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 (V δ 1) variable region gene segment, whereas γ/δ T cells in the circulation predominantly express the TCRDV2 (V δ 2) 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 (V δ 3) and TCRAV (V α) 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 (V δ 2)² gene segment (10, 11), whereas TCRDV1 (V δ 1) 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 (V α) 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).

δ TCRs 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 V δ 1 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.

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

Table I. Oligonucleotides Used for PCR and Hybridization

Oligonucleotides	Sequence
TCRDC primer 1	GGGTCGACTTGGCTTCTGACTTCTTTGTGATT
TCRDC primer 2	TTATGAATGCGGCCGCTCTGTTATCTTCTGGATGACACG
Linear PCR, TCRDV1 primer*	ATAAGTCGACCTGTATGAAACAAGTTGGTGG
Linear PCR, TCRDV2 primer	ATAAGTCGACCACCCTCAGGTGCTCCATGAA
Linear PCR, TCRDV3 primer	ATAAGTCGACTGGTACTGCTCTGCACTTACG
TCRDC sequencing primer*	AACGGATGGTTTGGTATG
Hybridization probe, TCRDV1	CCTTATTCCGCCAGGGTTCTGATGAACAGAATGCAA
Hybridization probe, TCRDV2 [‡]	CCACCCTCAGGTGCTCCATGAA
Hybridization probe, TCRDV3	GCACGTCTTCAGTCCTTACTGGAGAGACTACCA

