Oligoclonality of Human Intestinal Intraepithelial T Cells

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Summary

T cells bearing the T cell receptor α/β (TCR-α/β) are the predominant lymphocyte population in the human intestinal epithelium. To examine if normal intestinal intraepithelial lymphocytes (IEL) have a TCR repertoire distinct from the TCR-α/β repertoire in peripheral blood lymphocytes (PBL), comparative analysis of relative Vβ gene usage in IEL and PBL was performed by quantitative polymerase chain reaction. In each of the six individuals examined, one to three Vβ families made up more than 40% of the total Vβ transcripts detected in the IEL, whereas there was a more even distribution of Vβ gene usage in the paired PBL. The predominant Vβ families, especially Vβ1, Vβ2, Vβ3, and Vβ6, were frequently shared among IEL of different individuals. PCR cloning and sequence analysis of the predominant Vβ6 family in two individuals revealed an identical V-D-J-C sequence in 13 of 21 clones obtained from one donor, and a different repeated sequence in 18 of 27 clones examined in the second donor. These data suggest that the Vβ skewing in IEL is due to an oligoclonal T cell expansion and may reflect the response of the intestinal mucosal immune system to a restricted set of as yet undefined antigens present in the gut.

Little is known about the function of lymphocytes at epithelial sites. Intestinal intraepithelial lymphocytes (IEL)[1] are a predominantly $CD4^-8^+$ subset of T cells localized throughout the epithelial lining of the gut (1). In the mouse, the majority of lymphocytes at epithelial surfaces such as the intestine use the TCR-γ/δ. Murine γ/δ IEL express two major TCR Vγ/Vδ pairs (Vγ5/Vδ4,6) and have extensive junctional diversity. As in the mouse, human TCR-γ/δ cells preferentially localize within the gut epithelium rather than in the lamina propria. However, the dramatic numerical epithelial predominance of γ/δ cells in mice (50–75% of the total IEL population) has not been observed in humans, where γ/δ T cells make up ~10% (range, 5–20%) of small bowel IEL and, according to one recent report, 37% (range, 13–87%) of large intestine IEL (2, 3). Thus, the majority of T cells in the adult human gut epithelium express the TCR-α/β.

The peripheral TCR-α/β repertoire is the result of a complex selection process in the thymus involving the recognition of self-MHC molecules (4). Alternatively, intestinal epithelial cells may share some differentiation-inducing capacities with thymic epithelial cells, leading to in situ TCR rearrangements on extrathymically derived IEL, as suggested by recent evidence of an extrathymic pool of TCR-α/β IEL in the murine gut (5). In addition to thymic and extrathymic selection, a skewed TCR-α/β repertoire may result from microbial or food antigen–driven expansion of intestinal lymphocytes. Evidence for the importance of TCR-α/β IEL in response to intestinal microorganisms is found in mice, where colonization by normal intestinal flora has little effect on TCR-γ/δ IEL, but sharply increases the number of TCR-α/β IEL (6). These findings suggest that T cells bearing TCR-α/βs may play the predominant immunological role in the human gut epithelium.

Here, the nature of the TCR repertoire of the predominant α/β receptor in the human gut was examined. We found evidence for skewed Vβ gene usage and oligoclonality as major characteristics of this T cell population.

[1] *Abbreviations used in this paper:* IEL, intraepithelial lymphocytes; LPL, lamina propria lymphocytes.

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Materials and Methods

Cell Isolation. Human colon samples and paired PBL were obtained from adult patients undergoing surgical resection for colorectal carcinomas and, in one individual, for familial polyposis coli. Small bowel tissue was obtained from a normal individual after a terminal traumatic event. IEL and lamina propria lymphocytes (LPL) were isolated from intestinal sections at least 10 cm away from any macroscopically detectable lesions, as previously described (3). Four of the five colon samples were obtained from German donors; the fifth colon sample as well as the small bowel sample were obtained from American subjects.

Monoclonal Antibodies. Freshly isolated cells were stained with mAbs Leu-4, Leu-3, and Leu-2a (anti-CD3, anti-CD4, and anti-CD8α, respectively; Becton Dickinson & Co., Mountain View, CA), BMA031 (anti-TCR-α/β; Behringwerke, Marburg, Germany), βF1 and TCR-δ1 (anti-TCR-α/β and anti-Cδ; T Cell Sciences, Cambridge, MA), HML-1 (directed to a molecule expressed on many epithelial lymphocytes, including 90% of IEL [1]), and 2ST8.5H7 (anti-CD8β [7]). mAbs LC4 (anti-Vβ5.1), 1C1 (anti-Vβ5.2 and Vβ5.3), 16G8 (directed to members of the Vβ8 family), S511 (directed to members of the Vβ12 family), and OT145 (anti-Vβ6.7a [8]) were obtained from T Cell Sciences.

