Clin Exp Immunol 1997; **107**:148–157

Distinct δ T cell receptor repertoires in monozygotic twins concordant for coeliac disease

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(Accepted for publication 29 August 1996)

SUMMARY

One of the hallmarks of coeliac disease, both active and treated, is an increased number and proportion of γ/δ intraepithelial T lymphocytes in the small intestinal mucosa, and an increased number of γ/δ T cells in the small intestinal mucosa of coeliac disease patients has been associated with the inheritance of specific HLA class II DQ alleles. Nonetheless, the contribution of genetic factors to the development of the T cell receptor (TCR) δ repertoire in coeliac disease is not known. We have assessed the contribution of genetic factors to development of the TCR δ repertoire in coeliac disease, by characterizing the junctional diversity of TCR δ transcripts expressed in the intestine and peripheral blood of a pair of monozygotic (MZ) twins concordant for coeliac disease. TCR Vδ1, Vδ2 and Vδ3 transcripts from small intestinal and colon biopsies, and from peripheral blood mononuclear cells, were amplified by polymerase chain reaction (PCR) and the complementarity determining region (CDR)3 domains of TCR δ transcripts were analysed by denaturing PAGE and direct nucleotide sequencing. The repertoire of TCR δ transcripts and CDR3 amino acid motifs in the intestine and peripheral blood of MZ twins concordant for coeliac disease exhibited no overlap. The TCR δ repertoire in each twin was oligoclonal, and complexity of the junctional regions of their TCR δ transcripts was typical of the repertoire in healthy adults. Thus, genetically identical individuals with coeliac disease have distinct, non-overlapping TCR δ repertoires. Moreover, genetic factors that determine disease susceptibility do not appear to select for specific TCR δ sequences or CDR3 amino acid motifs.

Keywords δ T cell receptor coeliac disease monozygotic twins T cell receptor repertoire γ/δ T cells

INTRODUCTION

Coeliac disease is characterized by damage to the mucosa of the small intestine and the malabsorption of most nutrients. Disease is activated by the ingestion of foods that contain wheat gliadins, rye secalins or barley hordeins [1,2]. Genetic factors are a major determinant of host susceptibility to coeliac disease [2]. In this regard, $\approx$ 75% of monozygotic (MZ) twins are concordant for disease [3]. Moreover, genes that map to the HLA class II D region determine susceptibility to coeliac disease. Thus, $>$ 90% of coeliac disease patients express an HLA class II DQ2 molecule encoded by the alleles DQA1*0501 and DQB1*0201, in *cis* or *trans*, and most of the remaining coeliac disease patients express a DQ8 molecule encoded by the alleles DQA1*0301 and DQB1*0302 [4–8]. Although these DQ alleles are necessary, they are not sufficient for the phenotypic expression of disease [2]. In contrast to MZ twins, concordance for coeliac disease among siblings who share one or both HLA haplotypes ranges from $\approx$25% to 40%, indicating the importance also of other genes in determining susceptibility to this disease [2,4,9].

Increased numbers of γ/δ intraepithelial lymphocytes (IEL) in the small intestine and an increased proportion of γ/δ IEL, relative to α/β IEL, are one of the hallmarks of coeliac disease [10–12]. IEL are located in the paracellular space between intestinal epithelial cells. T cells that express the γ/δ T cell receptor (TCR) are located mainly within the surface epithelium and variably comprise up to 10–30% of IEL in normal human small intestine and colon [13,14]. In contrast, T cells that express the α/β form of the TCR are abundant in the lamina propria and intraepithelial region. In addition to active coeliac disease, patients with treated coeliac disease are reported to have an increased proportion of γ/δ IEL to α/β IEL, and this finding has been used as a diagnostic adjunct for latent coeliac disease [15–19]. Furthermore, increased numbers of mucosal γ/δ T cells have been noted in otherwise

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healthy, first degree relatives of coeliac disease patients who carry the disease-associated HLA class II susceptibility alleles [20]. Increased numbers of γ/δ T cells in the intestinal mucosa do not appear to simply reflect mucosal inflammation, since γ/δ T cells are rarely increased in other chronic inflammatory mucosal states, such as tropical sprue, inflammatory bowel disease, or during microbial infection of the intestinal tract [10,15,21].

TCR δ-chains have the potential for extensive diversity through combinatorial joining of variable (V), diversity (D) and joining (J) gene segments, and by nucleotide insertions and deletions that occur at the junctions of these gene segments [22]. Most γ/δ IEL utilize the TCRDV1 [23] gene segment [10,14,24], while fewer cells express TCRDV2, TCRDV3 and TCRAV gene segments [24–26]. In contrast, most γ/δ T cells in the circulation express the TCRDV2 gene segment [27,28]. Similar to healthy individuals, most γ/δ T cells in the small intestine in coeliac disease express TCRDV1 [21,29].

In the present study, we characterized the TCR δ repertoire in a MZ twin pair concordant for coeliac disease in order to assess the contribution of genetic factors to the TCR δ repertoire in this disease. Based on an analysis of the complementarity determining region 3 (CDR3) domains of expressed TCR δ transcripts, we report herein that genetically identical individuals concordant for coeliac disease express distinct non-overlapping TCR δ repertoires.

MATERIALS AND METHODS

Subjects

BD and JD are 44-year-old male Caucasian MZ twins of Irish/German ancestry. Coeliac disease was diagnosed by small intestinal mucosal biopsy in BD at age 39 years, and in JD at 40 years. At the time of diagnosis, both twins had experienced a >5 kg weight loss and were markedly anaemic (haemoglobin 4·0 g/dl in BD, and 9·9 g/dl in JD), with combined iron deficiency and folate deficiency. Small intestinal mucosal biopsies from BD and JD revealed marked villous atrophy, crypt hyperplasia, abnormal morphology of the surface epithelial cells, increased crypt mitoses, an increased lymphocyte and plasma cell infiltrate in the lamina propria, and an increased density of IEL in the surface epithelium. Although BD and JD were treated with a gluten-free diet, they were only intermittently compliant with the diet. At the time of biopsy for the present study, BD was mildly anaemic (haemoglobin 12·8 g/dl) and iron-deficient (serum iron 29 μg/dl), whereas JD was no longer anaemic and iron studies were normal.

Neither JD nor BD had a past history or current evidence of dermatitis herpetiformis, diabetes mellitus or thyroid disease. Neither parent nor a younger brother, age 38 years, have coeliac disease. JD and BD resided in the same household until 18 years old.

