Evidence of T cell receptor β-chain patterns in inflammatory and noninflammatory bowel disease states

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[1]*Division of Gastroenterology, Brigham and Women's Hospital, Boston, Massachusetts 02115;* [2]*University of Bristol, Bristol BS2 8HW, United Kingdom;* [3]*Universitaetsspital, CH-8091 Zurich, Switzerland;* [4]*Universitit Wein, Vienna 1090, Austria;* [5]*Wayne State Medical Center, Detroit, Michigan 48201; and* [6]*Beth Israel Deaconess Medical Center, Boston, Massachusetts 02115*

Saubermann, Lawrence J., Christopher S. J. Probert, Andreas D. Christ, Andreas Chott, Jerrold R. Turner, A. Christopher Stevens, Steven P. Balk, and Richard S. Blumberg. Evidence of T cell receptor β-chain patterns in inflammatory and noninflammatory bowel disease states. *Am. J. Physiol.* 276 (*Gastrointest. Liver Physiol.* 39): G613–G621, 1999.—T cell activation, as defined by expression of relevant cell surface molecules, such as the interleukin-2 receptor (CD25), is increased in many chronic relapsing diseases, including inflammatory bowel disease (IBD). These T cells are generally activated through contact of their clonotypic T cell receptor (TCR) with a peptide antigen presented by a major histocompatibility complex molecule. One of the putative antigenic contact sites for the TCR is the third complementarity determining region (CDR3) of the TCR β-chain variable region (TCRBV). Therefore, analysis of the TCRBV CDR3 provides insight into the diversity of antigens encountered by a given T cell population. This study evaluated the TCRBV CDR3 usage of the activated intestinal lymphocytes from human subjects with IBD, diverticulitis (inflammatory control), and a normal tissue control. Public patterns, as demonstrated by shared TCRBV CDR3 amino acid sequences of activated intestinal T cell subpopulations, were observed. In particular, a public pattern of TCRBV22, a conserved valine in the fifth position, and use of TCRBJ2S1 or TCRBJ2S5 was present in three of four Crohn's disease subjects while not present in the ulcerative colitis subjects. However, the private patterns of TCRBV CDR3 region amino acid sequences were far more striking and easily demonstrated in all individuals studied, including a normal noninflammatory control. Thus we conclude that selective antigenic pressures are prevalent among an individual's activated intestinal lymphocytes.

Crohn's disease; ulcerative colitis; diverticulitis; interleukin-2

IDIOPATHIC INFLAMMATORY bowel disease (IBD) is a chronic inflammatory condition characterized by the presence of increased numbers of activated intestinal T lymphocytes (14, 33, 43, 47) and clonal expansions of specific intestinal T cell clones, as defined by an analysis of the germline configured clonotypic T cell receptor (TCR) (25, 40). These expansions can also be found in other relevant extraintestinal tissue sites, such as the peripheral blood and bile duct (2, 26, 44, 45). The presence of selective T cell expansions at involved tissue sites suggests discrete antigens might be involved in these disorders (13, 24).

A characteristic of the activated intestinal T cells in IBD, as well as T cells in similar chronic relapsing and remitting disorders such as multiple sclerosis or rheumatoid arthritis, is that the effector T cells exhibit surface activation markers, such as the interleukin-2 (IL-2) receptor. They also exhibit functional cytokine production, such as IL-2 (10, 14, 22, 32, 41, 48). Through in vitro growth in selective media, the activated T cell subsets from tissue sites can be examined for their TCR usage (10, 30, 58). This form of analysis has allowed identification of important T cell clones that may help to identify relevant antigens to these disorders (42, 55).

TCR analysis is based on the accepted notion that in order for the αβ T cell to become activated and execute its specific effector function(s), its TCR third complementarity determining region (CDR3) must contact a peptide fragment presented by a genetically defined major histocompatibility complex (MHC) molecule. As defined by X-ray crystallography and site-directed mutagenesis, one of the major sites of putative antigen contact for the TCR is the CDR3 of the TCR β-chain (TCRB) (20, 21, 31, 53). This hypervariable CDR3 (also known as the NDN region) is generated as a germline recombination event of multiple variable (V), diversity (D), joining (J), and constant (C) region gene segments with the addition of a limited number of nongermline encoded nucleotides (N-region additions). Thus the TCRB CDR3 represents the most hypervariable region of the TCR and contacts the peptide as well as the MHC (7). Therefore, examination of this region provides a measure of the antigenic experience of a particular T cell population.

An examination of CDR3 in murine models of T cell activation suggests responses to discrete antigens are manifest in terms of personal (or private) as well as shared (or public) responses when MHC usage is considered (15). A variety of examples of autoimmune and infectious diseases and in vitro analyses of T cell responses to model antigens suggest that this concept of private and public patterns of CDR3 usage can be readily translated to humans (9, 29, 57). As such, the identification of shared composition of CDR3s among a

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population of TCRs predicts that a response has occurred to a discrete antigen.

Multiple investigations into human disease and murine models of human disease have demonstrated the importance of T lymphocyte responses in infectious, neoplastic, and autoimmune conditions (1, 3, 6, 16, 27, 46, 50, 51). In the immunologic microenvironment of the human intestine, T cells are constantly activated in response to a variety of environmental antigens (39). However, no investigations, to date, have examined this activated subset of intestinal lymphocytes for evidence of TCR CDR3 amino acid homology in normal individuals or IBD subjects. If present, amino acid homologies would indicate that the activated subsets containing T cells are responding to frequently encountered and shared mucosal antigen(s). In this study, we investigated the activated IL-2 receptor-expressing intestinal T cell subpopulation, as defined by cultivation of freshly obtained tissue lymphocytes in recombinant IL-2 (rIL-2) to determine whether private or public patterns of T cell expansions could be observed in idiopathic inflammatory disease of the intestine, inflammatory disease of known etiology (diverticulitis), and in a normal intestinal mucosa.

