Distribution of Dominant T Cell Receptor β Chains in Human Intestinal Mucosa

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Summary

The majority of human intestinal intraepithelial lymphocytes (iIELs) are $CD8^+$ T cells that use the T cell receptor (TCR)-α/β. Previous studies have shown that iIELs isolated from segments of small intestine or colon contain one or several dominant α/β T cell clones. It is not known whether these clones expand only locally in response to a particular antigen or whether they are widely distributed throughout the intestine. To address this question, iIELs were purified from near the proximal and distal margins in a series of intestinal resections for noninflammatory diseases. TCR-β expression was then assessed by semiquantitative polymerase chain reaction amplification, analysis of N-region length, and DNA sequencing. The previously described oligoclonal expansion of iIELs was confirmed in each sample. Identical dominant clones were identified in the proximal and distal samples from most cases, including samples taken from sites as distant as the transverse and sigmoid colon or rectum. Distinct clones were found in only one case with samples from the terminal ileum and transverse colon. These results demonstrate that a relatively small number of widely dispersed T cell clones comprise the majority of cells in the human intestinal mucosa.

The intestinal mucosa contains a population of lymphocytes, termed intestinal intraepithelial lymphocytes (iIELs)[1], which are phenotypically distinct from the lymphocytes in the peripheral blood and primary lymphoid tissues. Most human iIELs are $CD45RO^+CD8^+$ T cells, which indicates that they have been previously activated and suggests that they recognize antigens presented by MHC class I or class I-like proteins (1–4). Evidence that iIELs are specialized to recognize a particular set of antigens is provided by analyses of their TCRs. Most human iIELs use the TCR-α/β, although use of the TCR-γ/δ in the colon is markedly increased relative to peripheral blood (2, 5, 6). DNA sequence analyses of the TCR-α/βs expressed by IELs in the human intestine has demonstrated that these cells are oligoclonal (7–9). iIELs using the TCR-γ/δ may be more heterogeneous, but do show a strong bias towards the use of particular Vγ and Vδ genes (6). These observations indicate that the TCR repertoire of human iIELs is relatively limited and suggest that the range of antigens recognized by these cells must be similarly limited.

Although one or several dominant iIEL clones using the TCR-α/β can be detected in surgical samples from multiple patients (7–9), whether these clones are widely disseminated within the intestinal mucosa is not clear. An alternative possibility has been that the oligoclonal expansion of iIELs detected in samples of human small and large intestine may be due to the local concentration of particular clones, with distinct dominant clones present in adjacent areas of intestine. To address this issue, this report examined the TCR-β chains used by iIELs extracted from near the proximal and distal margins of a series of intestinal resections. The results demonstrate that identical dominant clones are present among iIELs isolated from separate sections of intestine. These results put further constraints on the TCR repertoire of the intestinal mucosa and indicate that a relatively small number of T cells undergo dramatic clonal expansion and comprise the majority of the IELs in the human intestine.

Materials and Methods

Isolation of IELs. Human small and large intestines were obtained from patients undergoing surgery for a variety of conditions (see Table 1). In each case, a strip of mucosa was obtained from near the proximal and distal margins of the specimens. The iIELs were isolated from each strip separately using dithiothreitol and EDTA (10). The purity of each preparation was estimated by two-color immunofluorescence using directly conjugated antibodies

[1] *Abbreviation used in this paper:* iIELs, intestinal intraepithelial lymphocytes.

to CD3 (anti-CD3-PE; Becton Dickinson & Co., Mountain View, CA), CD4 (anti-CD4-FITC, Dako Corp., Carpinteria, CA), and CD8 (anti-CD8-FITC, Dako Corp.).

Semiquantitative PCR Amplification. RNA was isolated from ~10^6 iIELs and cDNA synthesis was carried out as described (7). The cDNA were PCR amplified in a series of 20-μl reactions containing 10 pmol of a Cβ antisense primer (GCCTTTTCCCTGTGGGAGAT), 10 pmol of a Vβ primer (see below), 0.1 mg/ml BSA, 0.2 mM dNTPs, and 0.5 U Taq polymerase. Each cycle was 94°C for 20 s, 55°C for 30 s, and 72°C for 60 s. Aliquots of 5 μl were withdrawn after 24 and 30 cycles and dot blotted with a ^{32}P-labeled internal antisense Cβ probe (GGCTCAAACACAGCGACCT). Quantitation was performed on a Phosphorimager (Molecular Dynamics Inc., Sunnyvale, CA), using the minimum number of cycles which yielded a clear signal. Each Vβ was expressed as a percent of the total product amplified by all of the Vβ primers.