Zygosity, HLA typing and coeliac antibodies

JD and BD were documented to be MZ twins by polymerase chain reaction (PCR) analysis of unlinked polymorphic short tandem repeat loci (probability of exclusion $<0{\cdot}000\ 01$) [30]. Their HLA class I type was A1, A24; B8, B7, as determined by microlymphocytotoxicity assay. Their HLA class II alleles were DRB1*0301, DRB1*1501; DRB3*0101, DRB5*0101; DQA1*0501, DQA1*01; DQB1*0201, DQB1*06 as determined by PCR [31,32]. Both subjects had circulating IgA antigliadin antibodies.

Intestinal biopsies and peripheral blood mononuclear cells

Mucosal biopsies, 2–3 mm in size, were obtained from the second portion of the duodenum and the sigmoid colon. Two biopsies were obtained from each of three sites in the duodenum and two biopsies were obtained from each of three sites in the sigmoid colon. Biopsies designated as $+3$ and $+10$ were obtained at a distance of 3 cm and 10 cm from biopsies labelled $+0$. One biopsy from each site was used for molecular analysis of the TCR δ repertoire and the second was used for histology. Two separate blood samples from each twin were obtained concurrently and peripheral blood mononuclear cells (PBMC) were separated as described [33]. JD and BD were studied on the same day.

Endoscopy revealed a loss of normal duodenal folds, consistent with coeliac disease, in both subjects [34]. Histological examination of the small intestinal mucosal biopsies from JD and BD, from each of the three sites, were also consistent with diagnosis of coeliac disease, and revealed total villous atrophy, crypt hyperplasia, increased crypt mitotic activity, increased lymphocytes and plasma cells in the lamina propria, an increased density of IEL, and a surface epithelium that varied from cuboidal to squamous. Biopsies from each of the three sites within the sigmoid colon were normal in both subjects, with the exception of scattered foci of increased IEL within the surface epithelial layer. All studies were approved by the UCSD Committee on Human Subjects.

RNA extraction, reverse transcription and PCR

Total RNA was extracted from mucosal biopsies and PBMC samples, and 1 μg was reverse transcribed in a 20-μl reaction as described before [33]. Aliquots (2 μl) of the reverse transcription (RT) reaction were amplified using 25 pmol of $5'$ primer for TCRDV1, DV2 or DV3, and 25 pmol of $3'$ primer for TCRDC (Cδ), resulting in PCR products of 150–200 bp in length. The primers were:

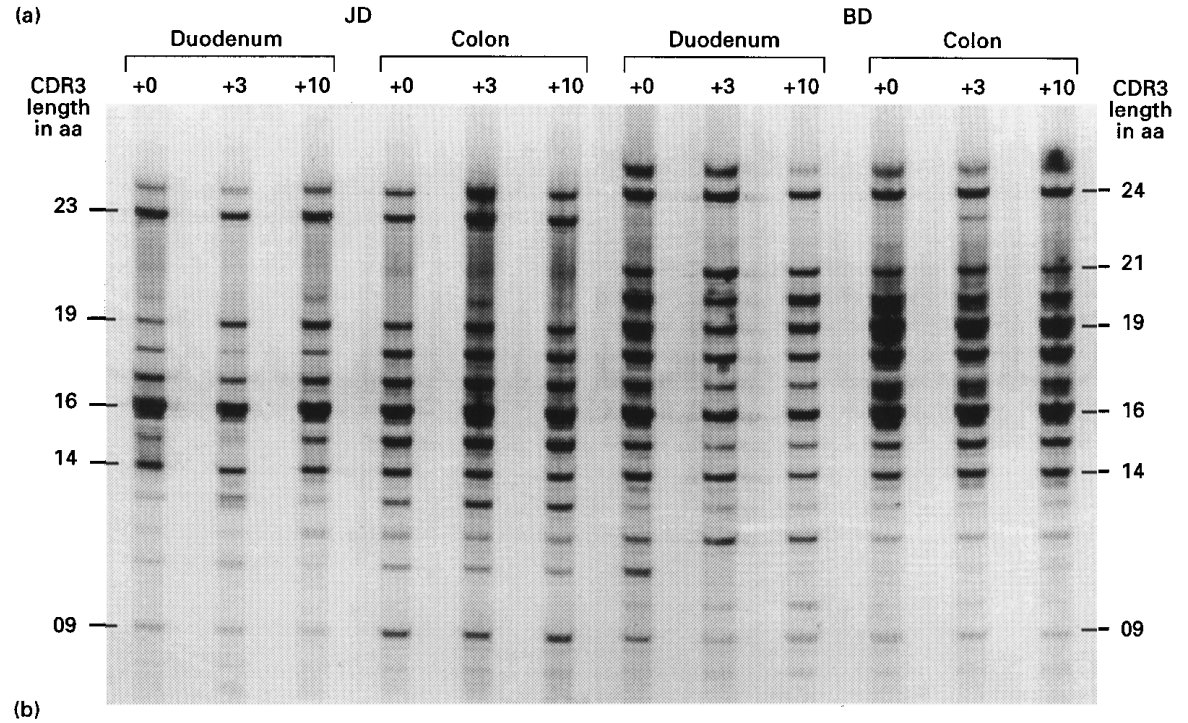
TCRDV1: CAGCCTTACAGCTAGAAGATTCAGC
TCRDV2: GCACCATCAGAGAGAGATGAAGGG
TCRDV3: TCACTTGGTGATCTCTCCAGTAAGG
TCRDC: AAACGGATGGTTTGGTATGAGGC

After an initial hot start, amplification consisted of 37–39 cycles of 40 s at 94°C, 50 s at 61°C, and 1 min at 72°C, followed by a final extension for 10 min at 72°C.

Analysis of CDR3 length distribution

Two microlitres of each PCR reaction containing amplified CDR3 domains of either Vδ1, Vδ2, or Vδ3 transcripts were mixed with formamide loading buffer and heat-denatured for 2 min at 95°C, after which PCR products were size separated on a 6% denaturing polyacrylamide gel and visualized by silver staining (Silver Sequence DNA staining reagents) under conditions recommended by the manufacturer (Promega, Madison, WI). Bands were photographed by exposing polyacrylamide gels for 8–15 s to Automatic Processor Compatible Film (Promega Silver Sequence). Each band contains sequences of identical CDR3 length and each band is separated by a distance corresponding to 3 bp (i.e. one codon). In a polyclonal TCR repertoire, the band pattern conforms to a bell-shaped distribution in which the greatest band intensity is at the modal length, with bands containing shorter and longer CDR3 regions being approximately equally distributed in decreasing frequency on either side. Band patterns were consistently reproducible when PCR amplification products from different RT reactions of the same RNA sample were analysed (data not shown).