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 V δ 1 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 μ g) was reverse transcribed in 20 μ l 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 μ l) was circularized according to Uematsu's protocol (30) as modified by us (31), using a ligation volume of 500 μ l. Circularized cDNA (20 μ l) was amplified by inverse PCR using TCRDC (C δ) 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 μ l containing 0.2 mM of each dNTP (Pharmacia P-L Biochemicals, Inc., Milwaukee, WI), 0.5 μ M 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 μ l 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 (Intelli-Genetics, Mountain View, CA). Nucleotide sequences were assigned to TCRDD (D δ) gene segments based on at least 3 bp identities (33). Gene segment assignments were as follows: TCRDJ1 (J δ 1), TCRDJ2 (J δ 2), and TCRDJ3 (J δ 3) were assigned according to Takihara et al. (34); TCRDJ4 (J δ 4) was assigned according to Davodeau et al. (35); TCRDV (V δ) 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 (V α) 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 chemiluminescent 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 V δ 1 or V δ 2 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 V δ 1 transcripts in human intestine and PBMC are oligoclonal (19, 20). In contrast, the V δ 2 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 V δ 2 transcripts were present in the colonic mucosa of each subject, and no overlap was noted in the transcripts among subjects. 88% of the V δ 2 transcripts in the colon mucosa were in frame. Moreover, within each subject, identical V δ 2 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), V δ 2 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 V δ 1 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 V δ 3 can also contribute to the δ TCR repertoire in some healthy individuals. Like V δ 2 transcripts, as shown in Fig. 3, V δ 3 transcripts in the colon were also oligoclonal, and in subject FA, identical transcripts could be detected 16 mo later. Further, most V δ 3 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 V δ 2 and V δ 3 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 CTCTGTGACACC	N/P	DD1 GAAATAGT	N/P	DD2 CCTTCCTAC	N/P	DD3 ACTGGGGGATACG	N/P	DD1: ACACCGATAAACTCAT DD2: CTCTGGGACACCCGA	I	BIOPSY II	III	FRAME
SUBJECT FA:													
C.2.FA1	CTCTGTGACACC	GTA					ACTGGGGGATACG		CTCTGGGACACCCGA	4/15	2/17	11/15	+
C.2.FA03	CTCTGTGAC						GGGG		CTCTGGGACACCCGA	2/15			+
C.2.FA13	CTCTGTGAC		TAG	G			CTGGGGGATAC		CTCTGGGACACCCGA	1/15			+
C.2.FA06	CTCTGTGAC	G	TAGT	GTTA			GGGGGAT		CTCTGGGACACCCGA	1/15			+
C.2.FA46	CTCTGTGAC	CCCCACGT					ACTGGGGGATACG	CAACGTAAGTCTCTGGG	CTCTGGGACACCCGA	1/15			+
PB.2.FA10*	CTCTGTGAC	C	AAT	T			CTGGGGGATACG	CTCAA	CTCTGGGACACCCGA			2/15	+
C.2.FA745	CTCTG	C					GGG	CCATTACACCGCGA	CTCTGGGACACCCGA			1/15	+
C.2.FA47	CTCTGTGACAC	GGGGGT					ACTGGGGGATACG	CTCTCTCAG	CTCTGGGACACCCGA	3/15	2/17	1/15	+
C.2.FA04	CTCTGTGACAC	GGATGGGT					ACTGGGGGATACG	AAGCGGG	CTCTGGGACACCCGA	3/15			+
C.2.FA07	CTCTGTGACAC	T			CTTC	GA	GGGG	GGC	CTCTGGGACACCCGA		13/17		+