Immunofluorescence and Flow Cytometry. Flow cytometric two-color analyses, carried out as described (9), showed that α/β T cells made up 92% or more of the $CD3^+$ cells in PBL, 77–98% in LPL, and 92–27% in IEL (32% in donor 1, 87% in donor 2, 27% in donor 3, 79% in donor 4, 92% in donor 5, and 88% in donor 6). Cell preparations were adequately pure, since the fraction of $HML\text{-}1^+$ cells was >90% or <0.5% in all IEL and PBL samples, respectively, and the CD4/CD8 ratio was <0.1 in IEL, >2.2 in LPL, and >1.6 in PBL.

Immunohistochemistry. Frozen sections of colon tissue from donor 2 were stained by the avidin-biotin complex method as reported (10). Reliability of the staining pattern for the different mAbs was monitored by staining of human thymus, tonsil, and lymph node. Quantitation of stained IEL was performed by differentially counting the positive lymphocytes and the epithelial cells in a blinded fashion, and was expressed as the number of stained lymphocytes/100 epithelial cells. More than 1,000 epithelial cells were counted in each tissue section stained. The exact binomial 95% confidence interval around the observed values was calculated, assuming uniform and random distribution of lymphocytes in adjacent tissue slices. Quantitation of stained lymphocytes in the tonsil sections, which included both T cell areas and B cell follicles, was performed by counting all positively stained lymphocytes with a reticular grid and calculating the results as the number of cells per mm^2.

Polymerase Chain Reaction. Isolation of total RNA, cDNA synthesis (from ~3 μg of total RNA), and PCR reactions were performed as described (9). Oligonucleotide primers included a panel of 22 Vβ-specific oligomers (corresponding to 20 Vβ families analyzed) and one of two antisense oligomers from the downstream Cβ region. Combined, these oligonucleotides have been shown to detect ~90% of the human Vβ genes (11). Sense and antisense Cα primers (5'CCAGAACCCTGACCCTGCCGTG 3' and 5'TATGGATCCGAGGGAGCACAGGCTGTCTT 3') were used to amplify and quantitate total Cα cDNA in all samples. cDNA samples were diluted before quantitative PCR amplification so that comparable amounts of Cα, as well as a linear phase of Cα and Vβ amplification, were obtained after 28 cycles in each sample. For each individual, analysis of PBL, LPL, and IEL were performed simultaneously and under identical conditions. PCR products were size separated on a 2% agarose gel, blotted onto Hybond-N (Amersham Corp., Arlington Heights, IL) membranes, and hybridized with γ-^{32}P-labeled internal Cβ probes (9), as well as an internal Cα probe (5' TTTAGAGTCTCTCAGCTGGTA 3'). Results were visualized by autoradiography and quantified directly with a Betascope blot analyzer (Betagen Corporation, Waltham, MA). Most samples were analyzed at least twice, and results showed minimal variations.

Validation of Quantitative PCR Analysis. Quantitative PCR analyses were validated as described (9). In addition, the percentage of TCR-α/β cells (mAb $BMA031^+$) bearing Vβ5.1, Vβ5.2/3, Vβ8, and Vβ12 was determined in several PBL, LPL, and IEL samples by two-color FACS® (Becton Dickinson & Co.) analyses using Vβ-specific mAbs. Results correlated well with the quantitative PCR Vβ values, expressed as a percentage of the sum of all Vβ transcripts measured. For example, the LPL sample of donor 3 showed the following results (mAb staining vs. quantitative PCR): Vβ5.1, 4.9% vs. 5.4%; Vβ5.2/3, 3.0% vs. 2.9%; Vβ8, 7.7% vs. 7.0%; Vβ12, 4.0% vs. 1.5%. Quantitative PCR results throughout the study are therefore expressed as: percentage Vβ = 100× (hybridization to one Vβ-specific PCR product/sum of all Vβ-specific hybridizations); except in Fig. 2, where Vβ and Cα were coamplified within the same tube, and Vβ usage was determined relative to the total Cα expressed, using the formula: percentage Vβ = 100× (hybridization to one Vβ-specific PCR product/total Cα-specific hybridization.