METHODS

Tissues. Fresh intestinal mucosa (~1 cm^2) was obtained from surgical resection specimens (*CD 1–3*, *UC 1–2*, *Div 1*) or from multiple endoscopic biopsies (*CD 4*, *UC 3*, *normal 1*). Four individuals with Crohn's disease (CD), three with ulcerative colitis (UC), one diverticulitis (Div), and one normal control (who underwent screening endoscopy) were involved in this study. Tissue was taken from grossly involved areas of colonic mucosa. In all cases, the human research committee granted approval and appropriate consents were obtained. Diagnoses were based on standard histopathological criteria.

Culture. Tissue biopsies for generation of activated T cells were immediately washed twice in sterile RPMI and then placed in 1 ml of culture medium containing RPMI 1640 (Cellgro, Herndon, VA) supplemented with the following: penicillin-streptomycin (10^5 U/l; Biowhittaker, Walkersville, MD), L-glutamine (2 mM; Cellgro), HEPES buffer (0.01 M; Cellgro), nonessential amino acids (1×; Cellgro), 10% normal human AB serum (Sigma, St. Louis, MO), rIL-2 (50 U/ml) kindly provided by Ajinomoto (Japan), and amphotericin B (0.5 μg/ml; Sigma). The tissue was then transferred to a 24-well flat-bottom sterile tissue culture plate (Becton Dickinson, Lincoln Park, NJ) and placed in a 5% CO_2 incubator at 37°C. Daily, the tissue was transferred to a new well containing 1 ml of fresh medium as previously described. After 14 days of incubation in this high rIL-2 environment, surviving cells were pooled, counted, and replated in a 96-well V-bottom plate (Becton Dickinson) with medium (as previously described) but with rIL-2 (10 U/ml; Ajinomoto), phytohemagglutinin-P (1.0 μg/ml; Sigma), and irradiated (5,000 rad) allogeneic peripheral blood mononuclear cells (10^5/well) for an additional 10 days of growth.

Cell sorting. Two aliquots of 5–8 × 10^6 cells were positively selected for either $CD4^+$ or $CD8^+$ cells, respectively, from the rIL-2 cultured T cell populations. Cells were initially pelleted and then resuspended in 1 ml of OKT4 and OKT8 hybridoma supernatants and incubated at 4°C for 45 min. Cells were then gently pelleted using a microcentrifuge at 5,000 rpm for 3 min, and the supernatants were removed. The cells were washed with 1 ml RPMI. Immunomagnetic goat anti-mouse beads (Dynal, Lake Success, NY) were added as per the manufacturer's instructions, and samples were gently rotated at 4°C for 1 h. After labeling, antibody-bound cells were positively selected with a magnet. This step was repeated to improve fraction purity to >95%, as evidenced by visual microscopic evaluation of magnetic bead-labeled cells. The positively selected T cell fractions were then snap frozen in liquid nitrogen and stored at −80°C.

Preparation of cDNA. Each positively selected cell fraction was subjected to mRNA extraction using the ultraspec mRNA isolation method (Biotecx, Houston, TX) as per the manufacturer's protocol. The mRNA was converted into cDNA by using 1 μl of MuMLV RT [New England Biolabs (NEB), Beverly, MA], 1.5 μl of oligo(dT) primer (100 mM), 1 μl of RNasin, a RNase inhibitor (NEB), 4 μl of 2.5 mM stock of equimolar dNTP (NEB), 2 μl of 0.1 M dithiothreitol (Sigma), 2 μl of 10× RT buffer (NEB), and 10 μl of RNase-free water. Forward and reverse primers specific for β_2-microglobulin were used in a 32-cycle PCR reaction to confirm the success of the RT reaction and assess the relative concentrations of cDNA products with each cycle consisting of 94°C for 20 s, 55°C for 30 s, 72°C for 60 s, and a final extension time of 10 min at 72°C.

CDR3 length displays. PCR amplification products were generated in the presence of ^{32}P by a 32-cycle PCR reaction (temperatures and times as previously described) using 24 separate Vβ primers (as previously published, Ref. 13) and a single consensus Cβ primer with ~1.0 pg of cDNA (13). The primers were selected to provide radioactive products encompassing the CDR3 of the TCR. These PCR products were then heat denatured and resolved on a 6% polyacrylamide gel (Sequagel, National Diagnostics, Atlanta, GA) at 95 W for 3 h to generate a CDR3 display and then developed by autoradiography. The results were similar to previously published CDR3 displays (13, 44)

Coresolution of CDR3 displays. TCR β-chain variable (TCRBV) PCR amplification products with dominant appearing bands were resolved together on another separate 6% polyacrylamide gel (National Diagnostics) to assess for coresolution of bands indicating the presence of similar length TCR products. Coresolving bands were then excised and placed in an elution buffer (0.5 M ammonium acetate, 0.1% SDS, 1 mM EDTA), which was shaken overnight at 37°C. Supernatants were collected, precipitated in 100% ethanol at −80°C overnight, centrifuged for 20 min at 14,000 rpm in a microcentrifuge at 4°C, washed in 70% ethanol with recentrifugation for 10 min at 14,000 rpm at 4°C, dried, and resuspended in 20 ml sterile, DNase-free water.

Reamplification of eluted cDNA. With the use of a nested Cβ primer, 5 μl of the eluted cDNA were further reamplified by another 26-cycle PCR reaction under the conditions previously described in the absence of ^{32}P. Reexposure on X-ray film of the original CDR3 display was performed to confirm appropriate band excision.

Subcloning and sequencing. The reamplified PCR products were ligated into the TA cloning vector (Invitrogen, San Diego, CA) and competent *Escherichia coli* transformed as per the manufacturer's instructions. Random colonies were isolated and grown in LB broth overnight in a 37°C shaker. Miniprepping was performed on cultured bacteria using the Qiaprep 8 plasmid protocol (Qiagen, Santa Clarita, CA). DNA was checked using optical density measurements at 260 λ to obtain relative concentrations for standardization in sequencing reactions. The Sequenase sequencing kit (US Biochemical, Cleveland, OH) was used to obtain TCR sequences as per

the manufacturer's recommendations. Sequences were interpreted using the CLONE 4 (Scientific and Educational Software) program.

