYFGPG	2.5	2
	1/31	11	CASS	EARTSVG	NEQFFGPG	2.1	2
	1/31	11	CASS	QGGG	NEKLFFGSG	1.4	1
Inactive							
	9/42	11	CASS	DSTSGRG	TDTQYFGPG	2.3	2
	8/42	11	CASS	GGQN	SGNTIYFGEG	1.3	1
	6/42	11	CASS	DQTGSITSGRVD	EQFFGPG	2.1	2
	5/42 (12%)	11	CASS	GGGG	NEKLFFGSG	1.4	1
	3/42	11	CASS	ELDRR	QETQYFGPG	2.5	2
	3/42	11	CASS	EQVL	GYTFGSG	1.2	1
	3/42	11	CASS	PGQGAD	TIYFGEG	1.3	1
	2/42	11	CASS	ESRDL	NTEAFFGQG	1.1	1
	2/42	11	CASS	LGLLAE	ETQYFGPG	2.5	2
	1/42	11	CASS	LRGPS	YEQYFGPG	2.7	2
Active							
	6/42 (14%)	16	CASS	QGPR	YEQYFGPG	2.7	2
	6/42 (14%)	16	CASS	LPGASD	DTQYFGPG	2.3	2
	6/42 (14%)	16	CASS	PGDSN	NSPLHFGNG	1.6	1
	5/42 (12%)	16	CASS	QGQ	NSPLHFGNG	1.6	1
	4/42	16	CASS	HPGLARPR	TDTQYFGPG	2.3	2
	4/42	16	CASS	QNTLRD	EQFFGPG	2.1	2
	3/42	16	CASS	PGLASVS	DTQYFGPG	2.3	2
	3/42	16	CASS	EP	NTEAFFGQG	1.1	1
	2/42	16	CASS	HQQY	TEAFFGQG	1.1	1
	1/42	16	CA	NTLGGGTEE	TQYFGPG	2.3	2
	1/42	16	CASS	QDGGY	QETQYFGPG	2.5	2
	1/42	16	CASS	QAGV	NIQYFGAG	2.4	2
Inactive							
	9/38	16	CASS	QHRVGA	YNEQFFGPG	2.1	2
	9/38	16	CASS	QDL	NSPLHFGNG	1.6	1
	5/38	16	CASS	QNTLRD	EQFFGPG	2.1	2
	5/38	16	CASS	RQGY	EQFFGPG	2.1	2
	4/38	16	CASS	QAGLLY	YGYTFGSG	1.2	1
	3/38	16	CASS	HPGLARPR	TDTQYFGPG	2.3	2
	2/38	16	CASS	PAGV	AKNIQYFGAG	2.4	2
	1/38	16	CASS	HRTSGF	YNEQFFGPG	2.1	2

*Clones preferentially expanded in the disease-active tissue are underlined, and their frequency in disease-active and disease-inactive tissues are given in parentheses.

one clone represented 92% of the entire BV15 repertoire in the disease-active tissue, but only 19% in the disease-inactive tissue (Table V). Similarly, in patient B, a single clone represented 61 vs. 12% of the entire BV11 repertoire (Table IV), in patient E, two clones represented 54 vs. 16% of the entire BV16 repertoire (Table VII), and in patient G two clones represented 53 vs. 15% of the entire BV2 repertoire for disease-active and disease-inactive tissue (Table VIII). In the other