(a)



(b)

CDR3 length	Duodenum		Colon		Duodenum		Colon		CDR3 length
	+0 cm	+10 cm	+0 cm	+10 cm	+0 cm	+10 cm	+0 cm	+10 cm	
23	J21	J21	J21	J21	B45	B45	B45	B45	24
19	*	*	*	*	B380	B380	B380	B380	21
16	J22	J22	J22	J22	*	B381	*	*	19
14	*	*	J39	J39	B54	B54	B54	B54	16
09	J24	J24	J24	J24	B47	B47	B47	B47	14
					B48	B48	B48	B48	09

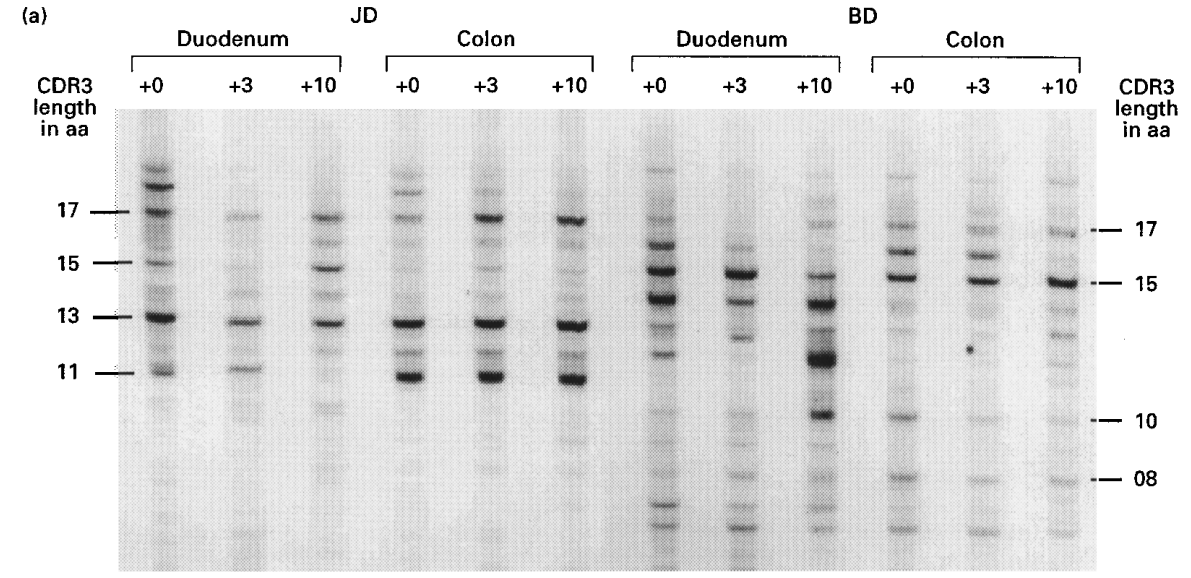
Fig. 1. (a) CDR3 length analysis of intestinal Vδ1 transcripts. The CDR3 regions of Vδ1 transcripts from JD and BD were amplified from duodenal and sigmoid colon biopsies, and size-fractionated by denaturing PAGE as described in Materials and Methods. Amplified products from JD and BD were run in parallel on the same gel. Lanes labelled +3 and +10 contain amplification products from biopsies obtained at a distance of 3 and 10 cm, respectively, from the biopsy labelled +0. The CDR3 lengths of the Vδ1 transcripts from both subjects ranged from 8 to 25 amino acids and the distribution of CDR3 lengths and variable intensity of the bands suggest that the Vδ1 repertoire in both subjects is restricted. As shown, band patterns in both the small intestine and sigmoid colon differed markedly between JD and BD. However, within each subject, similarity between the band pattern in the small intestine and sigmoid colon can be noted. (b) Clonal assignment and sequence analysis of CDR3 domains of Vδ1 transcripts. Bands of different CDR3 lengths were isolated from the gels (a), re-amplified by polymerase chain reaction (PCR) and sequenced. Transcripts are designated by the letter J or B followed by a number. *Transcripts where a sequence could not be obtained since those bands contained multiple different transcripts of identical CDR3 length. Although several Vδ1 transcripts of identical length were sequenced from both subjects, no Vδ1 transcripts were detected that were shared between the twins. As indicated, analysis often revealed a single transcript sequence in each band. Moreover, for each twin, identical Vδ1 transcripts were detected in the small intestine and sigmoid colon. Nucleotide sequences and deduced amino acid sequences of the transcripts are shown in Fig. 5.

Direct sequencing of PCR products

Individual bands were excised from the gels and incubated at room temperature in 50 μl sterile distilled H_2O, after which 5-μl aliquots were re-amplified for 30 cycles using the same primers and PCR conditions described above. Double-stranded PCR products were directly sequenced by the dideoxy chain termination method using a PCR sequencing kit (Amersham Life Sciences, Arlington, VA) and the TCRDC primer.

In many cases, one nucleotide sequence was obtained from a single isolated band of a given CDR3 length. This indicated that most transcripts within that band were identical. The existence within a band of multiple transcripts of the same CDR3 length but different nucleotide sequences did not yield a readable sequence, in the absence of further cloning. The TCR δ repertoire was defined as oligoclonal when several bands of different CDR3 lengths each contained a predominant sequence and the same sequences were detected in a separate biopsy or blood specimens.