The primers for Vβ1–20 were described previously (9). Additional primers used in this study were Vβ21, CTAGA(TC)GATTCACAGTTGCCTAA; Vβ22, CAGAGAAGTCTGAAATATTCGATGA; Vβ23, GATGCAGAGCGATAAAGGAAGCA; and Vβ24, CAAAGATTTTAACAATGAAGCAGACA.

Cloning and Sequencing PCR Products. The PCR products were cut with restriction enzymes recognizing unique sites in each Vβ or blunt-ended with T4 DNA polymerase. They were then digested with BglII (which cleaves at a conserved site in Cβ1 and Cβ2) and ligated into pBluescript (Stratagene, La Jolla, CA). Colonies were screened with an internal Cβ probe (GGCTCAAACACAGCGACCT) and multiple isolates were sequenced.

Analysis of PCR-amplified TCRs by Polyacrylamide Gel Electrophoresis. PCR-amplified TCRs were reamplified using a nested, ^{32}P end-labeled antisense Cβ primer (GGCTCAAACACAGCGACCT), as described previously (9). The PCR products were then heated denatured and separated on 6% DNA sequencing gels.

Results

iIEL Isolation from Small and Large Intestine. Table 1 shows a clinical summary of the surgical samples used for this study. In each case, the diagnosis was a noninflammatory disease and the mucosa was taken from a region of grossly normal intestine. In donors 1–5, the mucosal samples were from either the small or large intestine. In donors 6 and 7, the proximal sample was from the ileum and the distal sample was from the colon. The fraction of CD4$^+$ T cells was under 20% in the majority of cases, indicating that there was limited contamination from lamina propria lymphocytes. In donors 2 and 6, however, up to 40% of the T cells were CD4$^+$. This may reflect some contamination from the lamina propria or may be due to variability in the fraction of CD4$^+$ iIEL.

Analysis of iIEL Vβ Expression by Semiquantitative PCR Amplification in the Small or Large Intestine. cDNA was synthesized from each preparation and Vβ usage was assessed by semiquantitative PCR amplification, using a panel of Vβ-specific primers and a Cβ primer. Fig. 1 shows the pattern of Vβ usage by iIELs from the proximal and distal samples in donors 1–3. In donor 1, use of Vβ3 predominated in both the proximal and distal samples. In donor 2 there was a wider distribution of Vβ usage, but the patterns in both the proximal and distal samples were very similar. In donor 3, the use of Vβ6 was more prominent in the distal sample, but the relative use of the other Vβ families was similar.

Although these similarities would be consistent with the same clones being in the proximal and distal samples, they could instead reflect a bias introduced by positive selection for recognition of particular MHC molecules. Proximal and distal iIELs using prominent Vβ families were, therefore, analyzed further. This analysis was carried out by comparing N-region lengths and by DNA sequencing.

Detection and Comparison of Dominant Clones by Analysis of N-Region Length. The precise length of a TCR can be determined on DNA sequencing gels and depends upon the number of N-region insertions and deletions. Polyclonal populations on these gels generate a series of bands spaced every three base pairs, with a relatively normal distribution of band intensities (9, 11). In contrast, monoclonal or oligoclonal populations generate one or several dominant bands, respectively

Table 1. *iIEL Isolation from Proximal and Distal Sites*

Donor	Age	Sex	Clinical diagnosis	Proximal site	CD4$^+$	Distal site	CD4$^+$
1	NA	F	Jejunal bypass for obesity	Jejunum	NA	Jejunum	NA
2	80	F	Synchronous adenocarinomas of colonic splenic flexure and rectum	Transverse colon	14%	Proximal rectum	24%
3	68	M	Benign stricture with pill ulceration	Ileum	35%	Ileum	40%
4	43	M	Paraplegia requiring transverse colostomy	Transverse colon	19%	Sigmoid colon	8%
5	NA	F	Jejunal bypass for obesity	Jejunum	5%	Jejunum	6%
6	72	M	Moderately differentiated adenocarcinoma of cecum	Terminal ileum	40%	Transverse colon	27%
7	79	F	Moderately differentiated adenocarcinoma of R transverse colon	Terminal ileum	10%	Transverse colon	9%

The purity of each preparation was assessed by measuring the fraction of T cells that were CD4$^+$. NA, not available.

