The delta T cell receptor repertoire in human colon and peripheral blood is oligoclonal irrespective of V region usage.

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Research Article

The majority of gamma/delta T cell receptors (TCR) in the human intestinal mucosa are thought to use the TCRDV1 (V delta 1) variable region gene segment, whereas gamma/delta T cells in the circulation predominantly express the TCRDV2 (V delta 2) gene segment. delta T cell receptors that use the TCRDV2 variable region gene segment generally have been regarded as highly diverse, whereas those that use the TCRDV1 gene segment are oligoclonal, whether present in the intestinal tract or in peripheral blood. We report herein that oligoclonality is a general feature of the peripheral delta T cell receptor repertoire in healthy human adults, irrespective of the variable region used and regardless of whether gamma/delta T cells reside in the intestinal mucosa or in peripheral blood. In addition, the delta T cell receptor repertoire is shown to be highly compartmentalized between such sites as the colon and peripheral blood, relatively stable over at least a 10-16-mo period, and unique in each individual. Further, the spectrum of variable region genes used by delta T cell receptor transcripts in the human colon is greater than previously recognized. Thus, in addition to the TCRDV1 and TCRDV2 variable region gene segments, delta T cell receptors in normal intestinal mucosa can use TCRDV3 (V delta 3) and TCRAV (V alpha) gene segments which, in some individuals, comprise […]

The δ T Cell Receptor Repertoire in Human Colon and Peripheral Blood Is Oligoclonal Irrespective of V Region Usage

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Abstract

The majority of γ/δ T cell receptors (TCR) in the human intestinal mucosa are thought to use the TCRDV1 (Vδ1) variable region gene segment, whereas γ/δ T cells in the circulation predominantly express the TCRDV2 (Vδ2) gene segment. δ T cell receptors that use the TCRDV2 variable region gene segment generally have been regarded as highly diverse, whereas those that use the TCRDV1 gene segment are oligoclonal, whether present in the intestinal tract or in peripheral blood. We report herein that oligoclonality is a general feature of the peripheral δ T cell receptor repertoire in healthy human adults, irrespective of the variable region used and regardless of whether γ/δ T cells reside in the intestinal mucosa or in peripheral blood. In addition, the δ T cell receptor repertoire is shown to be highly compartmentalized between such sites as the colon and peripheral blood, relatively stable over at least a 10–16-mo period, and unique in each individual. Further, the spectrum of variable region genes used by δ T cell receptor transcripts in the human colon is greater than previously recognized. Thus, in addition to the TCRDV1 and TCRDV2 variable region gene segments, δ T cell receptors in normal intestinal mucosa can use TCRDV3 (Vδ3) and TCRAV (Vα) gene segments which, in some individuals, comprise a significant component of the mucosal δ T cell receptor repertoire. Our studies indicate that the potential of δ T cell receptors for extensive diversity is not reflected in the mature human repertoire. Moreover, these findings suggest a model wherein the δ T cell receptor repertoire in the colon and peripheral blood is shaped by selection and clonal expansion of γ/δ T cells that ultimately seed throughout the length of the colon mucosa and populate the circulation. (*J. Clin. Invest.* 1995. 96:1108–1117.) Key words: CDR3 length • intestinal mucosa • intraepithelial lymphocytes • inverse PCR • junctional regions

Introduction

T lymphocytes that express the γ/δ T cell receptor (TCR)[1] represent a lineage distinct from those that express the α/β TCR in terms of their development, selection within the thymus, and distribution in the periphery (1, 2). The putative restriction elements and spectrum of functional properties of γ/δ T cells are largely unknown but appear to differ in many respects from those of α/β T cells (1, 3, 4). In the periphery, γ/δ T cells in humans comprise ~5% of circulating T lymphocytes (5). These cells are more abundant in the human intestinal mucosa, where they are located predominantly within the surface epithelium. Thus, γ/δ T cells comprise 10–15% of intraepithelial lymphocytes (IEL) in the small intestine (6, 7) and as many as 40% of IELs in the colon (8, 9). γ/δ T cells in the human intestinal mucosa and peripheral blood appear to be relatively compartmentalized in that a majority of circulating γ/δ T cells in adults express the TCRDV2 (Vδ2)[2] gene segment (10, 11), whereas TCRDV1 (Vδ1) is the predominant variable (V) region expressed by γ/δ T cells in the intestinal mucosa (6, 9). With the exception of the TCRDV1 and TCRDV2, the extent to which γ/δ T cells in intestinal mucosal tissues utilize other V gene segments is not known.

The TCR δ locus in humans maps within the TCR α locus on chromosome 14 (12) and several TCRAV (Vα) gene segments are used by both TCR δ and TCR α transcripts in the blood (13–15). Despite this shared use of V genes, the restriction elements and ligands recognized by γ/δ T cells appear to differ from those recognized by α/β T cells, emphasizing the likely importance of junctional regions that encode the complementarity determining region (CDR) 3 for ligand recognition (16). The fact that the CDR3 domains of δ chains are generally longer and more variable in length than those of α and β chains has been interpreted to suggest that γ/δ T cells recognize ligands in a manner that is fundamentally different from that of α/β T cells, and perhaps more analogous to that of antibody (17).

δTCRs have the potential for extensive diversity through combinatorial joining of V, diversity (D), and joining (J) gene segments and by nucleotide insertions and deletions that occur at the junctions of these gene segments. The diversity of δ transcripts is further increased by the ability to use simultaneously more than one D gene segment (18). However, in the case of γ/δ T cells that use TCRDV1, this potential for diversity is not reflected in the mature repertoire in the intestine and peripheral blood. Thus, although their junctions are complex, the repertoire of Vδ1 transcripts expressed by those T cells is oligoclonal (19, 20). In contrast, circulating γ/δ T cells in the peripheral blood, which use TCRDV2, have been regarded as having a more diverse repertoire (21–26), although such diversity may decrease with age (27). Whether the repertoire of mucosal γ/δ T cells that use TCRDV2 and possibly other V region genes is polyclonal or restricted is unknown.

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1. *Abbreviations used in this paper;* CDR, complementarity determining region; D, diversity; IEL, intraepithelial lymphocyte; J, joining; TCR, T cell receptor; V, variable.

2. TCR gene segment nomenclature according to *Bull WHO.* 1993. 71:113–115.

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Table I. Oligonucleotides Used for PCR and Hybridization

Oligonucleotides	Sequence
TCRDC primer 1	GGGTCGACTTGGCTTCTGACTTCTTTGTGATTC
TCRDC primer 2	TTATGAATGCGGCCGCTCTGTTATCTTCTTGGATGACACG
Linear PCR, TCRDV1 primer*	ATAAGTCGACCTGTATGAAACAAGTTGGTGG
Linear PCR, TCRDV2 primer	ATAAGTCGACCACCCTCAGGTGCTCCATGAA
Linear PCR, TCRDV3 primer	ATAAGTCGACTGGTACTGCTCTGCACTTACG
TCRDC sequencing primer*	AACGGATGGTTTGGTATG
Hybridization probe, TCRDV1	CCTTATTCGCCAGGGTTCTGATGAACAGAATGCAA
Hybridization probe, TCRDV2‡	CCACCCTCAGGTGCTCCATGAA
Hybridization probe, TCRDV3	GCACTGTCTTCAGTCCTTACTGGAGAGACTACCA

SalI and NotI restriction sites are singly or underlined in bold, respectively. The TCRDC primer 1 is located in TCRDC exon 4, and the TCRDC primer 2 is located in TCRDC exon 1. The TCRDC primer 2 was used for inverse and linear PCR. *Reference 19. ‡Reference 63.

In the present study, we asked whether the entire δ TCR repertoire in adult human colon and peripheral blood is restricted, regardless of V region usage. As reported herein, we have characterized the junctional diversity and repertoire of δ TCR transcripts expressed in human colon and peripheral blood, and determined the full spectrum of V gene segment usage by δ TCR transcripts in normal human colon. The data demonstrate that the δ TCR repertoire in adult human intestine and peripheral blood is strikingly oligoclonal and relatively stable over time, irrespective of V region gene usage. Further, δ TCR transcripts expressed in human colon are shown to use a broader array of V region genes than previously recognized.

Methods

Colon biopsies and peripheral blood mononuclear cells. Biopsies of colon mucosa, 2–3 mm in size, were obtained from seven healthy, unrelated male subjects, ages 62–77, during routine screening using flexible endoscopy. Although mucosal biopsies contain both surface epithelium and lamina propria, γ/δ T cells within these biopsies are located largely, but not exclusively, within the intraepithelial region (8, 9). Endoscopic appearance of the mucosa was normal in all subjects. Whole blood samples were obtained concurrently and 10–16 mon later, and PBMC were separated as described previously (19). All studies were approved by the University of California at San Diego's Committee on Human Subjects. We previously reported on Vδ1 transcripts in four of the subjects used herein (FA, PL, PJ, and OJ) (19).