Nucleotide sequences were analysed using PC/Gene DNA analysis software (Intelligenetics, Mountain View, CA) and were assigned to TCRDD (Dδ) gene segments based on at least 3 bp identities [35]. Gene segment assignments were as follows: TCRDJ1, TCRDJ2 and TCRDJ3 were assigned according to Takihara *et al.* [36]; TCRDV gene segments were assigned according to Satyanarayana *et al.* (DV1) [37], Dariavach & Lefranc (DV2) [38] and Hata *et*



(b)

CDR3 length	Duodenum		Colon		Duodenum		Colon		CDR3 length
	+0 cm	+10 cm	+0 cm	+10 cm	+0 cm	+10 cm	+0 cm	+10 cm	
17	J65	J65	J65	J65	B72	B72	B72	B72	17
15	J77	J77	*	*	B208	*	B210	B210	16
14	J228	J228	J228	J228	B83	B83	*	B86	15
13	#	#	J93	J93	B89	B89	B89	B89	14
11	J172	J172	*	*	*	*	*	*	10
					*	B184	B184	B184	08

Fig. 2. (a) CDR3 length analysis of intestinal Vδ2 transcripts. Vδ2 transcripts from duodenal and sigmoid colon biopsies of JD and BD were analysed as described in Fig. 1a. The band patterns of the transcripts from the small intestine and colon biopsies from JD differed markedly from those of BD. However, for each subject the band patterns of the amplified transcripts from the separate colon biopsies were almost identical. (b) Clonal assignment and sequence analysis of CDR3 domains of Vδ2 transcripts. Data are presented as in Fig. 1. #Cases where a single sequence could not be deduced, but it could be determined that those bands contained transcripts that differed from those of identical length in other biopsies. As shown, no Vδ2 transcripts were shared by the twins. However, in each subject the Vδ2 repertoire was oligoclonal, with identical Vδ2 transcripts being present in the small intestine and sigmoid colon. Of note, in separate biopsies, bands of the same length but variable intensity often contained identical Vδ2 transcripts. Nucleotide sequences and translated amino acid sequences of Vδ2 transcripts are shown in Fig. 5.

al. (DV3) [39], and are equivalent to DV101S1, DV102S1A1T and DV103S1A1T, respectively [40].

CDR3 length

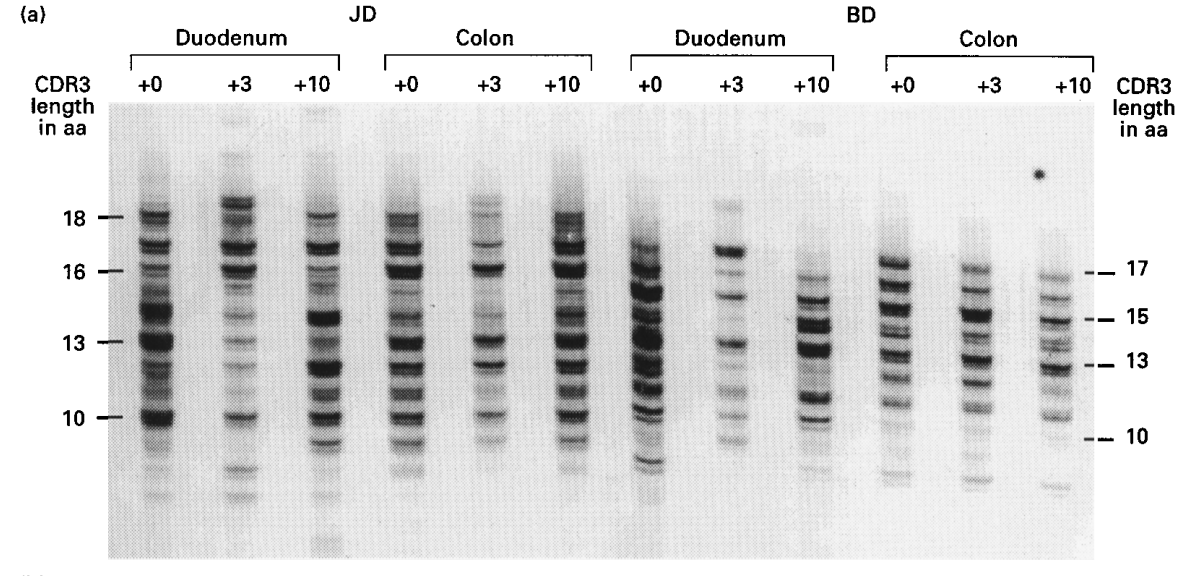
Lengths of the CDR3 domains of translated TCR δ transcripts were determined as described before [24,41]. Briefly, the distance was calculated between the conserved cysteine at position 88, which is encoded by the 3′ TCRDV region, and the conserved GXG triplet, which is encoded by all TCRDJ regions, and eight amino acids were subtracted [41–43].

RESULTS

We characterized the TCR δ repertoire in a pair of MZ twins concordant for coeliac disease (JD and BD) by analysing the spectrum of CDR3 lengths and the nucleotide sequence of TCR δ transcripts in the small intestine, sigmoid colon and peripheral blood. Vδ1, Vδ2 and Vδ3 transcripts from duodenal and sigmoid colon biopsies, and peripheral blood samples from each subject were amplified by PCR, after which PCR products were size-separated by denaturing PAGE and visualized by silver staining. In addition, multiple bands were excised from the gels and the CDR3 domains of δ transcripts were determined by direct sequencing. Adjacent bands contain δ transcripts which differ from each other by 3 bp (i.e. one codon) as confirmed by sequence analysis.

Analysis of CDR3 lengths of intestinal Vδ1 transcripts

Size separation of Vδ1 transcripts amplified from each duodenal and sigmoid colon biopsy of JD and BD yielded multiple bands (Fig. 1a). These transcripts encoded CDR3 domains ranging in length from 8 to 25 amino acids (see also Fig. 1b and Fig. 5). Variation in the distribution and intensity of transcripts of different CDR3 lengths and the repetitive pattern of the bands within each subject in multiple separate biopsies from the duodenum and sigmoid colon suggested the presence of a restricted Vδ1 repertoire in JD and BD. As shown in Fig. 1a, many of the Vδ1 transcripts in JD and BD were of identical size. However, there were marked differences in the overall band patterns between the twins in both the duodenum and sigmoid colon. We also noted that, within each subject, the band pattern of the amplified transcripts in the small intestine and colon were similar, suggesting that in each twin, identical Vδ1 transcripts might be present in the small intestine and colon.