RESULTS

Characterization of TCRBV CDR3 usage among activated intestinal T cell populations. To determine whether activated intestinal T cell populations were characterized by preferential TCRBV usage, TCRB CDR3 length displays, similar to those previously published (13, 44), were generated on activated intestinal T cells, which were enriched for $CD4^+$ and $CD8^+$ T cells by positive selection by immunomagnetic bead sorting. Activated T cells were obtained from one normal control individual and from regions of inflamed colonic tissue from four subjects with CD, three individuals with UC, and one with diverticulitis, by cultivating biopsies in rIL-2 for 2 wk and sorting the propagated cells into $CD4^+$ and $CD8^+$ enriched fractions. mRNA from these enriched cellular fractions was subjected to RT-PCR amplification followed by a PCR reaction utilizing a series of TCRBV region-specific primers in conjunction with a TCRBC region primer in the presence of [^{32}P]dCTP. The radiolabeled products were then resolved by PAGE. Although skewing, as defined by loss of normally observed Gaussian distribution of CDR3 TCRBV amino acid length was observed; there was no evidence of preferential particular TCRBV usage among the groups of study subjects (data not shown).

Characterization of CDR3 composition between individuals. Although the activated T cells were not characterized by preferential TCRBV usage, it was important to determine whether dominantly expressed TCRBVs, which were shared between study subjects, were characterized by common CDR3 usage. This would possibly indicate a shared response to a common antigenic determinant. cDNA from dominant and potentially clonally expanded bands from multiple study subjects were coresolved for similar length CDR3 amino acid sequences on a CDR3 display (Fig. 1). The extracted cDNA was reamplified using specific TCRBV primers, and random sequences were obtained from subcloned bacterial isolates. The deduced TCR amino acid sequences are shown in Tables 1 and 2 for the $CD4^+$ and $CD8^+$ T cell clones, respectively. The boundaries of the CDR3 were defined as the last two amino acids of the TCRBV region up to and including the conserved -FG- of the TCRBJ segment. This definition of the CDR3 is consistent with a previously published evaluation of TCRB sequences of normal human peripheral blood T lymphocytes (38). Table 3 presents a summary of shared TCR CDR3 patterns among the study subjects analyzed. Because the exact number and location of CDR3 residues that make contact with MHC peptide are unknown, we defined a similarity between TCRs if at least three amino acids were observed in the same position within the hypervariable NDN region. In addition, we analyzed the biophysical characteristics of the amino acid side chains of the CDR3 to provide an approximate tertiary view of this region, as previously described (38). This type of side-chain analysis provides another approach to examining the CDR3 composition by indicating possible steric and charge interactions with a putative MHC peptide complex because the CDR3 has been demonstrated to form a loop (7, 12, 38).

The majority of the shared patterns were observed among the $CD8^+$-enriched fraction of activated lymphocytes. Several of these patterns were observed between patient groups and were limited in scope. However, and more interestingly, this CDR3 TCRB region analysis

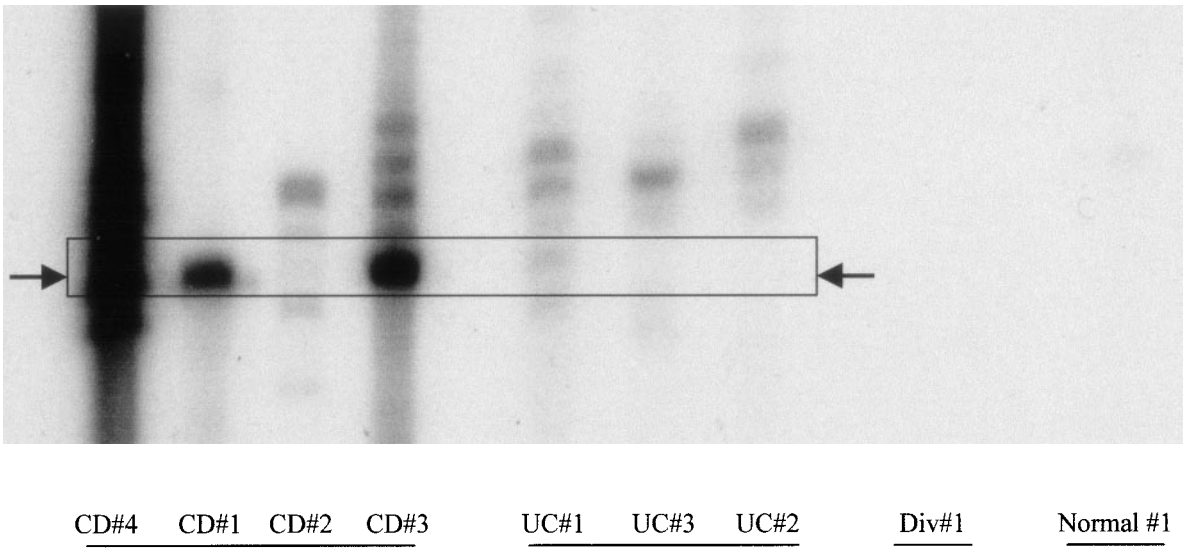

Fig. 1. Coresolution gel of T cell receptor (TCR)BV22 complementarity determining region (CDR3) of activated intestinal $CD8^+$ T cells between all study subjects. Radiolabeled PCR-amplified products were coresolved by PAGE, excised, reamplified using nested PCR, subcloned, and sequenced for analysis (see Table 2). Enclosed area, which is also depicted by arrows and represents the area sequenced, shows the dominant TCRBV22 band, which was present in 3 of 4 Crohn's disease (CD) individuals but which was not present in the ulcerative colitis (UC) cases. Also, there was no dominant expression of TCRBV22 for either the diverticulitis (Div) or normal control subject, as shown by the lack of cDNA present.