Sequence Analysis of PCR-amplified PCR Vβ6 Transcripts. Vβ6 family-specific PCR amplification of cDNA from IEL and paired PBL of donor 1 and from IEL of donor 2 were performed. To ensure the amplification of all members of the Vβ6 family, three Vβ6 subfamily-specific oligonucleotides were used as sense primers in equal concentrations (Vβ6.1/2/3, 5' GACAGGCCTGAGGGATCCGTCTC 3'; Vβ6.6/7, 5' GACAGGACTGGGGGATCCGTCTC 3'; and Vβ6.5/8/9, 5' GACAGGCCTAAGGGATCTTTCTC 3'. The amplified products were directionally cloned into M13 vectors and sequenced by the dideoxy chain termination method as previously reported (9).

Results and Discussion

Samples from five individuals were available for comparative study of PBL and colon-derived IEL and LPL. Surprisingly, an unequal expression of Vβ families was detected in the IEL. Vβ6 dominated in donor 1 (39%), in donor 2 (19%), in donor 3 (19%), and in donor 4 (23%). In addition to Vβ6, prominent Vβ families were Vβ2 and Vβ3 in donor 2 (15% each), Vβ3 in donor 3 (20%), and Vβ1 in donor 4 (15%). In a fifth donor, Vβ2 (27%) and Vβ3 (25%) again predominated while Vβ6 was not increased (Fig. 1, solid bars). Thus, in each donor, one to three Vβ families predominated and accounted for a mean of 43% of the total Vβ transcripts detected. This contrasted with the PBL, where Vβ expression was more evenly distributed over the different Vβ families (Fig. 1, hatched bars), in percentages similar to those shown in previous studies of PBL (9, 11). Interestingly, the predominant Vβ families (Vβ1, Vβ2, Vβ3, and Vβ6) in the IEL were shared by different individuals. Besides the major increases in Vβ1, Vβ2, Vβ3, and Vβ6, small increases of Vβ products in IEL compared with PBL were also noted, including Vβ5.1 and Vβ19 in donor 1, and Vβ10 in donor 5 (Fig. 1). Vβ13.1 was high in several IEL samples, but was also a substantial percentage of the total Vβ transcripts in the paired PBL samples. The Vβ repertoire of LPL closely