*Table V. CDR3 Sequences in Patient C**

	No.	BV family	BV	BD	BJ family	BJ	BC
Active							
	19/38 (50%)	11	CAS	KQGLR	NSPLHFGNG	1.6	1
	14/38 (37%)	11	CASS	LTGLGA	NYGYTFGSG	1.2	1
	5/38	11	CASS	EPQGG	SPLHFGNG	1.6	1
Inactive	7/43	11	CASS	EDRIP	SYEQYFGPG	2.7	2
	6/43	11	CAS	RQVN	TQYFGPG	2.3	2
	3/43	11	CASS	AGQLN	SGANVLTFGAG	2.6	2
	3/43	11	CASS	EMGR	TEAFFGQG	1.1	1
	2/43	11	CASS	EIGV	EQYFGPG	2.7	2
	2/43	11	CAS	PPAGGAE	DTQYFGPG	2.3	2
	2/43	11	CASS	EEG	ETQYFGPG	2.5	2
	2/43	11	CASS	GLLGRKG	TEAFFGQG	1.1	1
	2/43	11	CASS	DPAGRKG	TEAFFGQG	1.1	1
	1/43 (2%)	11	CASS	LTGLGA	NYGYTFGSG	1.2	1
	1/43	11	CAS	GRGAY	NSPLHFGNG	1.6	1
	1/43	11	CASS	RTGTL	NTEAFFGQG	1.1	1
	1/43	11	CASS	ESGRKA	EKLFFGSG	1.4	1
	1/43	11	CASS	EG	NQPQHFGDG	1.5	1
	1/43	11	CASS	T	QETQYGFPG	2.5	2
	1/43	11	CASS	DGQIV	QYFGPG	2.7	2
	1/43	11	CASS	HTGS	SYNEQFFGPG	2.1	2
	1/43	11	CASS	HTGSCC	NEQFFGPG	2.1	2
	1/43	11	CASS	PNRVLLQNRG	EQFFGPG	2.1	2
	1/43	11	CAS	QDRGKA	NTGELFFGEG	2.2	2
	1/43	11	CAS	GAGGWA	GELFFGEG	2.2	2
Active							
	34/37 (92%)	15	CATS	DPHRDPSG	EQFFGPG	2.1	2
	3/37	15	CATS	DFTSGS	QETQYFGPG	2.5	2
Inactive							
	6/31 (19%)	15	CATS	DPHDRPSG	EQFFGPG	2.1	2
	3/31	15	CATS	DPDTGAT	NEQFFGPG	2.1	2
	2/31	15	CATS	GGARQGSG	EQFFGPG	2.1	2
	2/31	15	CATS	DPIPGVMAS	EQFFGPG	2.1	2
	2/31	15	CATS	DPSSGES	SYNEQFFGPG	2.1	2
	2/31	15	CATS	DALAGGR	DTQYFGPG	2.3	2
	2/31	15	CATS	DSTLTFR	NQPQHFGDG	1.5	1
	1/31	15	CATS	DTGTSGS	NEQFFGPG	2.1	2
	1/31	15	CATS	GPGEDI	NEQFFGPG	2.1	2
	1/31	15	CATS	DTVSD	NEQFFGPG	2.1	2
	1/31	15	CATS	EHGTSGGYAF	NEQFFGPG	2.1	2
	1/31	15	CATS	DLSPRVTGG	EQFFGPG	2.1	2
	1/31	15	CATS	DRAGGL	QETQYFGPG	2.5	2
	1/31	15	CATS	DPRPGP	STDTQYFGPG	2.3	2
	1/31	15	CATS	DRSGDGGP	TDTQYFGPG	2.3	2
	1/31	15	CATS	TGRGGG	YEQYFGPG	2.7	2
	1/31	15	CATS	DSSGVS	YEQYFGPG	2.7	2
	1/31	15	CAT	AR	NYGYTFGSG	1.2	1
	1/31	15	CAT	AAGAA	NEKLFFGSG	1.4	1

*Clones preferentially expanded in the disease-active tissue are underlined, and their frequency in disease-active and disease-inactive tissues are given in parentheses.