Table 1. *Derived amino acid sequences for positively selected and activated CD4+ intestinal T cells*

Subject	TCRBV	TCRBJ	CDR3 Region	No.
		Crohn's disease		
CD 1	3.1	1.2	SSHRQGFGYN	9/36
CD 1	3.3	2.7	SSMIYEQY	9/36
CD 1	3.3	2.7	SSLIVKSYEQY	10/36
CD 1	17	1.1	SSVGQGSSEAF	2/36
CD 1	17	2.7	SSLDRSSYEQY	2/36
CD 1	21		SSLGGPINT	3/36
CD 1	21		SSLHGLVIC	1/36
CD 2	3.1	2.3	SSGANADTQY	9/51
CD 2	17	2.1	SSISAGWGEQF	12/51
CD 2	17	2.5	TTPWGRQETQY	22/51
CD 2	21	2.7	SSLDRGYYEQY	8/51
CD 3	3.1	1.1	IRTGRDTEAF	1/43
CD 3	3.1	1.1	SRPGTGGSAEAF	1/43
CD 3	3.1	1.1	SSSPANRDTEAF	1/43
CD 3	3.1	1.2	SRLGTDYGYT	1/43
CD 3	3.1	1.3	SSPGGISGNTIY	1/43
CD 3	3.1	1.4	SSLRALNEKLF	1/43
CD 3	3.1	1.5	SSSRTVSNLPQH	1/43
CD 3	3.1	1.6	SRPSGGDNSPLH	3/43
CD 3	3.1	2.1	SSLVGTSIV	1/43
CD 3	3.1	2.1	SSPPRSSYNEQF	1/43
CD 3	3.1	2.1	SSLLAGDNEQF	1/43
CD 3	3.1	2.3	SSFAAAQY	1/43
CD 3	3.1	2.7	SRGQFLEQY	1/43
CD 3	3.1	2.7	SSLNPYEQY	1/43
CD 3	3.3	1.1	SSFSLNTEAF	2/43
CD 3	3.3	1.2	SSLAPGFNYGYT	1/43
CD 3	3.3	1.2	SSLLRTGNYGYT	2/43
CD 3	3.3	1.2	SSLSGELF	1/43
CD 3	3.3	1.2	STRPTNYGYT	1/43
CD 3	3.3	1.2	SMSPGNYGYT	1/43
CD 3	3.3	1.6	SSLRSNNSPLH	1/43
CD 3	3.3	1.5	SRAGASNQPQH	1/43
CD 3	3.3	2.1	SRQTGVSEQF	1/43
CD 3	3.3	2.1	SSLGGKASCIEQF	1/43
CD 3	3.3	2.1	VSGGPLYNEQF	1/43
CD 3	3.3	2.2	SFRPGRTGELF	1/43
CD 3	3.3	2.3	SSLINDHPPTQY	1/43
CD 3	3.3	2.7	SSAAGTGAYEQY	1/43
CD 3	3.3	2.7	SSLAGREPSEQY	1/43
CD 3	3.3	2.7	SGASGQYEQY	1/43
CD 3	17	1.1	SSENLNTEAF	1/43
CD 3	17	1.1	SRPGLPSEAF	1/43
CD 3	17	1.6	SRPGAGSDSPLH	2/43
CD 3	17	1.6	SSAGPAGNSPLH	1/43
CD 3	17	2.1	SSMGGYNEQF	4/43
		Ulcerative colitis		
UC 1	3.1	1.1	SASQGRTEAF	1/22
UC 1	3.1	2.2	SSSTSTGELF	2/22
UC 1	3.3	1.1	PRAGANTEAF	1/22
UC 1	3.3	1.1	SSLRPNTEAF	5/22
UC 1	3.3	1.5	SSSSRGPQH	1/22
UC 1	3.3	2.3	SSFGGGDTQY	1/22
UC 1	12	1.3	IKGPVFYSGNTIY	2/22
UC 1	12	1.3	IGPGYQKSTYDQY	2/22
UC 1	12	2.3	ISTSGGDTDTQY	2/22
UC 1	13	2.1	SSEAPGPYNEQF	4/22
UC 1	13	2.1	SSTTASSYNEQF	1/22
UC 3	3.1	1.1	SSPLIDSEAF	1/14
UC 3	3.1	1.6	SFPGRVVGSPLH	1/14
UC 3	3.1	1.6	SSFPSGRSPLH	1/14
UC 3	3.1	1.6	SSSRQGRYNPPLH	1/14
UC 3	3.1	2.7	SSGAVSYEQY	3/14
UC 3	3.1	2.7	SSLGGTSGIQNIQY	1/14
UC 3	3.3	1.1	SKRMVDTEAF	1/14
UC 3	17	1.1	SSITAQGAF	1/14
UC 3	17	1.1	STGQGADAF	1/14
UC 3	17	1.3	SSLRLYSGNTIY	1/14
UC 3	17	1.6	SSRTVGGSPLH	2/14
		Diverticulitis		
Div 1	3.1	1.1	SSPGQGGGAF	1/6
Div 1	3.1	1.2	STGQNNSGYT	1/6
Div 1	3.1	2.3	SSFGTSAKY	1/6
Div 1	3.1	2.7	SSLRGDYEQY	2/6
Div 1	17	1.1	SSTLQGGTEAF	1/6

TCRB, T cell receptor β-chain (V = multiple variable and J = joining region gene segments). No., number of times the amino acid sequence was found on random sequencing of subcloned TCRBV sequences. CDR3 region, amino-acids between conserved -CA of TCRBV to conserved FG- of TCRBJ (38).

also detected a shared amino acid composition that was associated with the CD8+ T cell population of three of four CD subjects studied. This pattern was not present in a total of 51 sequences obtained from the UC subjects and was not present in the diverticulitis or normal control subject. The shared pattern identified in the CD patients consisted of TCRBV22, a conserved valine residue in the fifth position (shown in italics in Table 3), a highly conserved length, and either of the highly related junctional gene segments, TCRBJ2S5 or TCRBJ2S1. The side-chain analysis of this potential motif did not demonstrate any further indication of a shared pattern based on charge, although there was greater overlap in the two sequences sharing TCRBJ2S5.