SUBJECT LR:													
C.2.LR377	CTCTGTGACACC	TTAGG					TGGGGGATAC		CTCTGGGACACCCGA	3/13	5/20		+
C.2.LR395	CTCTGTGAC	GTCT			CTT	GT	ACTGGGGGATAC	CCGGTCGG	CTCTGGGACACCCGA	1/13	1/20		+
C.2.LR400	CTCTGTGAC	CGACGT					ACTGGGGGATACG	CA	CTCTGGGACACCCGA	1/13	1/20		+
PB.2.LR01*	CTCTGTGACACC	GTA					GGGGGATAC	AGGG	CTCTGGGACACCCGA		6/20		+
PB.2.LR03*	CTCTGTGACAC	GATGATGG					CTGGGGGATAC	AGCG	CTCTGGGACACCCGA	2/13			+
C.2.LR384	CTCTGTGACAC				TCC	CGGT	ACTGGGGGATAC	CCTACC	CTCTGGGACACCCGA	1/13			+
C.2.LR393	CTCTGTG	G			TTC	ACCGT	ACTGGGGGATACG	CCCGT	CTCTGGGACACCCGA		1/20		+
C.2.LR397	CTCTGTGACACC	CTGT			CTTCCTAC		CTGGGGAT	CTTTA	CTCTGGGACACCCGA		1/20		+
C.2.LR01	CTCTGTGACA	GT					ACTGGGGG	CCAGCCCTGG	CTCTGGGACACCCGA		2/20		+
C.2.LR387	CTCTGTGACAC	GGGTT					ACTGGGGGATACG		CTCTGGGACACCCGA	1/13	1/20		+
C.2.LR15	CTCTGTG	GGCC					GGG	TTTGCCGAAG	CTCTGGGACACCCGA	2/13			+
C.2.LR10	CTCTGTGAC	GGA					ACTGGGGGATAC	GT	CTCTGGGACACCCGA	1/13			+
C.2.LR388	CTCTGTGACAC	GGG	ATA		CCTT			TCCT	CTCTGGGACACCCGA	1/13			+
C.2.LR11	CTCTGTGACAC	GT					TGGG	ACTGCCAC	CTCTGGGACACCCGA		1/20		+
C.2.LR402	CTCTGTGACACC	GTGCA	GAA	GTT			CTGGGGGATAC	GGAGCT	CTCTGGGACACCCGA		1/20		+
SUBJECT OJ:													
C.2.OJ02	CTCTGTGACAC	TCGTGG					TGGGGGATACG		CTCTGGGACACCCGA	7/12	5/13		+
C.2.OJ06	CTCTGTGACACC						TGGGGGATAC	A	CTCTGGGACACCCGA	2/12	6/13		+
C.2.OJ13	CTCTGTGA	A	GAA						CTCTGGGACACCCGA	1/12			+
C.2.OJ01	CTCTGTGAC	CC					CTGGGG	CTCCGG	CTCTGGGACACCCGA	2/12	1/13		+
C.2.OJ12	CTCTGTGACA	G			CTAC			GACCCAGAG	CTCTGGGACACCCGA		1/13		+
SUBJECT PL:													
C.2.PL36	CTCTGTGACAC	GTA			TCC		GGGGG	CTGTAGGCTAATCT	CTCTGGGACACCCGA	8/18	3/12		+
C.2.PL13	CTCTGTGACACC	GG					CTGGGGGATAC	T	CTCTGGGACACCCGA	1/18	3/12		+
C.2.PL87	CTCTGTGACACC	G					TGGGGG	CTGTCT	CTCTGGGACACCCGA	1/18	1/12		+
C.2.PL92	CTCTGTGACACC	G						CTCTGG	CTCTGGGACACCCGA	2/18			+
C.2.PL08	CTCTGTGACACC	CT	GAA	TAG	C	TCCT	CTGGGGGATAC	CTGTCT	CTCTGGGACACCCGA		2/12		+
C.2.PL259	CTCTGTGACACC	GCACA					ACTGGGGGATAC	AACT	CTCTGGGACACCCGA		1/12		+
C.2.PL257	CTCTGTGAC	CCC	AGT		TCCT	C	GGGG	C	CTCTGGGACACCCGA		1/12		+
C.2.PL91	CTCTGTGACACC	GTGA			CTT		ACTGGGGGATACG	CGATTGGT	CTCTGGGACACCCGA	1/18			+
PB.2.PL254*	CTCTGTGACA	AGGT						AGCCCTGCCAAGCCCT	CTCTGGGACACCCGA	1/18			+
C.2.PL252	CTCTGTGACACC				CTTCCTAC			TCCT	CTCTGGGACACCCGA	1/18			+
C.2.PL90	CTCTGTGACAC	GCTGGAG					CTGGGGGATAC		CTCTGGGACACCCGA	1/18			+
C.2.PL256	CTCTGTGAC	ACCG					CTGGGG	TACCCGTTGG	CTCTGGGACACCCGA	1/18	1/12		+
C.2.PL253	CTCTGTGAC				CCTA	TACT	ACTGGGGGATACG		CTCTGGGACACCCGA	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 V δ 2 transcripts were in frame (repetitive transcripts were counted only once). These sequence data are available from EMBL/GenBank/DBJ 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 V δ 2 transcripts expressed by PBMC of adult humans are, in fact, oligoclonal and that identical transcripts can persist over time. Thus, dominant V δ 2 transcripts were present in repeated blood samples in most subjects, and, in each subject, V δ 2 transcripts identical to those in the initial blood sample could be detected 10–16 mo later. V δ 2 transcripts in