RNA extraction and reverse transcription. RNA was extracted from homogenized biopsies (28) or PBMC using an acid phenol method (29). Total cellular RNA (1–2 μg) was reverse transcribed in 20 μl using 100 ng of oligo(dT)$_{16}$ primer (Boehringer Mannheim, Indianapolis, IN) and RNase H$^-$ Moloney murine leukemia virus reverse transcriptase (Superscript; GIBCO BRL, Gaithersburg, MD) at 45°C under conditions recommended by the manufacturer.

Circularization of cDNA and inverse PCR. cDNA from the reverse transcription reaction (20 μl) was circularized according to Uematsu's protocol (30) as modified by us (31), using a ligation volume of 500 μl. Circularized cDNA (20 μl) was amplified by inverse PCR using TCRDC (Cδ) primers 1 and 2 oriented in inverse directions (Table I). Each primer contained SalI or NotI restriction site extensions to facilitate subsequent cloning. PCRs were performed in 100 μl containing 0.2 mM of each dNTP (Pharmacia P-L Biochemicals, Inc., Milwaukee, WI), 0.5 μM primers, and 2.5 U Taq Polymerase in buffer supplied by the manufacturer (Stratagene, La Jolla, CA). After an initial hot start, amplification cycles consisted of 35 s of denaturation at 94°C, 45 s of annealing at 60°C, and a 1-min extension at 72°C for 37–39 cycles, followed by a final extension for 10 min at 72°C. PCR products were size separated on a 1.25% NuSieve agarose gel (FMC Corp. Bio Products, Rockland, ME) and visualized by ethidium bromide staining. Bands of appropriate size were excised from gels, and DNA was purified by phenol extraction (32).

Linear PCR. For assessing the junctional regions of δ TCR transcripts, 2 μl of the reverse transcription reaction was amplified using 5′ primers specific for TCRDV1, DV2, or DV3 and a 3′ primer specific for TCRDC (Table I, TCRDC primer 2). Each primer contained SalI or NotI restriction site extensions. After an initial hot start, amplification consisted of 35 cycles of 1-min denaturation at 94°C, 1-min annealing at 58°C, and 1-min extension at 72°C, followed by a final extension for 10 min at 72°C. Products of duplicate reactions were combined, purified using PCR purification columns (QIAGEN Inc., Chatsworth, CA), and digested with SalI or NotI for subsequent cloning.

Cloning and analysis of PCR products. The products of circular and linear PCR were cloned into pBluescript SK+ (Stratagene). Recombinant plasmid DNA from color-selected colonies was sequenced by the dideoxy chain termination method using Sequenase (Amersham Corp., Arlington Heights, IL) and a TCRDC-specific sequencing primer (Table I). When cDNA from the same biopsy was amplified by inverse PCR and linear PCR, most of the junctional regions amplified were identical (data not shown).

Nucleotide sequences were compared using the programs Fastscan and Clustal contained in the PC/Gene DNA analysis software (IntelliGenetics, Mountain View, CA). Nucleotide sequences were assigned to TCRDD (Dδ) gene segments based on at least 3 bp identities (33). Gene segment assignments were as follows: TCRDJ1 (Jδ1), TCRDJ2 (Jδ2), and TCRDJ3 (Jδ3) were assigned according to Takihara et al. (34); TCRDJ4 (Jδ4) was assigned according to Davodeau et al. (35); TCRDV (Vδ) gene segments were assigned according to Satyanarayana et al. (DV1) (36), Dariavach and Lefranc (DV2) (37), and Hata et al. (DV3) (38). TCRAV segments were assigned according to Pluschke et al. (TCRAV14S2) (39), Wilson et al. (TCRAV19) (40), and Roman-Roman et al. (TCRAV25, TCRAV27, TCRAV28) (41).

The δ TCR repertoire in any subject was defined as oligoclonal in this study only if identical δ transcripts were detected in at least two different biopsies or blood samples from that individual.

Generation and screening of libraries of inverse PCR products. Color-selected colonies carrying inverse PCR products cloned into pBluescript SK+ were transferred in an ordered array to duplicate LB plates (32) supplemented with carbenicillin (Sigma Chemical Co., St. Louis, MO). To screen these libraries for the frequency of usage of different V regions, bacterial colonies were transferred to nylon membranes (Hybond N+; Amersham) and hybridized at 58°C with TCRDV1, DV2, or DV3 hybridization probes (2 picomoles/ml) (Table I) that were 3′ end-labeled with digoxigenin-11-dUTP (Boehringer Mannheim), under conditions recommended by the manufacturer.

Table II. Distribution of V Region Gene Usage Among Colonic δ TCR Transcripts

Subject	Colon Biopsy	% of Transcripts Using Indicated V Region Gene*				Number of Transcripts Analyzed
		DV1	DV2	DV3	AV‡	
OJ	I	98	2	0	0	138
	II	98	2	0	0	129
FA	I	85	10	5	0	120
	II	84	16	0	0	86
PL	I	80	14	5	1	77
	II	66	34	0	0	81
LR	I	45	3	32	20	31
	II	30	2	39	29	86
SR	I	87	8	5	0	40
PJ	I	65	24	9	2	63
SC	I	51	49	0	0	117

* Bacterial colonies carrying cloned δ TCR inverse PCR products from colonic biopsies were screened with TCRDV1, DV2, and DV3 specific oligonucleotide probes. Negative clones were further analyzed by sequencing to identify their V regions (see Methods). ‡The TCRAV (Vα) segments detected by sequencing were TCRAV14S2, AV19, and AV27 (subject LR; see also Fig. 4), AV25 (subject PL), and AV28 (subject PJ).

Bound probe was visualized by incubating the filters with anti-digoxigenin-alkaline phosphatase conjugate (Boehringer Mannheim), followed by the chemiluminiscent substrate CSPD® (Southern Light; Tropix Inc., Bedford, MA), according to the manufacturer's protocol, after which filters were exposed to Kodak XAR film (Eastman Kodak, Rochester, NY). Each filter contained, as a control, an array of bacterial colonies carrying cDNAs for TCRDV1, DV2, and DV3. Full-length cDNA inserts in recombinant plasmids from colonies that did not hybridize to the TCRDV1, DV2, or DV3 probes were sequenced to identify their V regions.

CDR3 lengths and statistical analysis. The length of the CDR3 domains of translated δ TCR transcripts were calculated from the most conserved cysteine encoded by the 3′ TCRDV region to the conserved GXG triplet encoded by the TCRDJ region as described by Rock et al. (17), and the significance of differences in the lengths of the CDR3 domain between δ chains using different V regions was assessed using the Mann-Whitney U test (42). This analysis also includes published δ chain sequences from healthy adult subjects (19, 20, 23, 43–46). Repetitive sequences were counted only once.