Notably, two other TCR CDR3s from the CD8+ T cells demonstrated homologous amino acid sequences, consisting of -LGQGG-, in the hypervariable NDN region including the use of TCRBJ1S6. Although, these five amino acids were identical to another NDN region observed among CD8+ T cells that our group has reported previously as part of a UC-associated pattern (13), the TCRBV usage was TCRBV13 in *normal subject 1* rather than TCRBV3 (13).

Characterization of CDR3s within individuals. Given the existence of shared TCR usage patterns among unrelated CD subjects, we sought the presence of intraindividual or personal (private) motifs. Extensive sequence analyses of the TCRs of two CD patients, one UC patient, one diverticulitis patient, and one normal control are shown in Table 4. Striking intraindividual TCR CDR3 amino acid sequence similarities were present among both the CD8+ and CD4+ fractions. Additionally, there was conserved CDR3 homology, individually, in all four subjects. In general, the amino acid homology appeared to increase within the hypervariable portion of the TCR CDR3 as the TCRBV and TCRBJ became more similar. As an example, in *CD 3*, the -EGL- related sequences (Table 4, shaded), -VGL- and -LGL-, including the related -QGM- that maintained the glycine followed by a hydrophobic residue in the fifth position (Table 4, see side-chain analysis), were part of a larger pattern that shared both TCRBV and TCRBJ regions (TCRBV22 and TCRBJ1S1, respectively). In comparison, although the -KEGLV- and

Table 2. *Derived amino acid sequences for positively selected and activated CD8$^+$ intestinal T cells*

Subject	TCRBV	TCRBJ	CDR3 Region	No.
		Crohn's disease		
CD 1	2.3	1.1	SARVGTRGTEAF	1/13
CD 1	8	2.7	SSLGRGNVVPYEQY	7/13
CD 1	22	2.1	SSEYVPAERF	4/13
CD 1	22	2.2	TGQTFTGELF	1/13
CD 2	3.1	1.4	SSPAGGWTEKLF	10/42
CD 2	3.3	1.1	SSSFGSTEAF	14/42
CD 2	8	1.1	SSFSTPGDGNTEAF	5/42
CD 2	9	2.2	SSLRTSGPNTGELF	3/42
CD 2	12	2.3	ISESAGTGYTDTQY	7/42
CD 2	13	2.1	TGNNEQF	3/42
CD 3	3.1	1.4	SRVGTTNEKLF	1/44
CD 3	3.1	2.1	SSSPGLRFNEQF	1/44
CD 3	3.1	2.7	SSLMTDSYEQY	6/44
CD 3	22	1.1	STLVGEENTEAF	1/44
CD 3	22	1.1	STLVGQENTEAS	3/44
CD 3	22	1.1	SSVGLNTEAF	4/44
CD 3	22	1.1	SSLGLNTEAF	1/44
CD 3	22	1.1	SSEGLNTEAF	3/44
CD 3	22	1.1	SSQGMNTEAF	1/44
CD 3	22	1.1	SSDWQLRTAAAF	1/44
CD 3	22	1.5	SSDSNQPQH	2/44
CD 3	22	1.6	RSQGASSPLH	1/44
CD 3	22	2.1	SRGRDNEQF	3/44
CD 3	22	2.1	SRGRDDEQF	1/44
CD 3	22	2.1	STDGDYLYNEQF	1/44
CD 3	22	2.1	STKEGLVYNEQF	1/44
CD 3	22	2.1	STKEGLEVLNEQF	1/44
CD 3	22	2.1	SSEVAGSYNEQF	1/44
CD 3	22	2.1	SSEMGAAPGNEQF	1/44
CD 3	22	2.3	RKPGLAGPDTQY	3/44
CD 3	22	2.3	SREARVQGTDTQY	1/44
CD 3	22	2.3	SSRNRGGSADTQY	3/44
CD 3	22	2.5	SSPRVVGETQY	1/44
CD 3	22	2.7	SSTLRQEQY	1/44
CD 3	22	2.7	SSELRTSYEQY	1/44
CD 4	3.1	1.2	SRFGGYT	3/38
CD 4	3.3	1.6	RPFGGGFPSNSPLH	2/38
CD 4	3.3	1.6	*SSLGQGGLPPLH*	1/38
CD 4	3.3	2.1	SSFSGPPGGTGDEQF	5/38
CD 4	3.3	2.3	IGDSTDTQY	5/38
CD 4	21	2.5	SSLEEGGQETQY	6/38
CD 4	3.1	2.7	SSLTQGTGPAEQY	5/38
CD 4	3.3	2.7	SSLLASEGLEQF	1/38
CD 4	4.1	1.1	SVELGPGAF	1/38
CD 4	3.3	2.7	SSAVQGGLIEQF	3/38
CD 4	3.3	2.7	SSSTNSGFHEQF	1/38
CD 4	22	2.1	SRSRVYNEQF	1/38
CD 4	22	2.5	SSPLVYETQY	4/38
		Ulcerative colitis		
UC 1	3.1	1.5	SSFSGGQPQH	5/48
UC 1	3.1	1.5	SSSWARAGNQPQH	2/48
UC 1	3.3	1.3	SRDWESTIY	5/48
UC 1	4.1	1.1	SVNPLNTEAF	1/48
UC 1	12.2	2.7	IGPGTEATYEQY	6/48
UC 1	17.1	1.1	SSTSGQAAAEAF	6/48
UC 1	21	2.3	SSLDGGSTDTQY	8/48
UC 1	22	1.1	SRGSNTEAF	5/48
UC 1	22	1.1	SRASNTEAF	1/48
UC 1	22	1.1	SRGTNTEAF	1/48
UC 1	22	1.1	SSGMDTEAF	4/48
UC 1	22	1.1	SILMNTEAF	1/48
UC 1	22	1.1	SVLMNTEAF	1/48
UC 1	22	1.1	SSGLNTEAF	1/48
UC 1	22	1.1	SGFLNTEAF	1/48
UC 2	3.1	2.3	SSHGVGTDTQY	2/24
UC 2	3.1	1.1	SSLRTGEAF	3/24
UC 2	3.3	1.2	SSPPVAYGYT	4/24
UC 2	22	2.7	SRGPGEQY	2/24
UC 2	22	2.1	SRGQGLNEQF	1/24
UC 2	22	2.6	SSLQLSGANVLT	2/24
UC 2	22	2.1	SRAQGLNEQF	1/24
UC 2	22	2.1	SSEAGVYNEQF	2/24
UC 2	22	1.2	SREGGSLYGYT	2/24
UC 2	22	2.2	SSSAQPTGELF	1/24
UC 2	22	2.1	SSENRGRDEQF	1/24
UC 2	22	2.3	STGGSTDTQY	1/24
UC 2	22	1.1	SSEQGVRDEAF	1/24
UC 2	22	1.1	SSVGEAF	1/24
UC 3	3.3	1.2	SSSRTGLGGYT	3/37
UC 3	3.3	1.2	SSKGGGYGYT	1/37
UC 3	3.3	2.1	SRQHWRPNYEQF	1/37
UC 3	3.3	2.2	SSLTAENTGELF	1/37
UC 3	3.3	2.2	SSLTGREGTGELF	1/37
UC 3	3.3	2.2	SSRCPGASTGELF	1/37
UC 3	3.3	2.7	SNLDGSYEQY	2/37
UC 3	3.3	2.7	SSCGGRHFREQY	1/37
UC 3	3.3	2.7	SSPDGTYEQY	1/37
UC 3	17	2.7	SSIGEGVEQY	1/37
UC 3	22	2.7	SSVLDSRIYEQY	1/37
UC 3	22	2.7	SSELDSRIYEQY	11/37
UC 3	22	1.1	SRTPGQGARDTEAF	1/37
UC 3	22	1.4	SSFRGRFEKLF	2/37
UC 3	22	2.7	SIQGPLYEQY	2/37
UC 3	22	1.1	SIELPGTEAF	3/37
		Diverticulitis		
Div 1	2.3	2.5	SASQTFGIETQY	1/1
		Normal		
Normal 1	13	1.1	SSFGGGEAF	1/26
Normal 1	13	1.1	TGTGLNTEAF	2/26
Normal 1	13	1.5	SSYPYQPQH	2/26
Normal 1	13	1.6	*STLGQGGSPLH*	1/26
Normal 1	13	2.1	SSPGLAGAEQF	2/26
Normal 1	13	2.5	SSYHGSDETQY	1/26
Normal 1	2	1.2	ARDVATDHANYGYT	2/26
Normal 1	2	1.2	ARDDATDHANYGYT	1/26
Normal 1	2	1.2	ARDVTTDHAHYGYT	1/26
Normal 1	2	1.4	GNGQGSVENEKLF	1/26
Normal 1	2	1.6	ARDTRTGGRGPLH	1/26
Normal 1	2	2.1	ASGAGLTYNEQF	1/26
Normal 1	2	2.2	ARDFANTGELF	1/26
Normal 1	2	2.3	SLADSAGNGHITQY	1/26
Normal 1	2	2.3	ARSAGGRGSTDTQY	3/26
Normal 1	2	2.3	AGGRGSTDTQY	1/26
Normal 1	2	2.7	ARDAGEWTYEQY	2/26
Normal 1	2	2.7	ARDTGEWTYEQY	2/26