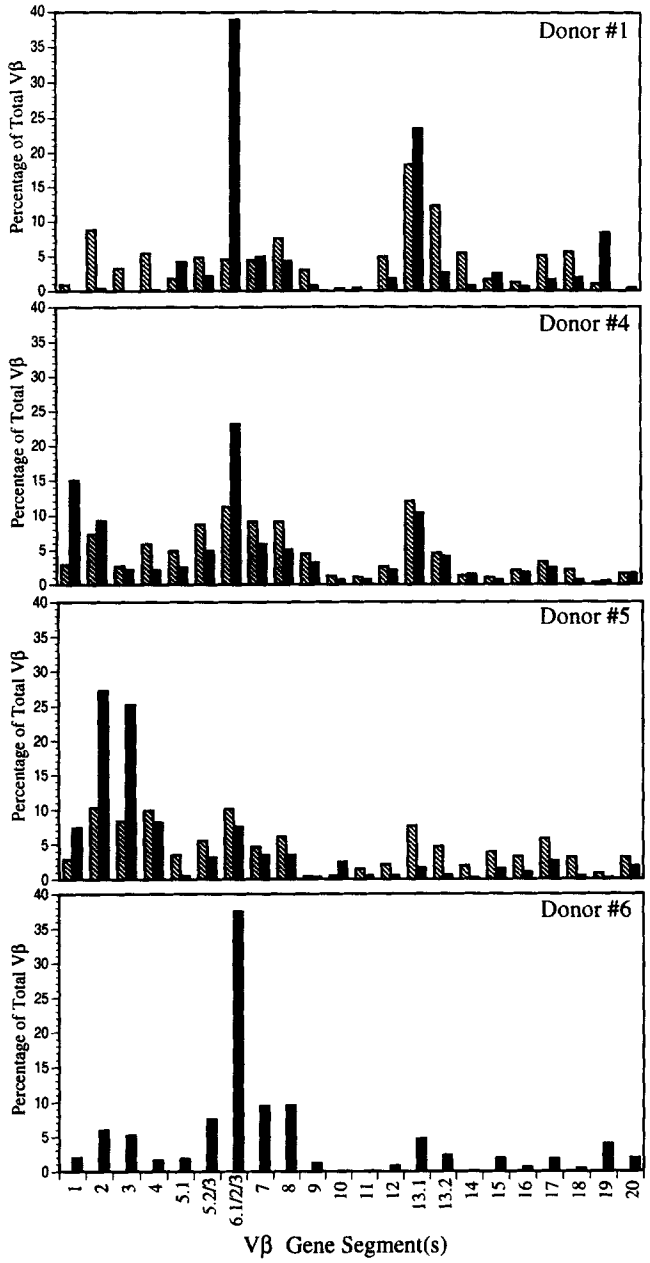

Figure 1. Skewed Vβ gene segment usage in human IEL. Relative Vβ gene usage in freshly isolated colon-derived IEL (*solid bars*) and paired PBL (*hatched bars*) from donors 1, 4, and 5, and in freshly isolated jejunum-derived IEL from donor 6. Vβ family (member) usage was determined by quantitative PCR and expressed as a percentage of the sum of total Vβ measured, as outlined in Materials and Methods. The axis represents the entire panel of TCR Vβ gene segment(s) measured.

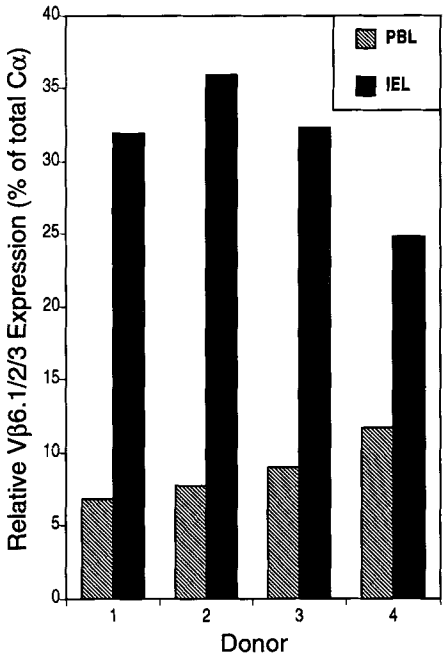

Figure 2. Vβ6.1/2/3 usage, expressed as a percentage of total Cα measured, is increased in IEL compared with paired PBL. PCR analysis of relative Vβ6.1/2/3 expression in freshly isolated colon-derived IEL (*solid bars*) and paired PBL (*hatched bars*) in donors 1–4. Vβ6.1/2/3 and total Cα products were coamplified by mixing Vβ6.1/2/3-Cβ and Cα-Cα primer pairs within the same tube. The obtained products (~220 and ~470 bp, respectively) were size separated and hybridized to internal Cβ and Cα probes, as described in Materials and Methods.

matched that of the PBL or showed results intermediate to those of IEL and PBL (data not shown). This finding is consistent with the previously reported polyclonality of LPL (12).

Since the above results were expressed as a percentage of the sum of all Vβ transcripts detected, the possibility existed that the observed predominance of Vβ1, Vβ2, Vβ3, and Vβ6 in IEL was an apparent increase, secondary to the presence of Vβ families not detected by the panel of Vβ primers used.

The measurement of total Cα would include transcripts from all α/β T cells present, including those whose Vβ genes might not have been detected. Therefore, additional experiments using quantitative PCR compared the Vβ transcripts relative to the total Cα transcripts obtained under identical conditions for IEL and PBL samples. In a representative experiment, Vβ6 and total Cα products from IEL and PBL populations of donors 1–4, coamplified by mixing the Vβ6.1/2/3-Cβ and the Cα-Cα primer pairs within the same tube, were compared. In contrast with the PBL populations where Vβ6.1/2/3 transcripts constituted 7–12% of the total Cα transcripts, the proportion of Vβ6.1/2/3 in the IEL was 25–36% of the total Cα expressed (Fig. 2). Thus, whether the relative quantities of Vβ products detected by PCR were compared with the sum of all Vβ products measured, or to the total amount of TCR Cα transcripts, Vβ1, Vβ2, Vβ3, and Vβ6 families were found to be increased in IEL compared with PBL.