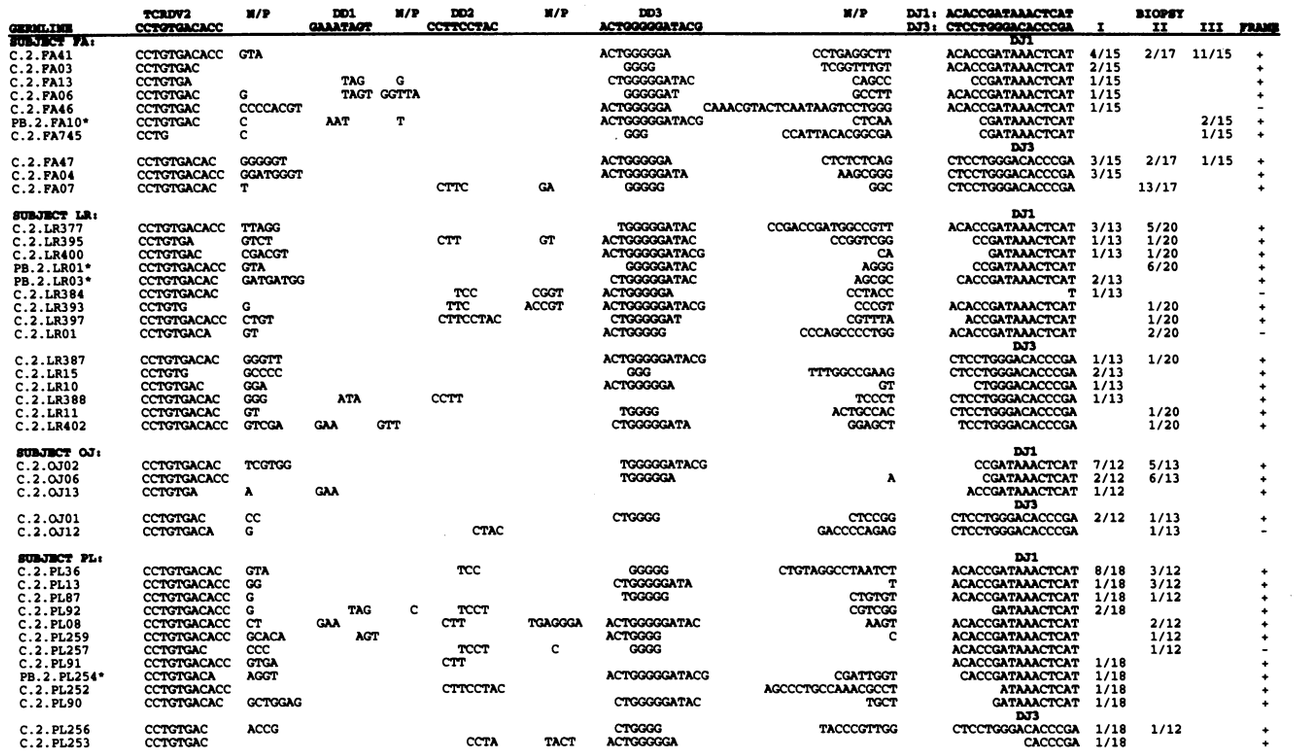
Results

Spectrum of V region usage by δ TCR transcripts in human colon. We first assessed the full spectrum of V genes that are expressed by γ/δ T cells in the colon. In this regard, prior studies that used immunohistochemistry or flow cytometry had identified γ/δ T cells that use Vδ1 or Vδ2 in the intestine (6, 9); however, antibodies are not available to identify γ/δ T cells that use other V gene segments. To determine the spectrum of V gene segments used by δ TCR transcripts in normal human colon, we used an inverse PCR method that allows equal amplification of δ TCR transcripts regardless of V region usage (30, 31). As shown in Table II, and consistent with prior immunohistochemical and flow cytometry studies (6, 9), TCRDV1 was the gene segment most frequently used by δ TCR transcripts in the colon, followed by TCRDV2. However, as shown herein, TCRDV3 and TCRAV gene segments are also used by mucosal δ TCR transcripts, albeit less frequently than TCRDV1 and TCRDV2. Yet in one subject (LR), TCRDV3 and TCRAV gene segments were used by > 50% of the δ TCR transcripts in colonic mucosa. These data indicate that the usage of V gene segments by δ TCR transcripts in human colon can be broader and more variable in healthy individuals than previously recognized.

δ TCR transcripts in human colon are oligoclonal irrespective of V gene segment usage. Our studies and others indicate that Vδ1 transcripts in human intestine and PBMC are oligoclonal (19, 20). In contrast, the Vδ2 repertoire in peripheral blood of healthy individuals has generally been regarded as highly diverse (21–26). To assess the junctional diversity of δ TCR transcripts in the colon which use TCRDV2, TCRDV3, or TCRAV gene segments, the junctional regions of δ transcripts using those gene segments were amplified and sequenced. As shown in Fig. 1, one or a few dominant Vδ2 transcripts were present in the colonic mucosa of each subject, and no overlap was noted in the transcripts among subjects. 88% of the Vδ2 transcripts in the colon mucosa were in frame. Moreover, within each subject, identical Vδ2 transcripts were detected in additional mucosal biopsies that were obtained at a distance of a few centimeters to over a meter from the first biopsy. Finally, when subject FA was rebiopsied 16 mo later (Fig. 1, biopsy III), Vδ2 transcripts identical to those noted in the initial biopsies were found, demonstrating the persistence of identical transcripts over time. As shown in Fig. 2, identical Vδ1 transcripts in the colon also persisted over time, a finding that parallels our prior data in small intestine (19).

The data in Table II demonstrate that Vδ3 can also contribute to the δ TCR repertoire in some healthy individuals. Like Vδ2 transcripts, as shown in Fig. 3, Vδ3 transcripts in the colon were also oligoclonal, and in subject FA, identical transcripts could be detected 16 mo later. Further, most Vδ3 transcripts (84%) in the colon were in frame. Finally, we note that TCR δ transcripts that use TCRAV gene segments in the colon are also oligoclonal (Fig. 4). In subject LR, analysis of junctional regions revealed identical transcripts using TCRAV14S2 and TCRAV27 gene segments in separate colon biopsies. All δ transcripts using TCRAV gene segments were in frame.

PBMC Vδ2 and Vδ3 transcripts are oligoclonal and differ from those in colon. Given the novel finding that δ transcripts in the colon that use TCRDV2 and TCRDV3 gene segments



GERMLINE	TCRDV2	N/P	DD1	N/P	DD2	N/P	DD3	N/P	DJ1: ACACCGATAAACTCAT DJ3: CTCCTGGGACACCCGA	I	BIOPSY II	III	FRAME
	CCTGTGACACC		GAAATAGT		CCTTCCTAC		ACTGGGGGATACG						
SUBJECT FA:									DJ1				
C.2.FA41	CCTGTGACACC	GTA					ACTGGGGGA	CCTGAGGCTT	ACACCGATAAACTCAT	4/15	2/17	11/15	+
C.2.FA03	CCTGTGAC						GGGG	TCGGTTTGT	ACACCGATAAACTCAT	2/15			+
C.2.FA13	CCTGTGA		TAG	G			CTGGGGGATAC	CAGCC	CCGATAAACTCAT	1/15			+
C.2.FA06	CCTGTGAC	G	TAGT	GGTTA			GGGGGAT	GCCTT	ACACCGATAAACTCAT	1/15			+
C.2.FA46	CCTGTGAC	CCCCACGT					ACTGGGGGA	CAAACGTACTCAATAAGTCCTGGG	ACACCGATAAACTCAT	1/15			-
PB.2.FA10*	CCTGTGAC	C	AAT	T			ACTGGGGGATACG	CTCAA	CGATAAACTCAT			2/15	+
C.2.FA745	CCTG	C					GGG	CCATTACACGGCGA	CGATAAACTCAT			1/15	+
									DJ3				
C.2.FA47	CCTGTGACAC	GGGGGT					ACTGGGGGA	CTCTCTCAG	CTCCTGGGACACCCGA	3/15	2/17	1/15	+
C.2.FA04	CCTGTGACACC	GGATGGGT					ACTGGGGGATA	AAGCGGG	CTCCTGGGACACCCGA	3/15			+
C.2.FA07	CCTGTGACAC	T			CTTC	GA	GGGGG	GGC	CTCCTGGGACACCCGA		13/17		+
SUBJECT LR:									DJ1				
C.2.LR377	CCTGTGACACC	TTAGG					TGGGGGATAC	CCGACCGATGGCCGTT	ACACCGATAAACTCAT	3/13	5/20		+
C.2.LR395	CCTGTGA	GTCT			CTT	GT	ACTGGGGGATAC	CCGGTCGG	CCGATAAACTCAT	1/13	1/20		+
C.2.LR400	CCTGTGAC	CGACGT					ACTGGGGGATACG	CA	GATAAACTCAT	1/13	1/20		+
PB.2.LR01*	CCTGTGACACC	GTA					GGGGGATAC	AGGG	CCGATAAACTCAT		6/20		+
PB.2.LR03*	CCTGTGACAC	GATGATGG					CTGGGGGATAC	AGCGC	CACCGATAAACTCAT	2/13			+
C.2.LR384	CCTGTGACAC				TCC	CGGT	ACTGGGGGA	CCTACC	T	1/13			-
C.2.LR393	CCTGTG	G			TTC	ACCGT	ACTGGGGGATACG	CCCGT	ACACCGATAAACTCAT		1/20		+
C.2.LR397	CCTGTGACACC	CTGT			CTTCCTAC		CTGGGGGAT	CGTTTA	ACCGATAAACTCAT		1/20		+
C.2.LR01	CCTGTGACA	GT					ACTGGGGG	CCCAGCCCCTGG	ACACCGATAAACTCAT		2/20		-
									DJ3				
C.2.LR387	CCTGTGACAC	GGGTT					ACTGGGGGATACG		CTCCTGGGACACCCGA	1/13	1/20		+
C.2.LR15	CCTGTG	GCCCC					GGG	TTTGGCCGAAG	CTCCTGGGACACCCGA	2/13			+
C.2.LR10	CCTGTGAC	GGA					ACTGGGGGA	GT	CTGGGACACCCGA	1/13			+
C.2.LR388	CCTGTGACAC	GGG	ATA		CCTT			TCCCT	CTCCTGGGACACCCGA	1/13			+
C.2.LR11	CCTGTGACAC	GT					TGGGG	ACTGCCAC	CTCCTGGGACACCCGA		1/20		+
C.2.LR402	CCTGTGACACC	GTCGA	GAA	GTT			CTGGGGGATA	GGAGCT	TCCTGGGACACCCGA		1/20		+
SUBJECT OJ:									DJ1				
C.2.OJ02	CCTGTGACAC	TCGTGG					TGGGGGATACG		CCGATAAACTCAT	7/12	5/13		+
C.2.OJ06	CCTGTGACACC						TGGGGGA	A	CGATAAACTCAT	2/12	6/13		+
C.2.OJ13	CCTGTGA	A	GAA						ACCGATAAACTCAT	1/12			+
									DJ3				
C.2.OJ01	CCTGTGAC	CC					CTGGGG	CTCCGG	CTCCTGGGACACCCGA	2/12	1/13		+
C.2.OJ12	CCTGTGACA	G			CTAC			GACCCCAGAG	CTCCTGGGACACCCGA		1/13		-
SUBJECT PL:									DJ1				
C.2.PL36	CCTGTGACAC	GTA			TCC		GGGGG	CTGTAGGCCTAATCT	ACACCGATAAACTCAT	8/18	3/12		+
C.2.PL13	CCTGTGACACC	GG					CTGGGGGATA	T	ACACCGATAAACTCAT	1/18	3/12		+
C.2.PL87	CCTGTGACACC	G					TGGGGG	CTGTGT	ACACCGATAAACTCAT	1/18	1/12		+
C.2.PL92	CCTGTGACACC	G	TAG	C	TCCT			CGTCGG	GATAAACTCAT	2/18			+
C.2.PL08	CCTGTGACACC	CT	GAA		CTT	TGAGGGA	ACTGGGGGATAC	AAGT	ACACCGATAAACTCAT		2/12		+
C.2.PL259	CCTGTGACACC	GCACA	AGT				ACTGGGG	C	ACACCGATAAACTCAT		1/12		+
C.2.PL257	CCTGTGAC	CCC			TCCT	C	GGGG		ACACCGATAAACTCAT		1/12		-
C.2.PL91	CCTGTGACACC	GTGA			CTT				ACACCGATAAACTCAT	1/18			+
PB.2.PL254*	CCTGTGACA	AGGT					ACTGGGGGATACG	CGATTGGT	CACCGATAAACTCAT	1/18			+
C.2.PL252	CCTGTGACACC				CTTCCTAC			AGCCCTGCCAAACGCCT	ATAAACTCAT	1/18			+
C.2.PL90	CCTGTGACAC	GCTGGAG					CTGGGGGATAC	TGCT	GATAAACTCAT	1/18			+
									DJ3				
C.2.PL256	CCTGTGAC	ACCG					CTGGGG	TACCCGTTGG	CTCCTGGGACACCCGA	1/18	1/12		+
C.2.PL253	CCTGTGAC				CCTA	TACT	ACTGGGGGA		CACCCGA	1/18			+