CDR3 region, amino acids between conserved -CA of TCRBV to conserved FG- of TCRBJ (38). No., number of times amino acid sequence found on random sequencing of subcloned TCRBV sequences. Italics and underlining, homology to UC-associated motif (13).

-KEGLEV- containing CDR3s shared the same TCRBV region and contain the -EGL- amino acid sequence (Table 4, bold), they exhibited less homology with the previous amino acid sequences and contained a different TCRBJ region gene product. Similarly, in the normal individual (*normal 1;* Table 4) and two UC subjects (*UC 1* and *UC 3*), there were also very conserved TCRB CDR3 patterns. A conserved amino acid sequence of -GGRG- (Table 4, bold) within the hypervariable CDR3 can be seen in one example, whereas throughout the sequences there was increasing homol-

Table 3. *Shared TCR patterns among activated intestinal CD8$^+$ and CD4$^+$ T cells*

Subject	TCRBV	TCRBJ	CDR3 Region	Side-Chain Analysis
			CD8$^+$ positively sorted T cells	
CD 4	3.3	1.6	SSLGQGGLPPLH	pphGpGGhPPhb
Normal 1	13	1.6	STLGQGGSPLH	pphGpGGpPhb
UC 3	3.3	2.7	SNLDGSYEQY	pphaGpYapY
UC 1	21	2.3	SSLDGGSTDTQY	pphaGGppappY
CD 4	3.1	1.2	SRFGGYT	pbhGGYp
CD 4	3.3	1.6	RPFGGGFPSNSPLH	bPhGGGhPpppPhb
Normal 1	13	1.1	SSFGGGEAF	pphGGGahh
CD 4	22	2.5	SSPL*V*YETQY*	ppPhhYappY
CD 3	22	2.5	SSPR*V*VGETQY*	ppPbhhGappY
CD 4	22	2.1	SRSR*V*YNEQF*	pbpbhYpaph
CD 1	22	2.1	SSEY*V*PAERF*	ppaYhPhaph
			CD4$^+$ positively sorted T cells	
CD 1	17	2.7	SSLDRSSYEQY	pphabppYapY
CD 2	21	2.7	SSLDRGYYEQY	pphabGYYapY

CDR3 region, amino acids between conserved -CA of TCRBV to conserved FG- of TCRBJ. TCRBD region is underlined. Side-chain analysis: uppercase letters = amino acids; lowercase letters = polar (p), hydrophobic (h), acidic (a), basic (b) (38). *Conserved valine (*V*) in fifth amino acid position of 3 of 4 CD subjects' TCRBV CDR3 regions.

ogy of the CDR3 when there was TCRBJ sharing. Also, as demonstrated in Table 4, these patterns appeared to be more evident among the activated CD8$^+$ T cell subsets than the CD4$^+$ T cell subset.