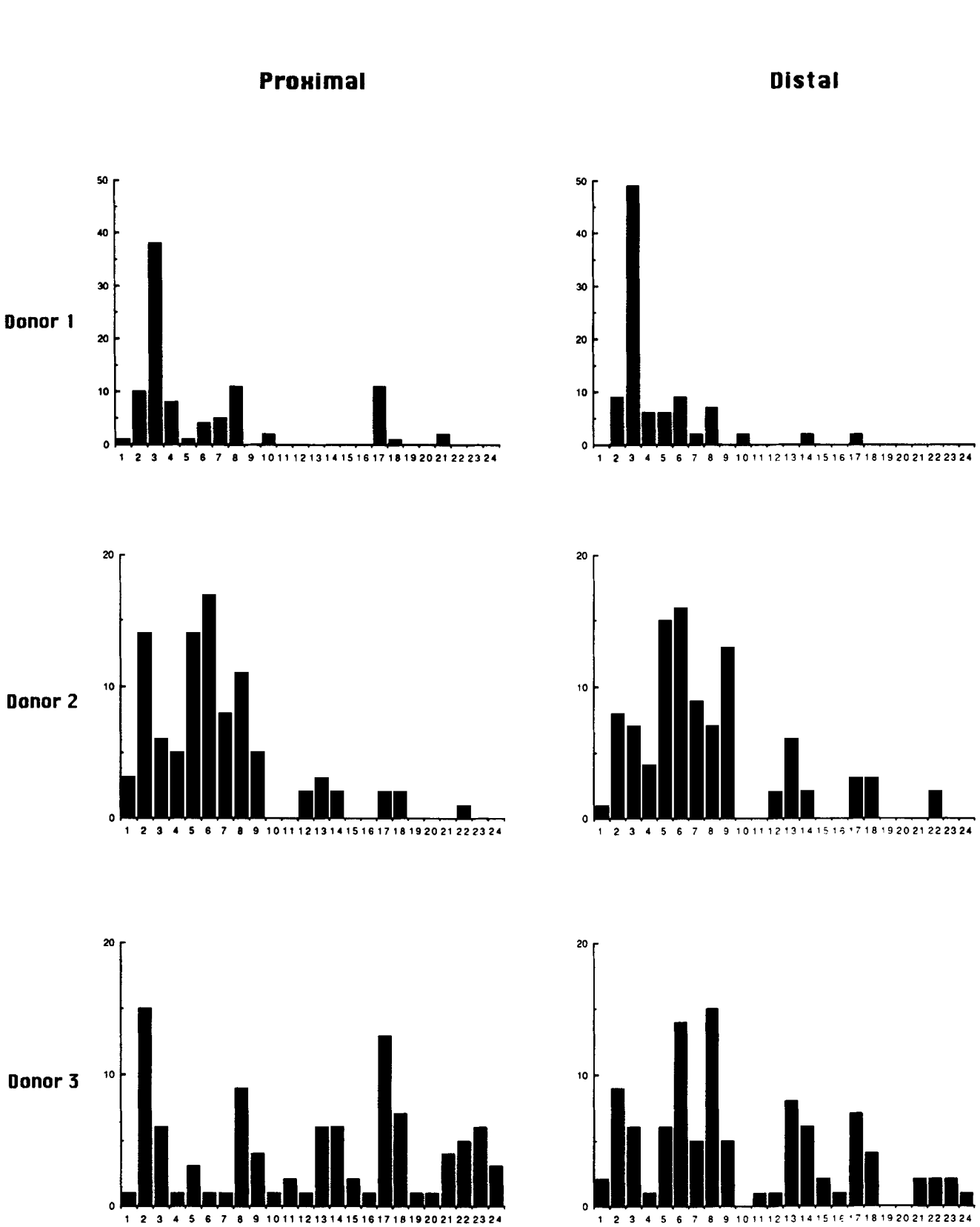

Figure 1. Semiquantitative PCR amplification of IELs. iIELs extracted from near the proximal or distal margins of the jejunum (donor 1), colon (donor 2), or ileum (donor 3) were examined. Vβ primers are shown on the x-axis and the percent of transcripts which amplified with each Vβ primer is indicated on the y-axis.