PBMC from subject PL were more diverse than those of the other subjects. Nonetheless, identical V δ 2 transcripts in subject PL also persisted over time. No transcripts were repeated among different individuals, and all V δ 2 transcripts from PBMC were in frame. Finally, we note that V δ 2 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 V δ 2 transcripts, repetitive V δ 3 transcripts were present in PBMC, and identical V δ 3 transcripts were consistently detected in a second blood specimen from the same individuals 10–16 mo

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

Figure 2. The V δ 1 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/DBJ under accession numbers L39468–L39474.

GERMLINE	TCRDV3	M/P	DD1	M/P	DD2	M/P	DD3	M/P	DD1	DD2	DD3	BIOPSY	FRAME
	CTTACTACTGTGCTTT		GAATATAGT		CCTTCCTAC		ACTGGGGGATACG		ACACCGATAAACTCAT			I	
	CTTACTACTGTGCTTT		GAATATAGT		CCTTCCTAC		ACTGGGGGATACG		CTTTGACACACCACT			II	
	CTTACTACTGTGCTTT		GAATATAGT		CCTTCCTAC		ACTGGGGGATACG		CTCTGGGACACCGGA			III	
SUBJECT FA:													
C.3. FA34	CTTACTACTGTGCTTT	GGACAAGTC			CCTTCCTAC		ATAC	CGTACTGTAAGTGTAG	CGATAAACTCAT	11/15	10/10	12/14	+
C.3. FA08	CTTACTACTGTGCTTT	AGGG					TGGGG	GATACACCGTTTCCG	CCGATAAACTCAT	3/15			+
C.3. FA659	CTTACTACTGTGCTTT	CAATGGA							CTCAT	1/15			+
C.3. FA33	CTTACTACTGTGCTTT	CTCTCT			CTT	T	ACTGGGGGATAC	AGG	CACCGATAAACTCAT			1/14	+
C.3. FA35	CTTACTACTGTGCTTT	CCCG			CCTTCCTAC	GTT	GGGGAT	CCCTTTAGAGGCTTT	CTCTGGGACACCGGA			1/14	-
SUBJECT LR:													
C.3. LR03	CTTACTACTGTGCTTT	CACA			CTAC	TGAA	GGGG	TTCG	ACTCAT	8/10	9/9		+
C.3. LR27	CTTACTACTGTGCTTT				CTAC	A	ACTGGGGGATAC		CGATAAACTCAT	2/10			+
SUBJECT OJ:													
C.3. OJ53	CTTACTACTGTGCTTT	AAGG			CTA	GGCA	GGGG	CCCC	ACACCGATAAACTCAT	2/8	6/11		+
C.3. OJ50	CTTACTACTGTGCTTT	ACCAA			CTTCC	GGCCGTCGG	ACTGGGGGATAC	T	GATAAACTCAT	3/8	3/11		+
C.3. OJ56	CTTACTACTGTGCTTT	CCG			TCCTAC	GCC	GGGG	T	ACACCGATAAACTCAT	1/8			+
C.3. OJ62	CTTACTACTGTGCTTT	TTCCGGCTGGGGTCTG			CTT	GCT	GGGGATAC	ACCCGT	ACACCGATAAACTCAT		1/11		+
C.3. OJ66	CTTACTACTGTGCTTT	AA			CTA	GC	TGGG		ACACCGATAAACTCAT		1/11		+
C.3. OJ524	CTTACTACTGTGCTTT	AAGGC	TAG	GCA			GGG	CCCC	ACACCGATAAACTCAT	1/8			-
C.3. OJ73	CTTACTACTGTGCTTT	CCAAGCCCC					GGGG	TCGCA	TGACGACCACT	1/8			+
SUBJECT PL:													
C.3. PL431	CTTACTACTGTGCTTT	CTTAACG			CCTA	GTTAAG	TGGGGGA	CTTTCT	ACACCGATAAACTCAT	4/11	2/12		+
C.3. PL427	CTTACTACTGTGCTTT	AAT				GAGA	GGGG		TAAACTCAT	1/11	4/12		+
C.3. PL440	CTTACTACTGTGCTTT	ACGC			TCCTAC	CT	CTGGGG	ACTCCCTCTGGGGG	ACACCGATAAACTCAT	1/11	2/12		+
C.3. PL514	CTTACTACTGTGCTTT	GTAGGGGGGT					ACTGGGGGATA	TGT	ACACCGATAAACTCAT	2/11			+
C.3. PL166	CTTACTACTGTGCTTT	C					GGGG	CC	ACACCGATAAACTCAT	1/11			+
C.3. PL430	CTTACTACTGTGCTTT	CCAGC	AATA		TTCTAC			CCCATGTCCGTTGT	ACCGATAAACTCAT	1/11			+
C.3. PL423	CTTACTACTGTGCTTT	CCCAGGGT					CTGGGGGATACG	TCATG	GATAAACTCAT	1/11			-
C.3. PL441	CTTACTACTGTGCTTT	CTTTGGCCT			TTCTA	ATC	CTGGGGAT		ACACCGATAAACTCAT		1/12		+
C.3. PL437	CTTACTACTGTGCTTT	TCCAC			TCC	CCC	TAC	CT	ACACCGATAAACTCAT		1/12		+
C.3. PL444	CTTACTACTGTGCTTT	AGG			TTCC			AT	ACCGATAAACTCAT		1/12		+
C.3. PL450	CTTACTACTGTGCTTT	GTAAAGATT			TTCC	GTC	ACTGGGGGATA	GTGGTA	AAACTCAT	1/12			+

Figure 3. The V δ 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 V δ transcripts were present in subject FA 16 mo later (biopsy III). 84% of the V δ transcripts were in frame (repetitive transcripts were counted only once). These sequence data are available from EMBL/Genbank/DBJ under accession numbers L39517–L39538.