DISCUSSION

Characterization of activated T cell populations on the basis of preferential growth in rIL-2 allows for the characterization of limited tissue samples such as biopsy materials. This form of in vitro analysis of activated T cells has been applied to a number of other disease conditions. It has been reported that these cell culture conditions may skew the TCR repertoire (17), but recent data suggest that rIL-2 promotes survival of activated T cells in vitro and in vivo (4, 52). Regardless of the in vitro cell culture conditions, the germline-configured TCR CDR3 will not undergo new rearrangements, and thus the presence of distinctive TCR CDR3 patterns indicates specific antigenic stimulation. With this approach, our results indicate that activated T cells are present in individuals with idiopathic IBD, diverticulitis, and in a normal healthy control. In addition, these studies show that the composition of these activated T cells, as defined by analysis of the CDR3s of the TCRB chains, is characterized by considerable personal (private) patterns of TCR usage. In the cases of CD, we also observed a shared (public) pattern of TCR usage marked by *1*) use of TCRBV22, *2*) a highly conserved length, *3*) either TCRBJ2S1 or TCRBJ2S5 gene segment usage, and *4*) a conserved valine at the fifth position of the CDR3.

The amino acid patterns of TCR CDR3 usage we observed among activated intestinal T cells are similar to patterns first reported by Kourilsky and colleagues (15). In their murine models of T cells contacting putative antigen(s) in the context of genetically determined MHC, they defined the responses of T cells in terms of private and public responses (15, 35). A private response was reflected by T cell expansions within a particular mouse, as determined by TCR usage, in response to peptide antigen(s). Between mice, there was also an apparent public T cell response to a particularly immunodominant peptide fragment that was also reflected in TCR usage (15, 35). Thus both the public and private patterns of TCR usage that we observed in human-activated intestinal T cells are similar to those described in murine models.

Table 4. *Intraindividual TCR patterns among activated intestinal CD8$^+$ and CD4$^+$ T cells*

Subject	TCRBV	TCRBJ	CDR3 Region	Side-Chain Analysis
			CD8$^+$ positively sorted T cells	
CD 3	22	1.1	SS**EGL**NTEAF	pp**aGh**ppahh
CD 3	22	1.1	SSVGLNTEAF	pp**hGh**ppahh
CD 3	22	1.1	SSLGLNTEAF	pp**hGh**ppahh
CD 3	22	1.1	SSQGMNTEAF	pp**pGh**ppahh
CD 3	22	2.1	STK**EGL**VYNEQF	pp**baGhh**Ypaph
CD 3	22	2.1	STK**EGL**EVLNEQF	pp**baGhahh**paph
CD 3	22	1.1	STLVGEENTEAF	pphhGaappahh
CD 3	22	1.1	STLVGQENTEAS	pphhGpappahp
CD 3	22	2.1	SRGRDNEQF	pbGbapaph
CD 3	22	2.1	SRGRDDEQF	pbGbaaaph
UC 1	22	1.1	SRGSNTEAF	pbGpppahh
UC 1	22	1.1	SRASNTEAF	pbhpppahh
UC 1	22	1.1	SILMNTEAF	phhhppahh
UC 1	22	1.1	SVLMNTEAF	phhhppahh
UC 3	22	2.7	SSVLDSRIYEQY	pphhapbhYapY
UC 3	22	2.7	SSELDSRIYEQY	ppahapbhYapY
Normal 1	2	1.2	ARDVATDHANYGYT	hbahhpabhpYGYp
Normal 1	2	1.2	ARDDATDHANYGYT	hbaahpabhpYGYp
Normal 1	2	1.2	ARDVTTDHAHYGYT	hbahppabhbYGYp
Normal 1	2	1.6	ARDTRT**GGRG**PLH	hbapbp**GGbG**Phb
Normal 1	2	2.3	ARSA**GGRG**STDTQY	hbph**GGbG**ppappY
Normal 1	2	2.3	A**GGRG**STDTQY	h**GGbG**ppappY
Normal 1	2	2.7	ARDAGEWTYEQY	hbahGaWpYapY
Normal 1	2	2.7	ARDTGEWTYEQY	hbapGaWpYapY
			CD4$^+$ positively sorted T cells	
CD 1	3.3	2.7	SSMIYEQY	pphhYapY
CD 1	3.3	2.7	SSLIYKSYEQY	pphhYbpYapY
CD 3	3.3	2.7	SSAAGTGAYEQY	pphhGpGhYapY
CD 3	3.3	2.7	SSLAGREPSEQY	pphhGbaPpapY
CD 3	3.3	2.1	SSLLAGDNEQF	pphhhGapaph
CD 3	17	2.1	SSMGGYNEQF	pphGGYpaph
CD 3	3.3	2.1	SSLGGKASCIEQF	pphGGbhpChaph
Div 1	17	1.1	SSTLQGGTEAF	ppphpGGpahh
Div 1	3.1	1.1	SSPGQGGGAF	ppPGpGGGhh

CDR3 region, amino acids between conserved -CA of TCRBV to conserved FG- of TCRBJ. TCRBD region underlined. See Table 3 for explanation of side-chain analysis (38). Div, diverticulitis. Shaded areas indicate EGL related sequences and bold areas indicate sequences as described in text.