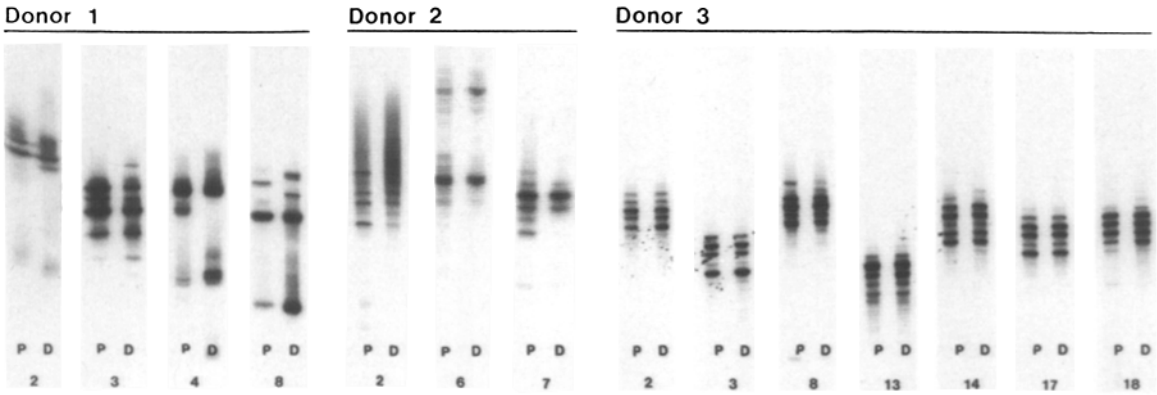

Figure 2. Comparison of iIEL clones using particular Vβs by analysis of N-region lengths. TCRs using particular Vβs were PCR amplified with a ^{32}P-labeled Cβ primer and analyzed on 6% DNA sequencing gels. The Vβ primer used for each pair is indicated. Lanes *P*, proximal; lanes *D*, distal. The wide distribution of bands in the Vβ6 lanes from donor 2 is due to the pooling of two Vβ6 primers at slightly different locations in order to amplify most members of the large Vβ6 family (9).

(9, 11). As we have shown previously, iIELs analyzed by this method generate one or several dominant bands with each Vβ tested, indicative of an oligoclonal population (9).

To determine whether identical dominant clones may be present in the proximal and distal samples, the size distributions of TCRs using prominent Vβ genes were compared. In donor 1, the TCRs using Vβ2, 3, 4, and 8 were examined. The size distributions of the major bands in the proximal and distal samples were similar using each of these primers (Fig. 2). The banding patterns in the proximal and distal samples from donors 2 and 3 were also similar (Fig. 2), as were the banding patterns from two additional donors (Fig. 3). These results suggested that some T cell clones were present at a high frequency in both the proximal and distal iIELs.

Comparison of Dominant Clones by DNA Sequencing. DNA sequencing was used to determine definitively whether identical dominant clones were present in the proximal and distal iIEL populations. TCRs using prominent Vβ genes were amplified by PCR and ligated into pBluescript. Multiple TCRs were then isolated from these libraries and sequenced. The results of this analysis are summarized in Table 2.