later (Fig. 6). However, 53% of PBMC V δ 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 V δ 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 V δ 1, V δ 2, and V δ 3 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 V δ 2/J δ 1 chains was significantly shorter than that of V δ 1/J δ 1 chains ($P < 0.001$), whereas the mean CDR3 length of V δ 3/J δ 1 chains did not differ significantly from that of V δ 2/J δ 1 chains. The difference between V δ 1 compared with V δ 2 and V δ 3 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. Chowder, W. Holtmeier, M. F. Kagnoff, and E. Morzycka-Wroblewska, manuscript in preparation). Rather, the difference in CDR3 lengths of V δ 1 and V δ 2 chains is best explained by differences in their D segment usage. As shown in Table IV, the majority of V δ 1 transcripts used TCRDD2 (D δ 2) and TCRDD3 (D δ 3) gene segments, while most of the V δ 2 transcripts used only TCRDD3, a finding also noted by others in regard to

GERMLINE	AV1482	TCCTGTGCTTATAGGAC	M/P	DD2	M/P	DD3	M/P	DD1	BIOPSY	FRAME
	AV19	CTACATCTGTGCTGTACG		CCTTCCTAC		ACTGGGGGATACG		ACACCGATAAACTCAT	I	
	AV27	GTCCCGTGGACTCGACC							II	
C.A. LR07		TTCTGTGCTTATAGGAC	GCGTATC	TCC	CACCTC	GGG	TTTT	ACACCGATAAACTCAT	3	2
C.A. LR02		TTCTGTGCTTATAGGAC	ACAG	CCTTCCTAC	CGT	ACTGG	CTCC	ACACCGATAAACTCAT		3
C.A. LR03		CTACATCTGTGCTGTCA	GATCCGG	CTTC	GGT	ACTGGG	CTA	ACCGATAAACTCAT		4
C.A. LR01		GTCCCGTGGACTCGACC		CCTTCCT		GGGGATACG	CCG	CCGATAAACTCAT	3	16
total:									6	25

Figure 4. δ TCR transcripts that use TCRAV ($V\alpha$) 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/DBJ under accession numbers L39614–L39619.

GENOTYPE	TCRDV2	N/P	DD1	N/P	DD2	N/P	DD3	N/P	DD4	1993	1994	FRAME
DJ1: ACACCGATAAACTCAT DJ2: CTCTGGGACACCCGA DJ3: CTCTGGGACACCCGA DJ4: CCAGACCCCTGATCTT												
SUBJECT FA:												
PB. 2. FA01	CCTGTGACACC	GTGGA			TCC	CT	ACTGGGGG	GGAAAACTGT	ACACCGATAAACTCAT	7/18	1/14	+
PB. 2. FA15	CCTGTGACACC	G					ACTGGGGG	GTCT	ACACCGATAAACTCAT	2/18	5/14	+
PB. 2. FA02	CCTGT	TTGTT					ACTGGGGG	AAG	CCGATAAACTCAT	2/18	1/14	+
PB. 2. FA11	CCTGTGACACC	GTACCCA							ACACCGATAAACTCAT	1/18		+
PB. 2. FA03	CCT				CCT	GTCTCTGT	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					TGGGGATACG	C	TAAACTCAT		1/14	+
PB. 2. FA611	CCTGTGACAC	TC			TCCTAC		GGGGATAC	AGG	CCGATAAACTCAT		1/14	+
PB. 2. FA05	CCTGTGACACC						CTGGGGGATACG	G	GACCCCTGATCTT	5/18	2/14	+
SUBJECT LR:												
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	+
PB. 2. LR04	CCTGTGACACC	GTGGG					ACTGGGGGATACG	CCCG	CCAGACCCCTGATCTT	1/27		+
SUBJECT PL:												
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	CTGGG					ACTGGGGGATAC	CC	ACACCGATAAACTCAT		1/10	+
PB. 2. PL683	CCTGTGACAC						ACTGGGGG	CCTTATGT	ACACCGATAAACTCAT		1/10	+
PB. 2. PL554	CCTGTGACACC	GTAGG					ACTGGGGGAT	GGGGCGA	ATAAACTCAT		1/14	+
PB. 2. PL550	CCTGTGAC	CCAACA					GGGGGAT	CGTCTCTATTACGAAGT	ACACCGATAAACTCAT		1/14	+
PB. 2. PL566	CCTGTGACAC	AG			TTCTTAC		TGGGGG	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	AAGAGGT	CCGATAAACTCAT		1/14	+
PB. 2. PL544	CCTGTGACACC	CTGAGGG					ACTGGGGG	CTCT	CAGCACAACT	1/10	1/14	+
PB. 2. PL269	CCTGTGAC	CGCGT					ACTGGGGGATAC	CCACGGG	CCTGGGACACCCGA	1/10		+
PB. 2. PL272	CCTGTGAC	GATGAC					GGGGG	CTTCTATT	CTCTGGGACACCCGA	1/10		+
PB. 2. PL539	CCTGTGAC	GAGC					GGGGG	CCCTCAT	CTCTGGGACACCCGA	1/10		+
PB. 2. PL555	CCTGTGACACC	GT					GGGGGAT	CCCG	CCCG		1/14	+
PB. 2. PL736	CCTGTGACAC						TGGGGGAT	GGAG	CTCTGGGACACCCGA		1/14	+
SUBJECT PJ:												
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	GGG	CT	ACCGATAAACTCAT		1/14	+
PB. 2. PJ711	CCTGTG						TGGGGGATA	AAGACGCCCTAG	CTTTGACAGCAAACT		1/14	+
PB. 2. PJ313	CCTGTG	TGGT					TGGGGGAT	TGAG	CTCTGGGACACCCGA	1/13		+