*Table VI. CDR3 Sequences in Patient D**

	No.	BV family	BV	BD	BJ	BJ family	BC
Active							
	6/40 (15%)	13S2	CASS	SGTSG	YNEQFFGPG	2.1	2
	5/40 (13%)	13S2	CAS	RPGTSG	YNEQFFGPG	2.1	2
	3/40	13S2	CASS	PGRTGT	TEAFFGQG	1.1	1
	3/40	13S2	CAS	RKGTSGH	NEQFFGPG	2.1	2
	2/40	13S2	CASS	PGLAG	TGELFFGEG	2.2	2
	2/40	13S2	CAS	TSSQRLV	TGELFFGEG	2.2	2
	2/40	13S2	CAS	TPSRGTG	ANVLTFGAG	2.6	2
	2/40	13S2	CASS	SLYV	NTEAFFGQG	1.1	1
	2/40	13S2	CASS	SGLAGA	NEQFFGPG	2.1	2
	1/40	13S2	CASS	AIRGP	NYGYTFGSG	1.2	1
	1/40	13S2	CASS	TGSLNY	YGYTFGSG	1.2	1
	1/40	13S2	CASS	YPTVG	NQPQHGGDG	1.5	1
	1/40	13S2	CA	TDSRSTGS	PQHFGDG	1.5	1
	1/40	13S2	CASS	TGTSG	YNEQFFGPG	2.1	2
	1/40	13S2	CAS	RAGTKL	NEQFFGPG	2.1	2
	1/40	13S2	CAS	LNQW	EQFFGPG	2.1	2
	1/40	13S2	CASS	YWLAL	QETQYFGPG	2.5	2
	1/40	13S2	CASS	YSWTSGGR	TQYFGPG	2.5	2
	1/40	13S2	CASS	YDTA	YEQYFGPG	2.7	2
	1/40	13S2	CASS	VF	YEQYFGPG	2.7	2
	1/40	13S2	CASS	LPQVGQGG	EQYFGPG	2.7	2
	1/40	13S2	CASS	YTRE	EQYFGPG	2.7	2
Inactive							
	3/38	13S2	CASS	YSGGH	TQYFGPG	2.3	2
	2/38	13S2	CAS	NAAGF	ETQYFGPG	2.5	2
	2/38	13S2	CAS	RPGTSGN	NEQFFGPG	2.1	2
	2/38	13S2	CAS	PDLR	GYTFGSG	1.2	2
	1/38	13S2	CASS	YRTGP	SYNEQFFGPG	2.1	2
	1/38	13S2	CASS	SDQLAGGD	NEQFFGPG	2.1	2
	1/38	13S2	CASS	YSRG	YNEQFFGPG	2.1	2
	1/38	13S2	CASS	YTPLAGGD	NEQFFGPG	2.1	2
	1/38	13S2	CASS	ARL	NEQFFGPG	2.1	2
	1/38	13S2	CASS	YGLTGA	YNEQFFGPG	2.1	2
	1/38	13S2	CAS	REL	NEEFFGPG	2.1	2
	1/38	13S2	CAS	RKGTSGH	NEQFFGPG	2.1	2
	1/38	13S2	CAS	YFGQESL	FFGPG	2.1	2
	1/38	13S2	CAS	T	SYNEQFFGPG	2.1	2
	1/38	13S2	CAS	TAPKKGPS	SYNEQFFGPG	2.1	2
	1/38	13S2	CA	TAGTGT	EQFFGPG	2.1	2
	1/38	13S2	CASS	YYFAYT	STDTQYFGPG	2.3	2
	1/38	13S2	CASS	YSTR	DTQYFGPG	2.3	2
	1/38	13S2	CASS	YREW	TDTQYFGPG	2.3	2
	1/38	13S2	CASS	SNAGLAGA	TDTQYFGPG	2.3	2
	1/38	13S2	CASS	YPIAG	AKNIQYFGAG	2.4	2
	1/38	13S2	CASS	RPAG	TQYFGPG	2.5	2
	1/38	13S2	CAS	KKHWDRDSA	GYTFGSG	1.2	1
	1/38	13S2	CASS	GQSI	NQPQHFGDG	1.5	1
	1/38	13S2	CASS	YRDRYY	QPQHFGDG	1.5	1
	1/38	13S2	CASS	RM	NTEAFFGQG	1.1	1
	1/38	13S2	CAS	RTGV	NTEAFFGQG	1.1	1
	1/38	13S2	CAS	RVPPQDM	NTEAFFGQG	1.1	1
	1/38	13S2	CAS	NPLDTRDL	NTEAFFGQG	1.1	1
	1/38	13S2	CAS	RTDH	YEQYFGPG	2.7	2
	1/38	13S2	CAS	RESGGAGRD	EQYFGPG	2.7	2
	1/38	13S2	CAS	YSIGGTS	YEQYFGPG	2.7	2
	1/38	13S2	CAS	RKPTGAS	YEQYFGPG	2.7	2