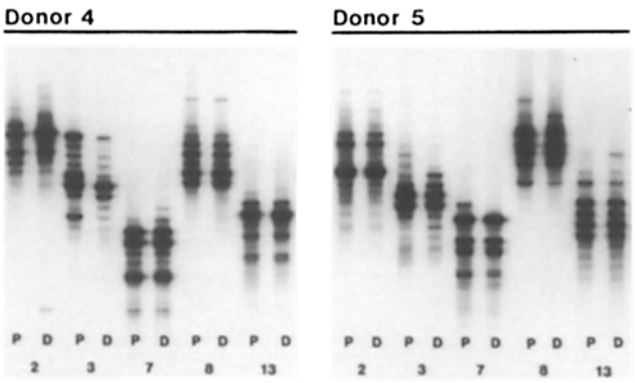

Figure 3. Comparison of additional iIELs by analysis of N-region length. TCRs using five arbitrarily chosen Vβs (2, 3, 7, 8, and 13) were analyzed. Lanes *P*, proximal; lanes *D*, distal.

In donor 1, a single Vβ3 clone comprised the majority of Vβ3 isolates from both the proximal and distal iIELs. As ~40% of the proximal and distal iIELs used Vβ3 (Fig. 1), this result indicated that up to 20–30% of the proximal and distal iIELs were derived from this single clone. An identical dominant clone using Vβ4 (Vβ4.3–Jβ2.2) in the proximal and distal sample was similarly identified. Two additional Vβ4 clones, each using Vβ4.1 and Jβ2.7, were unique to the proximal or distal iIEL population. Finally, a single dominant Vβ8 was found in both samples. These results confirm that the iIELs from this donor were oligoclonal and that several clones were present at a high frequency at two distinct sites.

In donor 2, TCRs using Vβ6 and 7 were analyzed. Three clones using Vβ6 were identified in both the proximal and distal samples, although the majority of Vβ6 isolates from this donor were identified only once in the proximal or distal sample (Table 2). An inframe TCR using Vβ6 with a highly conserved cysteine replaced by arginine was found in both the proximal and distal samples. This particular Vβ6 gene is probably nonfunctional. The relative diversity of TCRs using Vβ6 may reflect the large size of this Vβ gene family. In contrast, the clones using Vβ7 in the proximal and distal samples were identical. In donor 3, identical clones using Vβ2 and 3 were similarly identified (Table 2).

Analysis of iIELs from the Small and Large Intestine of the Same Patient. In two patients with carcinomas in the right colon, it was possible to obtain samples from the small intestine (ileum) and colon (donors 6 and 7). The Vβ distribution in these donors, based upon semiquantitative PCR amplification, is shown in Fig. 4. In donor 6 the relative usage of each Vβ family was similar in the ileum and colon. In donor 7, the ileum and colon Vβ usage appeared more distinct.

The N-region lengths for TCRs using several prominent Vβ families were next determined in these samples. In donor 6, TCRs using Vβ3, 5, 8, 14, and 23 gave different patterns, indicating that the dominant clones using these Vβs in the

Table 2. *Sequence Analysis of Proximal and Distal ilELs*

Donor	V	N	J	Proximal	Distal	Frame
1	Vβ3-CASS	LVR	NTGELFFG-Jβ2.2	12/17	9/14	+
	Vβ3-CA	RPGQGVI	QYFG-Jβ2.7	1/17	3/14	+
	Vβ3-CASS	LTSGL	YNEQFFG-Jβ2.1	2/17	0/14	−
	Vβ4.3-CSA	PKQGA	NTGELFFG-Jβ2.2	9/18	6/11	+
	Vβ4.1-CSV	EGDSY	YEQYFG-Jβ2.7	7/18	0/11	+
	Vβ4.1-CSV	LGTGGDSVT	YEQYFG-Jβ2.7	0/18	4/11	+
	Vβ4.1-CSV	VPGLDPP	DTQYFG-Jβ2.3	2/18	0/11	+
	Vβ8-CAS	IEGEVS	GYTFG-Jβ1.2	11/11	11/11	+
2	Vβ6-CAS	IRPTGGG	YGYTFG-Jβ1.2	3/12	2/13	+
	Vβ6.6-CASS	LVYGN	EAFFG-Jβ1.1	1/12	1/13	+
	Vβ6-RASS	PSRQACLD	GYTFG-Jβ1.2	1/12	2/13	−
	Vβ7-CASS	QDISGGA	NEQFFG-Jβ2.1	3/4	3/5	+
	Vβ7-CASS	QDDGGVA	TIYFG-Jβ1.3	1/4	1/5	+
3	Vβ2.3-CSA	TEGP	YGYTFG-Jβ1.2	1/9	2/8	+
	Vβ2.3-CSA	RGQPV	NEKLFFG-Jβ1.4	3/9	1/8	+
	Vβ3-CASS	TGGRW	QFFG-Jβ2.1	3/5	2/5	+
	Vβ3-CASS	LGVGSRI	FG-Jβ2.5	0/5	2/5	+