We have previously reported on an apparent shared or public TCR CDR3 amino acid pattern observed within the $CD8^+$ T cell subset in approximately one-half of the UC subjects undergoing colectomy for refractory disease (13). Uses of *1*) TCRBV3, *2*) TCRBJ1S6, and *3*) a given NDN region length of five amino acids have characterized the identified pattern (13). In the present study, this particular pattern was not identified among the three UC cases but was present in one CD subject (*CD 4*). This absence in the UC cases may represent a sampling error or be related to disease severity, as only five of nine UC colectomy cases were originally found to have this motif (13). On-going prevalence studies also indicate that this motif can be found in CD but at a reduced frequency (unpublished results, communication from C. S. J. Probert). It is also possible that these abnormalities represent UC-like disease, given the known overlap that can occur between CD and UC. In the normal control subject, the amino acid sequence -LGQGG- within the NDN region was identical to one of the sequences described in our original report (13), although the TCRBV was different. It is unknown as to whether $CD8^+$ T cell clones expressing this receptor sequence may recognize a similar antigenic epitope common to mucosal tissues or the receptor pattern of TCRBV3, TCRBJ1S6, and a conserved length in UC subjects defines a separate and specific epitope.

Public patterns of TCR usage in the murine population are easier to recognize than in the human population, most likely due to the inbred genetic homogeneity of the murine models and the influence of the MHC. A clear example of this shared TCR CDR3 homology can be observed in a murine model of allograft rejection whereby T cell clonal expansions can be specifically shown to develop in vivo as well as demonstrate functional reactivity in in vitro mixed lymphocyte reactions (18). An example of this MHC influence in humans can also be seen in infectious disorders in which the antigen(s) is/are known. For example, in Epstein-Barr virus infection, which becomes a latent and persistent viral infection in humans, MHC-restricted $CD8^+$ cytotoxic T cell clones have been detected in five MHC-identical, unrelated subjects, which respond to a particular dominant epitope of the virus and have similar TCR CDR3 sequences (5). In another human condition, multiple sclerosis, in which there is a presumed antigenic response to myelin basic protein, MHC-restricted T cell clones with similar TCR sequences have also been identified. This indicates a shared reactivity to an immunodominant fragment of myelin basic protein and further demonstrates a public response to a particular antigen-MHC complex (56).

In the present study, despite coresolution of dominant appearing TCRBV region usage between individuals, few public CDR3 amino acid sequences were determined. Nonetheless, a pattern among three of four CD subjects that utilized TCRBV22 and a conserved valine residue at the fifth NDN position was present. This pattern will require further evaluation to determine its prevalence and significance in intestinal T cell populations. It is possible that the selective expansion and activation of these T cell clones may represent a response to a common antigenic epitope in CD.

The intestinal subpopulations of activated T cells from diseased and nondiseased individuals showed T cell expansions with no apparent predominance of TCRBV usage. The presence of such clonal expansions of T cells, especially in the $CD8^+$ subsets, in normal individuals is not surprising and is consistent with other experimental data (19, 28). The lack of predominance of a particular TCRBV region associated with IBD may be indicative of the low number of study subjects but is consistent with the findings of other groups (23, 40). This lack of a shared dominant TCRBV usage argues against a significant role of superantigens in the molding of intestinal T cell repertoire, either in health or disease. Alternatively, the differences in TCRBV usage may reflect the role of the MHC in these observed T cell expansions. Support for this hypothesis comes from Silver and colleagues (2) who have demonstrated the influence of MHC in determining TCR repertoire in the peripheral blood from healthy control subjects. A further indication of the influence MHC on selecting TCRBV usage can be inferred by evaluation of discordant monozygotic CD twin pair's peripheral blood lymphocytes (26). There were no significant differences in TCRBV usage between twins, yet the patterns were "strikingly similar" between them (26).

In contrast to the public TCR CDR3 amino acid patterns, the personal (private) patterns we observed among the activated T cells in the inflammatory and noninflammatory study groups were striking. These private amino acid sequence patterns most likely indicate selective pressure on an individual's intestinal T cell subpopulation. This selective pressure presumably is in response to antigens that are prevalent within the mucosal tissues at the time of the study. Supportive of this concept are studies by Davis and colleagues (36), who repeatedly exposed mice to an antigen and demonstrated over time how similar the TCR CDR3 amino acid usage became within the T cell subpopulation of a particular mouse strain that exhibited an activated phenotype. We were able to detect increasing homology within the hypervariable NDN region among derived amino acid sequences as they increasingly shared both TCRBV and TCRBJ regions. This would be consistent with a "best fit" model of the TCR selectively expanding against a given putative MHC-peptide epitope. There were other sequences that did not increase homology within the NDN region, although they shared TCRBV and TCRBJ regions, which possibly indicate other relevant TCRs whose patterns were not as discernible. The recognition of a private-patterned response to antigen(s) may assist in defining possibly biologically relevant antigen(s) in mucosal disease, given increasing improvements in determining the cognate antigens of particular T cell clones (49, 50).

The private TCR responses in an apparent normal-appearing intestinal tissue sample would presumably indicate that the normal activated intestinal lymphocytes are undergoing selective responses to particular antigen(s) as part of a normal physiological process.

Whether this activation mechanism occurs directly through contact with luminal- (environmental and/or infectious) or tissue-related (autoimmune) antigens is unknown. Recently, astrovirus-specific and reactive $CD4^+$ T cell clones were identified from normal human small intestine, suggesting that the memory T cells within the intestine represent T cells specific for remote antigen exposure(s) (37). Whether the TCR patterns observed here represent activation of these T cell clones or newly recruited T cells responsive to recent antigenic exposures is unknown. Notably, similar types of patterns can be observed in other presumably activated and expanded T lymphocyte populations in other disease conditions of autoimmune, infectious, and neoplastic states (8, 11, 29, 34, 42, 54).

In conclusion, both public and private amino acid sequence patterns can be found within TCRB CDR3s of activated intestinal mucosal T lymphocyte subpopulations. Evaluation of the antigen(s) recognized by T cells expressing these TCR patterns may provide further insight into the apparent immunologically mediated destructive processes associated with diseases such as IBD, as well as provide further insights into the role of activated T lymphocytes within the normal gastrointestinal tract.

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