*Clones preferentially expanded in the disease-active tissue are underlined, and their frequency in disease-active and disease-inactive tissues are given in parentheses.

two cases (BV16 in patient B and BV13S2 in patient D), evidence of expansion was less striking although the dominant clones present in the disease-active tissue were completely absent from the disease-inactive tissue. Thus, in all seven cases representing five different patients, there was a clear difference in the distribution of TCR in disease-active and disease-inactive tissue, as well as evidence of preferential expansion of a limited number of TCR in the disease-active tissue.

Discussion

Previous studies from this laboratory in which the TCR repertoires of LPL and PBL within the same individual were compared for both CD and control groups had suggested that in CD there is a dramatic change in the TCR repertoire of $CD4^+$ LPL. To confirm this observation and to determine whether the observed changes in the repertoire displayed restricted heterogeneity indicative of an antigen-specific response, the TCR repertoires of LPL isolated from both disease-active and disease-inactive colonic tissue of seven CD patients were compared. Disease-inactive tissue was independently defined by a pathologist as tissue that showed no gross or histological evidence of inflammation. By comparing disease-active and disease-inactive tissue within the same individual, we could eliminate the possibility that any observed differences in repertoire were caused by individual genetic differences or mode of ther-

*Table VII. CDR3 Sequences in Patient E**

	No.	BV family	BV	BD	BJ	BJ family	BC
Active							
	11/33 (33%)	16	CASS	KRDRMK	TQYFGPG	2.5	2
	7/33 (21%)	16	CAS	RKIGGRH	YGYTFGSG	1.2	1
	3/33	16	CASS	QDPGRM	EKLFFGSG	1.4	1
	2/33	16	CASS	QAIGDT	GNTIYFGEG	1.3	1
	1/33	16	CASS	PQRRGY	QETQYFGPG	2.5	2
	1/33	16	CASS	RTVS	YGYTFGSG	1.2	1
	1/33	16	CASS	QVSSN	NEQFFGPG	2.1	2
	1/33	16	CASS	QMSGTG	EQFFGPG	2.1	2
	1/33	16	CASS	RQY	EQFFGPG	2.1	2
	1/33	16	CAS	RQNPHS	GELFFGEG	2.2	2
	1/33	16	CASS	RRLAG	TDTQYFGPG	2.3	2
	1/33	16	CAS	RQHFGQGH	SYEQYFGPG	2.7	2
	1/33	16	CASS	PELEGA	PLHFGNG	1.6	1
	1/33	16	CASS	LNGQAAG	QPQHFGDG	1.5	1
Inactive							
	3/31 (10%)	16	CASS	KRDRMK	TQYFGPG	2.5	2
	3/31	16	CASS	QLTSY	YNEQFFGPG	2.1	2
	2/31	16	CASS	QFLSGGR	NEQFFGPG	2.1	2
	2/31 (6%)	16	CAS	RKIGGRH	YGYTFGSG	1.2	1
	2/31	16	CASS	RTSGTS	TDTQYFGPG	2.3	2
	1/31	16	CASS	QDPGRM	EKLFFGSG	1.4	1
	1/31	16	CASS	QDIGPG	EKLFFGSG	1.4	1
	1/31	16	CASS	QATGSSALA	TNEKLFFGSG	1.4	1
	1/31	16	CASS	PRVNS	NEKLFFGSG	1.4	1
	1/31	16	CAS	RGYSGS	YGYTFGSG	1.2	1
	1/31	16	CAS	PAAKNV	YGYTFGSG	1.2	1
	1/31	16	CASS	QLLAG	QETQYFGPG	2.5	2
	1/31	16	CASS	PRDKMN	TQYFGPG	2.5	2
	1/31	16	CASS	QIGVR	YNEQFFGPG	2.1	2
	1/31	16	CASS	QGRDD	EQFFGPG	2.1	2
	1/31	16	CASS	QVW	NTEAFFGQG	1.1	1
	1/31	16	CASS	QVEGQG	AFFGQG	1.1	1
	1/31	16	CASS	QQV	QGELFFGEG	2.2	2
	1/31	16	CASS	RDA	GELFFGEG	2.2	2
	1/31	16	CASS	QGFRRDA	GELFFGEG	2.2	2
	1/31	16	CASS	FCYR	STDTQYFGPG	2.3	2
	1/31	16	CASS	LLAGF	TDTQYFGPG	2.3	2
	1/31	16	CASS	QVTIR	DTQYFGPG	2.3	2
	1/31	16	CASS	LTRGV	EQYFGPG	2.7	2