Figure 5. The V δ 2 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 V δ 2 repertoire was oligoclonal in subjects PJ, FA, and LR and somewhat more diverse in subject PL. Of note, V δ 2 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/DBJ 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 V δ 2 transcripts used TCRDJ3 (J δ 3) gene segments, and, as indicated in Table III, the CDR3 domain of V δ 2/J δ 3 chains was ~2 amino acids longer than that of V δ 2/J δ 1 and, in this regard, similar to that of V δ 1/J δ 1 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 V δ 2/J δ 1 PBMC transcripts, a finding also noted by others (47, 48). This was also the case for 65% of V δ 2/J δ 1 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 (V α) 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 V δ 1 or V δ 2 (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

GERMLINE	TCRDV3	N/P	DD1	N/P	DD2	N/P	DD3	N/P	DJ1: ACACCGATAAATCAT DJ3: CTCCTGGGACACCGGA DJ4: CCAGACCCCTGATCTT	PBMC	1993	1994	FRAME
SUBJECT LR:	CTTACTACTGTGCCTTT		GAAATAGT		CCTTCCTAC		ACTGGGGATACG		DJ1				
PB.3.LR474	CTTACTACTGTGCCTT	CTCCC			CCTTCCTAC	GGG	TGGGGGATACG	TCG	GATAAATCAT	1/11	3/16	+	
PB.3.LR480	CTTACTACTGTGTCC	C			CCTT	GGG	GGG	CTTGACCTTGGAGGGAC	ACACCGATAAATCAT	2/11	1/16	+	
PB.3.LR477	CTTACTACTGTGCC	AACCCGCAAGACTGCCGCT			CCT	T	ACTGGGGAT	GATGT	ACACCGATAAATCAT	2/11	1/16	-	
PB.3.LR483	CTTACTACTGTGCCTT	CCCCCAA				TC	TGGGGGATAC	AAAG	ACACCGATAAATCAT	1/11	1/16	-	
PB.3.LR496	CTTACTACTGTGCCTTT				TCC	AC	GGGGGATAC	CT	ACACCGATAAATCAT		2/16	+	
PB.3.LR500	CTTACTACTGTGCCTT				CCTAC	GT	TGGGGGA	CCTCTTACGGGAT	ACACCGATAAATCAT		2/16	-	
PB.3.LR502	CTTACTACTGTGCCTTT	CCCGGACC			TCC	GACTCAT	CTGGGGGA	GCTAGACGGGGTTT	ACACCGATAAATCAT		1/16	+	
PB.3.LR506	CTTACTACTGTGCCTTT	CAG			CCT	CTGGACAGGGGA	TGGGGG	G	ACACCGATAAATCAT		1/16	+	
PB.3.LR507	CTTACTACTGTGCCTTT	AGTTTGGGGTT			CTAC	GGCGT	ACTGGGG	ATA	ATAAATCAT		1/16	+	
PB.3.LR504	CTTACTACTGTG	TTGGGTTC			CCT		ACTGGGGGA	CCTGGGGT	ACACCGATAAATCAT		1/16	-	
PB.3.LR479	CTTACTACTGTGCCTTT	A	GAAA		CCTAC		TGGG	TGA	ACACCGATAAATCAT	1/11	-	-	