Figure 1. The δ TCR repertoire in colonic mucosa is oligoclonal and relatively stable over time. Biopsies I and II were obtained concurrently and were ~ 1–1.5 m apart in subjects LR and OJ, and ~ 2–3 cm apart in subjects FA and PL. In subject FA, an additional biopsy of colonic mucosa (III) was obtained from the sigmoid colon 16 mo later. Numbers refer to the fraction of transcripts that carry the indicated junctional regions. Sequences in and out of frame are indicated by (+) and (−), respectively, and germline sequences are indicated at the top in boldface type in this and subsequent figures. As shown, identical transcripts were found in biopsy I and II in each of the four subjects, and none of the transcripts were shared among subjects. In subject FA, identical transcripts were detected 16 mo later (biopsy III). Asterisks indicate transcripts present also in the PBMC of these subjects (see Fig. 5). 88% of the Vδ2 transcripts were in frame (repetitive transcripts were counted only once). These sequence data are available from EMBL/GenBank/DDBJ under accession numbers L39475–L39513.

were oligoclonal, we next asked whether transcripts that use those gene segments in the circulation were also oligoclonal. In this regard, δ transcripts that use TCRDV1 were known to be oligoclonal in the intestine and circulation (19, 20). In contrast, γ/δ T cells in the circulation that use TCRDV2 had previously been thought to be highly diverse (21–26). The data in Fig. 5 show that Vδ2 transcripts expressed by PBMC of adult humans are, in fact, oligoclonal and that identical transcripts can persist over time. Thus, dominant Vδ2 transcripts were present in repeated blood samples in most subjects, and, in each subject, Vδ2 transcripts identical to those in the initial blood sample could be detected 10–16 mo later. Vδ2 transcripts in PBMC from subject PL were more diverse than those of the other subjects. Nonetheless, identical Vδ2 transcripts in subject PL also persisted over time. No transcripts were repeated among different individuals, and all Vδ2 transcripts from PBMC were in frame. Finally, we note that Vδ2 transcripts in the PBMC showed very little overlap with those in the colon. It is likely that the few transcripts that were shared between PBMC and colon reflect the presence of small amounts of blood known to be present in mucosal biopsy specimens.

Like Vδ2 transcripts, repetitive Vδ3 transcripts were present in PBMC, and identical Vδ3 transcripts were consistently detected in a second blood specimen from the same individuals 10–16 mo

GERMLINE	TCRDV1	N/P	DD1	N/P	DD2	N/P	DD3	N/P	DJ1: ACACCGATAAACTCAT DJ2: CTTTGACAGCACAACT	I	BIOPSY II	III	FRAME
	CTCTTGGGGAACT		GAAATAGT		CCTTCCTAC		ACTGGGGGATACG						
									DJ1				
C.1.FA17	CTCTTGGGG	CCCCG			CCTTCCT	CTTTAT	ACTGGGGGATAC	AA	ACCGATAAACTCAT	5/14	4/19	1/9	+
C.1.FA01	CTCTTGGGGA	CCTTC	TAGT	GGT	CCTTCC		GAT	TAGGATCAGAGGTC	CCGATAAACTCAT	5/14	5/19	6/9	+
C.1.FA07	CTCTTGGGG	GCAGG			CCTTCCTAC	TTCGTA	GGGG	CGT	ACACCGATAAACTCAT		1/19	2/9	+
C.1.FA13	CTCTTGGGGAACT				CTAC	C	CTGGGG	TCACTCCGT	ACACCGATAAACTCAT		3/19		+
C.1.FA06	CTCTTGGGGAA	GA			TCC	CG	ACTGGGGGATAC	CGGTGT	ACACCGATAAACTCAT		2/19		+
C.1.FA14	CTCTTGGGGAAC				CCTTCCT			CCAAAAACCGT	CCGATAAACTCAT		2/19		+
									DJ2				
C.1.FA30	CTCTTGGGGAAC	ACGACGCAG			CCTTCC	GATGCCCGATTGG	ACTGGGGGATACG	CGA	CTTTGACAGCACAAC	4/14	2/19		+

Figure 2. The Vδ1 repertoire in colonic mucosa is oligoclonal and relatively stable over time. Biopsies I and II, ~ 2–3 cm apart, were obtained concurrently from subject FA. Biopsy III was obtained from the same region of colon, 16 mo later. Numbers refer to the fraction of transcripts that carry the indicated junctional regions. As shown, each biopsy contained repetitive transcripts, and all transcripts were in frame. Identical transcripts were shared among biopsies, and some of the transcripts present in the initial two biopsies were detected again in biopsy III, 16 mo later. These sequence data are available from EMBL/GenBank/DDBJ under accession numbers L39468–L39474.