*Clones preferentially expanded in the disease-active tissue are underlined, and their frequency in disease-active and disease-inactive tissues are given in parentheses.

apy. Furthermore, since LPL from disease-active and disease-inactive tissues were derived from adjacent surgical specimens, we minimized repertoire differences that might be caused by cellular compartmentalization, such as those we have previously documented for T cells from peripheral blood and the lamina propria compartment (24). This was confirmed by our comparison of LPL isolated from two adjacent segments of disease-active tissue of several CD patients, where we observed very few differences in CDR3 length profiles (data not shown) and repertoire (Table III).

When the repertoires of LPL from disease-active and disease-inactive tissue were compared within the same individual, we observed major differences in the $CD4^+$ population for all seven CD patients (Fig. 1 and Table II). In contrast, the repertoires of $CD8^+$ LPL from the disease-active and disease-inactive tissue of the same individual were strikingly similar. These observations confirm our previous conclusion that CD is accompanied by repertoire changes that occur predominantly in $CD4^+$ LPL (24). These studies also reveal that although each patient is characterized by a unique pattern of repertoire differences, there are several BV segments that uniformly increase in the disease-active tissue of all seven CD patients relative to disease-inactive tissue. For all seven CD patients, there were significant increases ($> 50\%$) in the levels of BV11, 13S2, 15, and 17 (Table II). In addition, BV16 increased in six of the seven patients. In many of these cases, the increases were two- or threefold (Table II).

The increases in the relative levels of RNA encoding several different BV segments in the disease-active tissue relative to disease-inactive tissue suggested that there was a preferential activation and/or proliferation of T cells expressing these BV segments. To determine whether this preferential activation might be restricted to T cells expressing a small number of different receptors (i.e., oligoclonal), we used an assay that we have previously used to detect oligoclonality in PBL and LPL (30, 31). This analysis revealed evidence of oligoclonality that was unique to the disease-active tissue of six of the seven patients; only in patient A was there no evidence of oligoclonality by this assay (Fig. 2). Sequence analysis of BV segments that displayed restricted heterogeneity patterns unique to the disease-active tissue confirmed the CDR3 length analysis data;