PB.3.LR476	CTTACTACTGTGCCTT	ATTGAGATCGT					ACTGGGGGATACG		CCGATAAATCAT	1/11	-	-	
PB.3.LR478	CTTACTACTGTGCCTTT	AG	AGT	GGGC	CCTAC	GATGGATG	TGGGGG	GTTCTTACG	CCGATAAATCAT	1/11	+	+	
PB.3.LR484	CTTACTACTGTGCCT	C					GGGGG	GTTAG	DJ3				
PB.3.LR475	CTTACTACTGTGCCTT	CTGGGGGT			TTCC	CGCCTA	ACTGGGGAT	CAC	DJ4				
SUBJECT PL:	CTTACTACTGTGCCTT				CTTC	AGT	ACTGGGGGATA	TCG	DJ1				
PB.3.PL345	CTTACTACTGTGCCTT	CAAGGGGTG					TGGGGGATAC	TTTACAA	TAAATCAT	3/11	4/16	+	
PB.3.PL339	CTTACTACTGTGCCTT	CG	TAG			CGCCATCTCGG	ACTGGGG	GGAG	TAAATCAT	2/11	1/16	+	
PB.3.PL342	CTTACTACTGTGCCTTT				CTAC	C	GGGGATC	GGAG	ACACCGATAAATCAT	1/11	1/16	+	
PB.3.PL347	CTTACTACTGTGCCTTT	CC	TAG	G	CCTT		ACTGGGGATC	GGCCGAAG	AACTCAT	3/11	3/16	-	
PB.3.PL341	CTTACTACTGTGCCTTT	CATATGGCT	ATAG		CCTT	TTT	ACTGGGGATC	ACCACCTGGGG	ACTCAT	1/11	-	-	
PB.3.PL340	CTTACTACTGTGCCTTT	GAA				TTT	ACTGGGGATC	CTTTGACCC	ATAAATCAT	1/11	+	+	
PB.3.PL364	CTTACTACTGTGCCTTT				TAC	GT	ACTGGGG	ATAAAG	ACACCGATAAATCAT		2/16	+	
PB.3.PL367	CTTACTACTGT							GT	ATAAATCAT		1/16	+	
PB.3.PL371	CTTACTACTGTGCCTT	ACCTA			CTTCCTAC	TCTGATTTT	ACTGGGG	TGATAAG	ACCGATAAATCAT		1/16	+	
PB.3.PL374	CTTACTACTGTGCCTTT	CTCAG			TTCC	CGT	ACTGGGGGATACG	T	ACACCGATAAATCAT		1/16	-	
SUBJECT FA:	CTTACTACTGT				CTTC		GGGGG	GTCCGAGTTTCTGGGGGGTCTG	DJ1				
PB.3.FA635	CTTACTACTGT	TCCTC	TAG	CGAC	CCTTC		CTGGG	TTTCGGGGTAGG	ACACCGATAAATCAT		4/12	-	
PB.3.FA644	CTTACTACTGTGCC	CTCAAG			CCTTCCTA	A	TGGGGGATACG	GGAG	TAAATCAT	1/12	+	+	
PB.3.FA634	CTTACTACTGTGCCTTT						ACTGGGGATAG	GGGGGGT	CGATAAATCAT	1/12	+	+	
PB.3.FA632	CTTACTACTGTGCCTTT	AAATA	AGATTGGGA	TTCTAC	GGCT		ACTGGGGGATAG		ACACCGATAAATCAT	1/12	-	-	
PB.3.FA639	CTTACTACTGTGCCTTT	AACGG					GGG	CTACAAGTGGTTACGCTTAGAGT	ACACCGATAAATCAT	1/12	-	-	
PB.3.FA643	CTTACTACTGTGCC	GTCTCA			CCTTCCTA	AGTTGTT	TGGGG	GGGACGGGAACGTGT	ACCGATAAATCAT	1/12	-	-	
PB.3.FA633	CTTACTACTGTGC	TAAAG			TCCT	GATATGG	ACTGGGG	GGTTC	ATAAATCAT	1/12	-	-	
PB.3.FA638	CTTACTACTGTGC	TTATCAA			CCT	GG	TGGGG	GGTTC	GATAAATCAT	1/12	-	-	
PB.3.FA645	CTTACTACTGTGCCTTT	TGA							AACTCAT	1/12	-	-	