GERMLINE	TCRDV3	N/P	DD1	N/P	DD2	N/P	DD3	N/P	DJ1: ACACCGATAAACTCAT DJ2: CTTTGACAGCACAACT DJ3: CTCCTGGGACACCCGA	I	BIOPSY II	III	FRAME
	CTTACTACTGTGCCTTT		GAAATAGT		CCTTCCTAC		ACTGGGGGATACG						
SUBJECT FA:									DJ1				
C.3.FA34	CTTACTACTGTGCCTTT	GGACAAGTC			CCTTCCTAC		ATAC	CGTACTGTAAGTGTAG	CGATAAACTCAT	11/15	10/10	12/14	+
C.3.FA08	CTTACTACTGTGCCTTT	AGGG					TGGGGG	GATACACCGTTTCCG	CCGATAAACTCAT	3/15			+
C.3.FA659	CTTACTACTGTGCCTT	CCATGGA					GGG		CTCAT	1/15			+
C.3.FA33	CTTACTACTGTGCC	CTCTCT			CTT	T	ACTGGGGGATAC	AGG	CACCGATAAACTCAT			1/14	-
									DJ3				
C.3.FA35	CTTACTACTGTGCCTT	CCCG			CCTTCCTAC	GTT	GGGGAT	CCCTTTAGAGGCTTT	CTCCTGGGACACCCGA			1/14	-
SUBJECT LR:									DJ1				
C.3.LR03	CTTACTACTGTGCC	CACA			CTAC	TGAA	GGGG	TTCG	ACTCAT	8/10	9/9		+
C.3.LR27	CTTACTACTGTGCC				CTAC	A	ACTGGGGGATAC		CGATAAACTCAT	2/10			+
SUBJECT OJ:									DJ1				
C.3.OJ53	CTTACTACTGTGCCTTT	AAGG			CTA	GGCA	GGGG	CCCC	ACACCGATAAACTCAT	2/8	6/11		+
C.3.OJ50	CTTACTACTGTGCCT	ACCAA			CTTCC	GGCCGTCGG	ACTGGGGGATAC	T	GATAAACTCAT	3/8	3/11		+
C.3.OJ56	CTTACTACTGTGCCT	CCG			TCCTAC	GCC	GGGGG	T	ACACCGATAAACTCAT	1/8			+
C.3.OJ62	CTTACTACTGTG	TTCCGGCTGGGGTCGT			CTT	GCT	GGGGATAC	ACCCGT	ACACCGATAAACTCAT		1/11		+
C.3.OJ66	CTTACTACTGTGCCTT	AA			CTA	GC	TGGG		ACACCGATAAACTCAT		1/11		+
C.3.OJ524	CTTACTACTGTGCCTTT	AAGGC	TAG	GCA			GGG	CCCC	ACACCGATAAACTCAT	1/8			-
									DJ2				
C.3.OJ73	CTTACTACTGTGCCTT	CCAAGCCCCC					GGGG	TCGCAA	TGACAGCACAACT	1/8			+
SUBJECT PL:									DJ1				
C.3.PL431	CTTACTACTGTGCCTT	CTTAACG			CCTA	GGTTAAG	TGGGGGA	CTTTTCT	ACACCGATAAACTCAT	4/11	2/12		+
C.3.PL427	CTTACTACTGTGCCTTT		AAT			GAGA	GGGG		TAAACTCAT	1/11	4/12		+
C.3.PL440	CTTACTACTGTGCCTTT	ACGC			TCCTAC	CT	CTGGGG	ACTCCCTCCTGGGGGG	ACACCGATAAACTCAT	1/11	2/12		+
C.3.PL514	CTTACTACTGTG	GTAGGGGGGT					ACTGGGGGATA	TGT	ACACCGATAAACTCAT	2/11			+
C.3.PL166	CTTACTACTGTGCCTTT	C					GGGGG	CC	CACCGATAAACTCAT	1/11			+
C.3.PL430	CTTACTACTGTGCC	CCAGC	AATA		TTCCTAC			CCCATGTCCGTTGT	ACCGATAAACTCAT	1/11			+
C.3.PL423	CTTACTACTGTGCCT	CCCAGGGT					CTGGGGGATACG	TCATG	GATAAACTCAT	1/11			-
C.3.PL441	CTTACTACTGTGCC	CTTTTGCCCT			TTCCTA	ATC	CTGGGGGAT		ACACCGATAAACTCAT		1/12		+
C.3.PL437	CTTACTACTGTGCCTTT	TCCAC			TCC	CCC	TAC	CT	ACACCGATAAACTCAT		1/12		+
C.3.PL444	CTTACTACTGTGCCTTT	AGG			TTCC			AT	ACCGATAAACTCAT		1/12		+
C.3.PL450	CTTACTACTGTGCC	GTTAAGATT			TTCC	GTC	ACTGGGGGATA	GTGGTA	AAACTCAT		1/12		+

Figure 3. The Vδ3 repertoire in colonic mucosa is oligoclonal and relatively stable over time. Biopsy I and II from each subject were obtained concurrently and were ~ 1–1.5 m apart in subjects LR and OJ, and ~ 2–3 cm apart in subjects FA and PL (see also Fig. 1). Biopsy III in subject FA was obtained 16 mo later. As shown, identical transcripts were detected in biopsy I and II from each of the four subjects. In addition, identical dominant Vδ3 transcripts were present in subject FA 16 mo later (biopsy III). 84% of the Vδ3 transcripts were in frame (repetitive transcripts were counted only once). These sequence data are available from EMBL/Genbank/DDBJ under accession numbers L39517–L39538.

later (Fig. 6). However, 53% of PBMC Vδ3 transcripts were out of frame. Nonetheless, some of these out-of-frame sequences were also found repetitively and could be detected again in blood samples obtained from the same subject ~ 1 yr later. It is likely that these out-of-frame Vδ3 transcripts are derived from cells in which the functional T cell receptor transcript is encoded by the other TCRD allele or by a TCRA allele.

CDR3 length distribution and molecular features of δ TCR transcripts. γ/δ T cells appear to interact with ligands in a manner fundamentally different from that of α/β T cells (3, 4). Consistent with this, the TCR CDR3 domain, which is thought to be important for ligand recognition, is significantly longer and more variable in length in δ than in α or β chains (17). Prior studies have not addressed possible differences in the CDR3 lengths of δ chains that express different V regions. We analyzed the average length and range of lengths of the CDR3 domain of translated Vδ1, Vδ2, and Vδ3 transcripts from colon and peripheral blood. This analysis used both our data and published data from healthy adult subjects (19, 20, 23, 43–46). As shown in Table III, there was no significant difference in the mean or range of CDR3 lengths between colonic and PBMC δ chains translated from transcripts that use the same V region gene segment. Moreover, different dominant transcripts that use the same V region did not code for a specific CDR3 length. However, as shown in Table III and Fig. 7, the mean CDR3 length of PBMC and mucosal Vδ2/Jδ1 chains was significantly shorter than that of Vδ1/Jδ1 chains ($P < 0.001$), whereas the mean CDR3 length of Vδ3/Jδ1 chains did not differ significantly from that of Vδ2/Jδ1 chains. The difference between Vδ1 compared with Vδ2 and Vδ3 chains cannot be fully accounted for by the fact that 3′ of the conserved cysteine, germline TCRDV1 gene segments are five or six nucleotides longer than TCRDV2 or TCRDV3, respectively, because, in the fully assembled TCRDV1 segment, this difference is often eliminated by more extensive trimming of TCRDV1 ends (Y. Chowers, W. Holtmeier, M. F. Kagnoff, and E. Morzycka-Wroblewska, manuscript in preparation). Rather, the difference in CDR3 lengths of Vδ1 and Vδ2 chains is best explained by differences in their D segment usage. As shown in Table IV, the majority of Vδ1 transcripts used TCRDD2 (Dδ2) and TCRDD3 (Dδ3) gene segments, while most of the Vδ2 transcripts used only TCRDD3, a finding also noted by others in regard to

GERMLINE	AV14S2 AV19 AV27	TTCTGTGCTTATAGGAGC CTACATCTGTGCTGTCACG GTGCCGTGGACTCGACC	N/P	DD2 CCTTCCTAC	N/P	DD3 ACTGGGGGATACG	N/P	DJ1 ACACCGATAAACTCAT	BIOPSY I	BIOPSY II	FRAME
C.A.LR07		TTCTGTGCTTATAGGAGC	GCGTATC	TCC	CACCCTC	GGGG	TTTT	ACACCGATAAACTCAT	3	2	+
C.A.LR02		TTCTGTGCTTATAGGAGC	ACAG	CCTTCCTAC	CGT	ACTGG	CTCC	ACACCGATAAACTCAT		3	+
C.A.LR03		CTACATCTGTGCTGTCA	GATCCGG	CTTC	GGT	ACTGGG	CTA	ACCGATAAACTCAT		4	+
C.A.LR01		GTGCCGTGGACTCGACC		CCTTCCT		GGGGATACG	CCG	CCGATAAACTCAT	3	16	+
								total:	6	25	

Figure 4. δ TCR transcripts that use TCRAV (Vα) gene segments are oligoclonal. Biopsies were obtained from the sigmoid colon (biopsy I) and cecum (biopsy II), ~ 1.5 m apart, in subject LR. As shown, transcripts C.A. LR07 (TCRAV14S2) and C.A. LR01 (TCRAV27) were present in biopsies from different regions of the colon. The number of transcripts having each junctional sequence are indicated. The 3′ ends of most germline TCRAV segments are not known and are, therefore, assigned arbitrarily (39–41). Based on a comparison with published sequences (39, 62), we suggest the TCRAV14S2 germline sequence is at least nine nucleotides larger than previously described. These sequence data are available from EMBL/GenBank/DDBJ under accession numbers L39614–L39619.