*Table VIII. CDR3 Sequences in Patient G**

	No.	BV family	BV	BD	BJ	BJ family	BC
Active							
	13/34 (38%)	2	CS	ARGD	SNQPQHFGDG	1.5	1
	5/34 (15%)	2	CS	ARNPRAGG	TDTQYFGPG	2.3	2
	4/34	2	CAS	RKIGGRH	YGYTFGSG	1.2	1
	2/34	2	CS	ARDASGTV	YNEQFFGPG	2.1	2
	1/34	2	CS	ALFRTGPG	QPQHFGDG	1.5	1
	1/34	2	CS	ANTGPA	QPQHFGDG	1.5	1
	1/34	2	CS	ARC	DTQYFGPG	2.3	2
	1/34	2	CS	ARDAGGAK	DTQYFGPG	2.3	2
	1/34	2	CS	ASRLAGGF	YNEQFFGPG	2.1	2
	1/34	2	CS	ARAGPGY	EQYFGPG	2.7	2
	1/34	2	CS	ATLP	QETQYFGPG	2.5	2
	1/34	2	CS	GGV	QETQYFGPG	2.5	2
	1/34	2	CS	ARTAG	GNTIYFGEG	1.3	1
	1/34	2	CS	ASPTGR	GNTIYFGEG	1.3	1
Inactive							
	4/27 (15%)	2	CS	ARGD	SNQPQHFGDG	1.5	1
	4/27	2	CS	APTGTA	NYGYTFGSG	1.2	1
	3/27	2	CS	ARAP	QDPQHFGDG	1.5	1
	2/27	2	CS	ASGDT	QYFGPG	2.7	2
	2/27	2	CS	ARAKRTGK	NEKLFFGSG	1.4	1
	2/27	2	CS	ARDTGF	SGNTIYFGEG	1.3	1
	1/27	2	CS	GGV	QETQYFGPG	2.5	2
	1/27	2	CAS	RKIGGRH	YGYTFGSG	1.2	1
	1/27	2	CS	ARDPGAT	NEKLFFGSG	1.4	1
	1/27	2	CS	ARGRYR	EQYFGPG	2.7	2
	1/27	2	CS	AKRLASS	YNEQFFGPG	2.1	2
	1/27	2	CAS	ASLP	YGYTFGSG	1.2	1
	1/27	2	CS	ARQRGGEG	SDTQYFGPG	2.3	2
	1/27	2	CS	ASIKRG	TDTQYFGPG	2.3	2
	1/27	2	CS	AGGGRG	GYTFGSG	1.2	1
	1/27	2	CS	ASQGAR	SDTQYFGPG	2.3	2

*Clones preferentially expanded in the disease-active tissue are underlined, and their frequency in disease-active and disease-inactive tissues are given in parentheses.

the TCR sequences present in disease-active tissue were distinct from those present in disease-inactive tissue, and there was evidence of selective expansion of a small number of T cells (Tables IV–VIII). In a few cases, TCR that were greatly expanded in the disease-active tissue were also observed in the disease-inactive tissue, although at a much lower frequency, suggesting that even in disease-inactive tissue there is already a partial expansion of T cells expressing these receptors. It is very likely that continued activation and expansion of T cells bearing these receptors are responsible for much of the inflammation in the disease-active tissue, although it is not clear why these T cells should suddenly become activated.

One possibility, which is suggested by the large increases in the levels of several BV segments in disease-active tissue relative to disease-inactive tissue, is activation by one or more "superantigens" that are known to activate polyclonal T cells in a BV-specific fashion (33, 34). Although the polyclonality of these BV segments of individual A is consistent with a superantigen-like response, the extreme oligoclonality observed in some of the other patients argue against a superantigen-like response. It may be that both superantigens and antigen-specific responses contribute to the inflammation observed in CD. Alternatively, there may be several different etiologies for CD. Such a scenario, in which there are different etiologies leading to the same gross manifestations, have been proposed for other autoimmune diseases (35–37), and are supported by animal models of inflammatory bowel disease where different defects in or insults to the immune system lead to diseases with grossly similar characteristics (38–45). Further analysis of the inflammatory response in additional CD patients may allow us to distinguish between these various models and provide a better understanding of the etiology of CD.

In summary, our data suggest that at least in some CD patients there is evidence of a highly restricted T cell response. It remains to be determined whether these T cells play a primary role in initiating the disease process or are secondary to the initial event. Nevertheless, our identification of selectively expanded T cells suggests that it may be possible to identify the antigen(s) responsible for much of the inflammation in CD, and opens the door to the possibility of therapy aimed at tolerizing the patient to these antigens.

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