(b)

CDR3 length	Duodenum		Colon		Duodenum		Colon		CDR3 length
	+0 cm	+10 cm	+0 cm	+10 cm	+0 cm	+10 cm	+0 cm	+10 cm	
18	J168	J168	J168	J168	*	*	*	*	17
17	J196	J196	J196	J196	B122	*	B124	B124	16
16	*	*	*	*	B128	B128	#	#	15
13	J134	*	J134	J134	B140	B140	B142	B142	13
11	J180	J180	J180	J180	B148	B148	#	#	11
10	J152	J152	*	*	B158	B158	#	#	10

Fig. 3. (a) CDR3 length analysis of intestinal Vδ3 transcripts. Like Vδ1 and Vδ2 transcripts, the band patterns of Vδ3 transcripts among the colon biopsies were similar within each subject, but differed between JD and BD. However, the band patterns of duodenal Vδ3 transcripts among the biopsies in each individual exhibited greater differences. Bands that, in some lanes, appear as closely running doublets may result from non-uniform addition by Taq polymerase of non-template encoded adenosine at the 3′ end [56] or incomplete denaturation, since sequence analysis of excised doublets revealed single sequences. (b) Clonal assignment and sequence analysis of CDR3 domains of Vδ3 transcripts. Data are presented as in Figs 1 and 2. No Vδ3 transcripts were shared by the twins but, like Vδ1 and Vδ2, the Vδ3 repertoire in the small intestine and sigmoid colon was oligoclonal in each subject. There was marked overlap in the Vδ3 repertoire in the small intestine and sigmoid colon of JD, but not of BD. Nucleotide sequences and translated amino acid sequences of Vδ3 transcripts are shown in Fig. 5.

The Vδ1 repertoire is oligoclonal but non-overlapping between JD and BD

To determine if PCR products of the same CDR3 length in JD and BD encoded identical or different transcripts, a number of bands were isolated from the gels and directly sequenced. As summarized in Fig. 1b, many of the excised bands contained transcripts with a single junctional sequence (see also Fig. 5), a finding which was independent of band intensity (e.g. CDR3 length 09). Moreover, of the transcripts sequenced, none was shared by the twins.

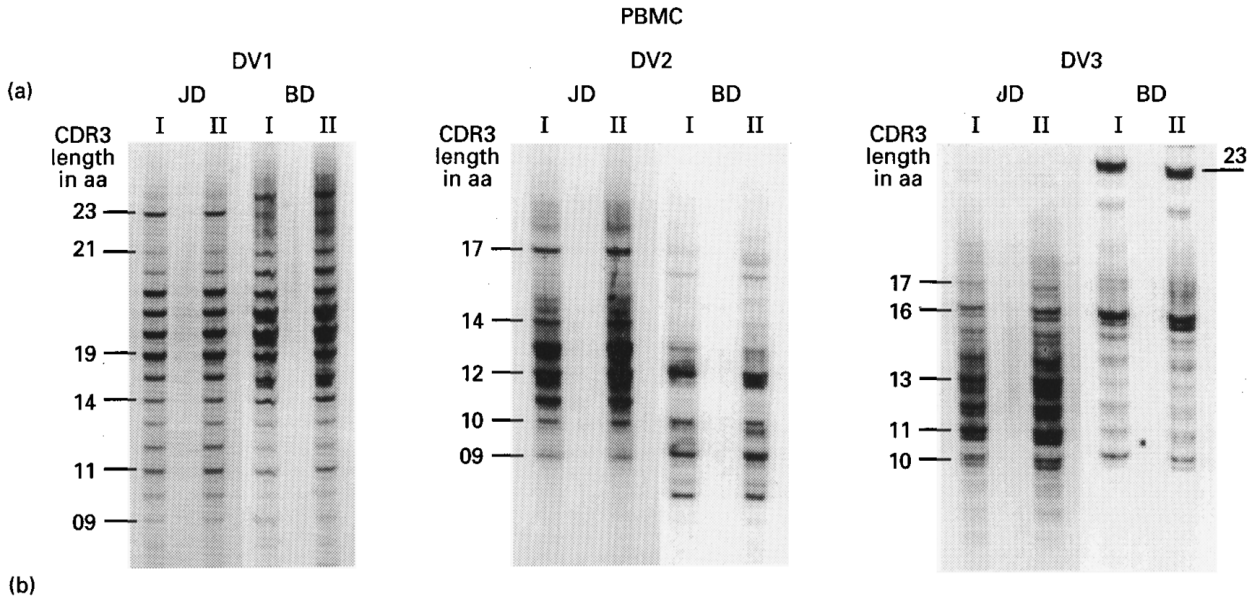
Sequence analysis confirmed the oligoclonal nature of the Vδ1 repertoire in each twin, as identical transcripts could be detected in biopsies from distinct sites. In addition, sequence analysis confirmed an overlap in the Vδ1 TCR repertoire between the small intestine and sigmoid colon of each twin, as suggested by PAGE (e.g. in JD, the most intense band at a CDR3 length of 16 amino acids contained a single sequence in all biopsies). The latter finding contrasts with our prior studies in three healthy adults [33] where the Vδ1 repertoire differed markedly between the small intestine and colon.

Vδ2 and Vδ3 CDR3 lengths in the intestine

Size separation of amplified Vδ2 and Vδ3 transcripts from the small intestine and sigmoid colon resulted in bands having CDR3 lengths ranging from 6 to 19 amino acids (Figs 2a and 3a). The band patterns of Vδ2 and Vδ3 transcripts suggested that, like Vδ1, these repertoires were also restricted. The band patterns of Vδ2 transcripts in each twin were similar among the three separate colonic biopsies, although they differed between the twins (Fig. 2a). Such was also the case for Vδ3 transcripts (Fig. 3a). Transcripts from the three separate duodenal biopsies from each twin yielded band patterns that appeared to be somewhat different from each other.

Sequence analysis of the Vδ2 and Vδ3 transcripts in the small intestine and colon

As shown in Figs 2b, 3b and 5, we found no Vδ2 or Vδ3 transcripts that were shared by the twins. However, in each twin, the Vδ2 and Vδ3 repertoire was oligoclonal. In addition, within each twin there was an overlap of Vδ2 transcripts between the small intestine and colon. Although this was also the case for small intestinal and



(b)

CDR3 length	JD I	JD II	BD I	BD II
24	ND	ND	*	*
23	J21	J21	*	*
21	ND	ND	*	*
17	ND	ND	B65	B65
16	*	*	B63	B63
14	J39	J39	B187	B187
11	ND	ND	B61	B61
09	J24	J24	*	*

CDR3 length	JD I	JD II	BD I	BD II
17	J69	J69	*	*
14	*	*	ND	ND
13	J95	J95	ND	ND
12	J106	J106	B713	B713
11	*	*	ND	ND
10	ND	ND	*	*
09	ND	ND	B112	B112

CDR3 length	JD I	JD II	BD I	BD II
23	ND	ND	B115	B115
17	J196	J196	*	*
16	J120	J120	B127	B127
13	J138	J138	*	*
11	J146	J146	B167	B167
10	*	*	B162	B162