GERMLINE	TCRDV2 CCTGTGACACC	N/P	DD1 GAAATAGT	N/P	DD2 CCTTCCTAC	N/P	DD3 ACTGGGGGATACG	N/P	DJ1: ACACCGATAAACTCAT DJ2: CTTTGACAGCACAACT DJ3: CTCCTGGGACACCCGA DJ4: CCAGACCCCTGATCTT	PBMC 1993	PBMC 1994	FRAME
SUBJECT FA:									DJ1			
PB.2.FA01	CCTGTGACACC	GTGGA					ACTGGGGGA	GGAAAAACTGT	ACACCGATAAACTCAT	7/18	1/14	+
PB.2.FA15	CCTGTGACACC	G			TCC	CT	ACTGGGGGA	GTCT	ACACCGATAAACTCAT	2/18	5/14	+
PB.2.FA02	CCTGT	TTGTT					ACTGGGGGATA	AAG	CCGATAAACTCAT	2/18	1/14	+
PB.2.FA11	CCTGTGACACC	GTACCCA							ACACCGATAAACTCAT	1/18		+
PB.2.FA03	CCT				CCT	GTTCTCGT	GGGGATACG	CGGT	ACACCGATAAACTCAT	1/18		+
PB.2.FA10*	CCTGTGAC	C	AAT	T			ACTGGGGGATACG	CTCAA	CGATAAACTCAT		1/14	+
PB.2.FA604	CCTGTGACACC	GT			CTT	GGGCCGT	ACTGGGGGATACG	CGTATCCCGAG	ACCGATAAACTCAT		1/14	+
PB.2.FA617	CCTGTGAC		GAA	GT			ACTGGGGG	TCCTTGG	CGATAAACTCAT		1/14	+
PB.2.FA618	CCTGTGACA	TTGT					TGGGGGATACG	C	TAAACTCAT		1/14	+
PB.2.FA611	CCTGTGACAC	TC			TCCTAC		GGGGGATAC	AGG	CCGATAAACTCAT		1/14	+
									DJ4			
PB.2.FA05	CCTGTGACACC						CTGGGGGATACG	G	GACCCCTGATCTT	5/18	2/14	+
SUBJECT LR:									DJ1			
PB.2.LR03*	CCTGTGACAC	GATGATGG					CTGGGGGATAC	AGCGC	CACCGATAAACTCAT	19/27	5/11	+
PB.2.LR01*	CCTGTGACACC	GTA					GGGGGATAC	AGGG	CCGATAAACTCAT	3/27	4/11	+
PB.2.LR213	CCTGTGACACC	GT					TGGGG	CTTCTT	ACACCGATAAACTCAT		2/11	+
PB.2.LR06	CCTGTGACACC	GT					CTGGGGGATAC	AG	ACCGATAAACTCAT	3/27		+
PB.2.LR07	CCTGTGA	T			CTTC	TGACT	GGGGATACG	GG	TAAACTCAT	1/27		+
									DJ4			
PB.2.LR04	CCTGTGACACC	GTCGG					ACTGGGGGATACG	CCCG	CCAGACCCCTGATCTT	1/27		+
SUBJECT PL:									DJ1			
PB.2.PL549	CCTGTGACACC	G					TGGGGGATACG	CGATCC	AAACTCAT	1/10	3/14	+
PB.2.PL679	CCTGTGA	TCG					ACTGGGGG	TTGAGGA	CGATAAACTCAT	2/10		+
PB.2.PL262	CCTGTGAC	TC					ACTGGGGG	TAC	ACACCGATAAACTCAT	1/10		+
PB.2.PL689	CCTGTGACACC	CTGCG					ACTGGGGGATAC	CC	ACACCGATAAACTCAT	1/10		+
PB.2.PL683	CCTGTGACAC						ACTGGGGGA	CCTTATGT	ACACCGATAAACTCAT	1/10		+
PB.2.PL554	CCTGTGACACC	GTAGG					ACTGGGGGAT	GGGGCGA	ATAAACTCAT		1/14	+
PB.2.PL550	CCTGTGAC	CCAACA					GGGGGAT	CGTCTCTCATTACGAAGT	ACACCGATAAACTCAT		1/14	+
PB.2.PL566	CCTGTGACAC	AG			TTCCTAC		TGGGGGA	GAGT	ACACCGATAAACTCAT		1/14	+
PB.2.PL254*	CCTGTGACA	AGGT					ACTGGGGGATACG	CGATTGGT	CACCGATAAACTCAT		1/14	+
PB.2.PL706	CCTGTGAC	GGGTT					ACTGGGGGATA	GA	ACCGATAAACTCAT		1/14	+
PB.2.PL726	CCTGTGACA	TG					CTGGGG	ACCTCAT	ACACCGATAAACTCAT		1/14	+
PB.2.PL735	CCTGTGACACC	G					TGGGGGAT	T	CCGATAAACTCAT		1/14	+
PB.2.PL737	CCTGTGAC	C			CCTT		ACTGGGGGATAC	AAGAGGGT	CCGATAAACTCAT		1/14	+
									DJ2			
PB.2.PL544	CCTGTGACACC	CTGAGGG					ACTGGGGG	CTCT	CAGCACAACT	1/10	1/14	+
									DJ3			
PB.2.PL269	CCTGTGAC	CGCGT					ACTGGGGGATAC	CCACGGGG	CCTGGGACACCCGA	1/10		+
PB.2.PL272	CCTGTGAC	GATGAC					GGGGGA	CTTCTATT	CTCCTGGGACACCCGA	1/10		+
PB.2.PL539	CCTGTGAC	GAAGC					GGGGGA	CCCCTCATT	CTCCTGGGACACCCGA	1/10		+
PB.2.PL555	CCTGTGACACC	GT					GGGGAT	CCCCG	CCCGA		1/14	+
PB.2.PL736	CCTGTGACAC						TGGGGGAT	GGAG	CTCCTGGGACACCCGA		1/14	+
SUBJECT PJ:									DJ1			
PB.2.PJ301	CCTGTGAC	CT					ACTGGG	AGTCC	ACACCGATAAACTCAT	11/13	12/14	+
PB.2.PJ307	CCTGTGACAC	AGG					TGGGGG	TATTCTACTACCAT	ACACCGATAAACTCAT	1/13		+
PB.2.PJ713	CCTGTGAC	CGAG				TAC TGTTAGGA	GGGG	CT	ACCGATAAACTCAT		1/14	+
									DJ2			
PB.2.PJ711	CCTGTG						TGGGGGATA	AAGACGCCCTAG	CTTTGACAGCACAACT		1/14	+
									DJ3			
PB.2.PJ313	CCTGTG	TGGT					TGGGGGAT	TGAG	CTCCTGGGACACCCGA	1/13		+

Figure 5. The Vδ2 repertoire in peripheral blood is oligoclonal and relatively stable over time. PBMCs were obtained at the same time as colonic biopsies in four subjects (1993). In addition, PBMCs were obtained from these subjects 10–16 mo later (1994). As shown, identical transcripts were present in both blood samples. The Vδ2 repertoire was oligoclonal in subjects PJ, FA, and LR and somewhat more diverse in subject PL. Of note, Vδ2 transcripts rarely used the TCRDD2 gene segment. Transcripts indicated by an asterisk were also present in the colonic mucosa of these subjects (see also Fig. 1). No sequences were shared among subjects. These sequence data are available from EMBL/Genbank/DDBJ under the accession numbers L39539–L39579.

peripheral blood (21). This finding was true both for in-frame and out-of-frame sequences, suggesting that this difference is due to differential TCRDD2 usage during V-D-J recombination rather than to selection at the cellular level. 30% of colonic Vδ2 transcripts used TCRDJ3 (Jδ3) gene segments, and, as indicated in Table III, the CDR3 domain of Vδ2/Jδ3 chains was ~ 2 amino acids longer than that of Vδ2/Jδ1 and, in this regard, similar to that of Vδ1/Jδ1 chains. This difference can be attributed to the fact that the TCRDJ3 gene segment encodes a product that is three amino acids longer than the one encoded by TCRDJ1.

Amino acid sequences encoded by δ TCR transcripts. A hydrophobic valine, leucine, or isoleucine was encoded by the first codon of the junctional region in 85% of Vδ2/Jδ1 PBMC transcripts, a finding also noted by others (47, 48). This was also the case for 65% of Vδ2/Jδ1 mucosal transcripts sequenced herein. Comparison of the amino acid sequences of the translated junctional regions of the δ TCR transcripts did not reveal a common motif shared by most transcripts using the same V region, although groups of two to four translated transcripts that differed from each other by only one or two amino acids were noted (data not shown; translated amino acid sequences are available upon request).

Discussion

The results herein demonstrate that oligoclonality is a general feature of the δ TCR repertoire in the colon and peripheral blood of healthy adults, irrespective of V region usage. These data favor a model wherein the adult human δ TCR repertoire is shaped by selection and clonal expansion of γ/δ T cells that seed throughout the length of the colon and populate the circulation. Since the repertoire of δ TCR transcripts in the intestinal mucosa differs from that in the circulation, it is possible that different ligands and/or factors drive the expansion of the γ/δ T cells present in those sites.