In addition to the colon-derived IEL populations studied above, a small bowel preparation from a healthy donor in whom no PBL were available for comparison was also examined. Unequal expression of Vβ gene segments in the jejunum-derived IEL was again noted, with Vβ6 (38%) as the predominantly expressed Vβ family (Fig. 1, donor 6). Analysis of a jejunum-derived IEL cell line from another healthy donor maintained in culture for 4 wk was also performed. This line expressed 80% HML-1$^+$ cells and >90% CD4$^-$8$^+$ cells at

	Vβ	N	Dβ1.1	N	Dβ2.1	N	Jβ	Cβ	frame	#
			gggacaggggc		gggactagcgggaggg					
					g					
a IEL donor #1										
6.2/3	AGC	TTACACGT	GACAGGG			CGTAGAAA	1.1(-1)	1	+	13
6.4	AGC	CCC	GGGACAGGG			AACG	1.5(-2)	1	+	1
6.5/8/9	AGC	GC	GACAGGG			TTTC	1.2(-3)	1	+	1
6.5/8/9	AGC	GCAT	CAGGGG	CGTATAT	GAGG		2.5(-5)	2	+	1
6.5/8/9	AGC	TTA			GAGG	A	2.7(-3)	2	+	1
6.5/8/9	AGC	TCAACG			CTAGCGGGAGG		2.5(-4)	2	+	1
6.5/8/9	AGC	CCG	CAGGG			CGAAGATTC	1.2(-6)	1	-	1
6.6/7	AGC	TCGG					2.1(-5)	2	+	1
6.6/7	AGC	CCAA	GGACAGGG				2.5(-2)	2	+	1
6.6/7	AGC	T			GGACTAGCG	AGAG	2.1(-6)	2	+	1
b PBL donor #1										
6.2/3	AGC	TTAGTT	CAGGG	TTTA	GGACT	T	2.5(-2)	2	+	1
6.2/3	AGC	TTAGATTA					2.2(-7)	2	+	1
6.2/3	AGC	CCCC	GGGACAG			T	2.1(-4)	2	+	1
6.4	AGC	TTAAG			GGGAG	AAGC	2.3(-2)	2	+	1
6.4	AG	TC			GCGGGA	CGATAG	2.7(-2)	2	+	1
6.4	AGC	TT	GGGGC			AGA	2.7(-2)	2	+	1
6.5/8/9	AGC	TCCGCCCTATTTTATACT			CGGG	TCCACGCTTTTTT	2.1(-3)	2	+	3
6.5/8/9	AGC	GTC			GGAC	ATTTTA	2.1(-2)	2	+	1
6.5/8/9	AGC	TTAGCGGCCCC	GGGACAGG			TGGCG	1.5(-7)	1	+	1
6.5/8/9	AGC	TTAGTT	AGGG			AAATCA	1.2(-9)	1	+	1
6.5/8/9	AGC	CATTTCCTAACGTT			CGGG		2.7(-6)	2	-	1
6.5/8/9	(-8)GTCGTGG				GGGAGG	T	2.7	2	+	1
6.6/7	AG	TCAC	GGACAGGG			AC	1.6(-8)	1	+	1
6.6/7	AGC	TTGACCGG			AGCGGGAGG	AGGTGG	2.1(-9)	2	+	1
6.6/7	AGC	TTATCC			GGGACTAGCGGGAGG	TTCTTT	2.7(-4)	2	+	1
6.6/7	AGC	T					2.5	2	+	1
6.6/7	AGC	ACAA	GACAGG			CG	2.1(-1)	2	+	1
c IEL donor #2										
6.1	AGC	TT			GGGGG	TCCCCCTG	2.7(-5)	2	-	1
6.1	AGC	TTAG			GGGGGGG	TCC	2.5(-1)	2	+	1
6.2/3	AGC	CAAG			TAGCGGGG	AGT	2.3(-3)	2	+	2
6.2/3	AGC					GCCCCTTGG	2.2(-5)	2	+	1
6.2/3	AGC	TTAGAGTCT	CAGG			A	2.7(-3)	2	+	2
6.4	AGT	CTA			GGACTAGCGG	A	2.1(-4)	2	+	1
6.5/8/9	AGC	AGCAC			GACTAGCGGGGGGG		2.3(-4)	2	+	1
6.5/8/9	AGC	TT	GGGACAGGG			CC	1.1	1	+	1
6.6/7	AGC	TTAGG			GGGGGGG	CCA	2.5(-2)	2	+	18

d	Vβ	D-J	Cβ
donor #1	C-A-S-S-	L-H-V-T-G-R-R-K-N-T-E-A-F-F-G-Q-G-T-R-L-T-V-V-	E-D-L-N-K-
donor #2	C-A-S-S-	L-G-G-G-P-Q-E-T-Q-Y-F-G-P-G-T-R-L-L-V-L-	E-D-L-K-N-