All sequences identified more than once in the proximal or distal samples are reported. Amino acid sequences are in single letter code. TCRs are inframe (+) or not inframe (−) as indicated.

ileum and colon were distinct (Fig. 5). In contrast, predominant TCRs of the same size using Vβ1 and 2 were identified. To determine whether these were from the same clone in the ileum and colon, TCRs using Vβ1 and 2 were cloned and sequenced. Table 3 shows that the clones using these two TCRs were distinct in the ileum and colon.

In donor 7, fewer bands appeared in the proximal sample from the ileum than in the distal colon sample in most cases. However, the major bands using each Vβ from the ileum were of the same size as predominant bands in the colon, indicating that some dominant clones could be present in both samples. Sequencing of TCRs using Vβ3 and 8 demonstrated that, in contrast to the result in donor 6, identical clones were indeed present in both the ileum and colon (Table 3).

Discussion

Previous studies demonstrated that $CD8^+$ iIELs using the TCR-α/β from normal human small intestine and colon were oligoclonal (7–9). The distribution of these clones, whether each was concentrated in one small section of the intestine or whether they were more widespread, was not determined. To address this question, iIELs were isolated from normal appearing mucosa near the proximal and distal margins of intestinal resections in a series of patients. T cell clones in these iIELs were identified and compared by semiquantitative PCR amplification, determination of N-region length and DNA sequencing of their TCR-β chains. These studies revealed identical dominant TCR-β chains in the proximal and distal iIEL samples in all but one donor. This demonstrates that particular dominant iIEL clones are widespread, as the incorporation of many N-region additions makes it unlikely that these TCR-β chains represent invariant rearrangements in distinct clones associated with multiple different TCR-α chains. The wide distribution of dominant clones indicates that a relatively small number of T cells undergo dramatic clonal expansion, presumably in response to one or a limited number of intestinal antigens.

The iIELs in this study were isolated from various portions of the small intestine and colon (see Table 1). In every sample studied there was evidence of oligoclonal expansion, with no striking differences in the approximate number of clones present. The data from the colon in Tables 2 and 3 (donors 2, 6, and 7) indicate that one or two clones comprised the majority of T cells using particular Vβs in most cases. This is similar to the degree of diversity in the small intestine observed in this report and previously (7, 9). The similarity in TCR diversity is in contrast to the markedly different antigenic environments in the lumen of the small