We previously reported that TCR δ transcripts in the intestine that use TCRDV1 are oligoclonal (19). To determine whether δ transcripts in the intestine that use other V genes are also oligoclonal, the frequency with which colonic δ transcripts used different V regions was first assessed. Although TCRDV1 and, to a lesser extent, TCRDV2 were the major gene segments used by γ/δ T cells in the colon of most subjects, γ/δ T cells in the colon of some healthy individuals also variably used TCRDV3 and TCRAV (Vα) gene segments. In one individual, these non-TCRDV1, non-TCRDV2 gene segments were used by the majority of δ TCR transcripts. Including the data herein, members of at least 11 TCRAV gene families have now been shown to recombine with TCR δ gene segments (13–15). These findings are consistent with those of others using immunohistochemistry and flow cytometry to report that γ/δ cells in the small intestinal mucosa and colon express predominantly Vδ1 or Vδ2 (6, 7, 9). However, those two V regions did not always account for the total number of mucosal T cells expressing γ/δ TCR (6, 49, 50). Although, in the past, γ/δ T cells in the

	TCRDV3	N/P	DD1	N/P	DD2	N/P	DD3	N/P	DJ1: ACACCGATAAACTCAT DJ3: CTCCTGGGACACCCGA DJ4: CCAGACCCCTGATCTT	PBMC		
GERMLINE	CTTACTACTGTGCCTTT		GAAATAGT		CCTTCCTAC		ACTGGGGGATACG			1993	1994	FRAME
SUBJECT LR:									DJ1			
PB.3.LR474	CTTACTACTGTGCCTT	CTCCC			CCTTCCTAC	GGG	TGGGGGATACG	TCG	GATAAACTCAT	1/11	3/16	+
PB.3.LR480	CTTACTACTGTTCC	C			CCTT		GGG	CTTGACCTTGGAGGGAC	ACACCGATAAACTCAT	2/11	1/16	+
PB.3.LR477	CTTACTACTGTGCC	AACCCGCAGGACTGCCGCT			CTT	T	ACTGGGGGAT	GATGT	ACACCGATAAACTCAT	2/11	1/16	-
PB.3.LR483	CTTACTACTGTGCCT	CCCCCCAA			CCTA	TG	TGGGGGATAC	AAAG	ACACCGATAAACTCAT	1/11	1/16	-
PB.3.LR496	CTTACTACTGCGCCTTT				TCC	AC	GGGGGATAC	CT	ACACCGATAAACTCAT		2/16	+
PB.3.LR500	CTTACTACTGTGCCTT				CCTAC	GT	TGGGGGA	CCTCTTACGGGAT	ACACCGATAAACTCAT		2/16	-
PB.3.LR502	CTTACTACTGTGCCTTT	CCCGGACC			TCC	GACTCAT	CTGGGGGA	GCTAGACGGGGTTT	ACACCGATAAACTCAT		1/16	+
PB.3.LR506	CTTACTACTGTGCCTTT	CAG			CCT	CTGGACAGGGGA	TGGGGG	G	ACACCGATAAACTCAT		1/16	+
PB.3.LR507	CTTACTACTGTGCCTTT	AGTTTTGGGGTT			CTAC	GGCGT	ACTGGGG	ATA	ATAAACTCAT		1/16	+
PB.3.LR504	CTTACTACTGTG	TTGGGTTC			CCT		ACTGGGGGA	CCTGGGGT	ACACCGATAAACTCAT		1/16	-
PB.3.LR479	CTTACTACTGTGCCTTT	A GAAA			CCTAC		TGGG	TGA	ATAAACTCAT	1/11		-
PB.3.LR476	CTTACTACTGTGCCTT	ATTTGAGATCGT					ACTGGGGGATACG		CCGATAAACTCAT	1/11		-
PB.3.LR478	CTTACTACTGTGCCTTT	AG	AGT GGGC		CCTAC	GATGGATG	TGGGGG	GTTCTTACG	CCGATAAACTCAT	1/11		+
									DJ3			
PB.3.LR484	CTTACTACTGTGCCT	C					GGGGG	GTTAG	CTCCTGGGACACCCGA	1/11		-
									DJ4			
PB.3.LR475	CTTACTACTGTGCCTT	CTGGGGGGT			TTCC	CGCCTA	ACTGGGGGAT	CAC	CCAGACCCCTGATCTT	1/11	2/16	+
SUBJECT PL:									DJ1			
PB.3.PL345	CTTACTACTGTGCCTT				CTTC	AGT	ACTGGGGGATA	TCG	TAAACTCAT	3/11	4/16	+
PB.3.PL339	CTTACTACTGTGCCT	CAAGGGGTTG					TGGGGGATAC	TTTTACAA	TAAACTCAT	2/11	1/16	+
PB.3.PL342	CTTACTACTGTGCCTT	CG	TAG			CGCCCATCTCGG	ACTGGGGG	GGAG	ACACCGATAAACTCAT	1/11	1/16	+
PB.3.PL347	CTTACTACTGTGCCTTT				CTAC	C	GGGGATC	GCCCGAAG	AACTCAT	3/11	3/16	-
PB.3.PL341	CTTACTACTGTGCCTTT	CC	TAG G		CCTT		ACTGGGGGATC	ACCACCTGGGGG	ACTCAT	1/11		-
PB.3.PL340	CTTACTACTGTGCCTTT	CATATGGCT	ATAG		CCTT	TTT	ACTGGGGGATC	CTTTTGACCC	ATAAACTCAT	1/11		-
PB.3.PL364	CTTACTACTGTGCCTTT	GAA					ACTGGGG	ATAAAG	ACACCGATAAACTCAT		2/16	+
PB.3.PL367	CTTACTACTGT					TAC	GT	GT	ATAAACTCAT		1/16	+
PB.3.PL371	CTTACTACTGTGCCTT	ACCTA			CTTCCTAC	TCTGATTTT	ACTGGGGG	TGATAAG	ACCGATAAACTCAT		1/16	+
PB.3.PL374	CTTACTACTGTGCCTTT	CTCAG			TTCC	CGT	ACTGGGGGATACG	T	ACACCGATAAACTCAT		1/16	-
SUBJECT FA:									DJ1			
PB.3.FA635	CTTACTACTGT				CTTC		GGGGG	GTCCGAGGTTTCTGGGGGGTCGT	ACACCGATAAACTCAT		4/12	-
PB.3.FA644	CTTACTACTGTGCC	TCTCC	TAG CGAC		CCTTC		CTGGGG	TTTCGGGGTAGG	TAAACTCAT		1/12	+
PB.3.FA634	CTTACTACTGTGCCTT	CTCAAG			CCTTCCTA	A	TGGGGGATACG	GGAG	CGATAAACTCAT		1/12	+
PB.3.FA632	CTTACTACTGTGCCTTT		AAATA AGATTGGGA		TTCCTAC	GGCT	ACTGGGGGATAG	GGGGGGGT	ACACCGATAAACTCAT		1/12	-
PB.3.FA639	CTTACTACTGTGCCTTT	AACGG					ACTGGGGGATAC		ACACCGATAAACTCAT		1/12	-
PB.3.FA643	CTTACTACTGTGCC	GTCTCA					GGG	CTACAAGTGGTTACGCCTTAGAGT	ACACCGATAAACTCAT		1/12	-
PB.3.FA633	CTTACTACTGTGC	TTAAG			CCTTCCTA	AGTTGTT	TGGGGG	GGGACGGGAACTGTT	ACCGATAAACTCAT		1/12	-
PB.3.FA638	CTTACTACTGTGC	TTATCAA			TCCT	GGTATGG	ACTGGGGG	GGAAACA	GATAAACTCAT		1/12	-
PB.3.FA645	CTTACTACTGTGCCTTT	TGA			CCT	GG	TGGGGG	GGTTG	AACTCAT		1/12	-

Figure 6. The Vδ3 repertoire in peripheral blood is oligoclonal and relatively stable over time. PBMC were obtained at the same time as colonic mucosal biopsies in subjects LR and PL (1993) as well 10–16 mo later (1994). In subject FA, the blood sample (1994) was obtained concurrently with that of FA's biopsy III. Identical transcripts were found 10–16 mo apart in subjects LR and PL. Note the high frequency (53%) of out-of-frame sequences, three of which were also detected 10–16 mo later. These sequence data are available from EMBL/Genbank/DDBJ under accession numbers L39580–L39613.

intestine-expressing V regions other than Vδ1 and Vδ2 were reported in subjects with celiac disease (50, 51), the present findings indicate there can be considerable variability in the expression of a number of Vδ gene segments, including non-TCRDV1 and non-TCRDV2 segments, within healthy subjects.