Figure 6. The V δ 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/DBJ under accession numbers L39580–L39613.

intestine-expressing V regions other than V δ 1 and V δ 2 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 V δ 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 identi-

cal 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 $\pm$ SEM*	Number of Transcripts Analyzed
V δ 1/J δ 1, colon [†]	9–23	15	15.0 $\pm$ 0.3	116
V δ 1/J δ 1, PBMC [‡]	8–22	15	14.9 $\pm$ 0.4	61
V δ 2/J δ 1, colon [§]	6–17	12	12.1 $\pm$ 0.5	30
V δ 2/J δ 1, PBMC [§]	8–18	12	12.3 $\pm$ 0.2	197
V δ 2/J δ 3, colon				
+ PBMC [¶]	10–18	14	14.3 $\pm$ 0.5	17
V δ 3/J δ 1, colon				
+ PBMC [¶]	3–18	12	11.5 $\pm$ 0.6	39

* The mean CDR3 length of V δ 2/J δ 1 chains in colon and PBMC was significantly shorter than that of V δ 1/J δ 1 chains ($P < 0.001$). The mean CDR3 length of V δ 3/J δ 1 chains was significantly shorter than that of V δ 1/J δ 1 chains ($P < 0.001$). There was no significant difference in the mean CDR3 length of V δ 3/J δ 1 and V δ 2/J δ 1 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 V δ 2/J δ 3 or V δ 3/J δ 1 chains, colon and PBMC sequences were pooled.

Figure 7. CDR3 length of translated V δ 1/J δ 1 and V δ 2/J δ 1 transcripts from intestinal mucosa and PBMC. The histogram shows percentages of V δ 1/J δ 1 or V δ 2/J δ 1 chains having different CDR3 lengths. 177 V δ 1/J δ 1 and 227 V δ 2/J δ 1 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 V δ 2/J δ 1 sequences (12.3 $\pm$ 0.2) was significantly shorter than that of V δ 1/J δ 1 sequences (15.0 $\pm$ 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		Number of Transcripts Analyzed*
			D2	D3	
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 V δ 2 and V δ 3 transcripts, and it was known from prior studies (19, 20) that V δ 1 transcripts, which represent a minor component in peripheral blood, are oligoclonal. In addition, this may be the case for δ TCR that use TCRAV (V α) gene segments (15). However, we note that the degree of oligoclonality of δ transcripts in PBMC can vary among individuals, as exemplified by V δ 2 transcripts from subjects PL and PJ. Like V δ 1 (20), identical V δ 2 and V δ 3 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 (V γ 9/V δ 2) (10, 11), and the V δ 2 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 V δ 2 junctional sequences from an earlier report (23) also revealed the presence of repetitive V δ 2 transcripts in peripheral blood. Similar findings were noted in a recent study that examined age-related changes in the peripheral blood V δ 2 repertoire (27). Additional support for the oligoclonality of V γ 9/V δ 2 TCR in PBMC comes from studies of others who noted identical rearrangements in the V γ 9 chains that pair with V δ 2 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 V δ 2/J δ 1 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 V δ 2/J δ 1 transcripts were significantly shorter than those of V δ 1/J δ 1 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 V δ 1/J δ 1 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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