Figure 3. TCR Vβ6 junctional nucleotide sequences derived from PCR-amplified cDNA from IEL (*a*) and PBL (*b*) of donor 1, IEL of donor 2 (*c*), and the corresponding amino acid sequence of the junctional regions (*d*) of the predominant IEL clone in donors 1 and 2. Comparable amounts of cDNA from each source were PCR amplified, using a combination of Vβ6.1/2/3, Vβ6.5/8/9, and Vβ6.6/7 sense primers in equal concentrations. Obtained sequences are grouped according to Vβ6 family member usage. Family members Vβ6.2 and 6.3; Vβ6.5, 6.8, and 6.9; and Vβ6.6 and 6.7 cannot be distinguished based on the sequences obtained (25). The number of clones carrying an identical sequence are shown on the right. Numbers in parentheses indicate the amount of nucleotides deleted from the germline sequences. A + or − sign indicates whether sequences are in or out of frame, respectively. The germline Dβ1.1 and Dβ2.1 are shown at the top (22). D segments were assigned arbitrarily, based on the presence of four or more nucleotides colinear with germline Dβ sequences. In two instances, Dβ1.1 and Dβ2.1 appeared to be used in tandem. In donor 1, the predominant IEL clone used Vβ6.2 (or 6.3) rearranged to Jβ1.1/Cβ1. The predominant IEL clone in donor 2 used Vβ6.7 rearranged to Jβ2.5/Cβ2. In contrast with the clonal dominance in both IEL samples, the paired PBL sample of donor 1 contained few repeats.

the time of analysis, and Vβ6 (31%) and Vβ8 (25%) made up the majority of Vβ expressed (data not shown). Thus, skewing of the TCR-α/β repertoire relative to that in PBL was noted both in small and large bowel IEL, in samples from healthy donors as well as macroscopically normal bowel obtained from patients with malignant or premalignant lesions, and irrespective of the proportion of γ/δ cells present (8–73%). Furthermore, previous studies have shown that Vβ1, Vβ2, Vβ3, Vβ6, and Vβ8 were not expressed at higher levels in the $CD4^-8^+$ compared with the $CD4^+8^-$ PBL subsets (9, 13). Thus, the Vβ TCR skewing of IEL appears to be characteristic for the IEL in the gut rather than merely CD8 phenotype related.

Vβ families have been classified into two clusters, based on structural characteristics, including the ability of members of cluster I, but not cluster II, to form a salt bridge between the amino acids at positions 64 and 86. With the exception of Vβ3, each of the predominant human IEL families (Vβ1, Vβ2, and Vβ6), as well as Vβ8, which was found to be prevalent in the IEL cell line, are members of cluster I. Although other members of cluster I were not increased in human IEL, these data suggest some similarity with the situation in the chicken, where only Vβ gene products of cluster I are expressed by intestinal lymphocytes (14).

To assess whether the predominant Vβ families in the IEL population were expressed by clonal, oligoclonal, or polyclonal cell populations, the nucleotide sequences of randomly isolated cDNA clones from PCR-amplified material of the most predominant Vβ family, Vβ6, were determined in two IEL and one PBL sample. Surprisingly, a clonal population