The entire δ TCR repertoire in the colon was oligoclonal in the subjects studied herein, regardless of which V regions were used. Oligoclonality was demonstrated by the finding of identical dominant and rare transcripts that were repetitive throughout the colon. Further, the same transcripts were detected in the same individual over a year later. Moreover, these data support the notion that clones of γ/δ T cells are positively selected and expanded before they seed the length of the colon. The possibility that independent V-D-J rearrangements in distinct T cells scattered throughout the length of the colon result in identical δ TCR sequences at multiple sites can be excluded, given the extensive complexity of the junctions of the δ TCR transcripts

Table III. CDR3 Length of Translated δ TCR Transcripts

	Range	Median Length	Mean Length ±SEM*	Number of Transcripts Analyzed
Vδ1/Jδ1, colon‡	9–23	15	15.0±0.3	116
Vδ1/Jδ1, PBMC‡	8–22	15	14.9±0.4	61
Vδ2/Jδ1, colon§	6–17	12	12.1±0.5	30
Vδ2/Jδ1, PBMC§	8–18	12	12.3±0.2	197
Vδ2/Jδ3, colon + PBMC‖¶	10–18	14	14.3±0.5	17
Vδ3/Jδ1, colon + PBMC‖¶	3–18	12	11.5±0.6	39

* The mean CDR3 length of Vδ2/Jδ1 chains in colon and PBMC was significantly shorter than that of Vδ1/Jδ1 chains ($P < 0.001$). The mean CDR3 length of Vδ3/Jδ1 chains was significantly shorter than that of Vδ1/Jδ1 chains ($P < 0.001$). There was no significant difference in the mean CDR3 length of Vδ3/Jδ1 and Vδ2/Jδ1 chains ($P > 0.05$). ‡Sequences from published data (19, 20). §Sequences from data herein and published data (23, 43–46). ‖Sequences from data herein. ¶To obtain sufficient numbers for analysis of the CDR3 lengths of Vδ2/Jδ3 or Vδ3/Jδ1 chains, colon and PBMC sequences were pooled.

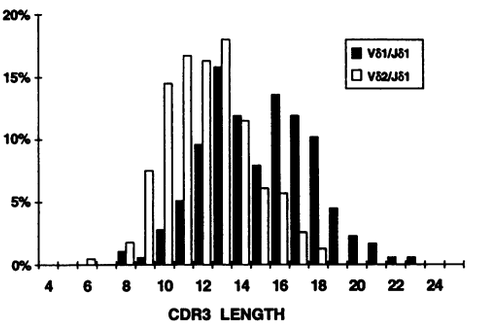


Figure 7. CDR3 length of translated Vδ1/Jδ1 and Vδ2/Jδ1 transcripts from intestinal mucosa and PBMC. The histogram shows percentages of Vδ1/Jδ1 or Vδ2/Jδ1 chains having different CDR3 lengths. 177 Vδ1/Jδ1 and 227 Vδ2/Jδ1 intestinal and PBMC chains were analyzed. Intestinal and PBMC TCR chains were combined for this analysis, and repetitive sequences were counted only once. As shown, the mean length of Vδ2/Jδ1 sequences (12.3±0.2) was significantly shorter than that of Vδ1/Jδ1 sequences (15.0±0.2) ($P < 0.001$) (see Table III). Sequences herein and published sequences from healthy adult subjects were included in this analysis (19, 20, 23, 43–46). CDR3 lengths were calculated as described by Rock et al. (17).

Table IV. D Segment Usage by Colon and PBMC δ TCR Transcripts

V Region	Site	Frame	% of Transcripts Using TCRDD Segment D2	% of Transcripts Using TCRDD Segment D3	Number of Transcripts Analyzed*
TCRDV1	Colon‡	+	77	90	112
	Colon‡	−	87	80	15
	PBMC‡	+	67	98	63
	PBMC‡	−	78	88	72
TCRDV2	Colon§‖	+	33	86	43
	PBMC§	+	34	94	111
	PBMC‡	−	35	100	17
TCRDV3	Colon‖¶	+	71	90	21
	PBMC¶	+	67	94	18
	PBMC¶	−	79	100	19

* Repetitive transcripts were counted only once. ‡Sequences from published data (19, 20, 23, 64). §Sequences from data herein and published data (23, 45). ‖Insufficient out-of-frame transcripts from colon using TCRDV2 and TCRDV3 were available for analysis. ¶Sequences from data herein.

reported herein. Although the expressed δ TCR differed among individuals, we do not know whether each individual responds to a distinct or an overlapping array of ligands.

A striking finding was that the δ TCR repertoire in the circulation is also oligoclonal. We documented this herein for Vδ2 and Vδ3 transcripts, and it was known from prior studies (19, 20) that Vδ1 transcripts, which represent a minor component in peripheral blood, are oligoclonal. In addition, this may be the case for δ TCR that use TCRAV (Vα) gene segments (15). However, we note that the degree of oligoclonality of δ transcripts in PBMC can vary among individuals, as exemplified by Vδ2 transcripts from subjects PL and PJ. Like Vδ1 (20), identical Vδ2 and Vδ3 transcripts persisted over at least a 10–16-mo period. In adults, a major fraction of γ/δ T cells in the circulation express TCRGV9/TCRDV2 (Vγ9/Vδ2) (10, 11), and the Vδ2 transcripts that encode this receptor have complex junctional regions (52), a finding confirmed in our study. Generally, this has been interpreted to indicate that those cells express a highly diverse repertoire (21–26). However, the data herein indicate that this is not the case. In support of our finding, our analysis of Vδ2 junctional sequences from an earlier report (23) also revealed the presence of repetitive Vδ2 transcripts in peripheral blood. Similar findings were noted in a recent study that examined age-related changes in the peripheral blood Vδ2 repertoire (27). Additional support for the oligoclonality of Vγ9/Vδ2 TCR in PBMC comes from studies of others who noted identical rearrangements in the Vγ9 chains that pair with Vδ2 chains in the peripheral blood (47, 53). We further note that oligoclonality of the TCR repertoire in the circulation does not appear limited to the γ/δ T cell lineage. Thus, the TCR β repertoire among some subpopulations of CD8+ T cells from the peripheral blood of healthy adults is also restricted (54, 55).

In light of the striking oligoclonality of the δ TCR repertoire in the mucosa and circulation, we searched for common amino acid motifs encoded by the junctional regions of translated δ TCR transcripts. In this regard, two recent studies demonstrated that the first codon of the junctional region encodes a hydrophobic valine, leucine, or isoleucine residue in > 90% of PBMC Vδ2/Jδ1 transcripts (47, 48). Since these amino acids were generally encoded by template-independent N nucleotides, this suggested ligand-mediated cellular selection of those TCR, analogous to that seen for TCR β transcripts in mice challenged with cytochrome C (56). Our results confirmed that observation but did not detect any additional shared motifs among these sequences. However, some translated δ TCR transcripts from different subjects differed in only one or two amino acids. Thus, it is conceivable that larger data bases of δ TCR transcripts will reveal the existence of common sequence or structural motifs.

The length and variability in length of the CDR3 domain of δ TCR chains have been reported to be greater than those of α and β chains, a finding interpreted to suggest that γ/δ T cells recognize ligands in a manner analogous to that of immunoglobulin (17). We compared the length and variability in length of the CDR3 domains of translated δ TCR transcripts that carry different V regions. These studies revealed that the CDR3 domains of Vδ2/Jδ1 transcripts were significantly shorter than those of Vδ1/Jδ1 transcripts. However, this difference was independent of whether these cells were present in the intestinal mucosa or circulation and reflected, in part, the less frequent use of the TCRDD2 gene segment by Vδ1/Jδ1 transcripts. Such differences were not secondary to selection at the cellular level because parallel findings were seen in both in- and out-of-frame sequences. Moreover, dominant δ TCR transcripts in the intestinal mucosa and circulation had no preferential CDR3 length.

Most γ/δ T cells in the intestinal mucosa are located in the intraepithelial region (8, 9). IELs are uniquely positioned to function as guardians of the paracellular space. Given the patchy distribution (i.e., one IEL per 6–10 epithelial cells in healthy individuals) and the apparently sessile nature of IEL, one might a priori predict that these cells would express an oligoclonal TCR repertoire that would enable them to respond rapidly to a limited array of broadly expressed microbial ligands, or self-ligands induced by cellular injury. This appears to be the case, because both the γ/δ IEL repertoire, as noted herein, as well as the α/β IEL repertoire, as noted by others, are oligoclonal (19, 57–60). The function of these cells may be to signal adjacent and underlying immune and inflammatory cells, for example, via cytokine release, to eliminate damaged or infected cells via cytotoxic responses or, as recently suggested for γ/δ T cells, to release mediators that enhance epithelial cell growth and repair (61). However, the finding that δ TCR in the circulation, like those in the intestinal mucosa, are also oligoclonal indicates that restriction of the TCR δ repertoire may be a general feature of γ/δ T cells, independent of their localization.

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