Fig. 4. (a) CDR3 length analysis of Vδ1, Vδ2 and Vδ3 transcripts from peripheral blood samples. Vδ1, Vδ2 and Vδ3 transcripts from two separate blood samples (designated I and II) from each subject were amplified and separated according to their CDR3 lengths, as described in Fig. 1a. The band patterns for all three Vδ families analysed differed between the twins. However, as shown, the band pattern of Vδ1 transcripts in the peripheral blood of JD was similar to that from the duodenum and sigmoid colon (compare with JD, Fig. 1a). (b) Clonal assignment and sequence analysis of CDR3 domains of Vδ1, Vδ2 and Vδ3 transcripts from peripheral blood samples. No Vδ transcript sequences were shared between the twins. However, the Vδ1, Vδ2 and Vδ3 repertoire in each twin was oligoclonal and, within each subject, identical δ transcripts were detected in separate blood samples from each subject. Several Vδ1 and Vδ3 transcripts from the peripheral blood were also found in the intestine of JD (see Fig. 1b). However, there was no overlap between intestinal and peripheral blood mononuclear cell (PBMC) Vδ transcripts in BD. Nucleotide sequences and translated amino acid sequences of Vδ3 transcripts are shown in Fig. 5. ND, Not done.

colonic Vδ3 transcripts in JD, Vδ3 transcripts in the small intestine of BD differed from those in the sigmoid colon.

The TCR Vδ1, Vδ2 and Vδ3 repertoire in peripheral blood is oligoclonal and shows no overlap between JD and BD

CDR3 length analyses of the Vδ1, Vδ2 and Vδ3 transcripts in two separate peripheral blood samples from each twin were performed in parallel. The band pattern of Vδ2 and Vδ3 transcripts suggested an oligoclonal repertoire (Fig. 4a), whereas the band pattern of Vδ1 transcripts was consistent with a more diverse repertoire.

To characterize further the Vδ1, Vδ2 and Vδ3 repertoire in the peripheral blood and to ask if there was overlap in the Vδ repertoire in the peripheral blood and intestine, we sequenced several bands containing PBMC transcripts that were of identical CDR3 lengths to those from the intestinal biopsies. As shown in Fig. 4b, no Vδ transcripts were shared between the twins. However, within each twin identical transcripts were found in separate blood samples, indicating the Vδ repertoire in peripheral blood was oligoclonal in JD and BD. Whereas in BD there was no overlap between transcripts in the peripheral blood and the intestine, in JD several Vδ1 and Vδ3 transcripts in PBMC were identical to those present also in the intestine (Figs 1b and 3b). This was not the case for Vδ2 transcripts which are the predominant Vδ transcripts in the peripheral blood [27].

Translation of nucleotide sequences from TCR Vδ1, Vδ2 and Vδ3 transcripts does not reveal a conserved amino acid sequence motif

As shown in Fig. 5, the Vδ1, Vδ2 and Vδ3 transcripts were all in frame. Junctional regions of the δ transcripts were complex, as shown by extensive nucleotide insertions and deletions at the junctions of V, D and J gene segments, and the extent of this complexity was comparable to that reported before in healthy adults [24,33]. Although DJ1 (Jδ1) was the predominant TCRDJ segment used, DJ2 or DJ3 segments were used by several Vδ1 and Vδ2 transcripts with long CDR3 domains. When nucleotide sequences were translated into corresponding amino acid sequences, analysis of the junctions did not reveal a common amino acid sequence motif.

		NUCLEOTIDE SEQUENCES						AMINO ACID SEQUENCES			
							DJ1:ACACCGATAAACTC			DJ1:	TDKLIFGKG
CDR3	TRAN-	V-DELTA 1	N/P	DD2	N/P	DD3	N/P DJ2:CTTTGACAGCACAA	DV1	JUNCTION	DJ2:	LTAQLFFGKG
LENGTH	SCRIPT	CTTGGGGAACT		CCTTCCTAC		ACTGGGGGATACG	DJ3:CTCCTGGGACACCC	CALGE		DJ3:	SWDTRQMFFGTG
24	B45	CTTGGGGAA	ACCTCCGGC	CCTTC TCACCCCCTAGTGGAGTCGT		ACTGGGGGATACG	TGGTTGGG CAGCACAA	CALGE	TSGPSHPLVESYWGIRGWA		AQLFFGKG
23	J21	CTTGGGGAACT	AAGCGG	CCTTCCTAC	CTACGTGGT	GGGATAC	ACCCCGAG CTCCTGGGACACCC	CALGE	LSGLPTYVVGYTPS		SWDTRQMFFGTG
21	B380	CTTGGGGAA	TTGGG	CCTTCC	CCATCACT	ACTGGGGGATA	GCACCTCCCGGGAGT ACCGATAAACTC	CALGE	LGLPHHYWGIAPPGS		TDKLIFGKG
19	B381	CTTGGGGAAC	CG	CCTA	GGAGAA	TGGGGGATAC	AAGGGGGGCTGCGT ACACCGATAAACTC	CALGE	PPRRMGDTRGAAY		TDKLIFGKG
17	B65	CTTGGGGA	TGTTCCCCGG	CCTTCCTAC	AAAT	ACTGGGGGATAC	CA GATAAACTC	CALG	DVPRPSYKYWGIP		DKLIFGKG
16	J22	CTTGGGGAA	ATTAGTCG	TTCCTAC	T	GGGGATAC	AGTA ACACCGATAAACTC	CALGE	ISRSYWGYSN		TDKLIFGKG
16	B54	CTTGGG	AACCCA	CTACG	TCCGT	ACTGGGGGA	GACCCT ACACCGATAAACTC	CALG	NPLRPYWGRPY		TDKLIFGKG
16	B63	CTTGGGGAACT	TT	CCTTCC	CGGCCT	ACTGGGGG	CTCT ACACCGATAAACTC	CALGE	LSFPAYWGLY		TDKLIFGKG
14	J39	CTTGGGGAAC	CGCCGCGCCA			GGGGG	CTGGGT ACACCGATAAACTC	CALGE	PPRQGAGY		TDKLIFGKG
14	B47	CTTGGGGA	T	CTTCCTACG		CTGGGGGATA	CCT ACACCGATAAACTC	CALG	DLPTLGDTY		TDKLIFGKG
14	B187	CTTGGGGAACT	C	CCT	GC	ACTGGGGGATACG	G ACACCGATAAACTC	CALGE	LPALGDTD		TDKLIFGKG
11	B61	CTTGGGGAAC	GAA			GGGGAT	TCG ACACCGATAAACTC	CALGE	RRGFD		TDKLIFGKG
09	J24	CT	ACGG			TGGGGGAT	CA ACACCGATAAACTC	CAL	RWGIN		TDKLIFGKG
09	B48	CTTGGG	AATGG			TGGG	ATCAAAGG TAAACTC	CALG	NGGIKG		KLIFGKG