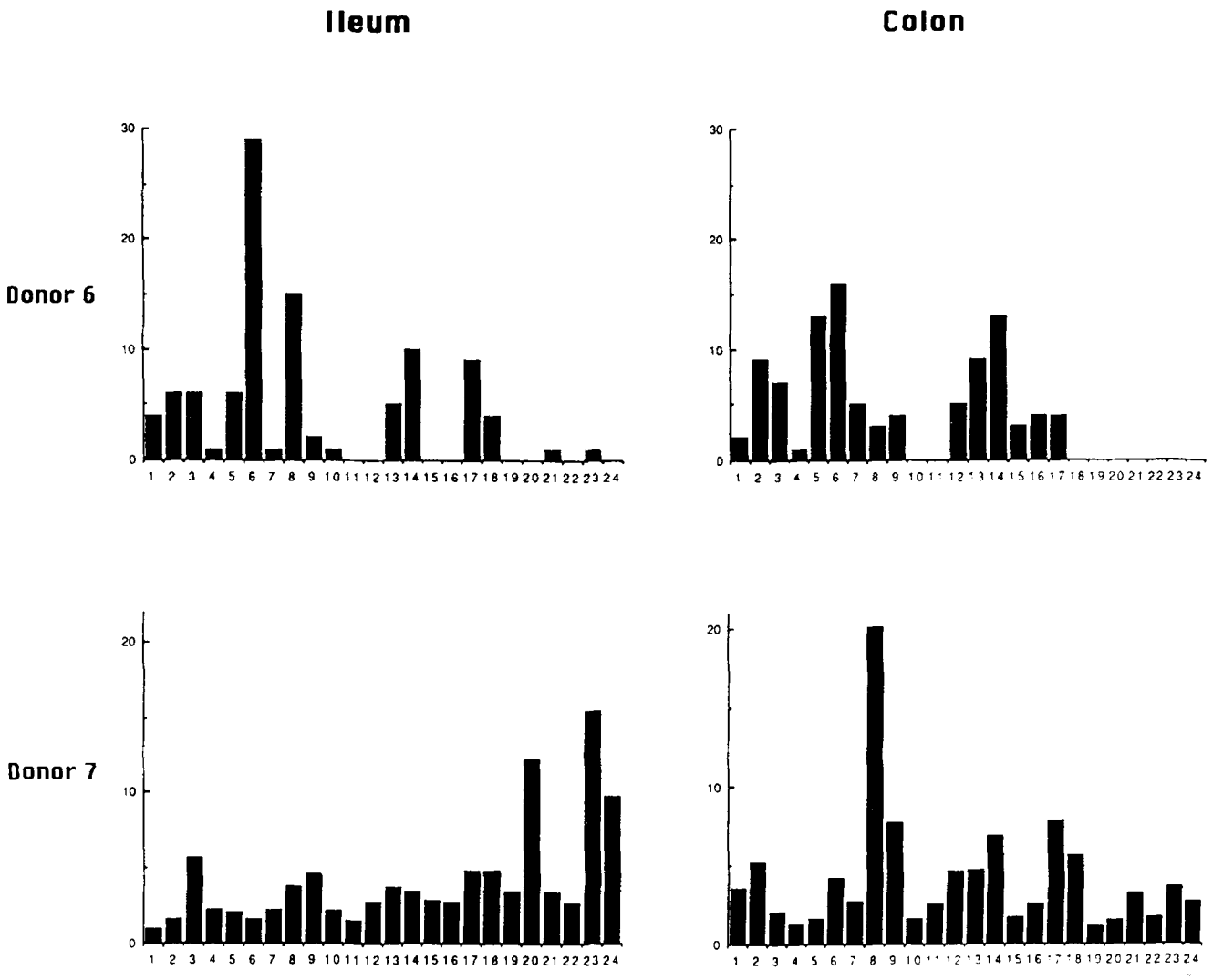

Figure 4. Semiquantitative PCR amplification of iIELs from the small intestine and colon. Two cases in which the proximal iIELs were from the ileum and the distal were from the transverse colon were analyzed.

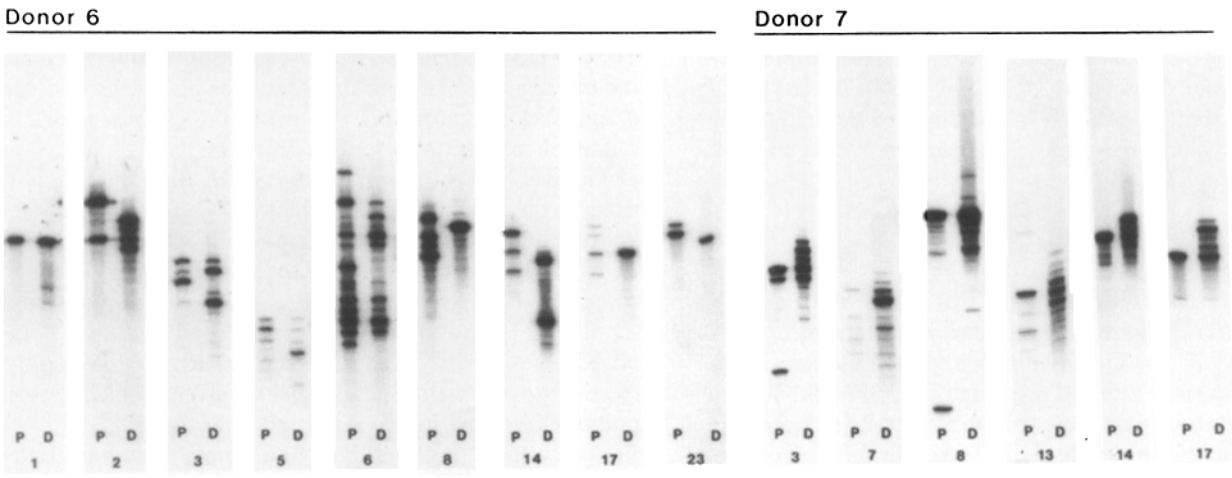

Figure 5. N-region length analysis of iIELs from donors 6 and 7. *P*, ileum; *D*, colon.