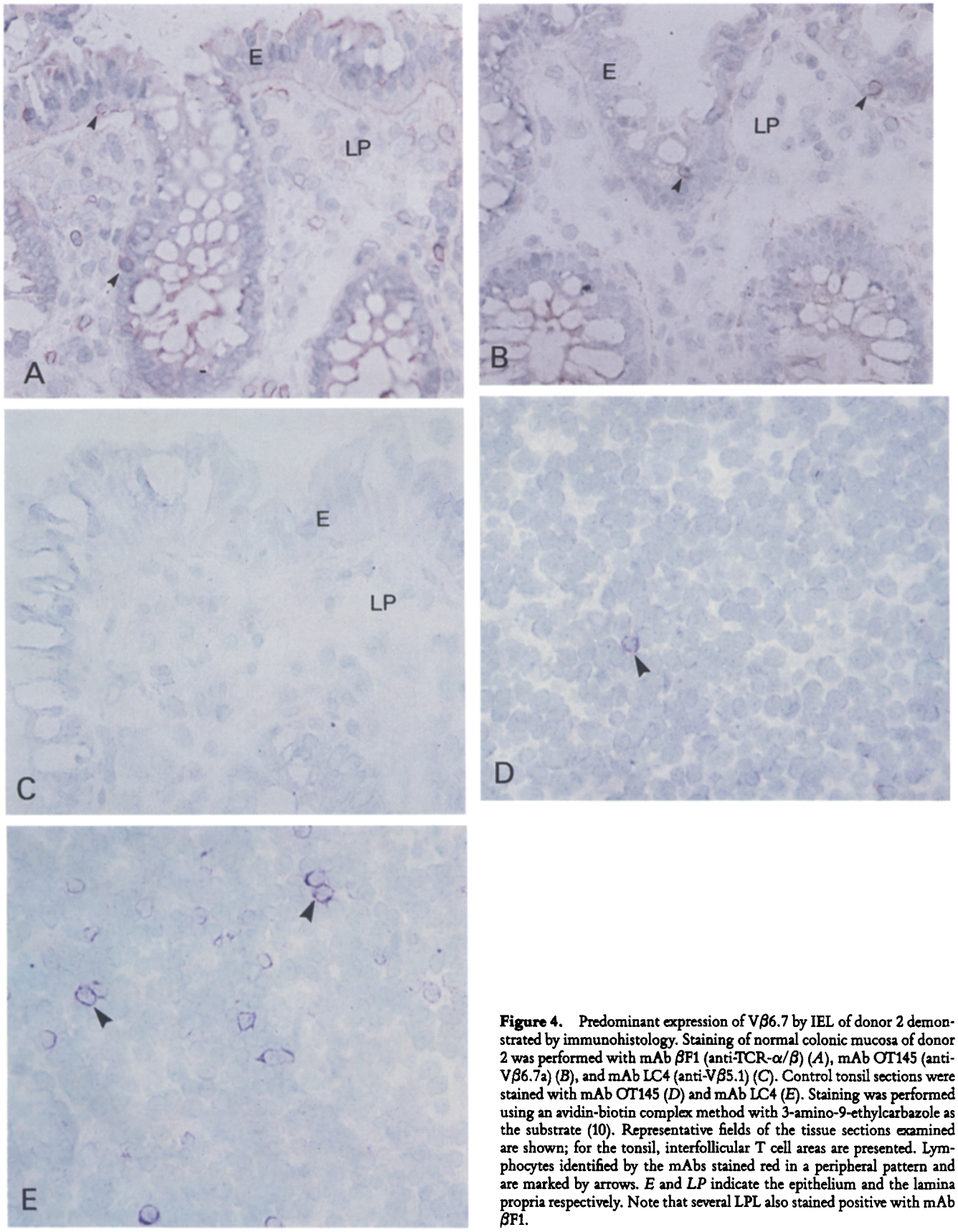

Figure 4. Predominant expression of Vβ6.7 by IEL of donor 2 demonstrated by immunohistology. Staining of normal colonic mucosa of donor 2 was performed with mAb βF1 (anti-TCR-α/β) (*A*), mAb OT145 (anti-Vβ6.7a) (*B*), and mAb LC4 (anti-Vβ5.1) (*C*). Control tonsil sections were stained with mAb OT145 (*D*) and mAb LC4 (*E*). Staining was performed using an avidin-biotin complex method with 3-amino-9-ethylcarbazole as the substrate (10). Representative fields of the tissue sections examined are shown; for the tonsil, interfollicular T cell areas are presented. Lymphocytes identified by the mAbs stained red in a peripheral pattern and are marked by arrows. *E* and *LP* indicate the epithelium and the lamina propria respectively. Note that several LPL also stained positive with mAb βF1.

was found in both of the IEL samples. Of 21 in-frame sequences of donor 1, 13 sequences were identical, as were 18 of 27 in-frame sequences of donor 2 (Fig. 3). In contrast, the paired PBL sample of donor 1 showed a polyclonal population, with few repeats in the 19 clones sequenced. Thus, the Vβ skewing in adult IEL appears to be caused by an oligoclonal T cell expansion. The predominant clones in the two IEL populations were different, as Vβ6.2 (or 6.3, as sequences are identical in the region obtained) rearranged to Jβ1.1/Cβ1 in donor 1, and Vβ6.7 (6.7a, 6.7b, or 6.6) rearranged to Jβ2.5/Cβ2 in donor 2 (Fig. 3). Vβ6.2/3 and Vβ6.7 differ in their first and second complementarity determining regions, as well as in the region shown to confer superantigen reactivity (15). The difference in Vβ6 family member usage between the two individuals may thus reflect the interaction of these Vβ products with distinct peptide/MHC complexes, or with distinct superantigens. Furthermore, the dissimilar junctional amino acid sequences and extensive junctional N segment insertions and germline nucleotide deletions of the clonally expanded IEL TCR-β sequences in the two donors suggest that the expanded IEL, unlike the murine dendritic epidermal cells, are unique in each individual and are not derived from an early fetal stage (2, 16). Regarding the origin of adult TCR-α/β IEL, two-color staining with CD8 mAbs revealed that the majority (70–95%) of the TCR-α/β IEL expressed CD8-α/β heterodimers (data not shown). Thus, if the type of CD8 co-receptor is indicative of the origin of IEL in humans, as has been suggested in the mouse (5), the majority of adult human TCR-α/β IEL studied here appear to be thymus derived.