							DJ1:ACACCGATAAACTC			DJ1:	TDKLIFGKG
CDR3	TRAN-	V-DELTA 2	N/P	DD2	N/P	DD3	N/P DJ3:CTCCTGGGACACCC	DV2	JUNCTION	DJ3:	SWDTRQMFFGTG
LENGTH	SCRIPT	TGTGACACC		CCTTCCTAC		ACTGGGGGATACG		CACDT			
17	J65	TGTGACACC		TTC	GCAGTCAGG	GGGGGATAC	CCCGAAACAG ACACCGATAAACTC	CACDT	FAVRGGYPETD		TDKLIFGKG
17	J69	TGTGAC	CCACTGGG	TCCT	CGTGG	TGGGGGATACG	AGGGAT ACACCGATAAACTC	CACD	PLGPRGGGYEGY		TDKLIFGKG
17	B72	TGTGAC	GAAGGAGGGT			ACTGGGGGATA	GGGTTG TCCTGGGACACCC	CACD	EGGYWGIGL		SWDTRQMFFGTG
16	B208	TGTGACACC	GGAAAGCA			CTGGGGGAT	GCCCTTGCACAATA CGATAAACTC	CACDT	GKHWGIALAQY		DKLIFGKG
16	B210	TGTGACACC	GTAGCGC	CCTAC	T	ACTGGGGGA	CCATGCCTAATCG TAAACTC	CACDT	VAPYYWGTMPNR		KLIFGKG
15	J77	TGTGAC	CTCGTCT	TTC	GAACGT	ACTGGGGGA	CTT ACACCGATAAACTC	CACD	LVFRTYWGTY		TDKLIFGKG
15	B83	TGTGACACC	G	TTCC	AGGGTGGT	ACTGGGG	CACGA ACACCGATAAACTC	CACD	TVPGWYWGTN		TDKLIFGKG
15	B86	TGTG	TGGGCTCGTT	TAC	T	ACTGGGGGATAC	AATAACGTCA ATAAACTC	CAC	VGSFTTGGYNNVN		KLIFGKG
14	J228	TGTGACACC	GCGACGGGGGT			ACTGGGGGATA	TTGG CGATAAACTC	CACDT	ATGVLGDIG		DKLIFGKG
14	B89	TGTGACAC	AG	CTTCCTAC	C	ACTGGGG	TGTAA ACACCGATAAACTC	CACDT	ASYHWGVN		TDKLIFGKG
13	J93	TG	GGGAGAACTGGC	TCCTAC			CCTCTCGT ACACCGATAAACTC	CA	WGELAPTLSY		TDKLIFGKG
13	J95	TGTGACAC	GGT			TGGGG	AATCTGGGAGGG ACACCGATAAACTC	CACDT	VGESGRD		TDKLIFGKG
12	B713	TGTGAC	TGCCGGT			CTGGGGGATAC	TACG CCGATAAACTC	CACD	CRSGGYYA		DKLIFGKG
12	J106	TGTGAC		TCC	GT	ACTGGGGGATA	GGT ACACCGATAAACTC	CACDT	RFPAG		TDKLIFGKG
11	J172	TGTGACAC	GAGAT	TTCC	GG	CTGG	CACCGATAAACTC	CACD	SVLGDRY		TDKLIFGKG
09	B112	TGTGACACC	GT			TGG	AGGAG CCGATAAACTC	CACDT	VGGA		DKLIFGKG
08	B184	TGTGACAC	TTTA	CTT			T CCGATAAACTC	CACDT	LLS		DKLIFGKG

							DJ1:ACACCGATAAACTC			DJ1:	TDKLIFGKG
CDR3	TRAN-	V-DELTA 3	N/P	DD2	N/P	DD3	N/P DJ3:CTCCTGGGACACCC	DV3	JUNCTION	DJ3:	SWDTRQMFFGTG
LENGTH	SCRIPT	TGTGCCTTTAG		CCTTCCTAC		ACTGGGGGATACG		CAF			
23	B115	TGTGCCTTTA	CATCGCT	CCTTCCTA	AGAGAGTTA	TGGGGGATACG	GTATTAAATAGACACGAGT ACACCGATAAACTC	CAF	TSLLPKRVMGDTVLNRHEY		TDKLIFGKG
18	J168	TGTGCCT	CCCCTATAATTTTTGGAA	CCTTCC	GGAGGTTAGGT	ACTGGGGGATACG	C TAAACTC	CA	SPIIFGTFRRLGTGGYA		KLIFGKG
17	J196	TGTGCCT	GCTTTAATCCCGA	TTCCT	TTCGGT	ACTGGGGGA	CAGAGT ACACCGATAAACTC	CA	CFNPDSFRYWGTEY		TDKLIFGKG
16	J120	TGTGCCTTTAG	CCTTG	CCTTCCTA		ACTGGGGGATACG	AGATTT ACACCGATAAACTC	CAF	SLAFLTGGYEIY		TDKLIFGKG
16	B122	TGTGCCTT	CAGGCT	TTCC	ACGAGACGACCT	TGGGGG	GACACCCCGGG CGATAAACTC	CAF	RLSTRRPWGDTQG		DKLIFGKG
16	B124	TGTGCCT	CAATCGTCT	CTTCC	AACCGCT	ACTGGGGGAT	CGCAAGCC CCGATAAACTC	CA	SIVSSNRYWGIVS		TDKLIFGKG
16	B127	TGTGCC	CGTGGG	TCC	CCCTCCCCCCGCGCG	CTGGGGG	CGCCGGCTGA CGATAAACTC	CA	RGSPSPRALGAPAD		DKLIFGKG
15	B128	TGTGCCT	AT	CTTCCT	CCTGATACT	ACTGGGGGATACG	GGT ACACCGATAAACTC	CA	YLPPDTTGGYGY		TDKLIFGKG
13	J134	TGTGCCTT	CCACGTAA	TAC	GT	ACTGGGGGATA	TT ACACCGATAAACTC	CAF	HVIRTGGYY		TDKLIFGKG
13	J138	TGTGCC	GCAA	CCT	CGT	ACTGGGGGATAC	TGGGGGACG CCGATAAACTC	CA	ATSYWGILGDA		DKLIFGKG
13	B142	TGTGCCTTT	G	CCTTCCTA	TTCGCA	GGGGGA	AGGT ACACCGATAAACTC	CAF	AFLFAGGRY		TDKLIFGKG
13	B140	TGTGCCTTTAG	ACC	CCTTCCTAC	GA	GGGGATACG	CCC CCGATAAACTC	CAF	RPLPTRGYAP		DKLIFGKG
11	J180	TGTGCCTTT	CGACC			CTGGG	CCCCGCCCG ACACCGATAAACTC	CAF	RPWAPPD		TDKLIFGKG
11	J146	TGTGCCT	CCCG	TTCC	GATATAGG	CTGGGGG	ACCGATAAACTC	CA	SRSDIGWG		TDKLIFGKG
11	B148	TGTGCCT	CCCGCTC			GGGGGA	CCCGCAGCACGA CGATAAACTC	CA	SRSGDPQHD		DKLIFGKG
11	B167	TGTGCCT	CT			ACTGG	CCTGCC CTCCTGGGACACCC	CA	STGLP		SWDTRQMFFGTG
10	J152	TGTGCCTT	CAGGCCG			TGGGGGATAC	ACACCGATAAACTC	CAF	RPWGIH		TDKLIFGKG
10	B158	TGTGCCTTTA	TAT	TTC	T	TGGGGG	GATCCG CGATAAACTC	CAF	IFLGGIR		DKLIFGKG
10	B162	TGTGCCTTT	CGG			TGG	ACTTATCTGTCCGT CGATAAACTC	CAF	RWTYLSV		DKLIFGKG