Table 3. *Sequence Analysis of ilELs from Ileum and Colon*

Donor	V	N	J	Proximal	Distal	F
6	Vβ1.1-CASS	RLAEY	YNEQFFG-Jβ2.1	3/4	0/5	+
	Vβ1.1-CASS	LGLR	NTGELFFG-Jβ2.2	0/4	5/5	+
	Vβ2.3-CSA	RDPQRGV	AKNIQYFG-Jβ2.4	4/5	0/5	+
	Vβ2.1-CSAS	IGGET	QYFG-Jβ2.5	0/5	2/5	+
7	Vβ3-CAS	TLAGI	YNEQFFG-Jβ2.1	6/6	4/5	+
	Vβ8-CASS	SLFGLGLD	EQFFG-Jβ2.1	5/6	5/6	+

All sequences identified more than once in either the ileum or colon are reported. Amino acid sequences are in single letter code. TCRs inframe (+) or not inframe (−) are indicated.

intestine versus the colon. This suggests that iIELs do not recognize primarily luminal antigens and supports the hypothesis that they recognize antigens synthesized by epithelial cells. The expression of such hypothetical epithelial target antigens may be due to infection or other stresses or could be developmentally programmed to insure the rapid turnover of these cells.

Recognition of antigens synthesized by epithelial cells is also consistent with the $CD8^+$ phenotype of most iIELs, which suggests that these cells recognize endogenous antigens presented by MHC class I or class I–like molecules. A minority of iIELs express CD4, however, and TCR expression by $CD4^+$ iIELs has not been studied specifically. Although predominant clones were readily detected in the iIEL preparations from donors 3 and 6, which contained up to 40% $CD4^+$ cells, it is possible that the $CD4^+$ T cells in these preparations were polyclonal. Analysis of purified $CD4^+$ iIELs is underway to determine if these cells are similar to $CD8^+$ iIELs or are polyclonal, the latter being consistent with recognition of diverse luminal antigens.

In two cases it was possible to examine iIELs from the small and large intestine of the same patient. In one such case, there was no overlap in the iIEL populations from the terminal ileum and transverse colon (donor 6). In the second case where cells from the small intestine and colon were compared (donor 7), several identical clones were found. The resections in both of these patients were for moderately differentiated adenocarcinoma and the iIEL samples in each case were from the terminal ileum and transverse colon. These results demonstrate that iIEL clones in the small intestine and colon may be nonoverlapping in some, but not all patients, and emphasize the potential variability when dealing with human pathology. Further studies of IELs from the small intestine versus the colon in patients with malignant and nonmalignant pathology should be informative. It will also be of interest to analyze IELs from other normal tissues to determine whether oligoclonal expansion is a common theme (12, 13) and whether there is overlap in T cell clones from other mucosal surfaces.

Monoclonal or oligoclonal T cell expansion occurs in some T cell populations from the peripheral blood of normal donors, including $CD4^-8^-$ (double negative) α/β T cells (11, 14–16), γ/δ T cells using the Vδ1 (17), or Vγ9 genes (18), and $CD8^+CD28^-$ α/β T cells in older donors (19). Similar to iIELs, these cells are presumably responding to chronic stimulation by a small number of pathogens or autoantigens. Although these cells have been detected in the peripheral blood, they may carry out their biological function in particular tissues. Candidate tissues include the skin for $CD4^-CD8^-$ α/β T cells (12, 13) and the colon for T cells using Vδ1 (6). The $CD8^+CD28^-$ TCR-α/β phenotype of the oligoclonal cells found in older donors is identical to the phenotype of most iIELs, which suggests that these cells in the peripheral blood may be derived from IELs in the intestine or other mucosa.

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