Some evidence for oligoclonality in tumor-infiltrating lymphocytes of nonintestinal tumors has been reported (17, 18). A relationship between the oligoclonality observed here and the presence of colorectal carcinoma in some of the patients cannot be totally excluded. However, the findings of Vβ skewing in IEL of healthy donors, the previously reported absence of oligoclonality in LPL of patients with colorectal carcinoma (12), and the substantial distance between the malignant lesions and the sections studied, make a causal relationship unlikely.

We were able to confirm the predominant expression of Vβ6.7 by IEL of donor 2 by performing immunohistochemistry using a Vβ6.7a-specific mAb (8). Staining of large bowel tissue of donor 2 with the Vβ6.7a-specific mAb was compared with staining with Vβ5.1, Vβ5.2/3, Vβ8, Vβ12, as well as TCR-β and CD3-specific mAbs. On average, 2.6 lymphocytes/100 epithelial cells and 2.8 lymphocytes/100 epithelial cells stained with βF1 and Leu-4 mAbs, respectively, consistent with the expected number of IEL in the large bowel (Fig. 4 *A*). Significantly, an average of 1.2 lymphocytes/100 epithelial cells or ~44% of the TCR-α/β IEL stained with the Vβ6.7a-specific mAb (95% confidence interval, 0.25–0.65), confirming the predominance of Vβ6.7 in this IEL sample (Fig. 4 *B*). In contrast, no IEL were identified in an area of >1,000 epithelial cells after staining with Vβ5.1- (Fig. 4 *C*), Vβ5.2/3-, Vβ8-, and Vβ12-specific mAbs. The opposite result was seen in control tonsil tissue, where the Vβ6.7 mAb identified proportionally fewer cells than did the other Vβ-specific mAbs; for instance, 53 lymphocytes/mm^2 and 158 lymphocytes/mm^2 were identified after staining with the Vβ6.7a mAb and the Vβ5.1 mAb, respectively (Fig. 4, *D* and *E*). The predominant staining of large bowel tissue of donor 2 with the Vβ6.7a-specific mAb also contrasted sharply with the two-color FACS® analysis of his paired PBL sample, which showed that only 3% of the TCR-α/β cells stained positive with the Vβ6.7a mAb. This is an intermediate level of Vβ6.7a usage in normal adult PBL (8, 13).

Based on the data obtained here, we suggest that intestinal IEL, which are known to be CD45RO$^+$ (19), may be predominantly stimulated by conventional antigens, resulting in clonal expansion of the antigen-specific T cells. In vivo and in vitro, preferential expression of one Vβ/Jβ and Vα/Jα product with limited heterogeneity in the junctional regions has been reported for T cells specific to cytochrome *c*, myelin basic protein, and myoglobin (20–22). Moreover, in long-term cultures of TNP-specific cytotoxic T cells, nearly half of the clones were found to use identical Vβ chain gene segments including the V-D-J junctional region (23). Thus, continuous stimulation by a small number of microbial antigens or self–stress antigens on intestinal epithelial cells might similarly lead to clonal proliferations of IEL. Whether antigen presentation predominantly occurs in Peyer's patches, from where lymphoblasts recirculate to the intestinal epithelium (1), or in the epithelium itself, by intestinal epithelial cells (24), remains to be determined.

While superantigens are likely to contribute to the adult IEL repertoire, they would not alone be expected to result in the oligoclonal population detected here. Rather, they might give rise to a polyclonal population with high junctional diversity while carrying the same Vβs (11, 15). However, a combination of superantigen-driven and conventional antigen-specific clonal expansions may result in the observed Vβ family predominance and oligoclonality of human IEL.

In conclusion, this study demonstrates the existence of a site-specific and oligoclonal TCR-α/β repertoire in human gut epithelium. The oligoclonality of IEL points to the presence of a restricted set of potent antigens in the gut that may be the ligands involved in the expansion of these T cells. Thus, the oligoclonality of IEL may be of major importance in providing an efficient immune response against these antigens.

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