Fig. 5. Nucleotide and amino acid sequence of the CDR3 regions of TCR Vδ1, Vδ2 and Vδ3 transcripts from intestinal and peripheral blood samples. The nucleotide and translated amino acid sequences of the CDR3 regions of Vδ1, Vδ2 and Vδ3 transcripts from JD and BD are shown. Transcript designations correspond to those given in Figs 1b–4b. Germ-line sequences are indicated at the top in bold. DD1 gene segments were infrequent and potential DD1 gene fragments are underlined within the N region. All Vδ1, Vδ2 and Vδ3 transcripts were in frame. Translation into the corresponding amino acid sequences [43], as shown to the right side of each nucleotide sequence, did not reveal a common amino acid motif in the CDR3 region.

DISCUSSION

Increased numbers and an increased proportion of γ/δ relative to α/β IEL in the small intestinal mucosa are a hallmark of coeliac disease, whether disease is active or in remission [15–17]. In addition, healthy first degree relatives of coeliac disease patients have increased numbers of γ/δ IEL in the small intestinal mucosa, and this correlates with whether or not those subjects express the HLA class II coeliac disease susceptibility alleles [20]. To assess if genetic factors that govern host susceptibility to coeliac disease also determine the γ/δ T cell receptor repertoire in coeliac disease, we characterized the junctional diversity of TCR δ transcripts expressed in the intestine and peripheral blood of a MZ twin pair who are concordant for coeliac disease. As shown herein, genetically identical individuals, concordant for coeliac disease, can express distinct, non-overlapping TCR δ repertoires. Thus, genetic factors that govern disease susceptibility do not appear to select for specific TCR δ sequences or CDR3 amino acid motifs.

The marked complexity of the TCR δ junctional sequences and oligoclonality of the TCR δ repertoire in the twins was typical of that reported by us and others in healthy adults, and appears to reflect positive selection and expansion of specific populations of T cells [24,33,44,45]. However, in contrast to healthy individuals, in whom the TCR δ repertoire is relatively compartmentalized with little overlap between the small intestine and colon ([33] and W. Holtmeier, unpublished data), we note there was marked overlap between the TCR δ repertoire in the small intestine and sigmoid colon of each twin. Although based strictly on the twin pair studied herein, this finding is nonetheless of interest in light of prior reports indicating that coeliac disease patients also have increased numbers of γ/δ IEL in the rectal mucosa [46]. Although mucosal damage in coeliac disease is generally confined to the proximal small intestine [3], proctitis and lymphocytic colitis have been noted in some coeliac disease patients [47,48] and, compared with healthy individuals, the rectal mucosa of coeliac disease patients has been reported to respond abnormally to experimental gluten challenge [49,50]. Thus, taken together, these studies suggest an underlying abnormality may affect the entire intestinal mucosa in coeliac disease.

Susceptibility to coeliac disease is strongly associated with the inheritance of specific HLA class II DQ molecules [5–8]. However, the γ/δ TCR repertoire does not appear to be shaped by MHC class I or class II molecules, and γ/δ T cells, unlike α/β T cells, do not appear to recognize peptides bound in the peptide binding groove of HLA class I or II molecules [51,52]. Although increased γ/δ T cells among healthy first degree relatives of coeliac disease probands correlated with the presence of specific DQ susceptibility alleles [20], these alleles, as shown herein, did not determine the expressed TCR δ repertoire.

Little is known regarding the role γ/δ T cells play in normal mucosal immunophysiology or in the pathogenesis of coeliac disease, and it is not known whether γ/δ T cells in coeliac disease recognize determinants on the disease-activating proteins or a different set of ligands. In contrast to α/β T cells, there is no proven paradigm for the nature of the ligands that are recognized by γ/δ T cells, although recent studies suggest a population of Vδ2-expressing γ/δ T cells can recognize small, non-peptide molecules [53,54]. However, recent reports suggest there are fundamental differences in the pathways by which ligands are processed and presented to γ/δ compared with α/β T cells [51,52,55], and it has been proposed that γ/δ T cells recognize ligands in a manner more analogous to that of antibody [41].

ACKNOWLEDGMENTS

This research was supported by National Institutes of Health grants DK35108 and DK47739. W.H. was supported by a Research Fellowship from the Crohn's and Colitis Foundation of America. D.L.R. was supported by NIH Training Grants DK07202 and CA09174. We thank Dr E. Morzycka-Wroblewska and Dr L. Eckmann for helpful comments on the manuscript.

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